Gordon Gordh
Simon McKirdy *Editors*

The Handbook of Plant Biosecurity

Principles and Practices for the Identification, Containment and Control of Organisms that Threaten Agriculture and the Environment Globally

Springer

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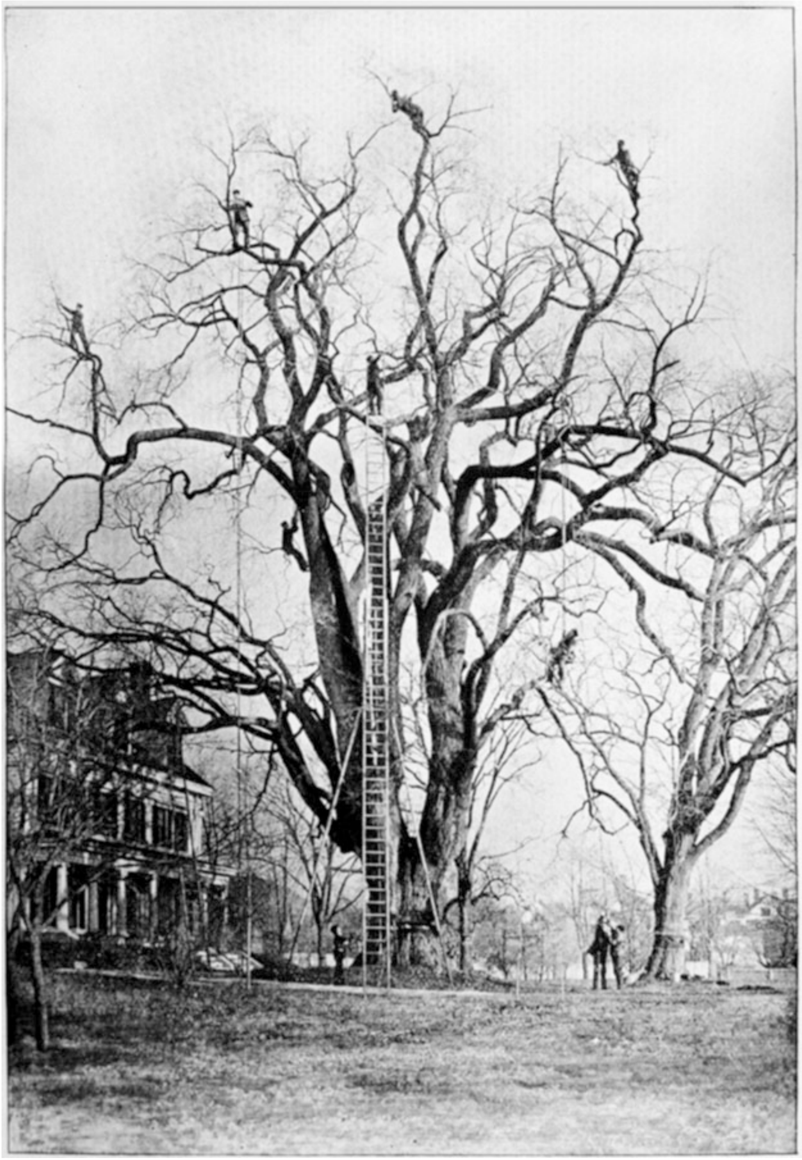

During 1893, European Gypsy Moth inspectors search for egg masses on an enormous elm tree at the Dexter mansion in Malden, Massachusetts. About 25 years earlier, EGM was purposefully introduced into the USA, escaped culture and continues to be a calamitous forest pest (Forbush and Fernald 1896. The Gypsy Moth. A report of the work of destroying the insect in the Commonwealth of Massachusetts, together with an account of its history and habits both in Massachusetts and Europe, PLATE XXXVI: Men at work on the Dexter elm, Malden. From a photograph. Porthetria dispar (Linn.). Wright & Potter Printing, Boston, 495 pp). Photo courtesy of David Lance, Plant Protection and Quarantine. APHIS USDA)

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Editors
Gordon Gordh
Plant Protection and Quarantine
USDA-APHIS
Raleigh, NC, USA

Simon McKirdy
Chevron Australia Pty Ltd
Perth, WA, Australia

Adjunct Professor
School of Veterinary and Life Sciences
Murdoch University
Murdoch, WA, Australia

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Foreword

As I write, I am acutely conscious of two key features of our global community:

- About one billion people around the world will go to bed hungry tonight, including over 150 million children. Hunger and malnutrition still persists despite our capacity to produce sufficient food for more than seven billion people.
- The food security of the world's population in both developed and developing nations is increasingly reliant on biosecure trade in plant and animal products and equally low risk movement of enormous volumes of non-food materials.

During the last 50 years, there have been outstanding developments in science and technology, communications and transport that have boosted our capacity to produce food and make it available in regions where it is most needed. Grain production has more than doubled and livestock production tripled.

In the next 40 years or less, the world's population will surpass nine billion people – which means two billion more people to feed. This is coupled with increasing average calorie intakes, changing diets and greater meat consumption in the developing countries.

Unfortunately, enough food to feed about three billion people is lost or wasted every year, with significant proportion of that loss due to pests. On its own, the rice blast fungus (*Magnaporthe oryzae*) is estimated to cause production losses which could otherwise have fed 60 million people. In some developing countries, 10–15 % losses of stored grains are common, and post-harvest crop losses to exotic pests and spoilage can exceed 50 %.

Studies indicate that if wheat losses in storage and in transit were reduced by 5 %, then the change would generate global benefits in excess of US$135 billion over a 30-year period. Furthermore, benefits of pre-border biosecurity measures that lower the probability of wheat stem rust (*Puccinia graminis*) race *Ug99* spreading throughout the world's major wheat growing regions could exceed US $0.7 billion per year.

Exotic pests are estimated to cost over US$1.4 trillion worldwide annually, and the social damage caused is disproportionately large in developing countries where hunger and poverty are a major concern. Often incursions of exotic pests result in production losses as well as trade restrictions that seriously impact on business continuity in a generally low profit margin food and agriculture sector.

Heavy reliance on continued improvement in crop yields is unlikely on its own to deliver global food security, particularly in an environment of scarce arable land, water, and nutrients for food production and more exposure of food production regions and entire supply chains to pest risks. Improvement in crop yields must be supported by biosecurity strategies that minimise pre- and post-harvest food destruction caused by pests.

Increased movement of people and commodities as a result of global population expansion and intensified trade and travel has escalated the risks to food production from exotic biosecurity threats. We see clear indications of significant increases in quantum and frequency of dispersal of potentially harmful organisms and enhanced vulnerability of ecosystems and food supply chains to biosecurity risks.

With a billion people travelling internationally, exponential increase in the movement of goods that are potential carriers of biosecurity threats, and advancement in transport technology that has reduced travel times, the vulnerability of nations to biosecurity risks has increased and is influenced by the risk status of other countries. Biosecurity risk management has undoubtedly emerged as an important international public benefit; the global community suffers as pests spread.

The globalisation of food production and distribution has enhanced potential for the dispersal of harmful organisms to new regions. Developing countries that have ineffective biosecurity systems face the daunting challenge of mitigating the ever-increasing biosecurity threats while accommodating trade that allows them to improve wealth creation and quality of life for their communities.

Some organisms affect the viability of food security systems, while others damage natural biodiversity. Spread of exotic pest species is widely recognised as one of the most significant threats to biodiversity. These pests can result in suppression and extinction of native flora and fauna that may be irreplaceable and highly valued by the local community. Introduced organisms which may be benign in their native environment may have latent potential to cause serious damage in the introduced environment.

In a globalised and interconnected world, the importance of biosecurity – safeguarding resources from biological threats – cannot be understated. Neither can the need for cooperation between organisations and countries with biosecurity expertise to deliver benefits to their own countries, developing countries and more biosecure trade.

In this context, the development of globally relevant biosecurity strategies has become a necessity to minimise the biosecurity risk exposure of global food security systems, natural biodiversity, lifestyle and human health and wellbeing. Continued and improved collaboration is needed on global biosecurity initiatives, agreements and legal frameworks and regulatory systems. This will continue to be an important way forward for sustaining and improving worldwide food security, biosecure trade and business and quality of life.

The Handbook of Plant Biosecurity is a vital step in this direction. It is a comprehensive book covering in depth a wide range of biosecurity subject matter across 23 chapters and 650 pages with contributions from 85 authors. While it is described as a '*Handbook*', it will serve as a valuable text for students of plant biosecurity internationally, particularly in Australia, New Zealand and the USA, and as reference work for biosecurity practitioners and others with a role or interest in minimising biosecurity risks.

This *Handbook* will be certain to be a useful teaching aid, given the breadth of the topics covered and the various author's diverse and individual expertise.

The editors, Gordon Gordh and Simon McKirdy, are congratulated for their vision, leadership and determination in putting together such an excellent publication that is the most comprehensive text on regulatory plant health produced to date. Congratulations also go to all contributors who have produced an important resource for all who work in the field and benefit from the dedicated work of the world's biosecurity specialists.

For years to come biosecurity scientists, academics, students, policy makers, and regulators and field staff worldwide will undoubtedly find this *Handbook* to be a very valuable source of information.

I am confident that *The Handbook of Plant Biosecurity* will deliver benefits to mankind across the globe and well into the future.

Rob Delane
Director General
Department of Agriculture and Food, Western Australia
Former Deputy Secretary, Biosecurity Services Group
Department of Agriculture, Fisheries and Forestry, Australia

Preface

When looking at the frontispiece of this Handbook, the aphorism "A picture is worth a thousand words" is *apropos* in many ways:

1. Invasive pests can be persistent and pernicious.
2. Human interventions can be comical.
3. After 120 years, biosecurity regulators still use tree-climbing technology to survey and mitigate some pest occurrences.
4. Biosecurity regulatory work can be challenging and hazardous but also effective and rewarding – compare the experiences with the European Gypsy Moth in North America and the Asian Longhorn Beetle (Chap. 16).

The genesis of this Handbook came from discussions among the editors, academics, and regulatory officials in Australia, Canada, New Zealand, and the USA on the ways in which information from the advances in science and technology is used to support the management of invasive plant pests. We also discussed the ways and the importance of communication among nations regarding the regulatory events and ongoing efforts to minimize the movement of unwanted plant pests (which incur diseases and induce weeds).

During the past 20 years, we have seen a dramatic increase in the impact of invasive alien species (IAS) upon agriculture and natural resources in all countries. This increase in IAS incursions can be attributed to the exponential growth in international trade and travel. While state and federal governments of many countries have redoubled their efforts to contain or eradicate invasive alien species, we have also noted that the public is generally unaware of the dangers posed by these species and the extent of regulatory work involved in the control or eradication of pests.

We perceived the need for an overview of regulatory work in plant biosecurity as practiced by local and national officials. We have called our efforts a "handbook" because we hope that the lay public, regulatory officers/administrators, scientists, the industry, and political leaders will use it as a reference work. We also hope that this Handbook will also serve as a textbook for students interested in pursuing a career in plant biosecurity work.

Acknowledgments

We acknowledge the contribution of technical support and information from many authors in many countries. More than 80 authors have contributed to the production of this Handbook with many technical contributors who have been recognized in the acknowledgments of individual chapters. We gratefully acknowledge the assistance of several people who have significantly contributed to the production of this Handbook. We especially wish to thank Ms. Ashley Jackson (CPHST APHIS USDA) for her help in formatting the text and figures. Ms. Bronwyn Jones (Australian Plant Biosecurity Cooperative Research Centre, Canberra) kindly reviewed and edited the references for each chapter. In addition, Dr. Christina Devorshak (CPHST APHIS USDA) provided critical assessment of Chap. 7. Finally, Dr. Jennifer Nicholson (CPHST APHIS USDA) evaluated critical issues discussed in the Handbook for Plant Protection and Quarantine, APHIS.

Raleigh, North Carolina — Gordon Gordh
Perth, Western Australia — Simon McKirdy

Contents

Chapter 1
An Introduction to Plant Biosecurity: Past, Present and Future

Philip E. Hulme

1.1 The Global Threat to Agriculture and the Environment Posed by Pests

Alien species comprise plants, animals and microbial pathogens introduced to a region through human activities and are the focal point of regulatory activities addressing plant biosecurity (Table 1.1). Alien species are often intentionally introduced to a new region to support farming, forestry, aquaculture and recreation because they may grow faster or larger (offering increased economic returns), can be used to consume, displace or suppress other species (biological control agents), or simply because people like them (pets and garden plants). Trade also may facilitate the spread of alien species directly through their unintentional introduction as contaminants of cargo or stowaways within different modes of transport (Hulme 2009a). Most alien species pose limited threats, and indeed often represent the cornerstone of agricultural production. Yet a few species cause economic or environmental problems and are classed as invasive pests (see Table 1.1 and Chap. 2 for definitions, and ISPM 5 for a glossary of regulatory terms). Invasive pests include weeds, plant pathogens and animals (invertebrate and vertebrate) that act in a negative way to consume or impact agricultural commodities and plant natural resources.) Terminology under the International Plant Protection Convention uses only the term “pest” to refer to the range of organisms that are harmful to plants. Authors sometimes use different terminology but the agreed and proper regulatory terminology is found in ISPM No. 5 (the Glossary).

Invasive alien species are now increasingly recognised as a major component of human-caused global environmental change, often resulting in a significant loss to economies, human health, biological diversity and ecosystem services

P.E. Hulme (✉)
The Bio-Protection Research Centre, Lincoln University,
PO Box 84, Christchurch, New Zealand
e-mail: philip.hulme@lincoln.ac.nz

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Table 1.1 Terms used to describe biological invasions (After Hulme 2007)

Term	Description
"Introduction"	The movement, by human agency, of a species, subspecies, or lower taxon (including any part, gametes or propagule that might survive and subsequently reproduce) outside its natural range (past or present). This movement can be either within a country or between countries
"Intentional introduction"	An introduction made deliberately by humans, involving the purposeful movement of a species outside of its natural range and dispersal potential. (Such introductions may be authorised or unauthorised.)
"Unintentional introduction"	An unintended introduction made as a result of a species utilising humans or human delivery systems as vectors for dispersal outside its natural range
"Alien species"	A species, subspecies, or lower taxon introduced outside its natural range (past or present) and dispersal potential (i.e. outside the range it occupies naturally or could not occupy without direct or indirect introduction or care by humans) and includes any part, gametes or propagule of such species that might survive and subsequently reproduce. Synonyms include: Non-native, non-indigenous and exotic species
"Naturalised species"	An alien species that becomes established in natural or semi-natural ecosystems with free-living, self-maintaining and self-perpetuating populations unsupported by and independent of humans
"Feral species"	A naturalised alien species that has reverted to the wild from domesticated stock e.g. has undergone change in phenotype, genotype and/or behaviour as a result of artificial selection in captivity
"Invasive alien species"	A naturalised alien species that is an agent of change, and threatens human health, economy and/or native biological diversity
"Pest"	A vertebrate or invertebrate organism that is damaging to livestock, crops, humans, or the environment
"Weed"	A plant considered undesirable or troublesome and not valued or wanted where it is found and often grows to the exclusion or injury of more desirable species
"Pathogen"	A microbe or microorganism (virus, bacterium, prion, or fungus) that causes disease in its animal or plant host

(Mack et al. 2000; Pimentel et al. 2005; Vilà et al. 2010). Preventing and mitigating these threats is commonly viewed as an international priority (McNeely et al. 2001; Simberloff and Rejmánek 2011).

The risk to agriculture posed by alien pests is well documented. One of the earliest and most dramatic examples involves the Grape Phylloxera (*Viteus vitifoliae* (Fitch)). This small sap-sucking insect feeds on the roots and leaves of grapevines thereby reducing the flow of water and nutrients. During the mid-19th century phylloxera, a native of North America, was accidentally introduced to Europe with American grapevines. North American grape species are at least partially resistant to phylloxera, but the European wine grape was highly susceptible leading to major deterioration in the vineyards. By the end of the nineteenth century, 65–90 % of all European vineyards had been destroyed. As a result of phylloxera and similar pest outbreaks, many governments established national quarantine and plant protection services. For example, the Netherlands Plant Protection Service was established in 1899; the Australian Commonwealth Quarantine Service came into operation in 1908; and the USA's Federal Horticultural Board was established in 1912 to enforce quarantine measures (Ebbels 2003).

How representative is phylloxera (see Chap. 20) of the kinds of threats faced by agriculture from pests? For the productive sectors (agriculture, horticulture and forestry) the impacts of pests are usually expressed as financial costs. Losses may be due to reductions in yields, additional costs of control and/or the opportunity cost of lost markets. Many studies have attempted to place a monetary value of the costs of management and lost production arising from alien pests (Pimentel et al. 2001, 2005; Colautti and Bailey 2003; Vilà et al. 2010). Considerable variation exists among studies in the assumptions made regarding cost calculations such that comparisons among studies are difficult. For example, the cost of ten of the most significant alien species in Canada has been estimated to be $175 million per year (Colautti and Bailey 2003), while the cost for a similar number of aliens in Europe is only $26.5 million per year (Vilà et al. 2010). However, often the opportunity cost arising from loss of market access is an order of magnitude higher than production losses or increased management costs (Hulme 2011a).

Yield losses attributable to animal pests (mostly arthropods that feed on the crop), weeds (usually annual grasses and herbs that compete for light, water and nutrients with the crop) and pathogens (a diverse group of organisms that reduce crop growth rates that includes fungi, bacteria, viruses, mycoplasma-like organisms, viroids and spiroplasmas) are a function of the crop and its management. Ten widespread crops in North America show annual yield losses of 8–20 %; plant pathogens on average exert the heaviest toll (Fig. 1.1). Without pest management, these losses would range from 51 % to 82 % and, in the absence of management, weeds would consistently pose the greatest burden on crop yields. This illustrates the critical role of pest management in safeguarding food supplies, especially targeting weeds. While benefits of management exist for all crops (Fig. 1.1), they are most marked for cotton (90 % reduction in yield losses) and least for wheat (65 %). This management comes at a price with the annual cost of weed management in crops in the USA amounting to $3 billion compared with $500 million each for animal pests and crop pathogens (Pimentel et al. 2005).

When the total cost in terms of yield losses and control expenditure of alien pests is estimated, then alien weeds and pathogens share the dubious honour of inflicting the greatest negative impact on crop production in developed and developing countries (Fig. 1.2). Comparison of economic losses to crops in the USA, UK, South Africa, Brazil and India highlight that nations also face different levels of exposure to alien pests that largely reflect the relative importance of agriculture to their economies (Pimentel et al. 2001). The impact of alien agricultural pests on yields may be around 50 % in the poorest countries (Oerke et al. 1994). Perrings et al. (2010) provide examples of pests that have had particularly severe effects on crop yields in the world's poorest region (Sub-Saharan Africa) that includes pathogens (Grey Leaf Spot, *Circosporda zeae-maydis* (Tehon & Daniels)),

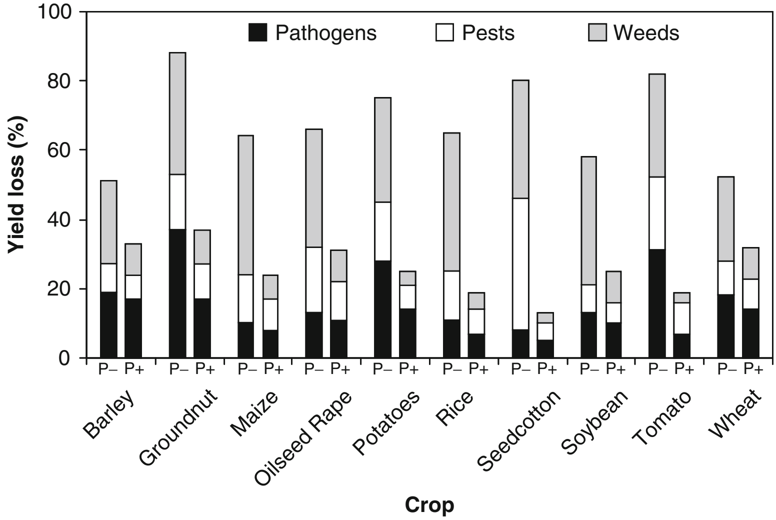

Fig. 1.1 Yield losses (%) attributable to weeds, pests and pathogens for ten crops in the USA in the presence (P+) and absence (P−) of protective measures (e.g. herbicide, pesticide, fungicide) (Data from the CABI crop protection compendium www.cabi.org/cpc/ accessed February 2009)

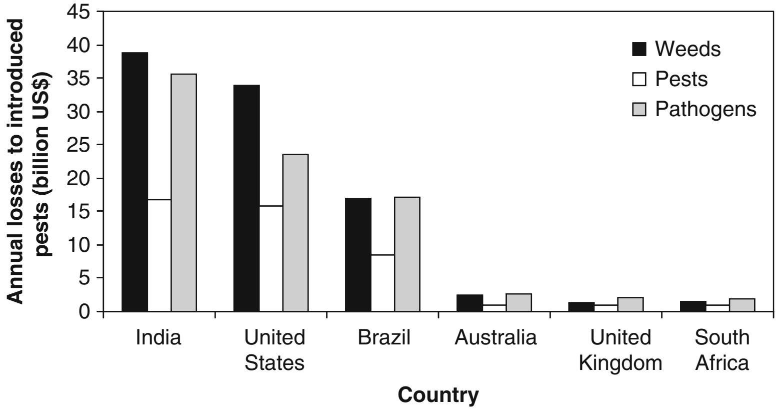

Fig. 1.2 Economic losses attributable to alien weeds, pests and pathogens of crops in the USA, India, South Africa, UK and Australia (Data from Pimentel et al. 2001)

weeds (Witchweed, *Striga hermontheca* (Delile) Benth.) and insect pests of crops (Cassava Mealybug, *Phenacoccus manihoti* Matile-Ferrero) and stored produce (Large Grain Borer, *Prostephanus truncatus* (Horn)).

Data presented by Pimentel et al. (2001) indicate that alien vertebrates are often perceived as only playing a small role in crop losses, though this is undoubtedly an underestimate of their impacts. Seed-feeding birds, particularly alien starlings

(*Sturnus vulgaris* Linn.) and sparrows (*Passer domesticus* Linn.), annually account for $1 billion of crop losses in the USA. Grazing of arable crops by rabbits (*Oryctolagus cuniculus* Linn.), is responsible for annual losses of $1.2 billion in the UK (Pimentel et al. 2001). Comparable data on vertebrates from other parts of the world are difficult to obtain; all three of these taxa have been introduced into Australia but reliable estimates of their impact on crop production are not available (Bomford and Hart 2002).

The economic costs to agriculture may not only result in the direct impact of competing weeds or phytophagous invertebrates on yield. Weed seeds can contaminate crops at harvest reducing their quality and requiring further expense for additional cleaning and processing (Chap. 21). Alien arthropods may also have indirect impacts by reducing pollinator numbers (e.g. Varroa Mite, *Varroa destructor* Anderson & Trueman), or by disrupting crop harvesting (e.g. mound building by the Red Imported Fire Ant, *Solenopsis invicta* Buren) (Chap. 7). Also, many insects act as vectors for plant pathogens. Phloem-feeding insects such as aphids, plant hoppers and leafhoppers transmit more than 150 different plant viruses; Corn Flea Beetles (*Chaetocnema pulicaria* Melsheimer) are responsible for spreading *Bacterium stewarti* (Smith), the bacterial pathogen of Stewart's Disease in corn. The Cabbage Root Fly (*Delia radicum* (Linn.)) spreads the fungal pathogen of Blackleg (*Phoma lingam* (Tode ex Fr.) Desm.) on oilseed rape.

When an alien pest becomes widely established in a new area such that eradication or regional containment is no longer feasible, its management usually becomes a private responsibility of the landowner. Loss of crop productivity and chemical control of pests are examples where producers bear the cost of an established alien species. In contrast, government is supposed to allocate resources for prevention of new problems and eradication of invasive alien species before they become permanently established. Thus, prioritisation must be made on the potential cost of species that have not become introduced into an area. Such estimates are often much larger than the cost estimates for established species (Table 1.2). Estimates for total current expenditure (Pimentel et al. 2001) due to all established arthropod pests of agricultural crops in Australia (~$1 billion) is little more than twice the total future costs of just three potential threats to agriculture (Table 1.2). The reason for the significantly higher weighting for potential threats is that while monetary estimates for established species include the costs due to management and loss of production they do not account for the impact of changes in market access. For example, in 1996 Karnal Bunt (*Tilletia indica* Mitra), a fungal pathogen of wheat, was discovered in grain grown in the south-western USA (Gullino et al. 2008). Subsequently, more than 50 countries adopted phytosanitary trade restrictions against the USA. Although the impact and cost of clean-up measures was limited, the loss of wheat exports amounted to over $250 million. This is a significant cost but only half the potential economic losses if such an incursion occurred in Australia (Table 1.2) Two pests of the potato (*Solanum tuberosum* Linn.) in the UK further illustrate the importance of accounting for the market. Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) and Potato Ring Rot (*Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff)

Table 1.2 Estimated potential costs per annum (US $) for selected threats to plant health

Species	Common name	Region	Average impact (US$ million) per annum	Reference
Leptinotarsa decemlineata	Colorado Potato Beetle	UK	0.2	Waage et al. (2005)
Clavibacter michiganensis subsp. *sepedonicus*	Potato Root Rot	UK	3.0	Waage et al. (2005)
Solenopsis invicta	Red Imported Fire Ant	Australia	251.1	Beale et al. (2008)
Tilletia indica	Karnal Bunt	Australia	448.0	Plant Health Australia (2009)
Toxotrypana curvicauda	Papaya Fruit Fly	Australia	68.0	Plant Health Australia (2009)
Varroa destructor	Varroa Mite	Australia	103.2	Beale et al. (2008)
Varroa destructor	Varroa Mite	New Zealand	15.4	Taylor and Gebbie (2000)
Teia anartoides	Painted Apple Moth	New Zealand	1.6	Taylor and Gebbie (2000)

Davis et al.) result in yield reduction. Only the ring rot bacterium can easily be spread in tubers and thus have a significant impact on potato seed exports (Table 1.2). These examples demonstrate that it is not wise to extrapolate from current management costs of established pests to predictions of costs of future threats.

The impact of invasive alien species on native plants and ecosystems includes the reduction in population size (sometimes to extinction) of threatened or endemic species; changes to the structure of plant and animal communities; alteration of ecosystem services (e.g. pollination, carbon sequestration) and dynamics (e.g. flood and fire frequency); hybridization and gene flow to native species; and the spread of pathogens (Levine and D'Antonio 2003; Hulme 2007; Kenis et al. 2009; Vilà et al. 2011). Several examples illustrate that alien pests pose a significant threat to native species. In the UK, the aquatic swamp stonecrop, *Crassula helmsii* (Kirk) Cockayne, threatens several rare species with local extinction (e.g. starfruit, *Damasonium alisma* Miller, Hampshire Purslane (*Ludwigia palustris* (Linn.) Elliott) and Pillwort (*Pilularia globifera* Linn.)). The evergreen shrub *Rhododendron ponticum* Linn. threatens one of the few endemic species to the UK, the Lundy Cabbage (*Coincya wrightii* (O.E. Schulz) Stace) (Hulme 2005). The Hemlock Woolly Adelgid (*Adelges tsugae* Annand) (a native of Japan) was first reported in the eastern USA in 1951 and is now threatening unique forest ecosystems by killing hemlock on a large scale, so that they are gradually replaced by other tree species. In particular, *A. tsugae* poses a major threat to the viability of Carolina Hemlock (*Tsuga caroliniana* Engelmann), a rare endemic tree species in the Appalachian

Mountains (Kenis et al. 2009). Alien goats (*Capra hircus* Linn.) are a major threat to native vegetation on many islands around the world. On the south Atlantic island of St Helena, goats have exterminated at least ten of the island's endemic plants (Lever 1994). The American Chestnut (*Castanea dentata* (Marshall) Borkhausen) was once one of the most important forest trees in eastern North America. The Chestnut Blight Fungus (*Cryphonectria parasitica* (Murrill) Barr) arrived on imported chestnut trees from Asia and was first observed killing American Chestnuts in 1904. By 1940, over 3.5 billion chestnut trees had been lost to the fungus and a dominant American tree species had become a threatened species (Loo 2009).

Alien pests have the potential to transform ecosystems by altering underlying biogeochemical, hydrological and/or geomorphological processes (Hulme 2007). Wholesale ecosystem modification occurred following colonisation of South African heathland (fynbos) by *Acacia* spp. Changes included augmentation of soil nutrients, changes in soil moisture, replacement of native plant species and facilitation of other alien weeds (Yelenik et al. 2004). In Spain, the Argentine Ant (*Linepithema humile* (Mayr)) displaces native invertebrates and vertebrates as well as impacts plants through disruption of myrmecochorous seed dispersal mutualisms (Kenis et al. 2009). The selective browsing of Red Deer (*Cervus elaphus* Linn.), in the forests of New Zealand has changed tree species and understory composition with consequences for leaf litter quality, decomposition rates and the litter-dwelling biota (Wardle et al. 2001). Originally from Eurasia, the White Pine Blister Rust Fungus (*Cronartium ribicola* J.C. Fischer) infects five-needled pines in western North America with cascading effects on montane ecosystems. Pine species occupy a critical niche in western ecosystems and are important in food chains of 110 animals as well as for slope stability, snow retention and watershed hydrology. Loss of pines will result in more homogeneous forests, changes in fire regimes, and reduced wildlife diversity (Loo 2009).

Hybridisation between alien and native species is a potentially serious threat to biodiversity as well as to domesticated plants and animals. A hybrid may exhibit new traits that enable it to occupy ecosystems from which either parent was previously absent. For instance, the North American cordgrass, *Spartina alterniflora* Loisel., hybridised with European *S. maritima* (Curtis) Fernald following its introduction into England and France. The allotetraploid hybrid cordgrass, *S. anglica* C.E. Hubbard, grows under a wider range of environmental conditions and has become a serious invasive alien species in coastal mudflats (Hulme 2007). Alternatively, hybridisation results in "genetic pollution" that threatens the integrity of native species. Where this involves the spread of maladaptive genes, lower hybrid performance could lead to progressive native population declines. The introduction in northwestern Europe of two southern subspecies of honeybee (*Apis mellifera ligustica* Spinosa and *A. m. carnica* Pollman) has led to large-scale gene flow and introgression between these subspecies and the native black honeybee, *A. m. mellifera* Linn. whose native populations are now threatened in northwest Europe (Kenis et al. 2009). Finally, hybrids may perform more vigorously than either parent such as the fungal pathogen responsible for Dutch

Elm Disease (*Ophiostoma ova-ulmi* Brasier & Mehotra). This pathogen is believed to have emerged as a hybrid between two existing, but less pathogenic, fungal pathogens *O. ulmi* (Buisman) Melin & Nannf. and *O. himal-ulmi* sp. nov. (Brasier 2001).

An alien species that arrives with its parasites/pathogens may impact native populations without affecting the alien host. For instance, Varroa Mite is a serious pest of honeybees that was first reported in brood cells of Asian Honeybee (*Apis cerana* Fabricius) drone larvae on Java. Subsequently, Varroa Mite shifted from *A. cerana* to cause serious damage to *A. mellifera* Linn. colonies in Europe. Similar consequences have occurred due to the tracheal parasitic mites (*Acarapis woodi* Rennie) of alien bumblebees that have parasitised native *Bombus terrestris* (Linn.) The alien shrub *Rhododendron ponticum* Linnaeus is a host of *Phytophthora ramorum* Werres et al., a causal agent of Sudden Oak Death in the USA (Chap. 17). Established populations of rhododendron have facilitated the spread of this disease to native beech trees, *Fagus sylvatica* Linn., in the UK (Hulme 2007).

Estimating the impact of biological invasions on the environment is much more complex, particularly if monetary values are sought. Non-market damages often are difficult to quantify because of the complex interactions among species in an ecosystem and a lack of information about the public's preferences across alternative ecological states. As a result, few studies provide estimates of non-market damages from invasive alien species (Hoagland and Jin 2006). However, the likelihood is that environmental costs of biological invasions will be perceived by policy-makers as being dwarfed by those borne by the primary production sectors, even where costs of control and eradication are similar (Smith and Petley 2009). As an example, weeds threaten one third of all New Zealand nationally threatened plant species. Estimates suggest that weeds currently degrade 17 % of the conservation estate corresponding to a total loss of native biodiversity equivalent to $4.4 billion (Williams and Timmins 2011). While an impressive sum, it is only equivalent to twice the annual cost of pastoral weeds to New Zealand (Bourdôt et al. 2007). Nevertheless, can we truly value the loss of biodiversity that can never be resurrected? Simply putting an economic value against it is dangerous. This disparity is undoubtedly a reflection of the inadequacy in which biodiversity and the natural environment is currently valued and this perspective may change as economists begin to value nature more appropriately (Dasgupta 2007).

The foregoing account does not provide an exhaustive list of plant biosecurity impacts but highlights that economic, environmental and social impacts are rarely additive due to the different assessment methods (e.g. monetary, species loss, human values) (Chap. 9). Species are likely to have consequences for more than one sector and in some cases may be positive in one yet negative in another. For example, the interrelationship between pests in agriculture and the environment has a long history. The introduction of wheat to North America was followed by the accidental introduction of the Stem Rust (*Puccinia graminis* C. H. Persoon) and later the deliberate introduction of the stem rust host, common barberry (*Berberis vulgaris* Linn.), for use in hedgerows. As the common barberry spread across eastern North America during the eighteenth century, partly through deliberate planting but also natural seed dissemination by birds, the association between the alien plant and prevalence of wheat stem rust became increasingly clear. During 1726–1772 legislative measures were passed by the colonists of New England, requiring or permitting the destruction of barberry bushes in Connecticut, Massachusetts and Rhode Island (Ebbels 2003). While science confirmed the association between barberry and stem rust in the late nineteenth century little control was practised until stem rust threatened the major wheat growing regions (Mack 2005). In 1918, barberry eradication laws were enacted to form barberry eradication areas, which covered virtually all the spring wheat-growing localities in the USA (Ebbels 2003). Large-scale surveillance, monitoring and control resulted in the destruction of 14 million barberry plants and the success illustrates the crucial need to understand environmental weeds, pathogens and crops as part of an integrated whole (Mack 2005). Given this long history of threats to plants in agriculture and the natural environment as well as the progressive development of national legislation to tackle these threats, why is the world facing increasing problems of invasive alien pests?

1.2 Trade, Travel, Transport and the Threat of Pests

Alien species are taxa that are introduced outside their natural range, either intentionally or unintentionally, by human agency and "natural" pathways (Table 1.1). The global pool of potential alien species, and the geographic and taxonomic pattern of biological invasions, is strongly shaped by trends in human trade and transport (Perrings et al. 2005; Meyerson and Mooney 2007). However, humans have transported and traded plant and animal species for millennia. Indeed, a defining moment in biological invasions dates to 1500 AD – a period associated with the end of the Middle Ages, the European re-discovery of the Americas, global exploration, the birth of colonialism and the start of radical changes in patterns of human demography, agriculture, trade and industry (Preston et al. 2004). Between the eighteenth and nineteenth centuries, the Industrial Revolution led to a period of increased international trade across most continents and facilitated by the construction of canals, highways and railways as well as the introduction of steamships (Findlay and O'Rourke 2007). The spread of European species worldwide was aided by 50 million Europeans who immigrated to distant shores between 1820 and 1930 taking with them, whether intentionally or by accident, numerous species (McNeely 2006). Yet, the highest rates of species introductions in Europe have occurred during the last 25 years (Hulme et al. 2009).

The recent increase in the threats posed to plant biosecurity mirrors the dramatic change in global trade. For example, the value of merchandise trade has increased markedly since the 1960s and particularly since the 1990s (Fig. 1.3a). Much of this growth can be attributed to trade in manufactured products, which now dominate global merchandise exports. In contrast, trade in agricultural raw materials and food

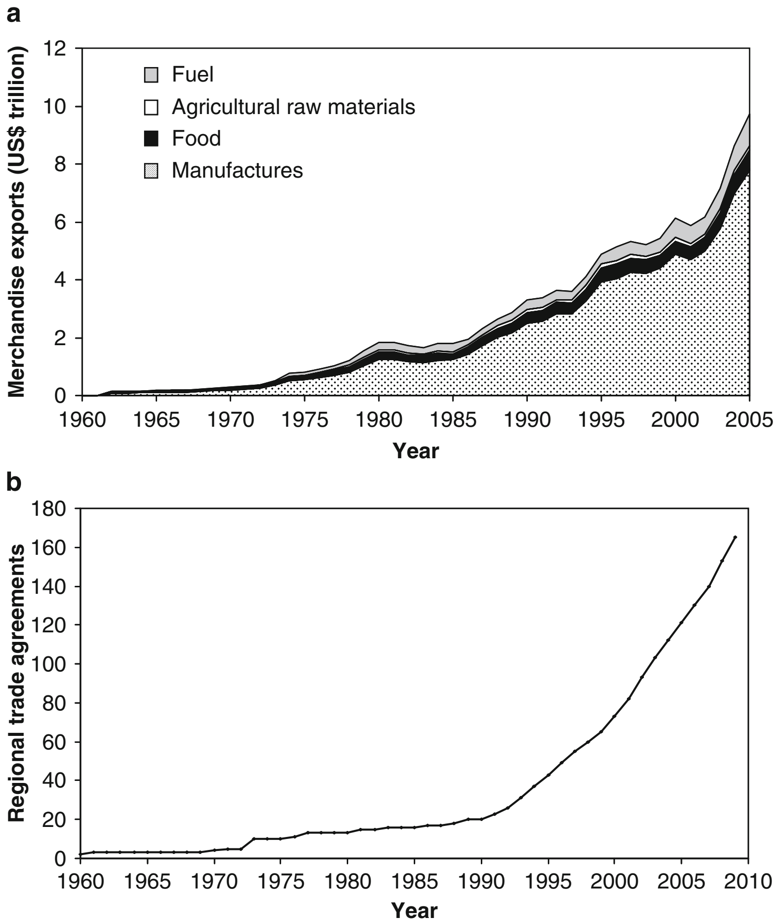

Fig. 1.3 Key temporal trends in the globalisation of trade since 1960 reflected in: (**a**) the increasing global value of different classes of merchandise exports and (**b**) the number of regional trade agreements (Data from World Bank 2008)

has grown at a much slower rate such that their share of global merchandise exports has declined over time. While international standards exist regarding the properties of agricultural raw materials and food, the heterogeneous nature of most manufactured commodities poses the greatest risk of alien species introductions. The value and volume of global trade is increasing. In 2006, more than 90 % of global trade was carried by sea with a cargo carrying fleet of over 50,000 ships transporting more than one million deadweight tonnes (IMO 2008). The increase in size, speed and number of the global cargo carrying fleet has led to a fourfold rise in the volume of global imports since the 1970s (Hulme 2009a). In 1973, container-ships were carrying four million TEUs (20 f. equivalent units). By 1983, transport rose to 12 million TEUs and by 2007 estimates show that global loaded container trade reached 141 million TEUs (UNCTAD 2007). Containers pose a novel problem. In addition to goods transported, alien pests can be associated as stowaways inside or on the surface of containers (Hulme et al. 2008). Thus, knowledge of the origin and transport history of the container (which may differ from the goods it contains) may be required to assess the risks of unwanted introductions.

Although a small player in terms of freight, aviation has surpassed shipping as the major form of international passenger transport. In 2006, 4.4 billion people passed through the world's main airports (ACI 2007). Also, airports facilitate greater penetration into continental regions than marine ports and worldwide numbers of the former exceed the latter more than 20-fold (Hulme 2009a). Increasing evidence shows that aviation is a more significant route of pest entry than might be expected based solely on cargo volumes. Of 725,000 pest interceptions at USA ports of entry between 1984 and 2000, 73 % occurred at airports compared with other ports (9 %) and twice as many pests associated with baggage than cargo (McCullough et al. 2006). While only a fraction of global commodities are transported by air, the speed of such transport means that many organisms unintentionally associated with cargo are capable of surviving long-distance intercontinental transport.

In addition to greater global trade, ties between nations have also been strengthened by globalisation, which is integrating the world economy and creating new international markets and partnerships (Hulme 2009a). A major trend in the trading system involves the proliferation of bilateral and regional trade agreements which aim to reduce tariffs and restrictions on trade between two or more nations within a certain region. The number of regional trade agreements (RTAs) has increased fourfold since 1990 and now they account for over 40 % of world trade (Fig. 1.3b). As a result, the distribution of global trade is heterogeneous with most trade occurring among members of particular RTAs. For example, 44 % of imports to the USA, Canada or Mexico stem from one or the other two NAFTA members, while intra-EU trade accounts for over 66 % of imports in the European Union (Perrings 2007). While these agreements may facilitate trade, they also facilitate the potential spread of pests. Most pests intercepted at the Canadian border originate in the USA, while most alien species in Europe have origins elsewhere in Europe (Hulme et al. 2009).

The recent increase in trade has resulted in a legacy of biological invasions. The magnitude of merchandise imports is a significant determinant of the number of species (Westphal et al. 2008; Desprez-Loustau 2009; Roques et al. 2009) as well as the rate of new species introductions (Levine and D'Antonio 2003) of diverse alien taxa. Less precise measures of trade and commerce, such as Gross Domestic Product (GDP, one component of which is net exports) correlate with the richness of alien spiders (Kobelt and Nentwig 2008), plants, birds, fish and mammals in Europe (Hulme 2007), plants in China (Liu et al. 2005) and fish across river basins worldwide (Leprieur et al. 2008). For alien plants the relationship between richness and GDP is stronger for island states than continents, reflecting the greater proportion of merchandise imports (38.0 % vs. 26.8 %) that contributes to their GDP (Hulme 2009a). Moreover, island ecosystems are often the most invaded and threatened worldwide (Donlan and Wilcox 2008). Further evidence of the direct role of trade stems from correlations between specific commodity sectors and the subsequent establishment of alien species via horticulture (Lambdon et al. 2008); the wild-bird trade (Carrete and Tella 2008), grain shipments (Shimono and Konuma 2008) and aquarium fish commerce (Gertzen et al. 2008). Knowledge of the volume, frequency, origin and destination of imports as well as the mechanism by which goods are transported probably will help better characterise risks. Indeed, these features of trade are now being integrated with aspects of species biology in attempts to prevent the entry of pest species (Hulme 2009a). However, a detailed assessment of information for timber pests entering Belgium, revealed that due to the temporal variation in trade from year to year as well as the political and economic aggregation of data, trade information is often of limited use for real-time risk assessment (Piel et al. 2008). An alternative approach is to characterise the mechanisms and historical trends in the way alien species have been moved around the world as a potential tool aimed at managing the risk of invasions.

In general, pathways describe the processes that result in the movement of alien species from one location to another. A recent framework identified six major pathways: Release, escape, contaminant, stowaway, corridor and unaided (Hulme et al. 2008). Many pests of agriculture were initially transported as commodities either to be introduced as a deliberate release (e.g. the starling *Sturnus vulgaris* Linnaeus in the USA) or subsequently escape from captivity (e.g. muskrat *Ondatra zibethicus* Linnaeus in the Czech Republic). Most major crops grown throughout the world are alien in the regions of major production e.g. oil seed rape (*Brassica napus* Linnaeus), wheat (*Triticum aestivum* Linnaeus), potato (*Solanum tuberosum* Linnaeus), oat (*Avena sativa* Linnaeus). In addition, many alien plants have been introduced to improve the forage content of pastures e.g. Alsike Clover (*Trifolium hybridum* Linnaeus), Lucerne (*Medicago sativa* Linnaeus), and Swamp Meadow Grass (*Poa palustris* Linnaeus). About 7 % of all alien plants established in the British Isles are feral crops, and several are widespread (Hulme 2005).

Many species are not intentionally transported but arrive as a contaminant of a commodity either as host-specific pathogens e.g. Chestnut Blight (*Cryphonectria parasitica* (Murrill) Barr) introduced to the USA through imported chestnut lumber/trees or arthropod pests e.g. Papaya Fruit Fly (*Toxotrypana curvicauda* Gerstaecker) within fresh fruit imported into Florida. Raw logs are a valuable and important international forestry commodity as well as a major source of alien species, especially insects and pathogens (Hulme 2009a). Accidental contamination of grain supplies or feedstuffs presents a diverse route for the introduction of alien plant species and has led to international standards of seed purity to combat this problem. About 14 % of alien plants in the British Isles have been introduced as seed contaminants (Hulme 2005). Moreover, today's cereal seed samples still are contaminated by alien seeds. Although contamination is often less than 1 %, the large numbers of seed sown each year can amount to a sizeable pool of introductions. Two common grain contaminants, Wild Oat (*Avena fatua* Linnaeus) and Field Speedwell (*Veronica persica* Poiret) are significant agricultural weeds with costs of control running to £100 million per annum in the UK (Hulme 2005).

Stowaways are directly associated with human transport but arrive independently of a specific commodity as organisms transported in cargo and airfreight. In Australia, during a 5 year period, over 5,500 invertebrates were found as stowaways on containers and cargo (Stanaway et al. 2001). Most insect stowaways were stored product pests, such as flour beetles, weevils, grain beetles, intercepted in empty shipping containers that contained residue from a previous consignment. A survey of Australian and New Zealand shipping containers concluded that 10–68 % (depending on origin) of containers carried stowaways posing quarantine risks on their outside surfaces e.g. Asian Gypsy Moth (*Lymantria dispar* Linnaeus) while 39 % had insects inside the containers including alien ants, wasps and beetles (Stanaway et al. 2001). The incursion of the Painted Apple Moth (*Teia anartoides* Walker) in New Zealand is believed to have arisen from eggs transported within shipping containers (Cook et al. 2002).

The corridor pathway highlights the role transport infrastructures play in the introduction of alien species. Linear features in a landscape, such as rivers, canals, roads and railways are often viewed as habitat corridors that help direct the movement of organisms through less hospitable habitat and may also facilitate the spread of alien organisms. In Canada, roads and road verges appear to have facilitated the spread of alien earthworms and the alien common reed, *Phragmites australis* (Cav.) Trin. ex Steud. (Hulme 2009a). Road density is a significant correlate of spatial patterns in the invasions of plants in China, insects in Europe (Roques et al. 2009) and Cane Toads (*Bufo marinus* (Linnaeus)) in Australia (Urban et al. 2008).

The unaided pathway describes situations where natural spread results in alien species arriving into a new region from a donor region where it is also alien. While this is a route for all taxa, it is particularly amenable for plant pathogens, of which the spores of many can travel considerable distances. The Coffee Rust (*Hemileia vastatrix* Berk. & Broome), a native of East Africa, spread from Angola to Brazil on transatlantic winds. Two species of poplar rust spread from Australia to New Zealand via high trajectory wind currents, while there are seasonal patterns of spread of the Wheat Stem Rust from Mexico to Canada (Viljanen-Rollinson et al. 2007). The Currant-Lettuce Aphid (*Nasonovia ribis-nigri* Mosley) migrated from New Zealand to Tasmania on low-level jet streams in January 2004 and rapidly spread throughout Australia (Cole and Horne 2006). Alien vertebrates can also spread across political and biogeographic boundaries without direct human assistance as in the case of the alien starling crossing from the USA to Canada or the muskrat spreading across the different countries of central Europe following escape from captivity in the Czech Republic (Hulme 2011a).

The importance of a particular pathway depends upon the biology and ecology of the taxon being considered (Hulme et al. 2008). Vertebrate pests have been introduced primarily through deliberate releases as game animals or biological control agents. Environmental weeds most frequently arise as escapes from gardens and horticulture (Hulme 2011b). Insect pests enter principally as contaminants of commodities, while pathogenic microorganisms and fungi are generally introduced as contaminants of their hosts (Hulme et al. 2008). However, even for a single pathway and taxon, probabilities can differ depending on the abundance of the organism in the area of origin, the number and location of arrival points at the destination, and whether the life cycle of an organism is of sufficient duration to extend beyond the time in transit (Hulme 2009a). In the absence of detailed data on rates of individual species introductions, accounting for pathways of introduction may be essential to disentangle the role of species and ecosystem traits in biological invasions as well as predict future trends and identify management options.

As the importance of international trade has increased, we see a progressive shift from *country independence* to *country interdependence* for effective management of plant pests. This shift enables governments to act on trade in order to protect human, animal or plant life or health, providing they do not discriminate or use this as disguised protectionism. The Agreement on the Application of Sanitary and Phytosanitary Measures (the "SPS Agreement") entered into force with the establishment of the World Trade Organisation on 1 January 1995. This binding agreement has had far reaching implications for the management of pests and diseases worldwide (Chap. 2). During the same period, awareness of the environmental consequences of globalisation culminated in the signing of the Convention on Biological Diversity (CBD) wherein "…each Contracting Party shall, as far as possible and appropriate, prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species" (CBD 1992). The CBD is a non-binding agreement but has had a major influence on conservation efforts and national policies (Le Prestre 2002). These perspectives are also influencing phytosanitary regulations established by the SPS Agreement with standards for plant pest risk analysis now also addressing risks to non-agronomic ecosystems (Shine 2007). The clear role of trade in weed, pest and pathogen introduction, the increasing momentum of economic globalisation and greater awareness of the interrelationships between environmental and economic sectors have progressively shaped the way governments address threats to plant protection. What was once only the responsibility of quarantine inspectors and plant protection officers has evolved in the last decade to become a discipline it its own right: Plant Biosecurity.

1.3 Plant Biosecurity: The Integration of Economic, Environmental and Political Perspectives Within Plant Protection

The term "biosecurity" refers to the research, procedures and policies that cover the exclusion, eradication or effective management of the risks posed by the introduction of alien plant pests, animal pests and diseases, animal diseases capable of transmission to humans (zoonoses), the introduction and release of genetically modified organisms (GMOs) and their products, and the introduction and management of invasive alien species and genotypes (Biosecurity Council 2003; Beale et al. 2008). In some sectors (particularly in the USA) bioterrorism is the greatest perceived biosecurity risk and biosecurity is defined as "… security against the inadvertent, inappropriate, or intentional malicious or malevolent use of potentially dangerous biological agents or biotechnology, including the development, production, stockpiling, or use of biological weapons, as well as natural outbreaks of newly emergent and epidemic diseases" (NRC 2006). However, conceptually bioterrorism is simply another pathway of introduction of unwanted pests and species. The intent, however, is quite different and the scale of damage, especially where pathogenic agents have been "weaponised" for improved delivery, dispersal and impact, potentially catastrophic (Gullino et al. 2008).

Biosecurity is a strategic and integrated approach that encompasses the policy and regulatory frameworks that analyse and manage risks in the sectors of food safety, human life and health, animal life and health, and plant life and health, including associated environmental risk. Broadly, biosecurity covers all activities aimed at managing the introduction of alien species to a particular region and mitigating their impacts should they become invasive. This includes the regulation of intentional (including illegal) and unintentional introductions and also the management of weeds, pests and pathogens by central and local government, industry and other stakeholders. The issues encompassed in biosecurity have traditionally been dealt with by different sectors such as plant health, animal health, human health and environmental protection. Each sector has its own regulatory framework addressing food safety laws, animal and plant quarantine, and pesticide regulations. The World Health Organization (WHO), Codex Alimentarius Commission (Codex), the International Plant Protection Convention (IPPC) and the World Organisation for Animal Health (OIE) provide international standards for human health, food safety, plant health, and animal health, respectively. The WHO is part of the Codex with the FAO. The IPPC, Codex, and OIE are all standard-setting organisations identified in the SPS Agreement of the WTO. The Convention on Biological Diversity sets non-binding protocols such as the Cartagena Protocols (CBD 1992). The Cartagena Protocol on Biosafety has been specifically developed in relation to protecting biological diversity from the potential risks posed by genetically modified organisms (GMO) resulting from modern biotechnology (CBD 2000). These international instruments have different histories, design, membership and legal status. This profusion of issues results in multiple regulatory systems that require high investment and recurrent costs. To be effective, biosecurity must take advantage of a more coherent, holistic approach that seeks synergies between the sectors at national and international levels and aims to shift the traditional focus on regulating individual organisms and sectors to ensuring confidence in the overall risk management framework. Models to rationalise regulatory functions among sectors in the quest for improved effectiveness and efficiency have appeared in several countries, most notably New Zealand (Biosecurity Council 2003) and Australia (Beale et al. 2008).

Strong sectorial identities exist that are associated with specific international standards, individual economic sectors, specific research communities and unique

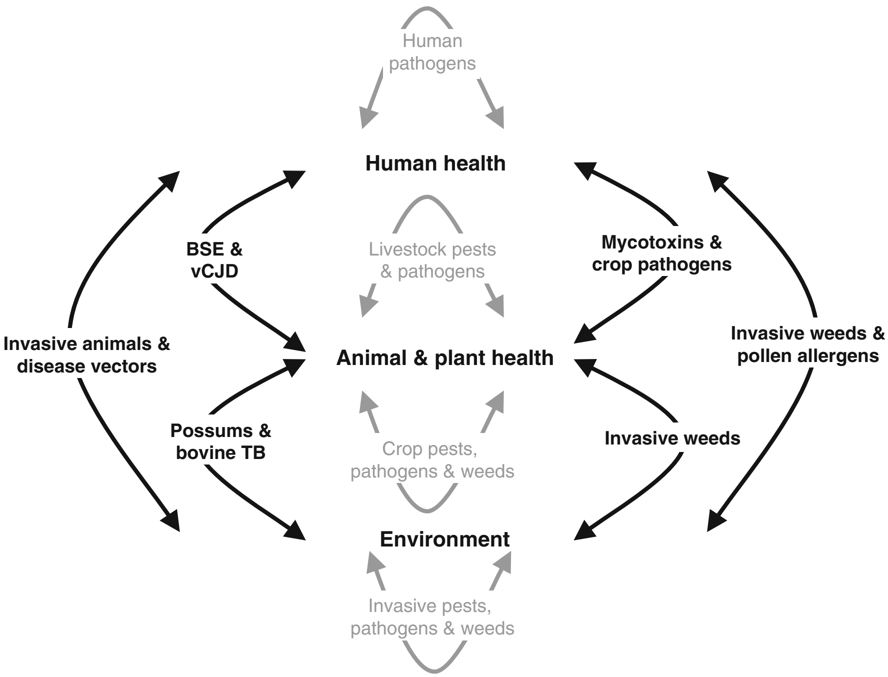

Fig. 1.4 Schematic representation of the multiple links among different biosecurity sectors. *Links in grey* show traditional responsibilities; *links in black* illustrate cross-sectorial threats

stakeholder involvement. *Human biosecurity* addresses zoonotic and emerging disease diagnosis and investigation; *animal biosecurity* deals with disease prevention and control in livestock production facilities; farms, fish farms and storage facilities; *plant biosecurity* aims to safeguard plant industries and crop production; *environmental biosecurity* is concerned with the protection of the environment and social amenity from the negative effects associated with invasive alien species. Yet, as the following examples illustrate, many biosecurity risks transcend the traditional boundaries of animal health, plant health, human health and the environment and call for a unified framework (Fig. 1.4).

The Common Brushtail Possum (*Trichosurus vulpecula* (Kerr)) is the most significant vertebrate pest in New Zealand, being a major ecological threat to indigenous biodiversity by feeding on the endemic fauna and flora, and representing significant economic threat as a vector for Bovine Tuberculosis (*Mycobacterium bovis* Karlson & Lessel) in cattle (McDowell et al. 2006). New Zealand's annual expenditure for Bovine Tuberculosis control is about NZ$83 m primarily targeting the culling of possums, an activity which also delivers biodiversity benefits. Aflatoxin-producing members of the fungus *Aspergillus* are widespread in nature and can colonize and contaminate cereal grain, oilseeds, spices, and tree nuts before harvest or during storage. Infection causes deterioration of seeds and yield losses, and also poses a risk to human health because high-level aflatoxin exposure produces an acute hepatic necrosis, cirrhosis, and/or carcinoma of the liver (D'Mello 2003). During 1977 and 1980, losses to individuals, firms and public expenditures due to aflatoxin contamination of maize in the south-eastern USA amounted to about US$ 200 million and US$ 238 million, respectively (Nichols 1983). Diseases of livestock, e.g. Bovine Spongiform Encephalopathy (BSE), may have significant consequences on human health e.g. variant Creutzfeldt-Jakob Disease (vCJD) through the consumption of contaminated food (Prusiner 1997). The cumulative budgetary cost of BSE has been estimated to be £3.5 billion (DTZ Pieda Consulting 1998) but the long-term socioeconomic costs of vCJD are unknown. Several alien weeds that have naturalised and established in native vegetation where they may replace native species also produce allergenic pollen that is a major cause of asthma and hay-fever e.g. birch *Betula pendula* Roth, ragweed, *Ambrosia artemisifolia* Linnaeus (D'Amato et al. 2007). Other weeds, while reducing native biodiversity also impact agricultural production. The Leafy Spurge (*Euphorbia esula* Linnaeus) infests 2 million ha of rangeland, pastures, hillsides, and riparian areas in North America where it reduces the plant species richness and diversity while at the same time is toxic to young cattle and reduces the value of grazing lands (Belcher and Wilson 1989). The annual losses across all agricultural sectors from Leafy Spurge have been estimated to be US$75 million for North Dakota alone (Leistritz et al. 1992). Finally some taxa pose important risks to the environment, plant, animal and human health. The two most common alien rats worldwide are the Black Rat (*Rattus rattus* (Linnaeus)) and the Brown Rat (*Rattus norvegicus* (Berkenhout)). Rat-borne diseases have claimed more human lives than all the wars in history combined (Meerburg et al. 2009). The omnivorous feeding habits of rats are also implicated in crop losses as well causing the decline of many small mammals, birds, reptiles and invertebrates. Their effect has been particularly severe on islands such as the Seychelles where rats have had more impact on endemic biodiversity than any other factor (Singleton et al. 2003).

Biosecurity involves the management of biological risks in a comprehensive manner to achieve food safety, protect animal and plant life and health, protect the environment and contribute to its sustainable use. Governments want to know the identity and nature of future alien species risks, and to estimate the nature and magnitude of the hazard, so as to anticipate and allocate resources efficiently. The diversity of potential risks, the difficulty of predicting potential harm for any species and the speed and stealth of many species in establishing and spreading pose a significant challenge to implementing individual biosecurity strategies for each potential invasive alien pest (Hulme 2011a). However, independently of whether an envelope in the post contains seeds of invasive alien weeds or anthrax spores, or if an insect is released for biological control or escapes from a quarantine facility, these threats to the environment and economy can be addressed through a common framework, known as the biosecurity continuum (Fig. 1.5). The continuum covers biosecurity activities offshore or pre-border in order to reduce the risks posed by introductions from other countries (Chap. 5), at a nation or region's borders to stop pests from entering a particular region (Chap. 6)

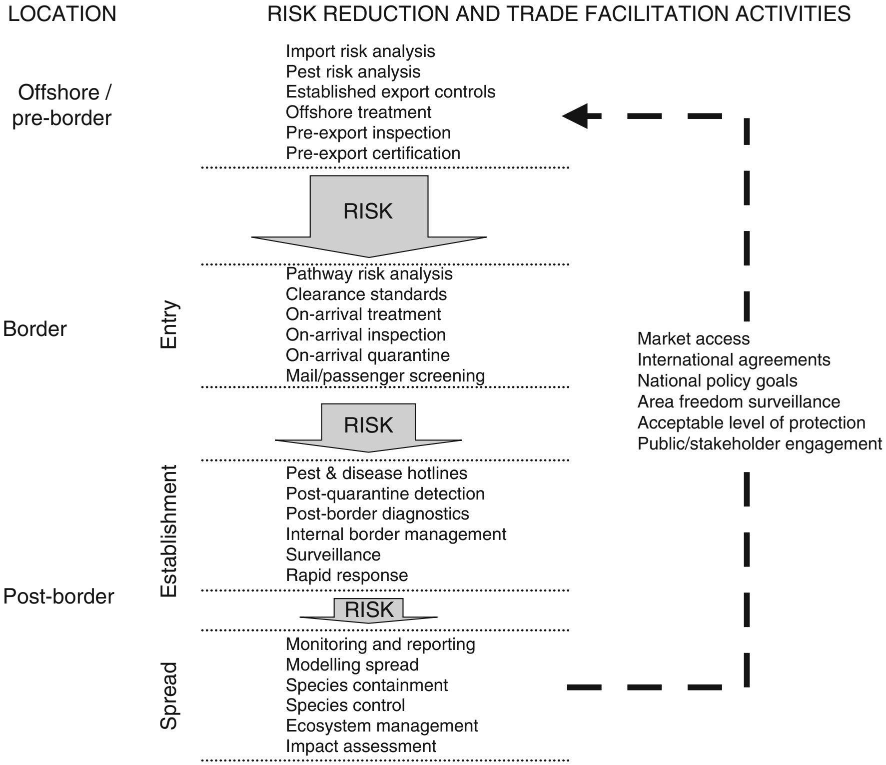

Fig. 1.5 An illustration of the key characteristics of the biosecurity continuum depicting the activities and responsibilities offshore, at the border and post-border

and within a region or post-border with the aim of finding and eradicating or managing risk organisms that have crossed the border and established in the region. To achieve optimal biosecurity requires knowledge and analysis of the diverse and complex risks along this continuum to identify, prioritise and apply measures in a coherent manner to progressively reduce the risks.

Pre-border activities include the development and review of biosecurity policies in order that commodities can be imported safely and with minimal restrictions to trade (Chap. 5). Through cooperation with international bodies such as WHO, OIE, Codex and regional plant protection organisations (RPPOs), countries can be informed of emerging pests relevant to human, animal and plant health. Import Risk Analysis (IRA) enables the risks associated with the import of a new commodity to be considered in a formal and transparent manner (Chap. 9). Risk analysis plays an important role in biosecurity with increasing attempts to harmonize terminology and methodology, while respecting the need for individual sectors to tailor risk analysis procedures to the characteristics of the risks involved (Hulme 2011a). An IRA usually involves the evaluation of biological and economic evidence to determine whether a pest should be regulated and the strength of any measures to be taken against it (Murray 2002; DAFF 2009). If the risks are believed to exceed an acceptable level of risk (often referred to as the appropriate level of protection), import of a product will not be allowed unless risk management measures are available and implemented to reduce the risk to an appropriate level. Three broad approaches have been adopted in biosecurity risk assessment: quantitative statistical models, semi-quantitative scoring, and qualitative expert assessment (Hulme 2011a). Unfortunately, problems in obtaining an objective measure of the hazards posed by alien species, challenges of predicting complex hierarchical and nonlinear systems, difficulties in quantifying uncertainty and variability, as well as cognitive biases in expert judgement, all limit the utility of current risk assessment approaches (Hulme 2012).

Not all biosecurity risks are easily identified a priori or managed pre-border and therefore inspections are required at the border (Chap. 6). The magnitude of the task is often daunting. In 1 year, the Australian Quarantine and Inspection Services (AQIS) screened 137 million mail items, 12.7 million incoming air passengers, 2 million cargo containers, 10.5 million air cargo consignments and 13,000 international vessels (Plant Health Australia 2009). Detector dogs and X-ray machines facilitate inspection at international airports, seaports, mail exchanges and container depots and, where goods are deemed high risk, they may be destroyed or treated. High risk and high-value animals (e.g. domestic pets), and plants (e.g. live orchids), may be held for further screening in post-entry quarantine to ensure pest-free status before release to the importer. However, the volume of goods and people moving across borders is a significant challenge for border inspections such that only a fraction of potential risk material is intercepted. Cost estimates for increasing manual inspections from the current rate of 2 % of containers up to 5 % of containers at the Port of New York and New Jersey would require an additional 400 inspectors at a cost of $1.2 million a month (Van Weele and Ramirez-Marquez 2011). Similarly, the indirect costs associated with increased uncertainty and burdens on the maritime supply chain are also significant, estimated at around $150 billion a year. As a consequence, considerable effort has been invested in developing tools to improve the operational efficiency of inspections (Van Weele and Ramirez-Marquez 2011).

No matter how successful pre-border and border activities might be, regulators cannot guarantee zero risk and as a result a range of post-border measures aiming to limit the impact of a pest should it be detected are needed. In many cases eradication is only possible if the pest is detected before it is widely spread (Rejmánek and Pitcairn 2002). Early detection and rapid response (EDRR) consists of search activities beyond the ports of entry, where search (and potentially removal) efforts are targeted toward areas where credible evidence suggests the presence of a biosecurity threat. Early detection enables action to be taken to prevent establishment and spread of pests, thereby reducing the potential long-term impacts and associated response and management costs. Rapid response systems should ensure outbreaks or incursions are dealt with quickly and efficiently with minimal impact on businesses and the community. Diagnostic services support these activities where the selection of appropriate management responses relies on the accurate identification of pests (Chap. 12). Advances in genomics and bioinformatics have resulted in rapid and relatively cheap genome sequencing of biosecurity risk organisms and analysis of these data has yielded extremely valuable information about the temporal and spatial origin, and movement, of pests (Cross et al. 2011). Continuing surveillance and monitoring are also important in the management of established pests (Chap. 11). The ability to predict the possible spread and impact of invasive alien pests is critical in designing and implementing cost-effective management programmes (Hulme 2006).

Although the biosecurity continuum is traditionally viewed as protecting a nation from the entry of invasive alien pests, it also has a clear role in supporting negotiations to maintain market access of its own exports (Fig. 1.5). As well as detection, an essential surveillance function is to demonstrate proof of freedom of a pest through structured surveys or other targeted methods. This is an increasing requirement for access to important international markets. Similarly, "due diligence" in managing biosecurity risks is expected of trading partners (e.g. pre-export certification, export controls etc.) and must also be applied at home. Importantly, the biosecurity continuum should facilitate international trade and not create unjustified barriers.

Biosecurity requires the bringing together of taxonomists, population biologists, statisticians, modellers, economists, chemists, engineers and social scientists to engage an agenda that is shaped by politics, legislation and public perceptions. Biosecurity is a young, evolving discipline that, in addition to the pressures arising from the increasing globalisation of the world economy, faces challenges of environmental change. Environmental change, particularly in relation to pollution, land use and climate, is a direct consequence of greater economic and geographical interconnectedness and has significant consequences for plant biosecurity. Economies have grown due to increased opportunities for trade and tourism, while human populations have spread into new habitats and this has raised the risk of pests spreading into the natural environment. Climate change further adds to the spread of pests by expanding species distribution ranges or habitats, changing migratory patterns, and increasing the probability of weather events that support the spread of vectors. The interactions between crops and pests are complex and poorly understood in the context of climate change (Gregory and Johnson 2009). In addition, land use change may be just as significant a driver of future biosecurity threats (Hulme 2009b).

The intensification of agriculture with concomitant increase in farm and field size, reliance on relatively few cultivars and homogenisation of the landscape has increased the potential impact of a pest incursion and complicates the ability to contain a pest in a single area. Genetic modification technologies may be a way to increase resistance of crops to pest. However, widespread planting of GM crops poses other potential threats such as the spread of novel genes to weeds (Warwick et al. 2009). As well as intensification, the diversification of agriculture towards new crops in particular biofuel species also poses novel risks to the environment. New crops possess traits that facilitate their spread into the natural environment (Raghu et al. 2006), and they may also act as alternative host species to crop pests (Spencer and Raghu 2009).

In the face of these multiple and poorly understood challenges, an effective biosecurity strategy together with the resources to predict, prevent and mitigate future threats will be crucial for a nation's food security and environmental protection. This is particularly the case for developing nations that face greater exposure to agricultural pests and also have rudimentary biosecurity systems in place to prevent threats or manage outbreaks (Perrings 2007). The challenge also applies to the USA (Lodge et al. 2006) and Europe (Hulme et al. 2009) where calls have been made for greater government investment across the biosecurity continuum.

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Chapter 2
The International Regulatory Framework

Lottie Erikson and Robert Griffin

Abbreviations and Definitions

APPPC	Asia and Pacific Plant Protection Commission
CA	Comunidad Andina
CBD	Convention on Biological Diversity
CEPM	Committee of Experts on Phytosanitary Measures
COSAVE	Comite Regional de Sanidad Vegetal Para el Cono Sur
CPM	Commission on Phytosanitary Measures
CPPC	Caribbean Plant Protection Commission
EPPO	European and Mediterranean Plant Protection Organisation
FAO	Food and Agriculture Organisation of the United Nations
IAPSC	Inter-African Phytosanitary Council
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
NAPPO	North American Plant Protection Organization
NPPO	National Plant Protection Organisation
OIE	Office International des Épizooties – the World Animal Health Organisation
OIRSA	Organismo Internacional Regional de Sanidad Agropecuaria
PFA	Pest Free Area – an area in which a specific pest does not occur and in which this condition is officially maintained

L. Erikson (✉) • R. Griffin
Plant Epidemiology and Risk Analysis Laboratory, Center for Plant Health Science and Technology, USDA-APHIS Plant Protection and Quarantine, Raleigh, NC 27606, USA
e-mail: Lottie.L.Erikson@aphis.usda.gov

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PPPO	Pacific Plant Protection Organisation
PRA	Pest Risk Analysis – the process of evaluating scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it
RPPO	Regional Plant Protection Organisation
SPS	Sanitary and Phytosanitary (as in WTO-SPS Committee)
TCP	Technical Cooperation Programme
WTO	World Trade Organisation

2.1 International Plant Protection Agreements

Pests and diseases have plagued agriculture since its beginning, but concerted legal action by governments to prevent or slow the introduction of pests and diseases is a relatively recent development (See Sect. 3.1). During the late nineteenth century, following several economically disastrous pest introductions in Europe, the need for voluntary coordination and cooperation by governments to restrict the movement of plant pests became apparent, especially in cases where geo-political boundaries and pest host ranges were inconsistent. Some notable events include Late Blight (*Phytopthora infestans* (Mont.) de Bary) of potatoes (1845), *Phylloxera* of grape-wine (1861), Downy Mildew (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni,) of grape-wine (1875), and Black Rot (*Guignardia bidwellii* (Ellis) Viala & Ravaz, (1892)) of grapes (1888), (Berg 1991).

The earliest international plant health agreement focused on a specific pest. *The International Convention on Measures to be taken against Phylloxera vastatrix* [currently *Daktulosphaira vitifoliae* (Fitch) [Hemiptera: Phylloxeridae]] was established in 1878 by Austria, Hungary, Germany, France, Belgium, Portugal, and Switzerland to address a pest of grapes accidentally introduced into France on infested grape vines imported from the USA (MacLeod et al. 2010). The original version of this convention concerned: (1) written assurance of pest-free status of grapevines traded internationally; (2) powers to inspect material at the border and take action on non-compliant material, and; (3) the requirement to establish an official government body to monitor implementation of the system. The convention was amended in 1881 and again in 1889.

The 1929 International Convention for the Protection of Plants had a broader scope which included pests other than *Phylloxera*. Although 46 countries participated, only 26 were signatories and only 12 ultimately ratified. The Convention was not effectively implemented in the years before and during the Second World War. After the war ended in 1945, the Food and Agriculture Organization (FAO) of the United Nations was established and member countries submitted proposals to FAO for the formation of an International Plant Protection Convention.

2.2 International Plant Protection Convention

The International Plant Protection Convention (IPPC) came into existence in 1952, superseding the previous international plant protection agreements discussed above,[1] after its adoption by the Sixth Session of the Food and Agriculture Organization of the United Nations (FAO) Conference in 1951 and its ratification by three signatory governments (Ceylon, Spain and Chile) in 1952.

The IPPC is a multilateral treaty deposited with the Director-General of the FAO and administered through the IPPC Secretariat located in FAO's Plant Protection Service. Currently (January 2013), 179 governments are signatories to the IPPC (see IPPC (https://www.ippc.int) for a current list of IPPC member countries.) The Convention provides a framework and forum for international cooperation, harmonization and technical exchange in collaboration with regional and national plant protection organizations.

The purpose of the IPPC is "to secure common and effective action to prevent the spread and introduction of pests and diseases of plants and plant products and to promote measures for their control" (Article I; IPPC 1997). International cooperation is critical for achieving the aims of the Convention. The IPPC encourages contracting parties to cooperate in exchanging information on pest occurrence, spread of outbreaks, to cooperate in special campaigns to combat serious pests where international action is needed, to cooperate in providing information for pest risk analyses, establishing Regional Plant Protection Organizations (RPPOs), developing standards, and to cooperate with other international organizations on matters covered by the Convention.

The Convention defines pest to include any species, strain, or biotype of plant, animal or pathogenic agent injurious to plants or plant products, and defines plants to include living plants, seed and germplasm (Article II; IPPC 1997). The IPPC applies to protection of cultivated plants and plant products, as well as to the protection of natural flora and organisms that can cause indirect damage to plants, including invasive species such as weeds. The relationship of the IPPC to other organizations with similar mandates is discussed in later sections of this chapter.

Provisions of the Convention also extend to storage places, packaging, conveyances, containers, soil and any other organism, object or material capable of harbouring or spreading plant pests, particularly where international transportation is involved. The Convention was revised in 1979 to update terminology and describe changes in model phytosanitary certificates, and was revised again in 1997 to align it with the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO-SPS Agreement) and to provide a mechanism for developing and adopting international standards for phytosanitary measures.

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[1] Including the International Convention respecting measures to be taken against *Phylloxera vasatrix* of 3 November 1881, the additional Convention signed at Berne on 15 April 1889, and the International Convention for the Protection of Plants signed at Rome on 16 April 1929. Article 10 of the IPPC, Substitution of prior agreements (FAO 1979).

The WTO-SPS agreement negotiations were begun in 1985, concluded in 1993, and resulted in amendments to the Convention (WTO 1994). The basic rules of international trade were negotiated under the World Trade Organization (WTO) General Agreement on Tariffs and Trade (GATT) in 1948. The GATT acknowledges the right of member countries to establish measures necessary to protect human, animal or plant life or health. The aim of the WTO-SPS Agreement is to ensure that this right is not misused and does not result in unnecessary barriers to international trade. The WTO-SPS Agreement encourages its member countries to base national SPS measures on international standards, guidelines and recommendations. It identifies standards developed by the IPPC as relevant for plant health, standards developed by the joint FAO/WHO *Codex Alimentarius* Commission as relevant for human health, and standards developed the World Organization for Animal Health (previously the Office International des Epizooties) as standards relevant for animal health.

When negotiations on the WTO-SPS Agreement began in 1986, the IPPC did not have an international secretariat or a mandate to develop international standards. In response to provisions in the SPS agreement, FAO established a secretariat for the IPPC in 1992 and formed the Committee of Experts on Phytosanitary Measures (CEPM) in 1993 to address the task of developing international standards. Negotiations on amendments to the IPPC began in 1995 and were finalized in 1997 when the FAO Conference adopted the New Revised Text of the IPPC. The revised convention came into force on October 2, 2005, after acceptance by two-thirds of contracting parties.

2.2.1 The 1997 Revised Text of the IPPC

The 1997 revision of the IPPC updated and strengthened the Convention by providing a mechanism for developing and adopting international standards and aligned terms and concepts with the WTO-SPS Agreement. It also included a greatly expanded and more precise set of definitions and created the possibility for the European Community to be a signatory. The revision established a Commission on Phytosanitary Measures, recognised the IPPC Secretariat and the IPPC's standard setting responsibilities, and required an official contact point for each member country. It also acknowledged Pest Risk Analysis (PRA) as the basis for technically justified measures and improved the format for phytosanitary certificates. The revision provided for the possibility of electronic certification, improved the dispute settlement mechanism, improved information sharing, and recognised regulated non-quarantine pests. Finally, it clarified the relationship to other international agreements and clarified obligations for risk analysis, surveillance, and information sharing.

2.2.2 Key Principles

The Convention does not contain a separate section on principles; however the first standard developed identifies principles that contracting parties should follow when imposing phytosanitary measures (ISPM 1, 2011). These include: Necessity (restrictive measures must be applied only when required by phytosanitary considerations); technical justification (measures must be based on sound science); transparency (measures must be published promptly and their rationale made available to other parties upon request); minimal impact (measures must be consistent with the risk and result in the minimum impediment to international movement of people and commodities); and non-discrimination (measures must not discriminate between different trading partners or between domestic producers and trading partners where identical or similar conditions prevail, unless there is technical justification).

2.2.3 Organization and Functions of the IPPC

The Commission on Phytosanitary Measures (CPM) is the governing body of the IPPC, and exists to promote implementation of the Convention's objectives. The CPM meets annually to review global plant protection needs, adopt International Standards for Phytosanitary Measures (ISPMs), establish procedures for dispute resolution and promote technical assistance to build phytosanitary capacity. The CPM is directed between sessions by a Bureau that provides advice, administration, and makes decisions. Basic funding and resources for the work programme of the CPM are provided through the FAO budget and through contributions and in-kind support from contracting parties.

The IPPC Secretariat is responsible for implementing decisions of the CPM and for coordinating the IPPC work programme which focuses on the development of International Standards for Phytosanitary Measures (ISPMs), the exchange of official information, and capacity building and technical assistance to develop the phytosanitary capacity of member countries to implement the IPPC and ISPMs.

The CPM has two subsidiary bodies: The Standards Committee (SC) and the Subsidiary Body on Dispute Settlement (SBDS) (Fig. 2.1). The SC oversees the standard-setting process and provides guidance and oversight to the Technical Panels (TP) and Expert Working Groups (EWG) whose primary task is to draft standards. The Standards Committee consists of 25 members from each of the seven FAO regions: Four each from Africa, Asia, Europe, Latin America and the Caribbean, and Near East; two from North America; and three from Southwest Pacific.

The SBDS was established in 2005 to assist contracting parties with disputes arising over the interpretation or application of the IPPC. The IPPC dispute settlement process focuses on the technical aspects of phytosanitary disputes. Unlike the WTO dispute settlement process, the IPPC dispute settlement process is non-binding (i.e. there is no mechanism to compel members to comply with recommendations arising from the process). While there have been numerous inquiries by contracting parties, no disputes have yet been concluded under the SBDS process.

The Strategic Planning and Technical Assistance Group (SPTA) is an informal working group that meets annually to deal with planning and prioritization of the work programme.

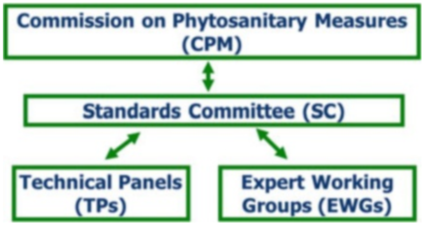

Fig. 2.1 IPPC standard setting bodies

National and Regional plant protection organizations (NPPOs and RPPOs) are important partners for achieving the goals and objectives of the Convention at national and regional levels. The Convention requires contracting parties to establish National Plant Protection Organizations and lists their principal responsibilities as: phytosanitary certification; surveillance; inspection and disinfestation of consignments; pest risk analysis; and protection of endangered areas.

RPPOs are inter-governmental organizations that coordinate the participation of NPPOs to achieve IPPC objectives by cooperating with countries in the region and with the IPPC to develop and implement ISPMs. Currently ten RPPOs exist: Asia and Pacific Plant Protection Commission (APPPC), Comunidad Andina (CA), Comite de Sanidad Vegetal del Cono Sur (COSAVE), Caribbean Plant Protection Commission (CPPC), European and Mediterranean Plant Protection Organization (EPPO), Inter-African Phytosanitary Council (IAPSC), North American Plant Protection Organization (NAPPO), Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), Pacific Plant Protection Organization (PPPO) and the Near East Plant Protection Organization (NEPPO). NEPPO held its first meeting in Rabat, Morocco, in October 2010. The meeting was attended by 16 Near East countries including, Algeria, Egypt, Iraq, Jordan, Kuwait, Libya, Morocco, Sultana of Oman, Pakistan, Sudan, Syria, Tunisia, Yemen, Cyprus, Lebanon, and Saudi Arabia.

2.2.4 Role of NPPOs in Regulatory Activities

NPPOs may have bilateral and multi-lateral responsibilities not specifically required by the Convention, which relate to addressing phytosanitary barriers to trade, and negotiating with trading partners regarding phytosanitary concerns that affect access to foreign markets for agricultural products. Specifically, NPPOs may engage in the following activities:

- Serve as the recognised National Plant Protection Organization, in accordance with the provisions of the International Plant Protection Convention and the

Sanitary/Phytosanitary Agreement of the World Trade Organization to negotiate risk mitigations that allows safe importations of foreign agricultural products. Implements and manages a national export certification programme.

- May serve as the official authority to negotiate technical conditions to address phytosanitary barriers to trade.
- May negotiate market access, expansion, and retention of agricultural products.
- May serve as a technical resource for interagency cooperators on phytosanitary issues.
- May develop, maintain, and use scientifically valid data systems to maintain a compilation of foreign import requirements to support export certification.

2.2.5 International Standards for Phytosanitary Measures (ISPMs)

International Standards for Phytosanitary Measures (ISPMs) are recognised as the basis for phytosanitary measures applied by members of the World Trade Organization under the WTO-SPS Agreement. ISPMs are adopted by contracting parties to the IPPC through the Commission on Phytosanitary Measures (CPM). Non-contracting parties to the IPPC are also encouraged to observe these standards.

ISPMs in themselves are not regulatory instruments but are implemented when countries establish phytosanitary measures within their national legislation. Countries may establish phytosanitary measures that provide a higher level of protection than ISPMs, as long as these national measures are technically justified. When national measures conform to IPPC Standards they benefit from a legal presumption of meeting the obligations of the WTO-SPS Agreement. This presumption can be challenged, but the challenger must show that measures are not technically justified and are more trade restrictive than necessary to protect plant health. Alternatively, when national requirements do not conform to IPPC Standards, the country must show that its measure(s) are technically justified and fully conform to the WTO-SPS Agreement (Stanton 2007).

ISPMs provide a useful framework for National Plant Protection Organizations (NPPOs), particularly when pest management systems and regulatory decision-making have an important role in international trade. As of November, 2011, 34 Standards had been adopted (see Table 2.1). Many more are in various stages of development. Adopted standards have been classified into the following categories: Procedures and references; pest surveillance; import regulations; compliance procedures; pest management; exotic pest response; and export certification (Hedley 2013). ISPMs such as the *Glossary* (ISPM 5) and *Phytosanitary treatments for regulated pests* (ISPM 28) are updated regularly. ISPM 28 contains 14 annexes to date, each representing a different phytosanitary

Table 2.1 International standards for phytosanitary measures and year of adoption, as of August 2012

ISPM no. and year	Title
ISPM 01:2006	Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade (originally adopted 1993, revised 2006)
ISPM 02:2007	Framework for Pest Risk Analysis (adopted 1995; revised 2007)
ISPM 03:2005	Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms (originally adopted 1996, revised 2005)
ISPM 04:1995	Requirements for the establishment of pest free areas
ISPM 05	Glossary of phytosanitary terms (updated as needed)
	Supplement 1 (2012) – guidelines on the interpretation and application of the concept of official control for regulated pests
	Supplement 2 (2003) – guidelines on the understanding of potential economic importance and related terms including reference to environmental considerations
	Appendix 1 (2009) – terminology of the Convention on Biological Diversity in relation to the Glossary of phytosanitary terms
ISPM 06:1997	Guidelines for surveillance
ISPM 07:2011	Phytosanitary certification system (originally adopted 1997, revised 2011)
ISPM 08:1998	Determination of pest status in an area
ISPM 09:1998	Guidelines for pest eradication programmes
ISPM 10:1999	Requirements for the establishment of pest free places of production and pest free production sites
ISPM 11:2004	Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms (originally adopted 2001, with supplements integrated 2003 and 2004)
ISPM 12:2011	Phytosanitary certificates (originally adopted 2001, revised 2011 by CPM-6)
ISPM 13:2001	Guidelines for the notification of non-compliance and emergency action
ISPM 14:2002	The use of integrated measures in a systems approach for pest risk management
ISPM 15:2009	Regulation of wood packaging material in international trade (originally adopted 2002, revised 2009)
	Annex 1 (2006) – approved measures associated with wood packaging materials (methyl bromide fumigation schedule modified in 2006)
ISPM 16:2002	Regulated non-quarantine pests: concept and application
ISPM 17:2002	Pest reporting
ISPM 18:2003	Guidelines for the use of irradiation as a phytosanitary measure
ISPM 19:2003	Guidelines on lists of regulated pests
ISPM 20:2004	Guidelines for a phytosanitary import regulatory system
ISPM 21:2004	Pest risk analysis for regulated non-quarantine pests
ISPM 22:2005	Requirements for the establishment of areas of low pest prevalence
ISPM 23:2005	Guidelines for inspection
ISPM 24:2005	Guidelines for the determination and recognition of equivalence of phytosanitary measures
ISPM 25:2006	Consignments in transit
ISPM 26:2006	Establishment of pest free areas for fruit flies (Tephritidae) Appendix 1 (2011) – Fruit fly trapping
ISPM 27:2006	Diagnostic protocols for regulated pests

(continued)

Table 2.1 (continued)

ISPM no. and year	Title
	DP 1:2010 – diagnostic protocol for *Thrips palmi* Karny
	DP 2:2012 – diagnostic protocol for Plum pox virus
	DP 3:2012 – diagnostic protocol for *Trogoderma granarium* Everts
ISPM 28:2007	Phytosanitary treatments for regulated pests
	PT 1: 2009 – irradiation treatment for *Anastrepha ludens*
	PT 2: 2009 – irradiation treatment for *Anastrepha obliqua*
	PT 3: 2009 – irradiation treatment for *Anastrepha serpentina*
	PT 4: 2009 – irradiation treatment for *Bactrocera jarvisi*
	PT 5: 2009 – irradiation treatment for *Bactrocera tryoni*
	PT 6: 2009 – irradiation treatment for *Cydia pomonella*
	PT 7: 2009 – irradiation treatment for Family Tephritidae (generic)
	PT 8: 2009 – irradiation treatment for *Rhagoletis pomonella*
	PT 9: 2010 – irradiation treatment for *Conotrachelus nenuphar*
	PT 10: 2010 – irradiation treatment for *Grapholita molesta*
	PT 11: 2010 – irradiation treatment for *Grapholita molesta* under hypoxia
	PT 12: 2011 – irradiation Treatment for *Cylas formicarius elegantulus*
	PT 13: 2011 – irradiation Treatment for *Euscepes postfasciatus*
	PT 14: 2011 – irradiation Treatment for *Ceratitis capitata*
ISPM 29:2007	Recognition of pest free areas and areas of low pest prevalence
ISPM 30:2008	Establishment of areas of low pest prevalence for fruit flies (Tephritidae)
ISPM 31:2008	Methodologies for sampling of consignments
ISPM 32:2009	Categorization of commodities according to their pest risk
ISPM 33:2010	Pest free potato (*Solanum* spp.) micropropagative material and minitubers for international trade
ISPM 34:2010	Design and operation of post-entry quarantine stations for plants
ISPM 35:2012	Systems approach for pest risk management of fruit flies (Tephritidae)
ISPM 36:2012	Integrated measures for plants for planting

treatment. In addition, the IPPC has coordinated the drafting of several explanatory documents to assist countries in understanding and implementing ISPMs and has adopted five recommendations on: information exchange by contracting parties, living modified organisms, threats to biodiversity, role of IPPC contact points, and reduction of the use of methyl bromide.

In 2008, the IPPC adopted a recommendation entitled *Replacement or Reduction of the Use of Methyl Bromide as a Phytosanitary Measure* (IPPC 2008a). Methyl bromide has been widely used as a pest control treatment because it offers broad spectrum control of insects, nematodes, weeds and pathogens (see Sect. 10.3.2). In the 1992 Copenhagen Amendment to the Montreal Protocol, methyl bromide was listed as an ozone-depleting substance subject to phase-out provisions of the Montreal Protocol, with exceptions in place for the use of methyl bromide for quarantine and pre-shipment purposes. The overall aim of the IPPC recommendation is to reduce methyl bromide emissions into the atmosphere and to urge IPPC members to develop national strategies regarding the use of methyl bromide as a phytosanitary measure in the following areas: replacing methyl bromide use; reducing methyl bromide use; physically reducing methyl bromide emissions; and accurately recording methyl bromide uses (IPPC 2008b).

Adopted ISPMs that deal with fundamental plant protection processes include:

- ISPM 1: Principles of plant quarantine as related to international trade;
- ISPM 2: Framework for pest risk analysis;
- ISPM 5: Glossary of phytosanitary terms;
- ISPM 6: Guidelines for Surveillance;
- ISPM 7: Export certification system (under revision as this draft was written);
- ISPM 12: Guidelines for phytosanitary certificate, (under revision);
- ISPM 9: Guidelines for eradication programmes;
- ISPM 11: Guidelines for pest risk analysis (risk assessment is discussed in Chap. 9), has been revised to include three annexes relating to environmental considerations – a fourth on plants as quarantine pests is under development;
- ISPM 15: Regulation of wood packaging material in international trade;
- ISPM 23: Guidelines for inspection.

2.2.6 The Process of Developing ISPMs

ISPMs are developed through a multi-stage process characterized by transparency and member participation. Member countries are encouraged to suggest topics for ISPMs, to nominate technical experts to develop initial drafts, and to comment on draft ISPMs at several points during their development. From drafting to final approval, the process of developing a standard can take 2 years or longer depending on factors such as priority, complexity, ability of member countries to reach consensus, and workload of the IPPC Secretariat. In recent years the IPPC programme of work has contained a backlog of nearly 100 topics in various stages of development.

The first step in the standards-development process is for the IPPC Secretariat to solicit topics for ISPMs from member countries that will help nations improve plant health and create a more equitable trading environment. Countries proposing topics submit a draft specification for the standard. Typically the IPPC Secretariat addresses a call for topics to National Plant Protection Organisations of member countries every 2 years during June. Topics are reviewed and prioritized by the Standards Committee.

Next, the IPPC Secretariat solicits nominations from member countries for international experts to draft ISPMs. The Standards Committee selects highly qualified subject matter experts from member countries to develop draft ISPMs. Drafts are submitted to the Standards Committee for review and approval.

Approved drafts are made available for country consultation for a 3-month period each year, typically from June through September. In recent years, a

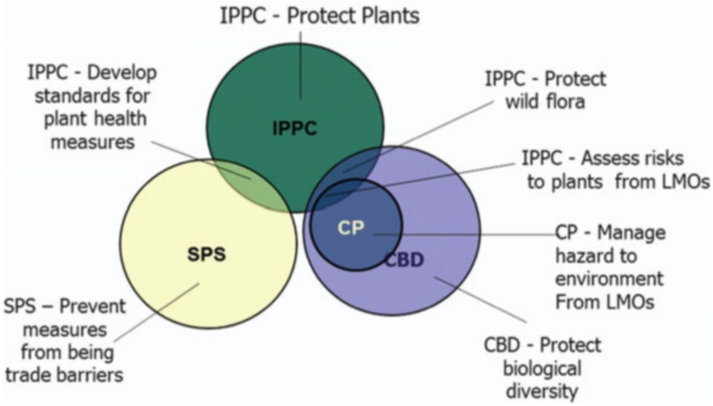

Fig. 2.2 IPPC relationship to selected international organisations

maximum of five Standards have been posted for country consultation each year. When the comment period ends, comments are compiled, reviewed by the Standards Committee, and incorporated into the draft as appropriate. Drafts are made available for a final round of member consultation immediately before adoption at the annual Commission on Phytosanitary Measures (CPM). If a Standard is not adopted it may be returned to the Standards Committee for further development or removed from the future programme of work.

Finally, Standards that are adopted by the CPM are published on the International Phytosanitary Portal by the IPPC Secretariat. The text of all adopted standards may be found on the IPPC portal at https://www.ippc.int/id/ispms.

2.2.7 IPPC Relationship to Other International Agreements

The IPPC has either a complementary relationship and/or overlapping mandates with other international agreements and conventions, including the WTO-SPS Agreement, the Convention on Biological Diversity, the Cartagena Protocol on Biosafety, and collaborates with them to achieve its plant protection goals (Fig. 2.2).

2.2.8 IPPC Relationship to the WTO-SPS Agreement

The SPS Agreement sets out the conditions under which national regulatory authorities may establish and enforce health and safety standards that directly or indirectly affect international trade. It attempts to prevent disguised trade restrictions by insuring that sanitary and phytosanitary measures are based on science and applied only to the extent necessary to protect human, animal or plant life, or health. Risk assessment (PRA) is the necessary foundation for all national SPS measures and is the yardstick by which SPS measures are appraised as necessary and justified. The nature and magnitude of the perceived risk must be clearly established so that any plant health measure(s) are commensurate with the risk. Measures should not arbitrarily discriminate between countries where identical or similar conditions prevail. Countries must notify their trading partners when they intend to establish SPS measures and seek their comments on proposed laws. Important principles in the SPS text include harmonization, equivalency, risk Assessment, pest or disease-free status, transparency, control, inspection, and approval procedures, technical assistance, special and differential treatment, consultations and dispute settlement, administration, and implementation (Stanton 2007).

The relationship of the IPPC to the WTO-SPS Agreement has been discussed to some extent in previous sections of this chapter. The IPPC came into force in 1952, before the adoption of the SPS Agreement in 1993. The IPPC was revised in 1997 to align it with terms and concepts in the WTO-SPS Agreement and to provide a mechanism for developing and adopting ISPMs. The IPPC scope and application extends beyond trade concerns to encompass a broad mandate for plant protection, and the IPPC is not under the jurisdiction of the WTO or the SPS Agreement. However, the WTO-SPS Agreement does have particular significance for the IPPC regarding the development of ISPMs affecting international trade.

The WTO-SPS Agreement calls for the harmonization of phytosanitary measures, i.e. that members base their phytosanitary measures on international standards. The IPPC began to develop international standards for phytosanitary measures after being recognised in the SPS Agreement as the relevant international organisation for plant protection standards. Many concepts articulated in the WTO-SPS Agreement (necessity, technical justification, transparency, minimal impact, non-discrimination) find expression in ISPM 1 – *Principles of Plant Quarantine as related to International Trade*. And many foundational ISPMs (ISPM 2 (revised 2007) Framework for Pest Risk Analysis and ISPM 4 (1995) Requirements for the establishment of Pest Free Areas) relate to WTO-SPS Agreement requirements – for example, the requirement that measures be based on Risk Assessment (Article 5) and that pest or disease free areas and areas of low pest or disease prevalence be recognised (Article 6).

2.3 Provisional Measures and the Precautionary Principle

"Precaution" is the concept of taking action to prevent harm. The precautionary approach as set forth in the Rio Declaration on Environment and Development (1992) endorses the use of government action to prevent dangers to the environment in the absence of full scientific certainty. The terms *precaution, precautionary approach, precautionary principle, or precautionary measure* do not appear in either the SPS Agreement or the IPPC. While both the Preamble and Article 3.3 of the SPS

Agreement explicitly recognise the right of Members to establish their own appropriate level of protection (which can be higher or more cautious than international standards), the design and implementation of any SPS measure reflecting a Member's appropriate level of protection must be based on a scientific assessment of the risks. The SPS Agreement does provide for use of *provisional measures* which are designed to facilitate trade in cases where uncertainty exists, specifically where relevant scientific evidence is insufficient (Article 5.7). It also identifies *emergency measures* which may be invoked when urgent problems of health protection arise (Annex B). The IPPC (Article 7) makes provision for emergency action based on the detection of a pest provided that the action is evaluated as soon as possible to ensure that it is justified. ISPM 13 refers to emergency actions for new or unexpected phytosanitary situations based on a preliminary PRA (Griffin 2000).

Under the WTO-SPS Agreement, a Member adopting a provisional measure must meet strict criteria – to seek the additional information necessary for a more objective assessment of risk, and to review the measure within a reasonable period of time. If challenged in the WTO dispute-settlement process, a Member imposing a provisional measure must demonstrate that the criteria in Article 5.7 have been met. Article 5.7 of the SPS Agreement has been invoked in two WTO plant health disputes (*Variety* and *Apples*) and in both cases the panels ruled that conditions for imposing provisional measures had not been met. What constitutes "a reasonable period of time" is to be established on a case-by-case basis with regard to the difficulty of obtaining the additional information needed to review the provisional measure (Stanton 2007).

In the *Hormones* case the "precautionary principle" was invoked by the European Community (EC) as a justification for not complying with the risk assessment provisions in Article 5 of the SPS Agreement. The US and Canada complaint against the EC's ban on imports of meat treated with growth-promoting hormones was the first SPS Agreement dispute referred to a dispute panel. The EC invoked the "precautionary principle" to defend its ban, arguing that the principle was a customary rule of international law. While Article 5.7 of the SPS Agreement permits members to take provisional actions in cases where relevant scientific information is not available, the EC did not invoke the Article 5.7 and clearly stated that the import ban was not a provisional measure. The Appellate Body did not take a position on the status of the "precautionary principle" in international law, but ruled that while governments may act from perspectives of prudence and precaution, the "precautionary principle" by itself does not override the provisions of the SPS Agreement (Articles 5.1 and 5.2) (Stanton 2003). (For more on the "precautionary principle", see Chap. 5.)

2.3.1 IPPC Relationship to the Convention on Biological Diversity (CBD) and the Cartagena Protocol

Significant areas of overlap exist in the mandates of the IPPC and The Convention on Biological Diversity (CBD). The CBD was adopted in 1992 with the aim of conserving biological diversity and, in the specific case of invasive alien species

Table 2.2 Revised IPPC Standards (ISPMs) relating to environmental, invasive species and CBD considerations[a]

ISPM 5 (2010)	Glossary of phytosanitary terms
	Supplement 2: 2002. Guidelines on the understanding of potential economic importance and related terms including reference to environmental considerations
	Appendix 1: 2009. Terminology of the convention on biological diversity in relation to the glossary of phytosanitary terms
ISPM 11 (2004)	
	Annex 1 of ISPM 11, Comments on the scope of the IPPC in regard to environmental risks
	Annex 2 of ISPM 11, Comments on the scope of the IPPC in regard to Pest Risk Analysis for living modified organisms
	Annex 3 of ISPM 11, Determining the potential for a living modified organism to be a pest
	Annex 4 of ISPM 11 currently under development, Pest Risk Analysis for intentionally imported plants as quarantine pests

[a]Adopted ISPMs may be downloaded from the IPPC website at https://www.ippc.int/id/ispms

(including weeds), protecting ecosystems, habitats or species. The IPPC mandate of plant protection extends beyond pests that directly affect cultivated plants to include protection of natural flora and organisms that can cause indirect damage to plants, including invasive alien plants or weeds.

Since 1992, the IPPC mandate relative to protection of the environment, invasive alien species, living modified organisms, and the role of the IPPC relative to the CBD have been clarified through working groups, through revision of international standards, and through a formal agreement between the IPPC and CBD to harmonize approaches regarding invasive alien species and plant pests (IPPC 1999, 2003). (See Table 2.2.)

Annex 1 of ISPM 11 clarifies the types of environmental risks that fall within the IPPC plant protection mandate to include: (1) Risks from pests that directly affect uncultivated or unmanaged plants, as in the case of forest disease and pests (example: Dutch Elm Disease, (*Ophiostoma novo-ulmi* (Brasier)); (2) risks from pests that indirectly affect plants, as in the case of weeds that could affect cultivated plants or wild flora through processes like competition (example: Purple Loosestrife (*Lythrum salicaria* Linnaeus) that competes in natural and semi-natural habitats); and (3) risks from pests that indirectly affect plants through effects on other organisms, as in the case of parasites of beneficial organisms. In addition, Annex 1 states that in order to protect the environment and biological diversity without creating disguised barriers to trade, environmental risks and risks to biological diversity should be analysed in a PRA.

2.3.2 IPPC Relationship to the Cartagena Protocol

The CBD conference of parties adopted a supplementary agreement known as the Cartagena Protocol on Biosafety that entered into force in 2003. The purpose of this agreement was to establish means to manage risks associated with *living* modified organisms (for example, seeds for planting rather than products for immediate consumption) resulting from biotechnology that are likely to have environmental impacts affecting biological diversity. The European Commission and the European Community member states have ratified this agreement. The United States is not a member of the Convention on Biological Diversity and therefore cannot sign the Cartagena Protocol.

The Protocol establishes an advance informed agreement approach to insure that countries are provided with the information necessary to make informed decisions before importation of LMOs. In addition, it refers to a precautionary approach and reaffirms the precautionary language of Principle 15 of the Rio Declaration on Environment and Development (1992) regarding action to prevent environmental degradation in the absence of full scientific certainty.

The scope of the IPPC in regard to risks from living modified organisms and the conduct of Pest Risk Analysis for living modified organisms is described in Annex 2 and 3 of ISPM 11. ISPM 11 relates to Pest Risk Analysis and it is acknowledged in Annex 2 that assessment of risks beyond the scope of the IPPC (i.e. human, animal health, or environmental) may be required for LMOs. Annex 3 of ISPM 11 identifies potential phytosanitary risks for LMOs (including changes in adaptive characteristics that may increase the potential for introduction and spread, adverse effects of gene follow or gene transfer, adverse effects on non-target organisms, genotypic and phenotypic instability), factors that may lead to further consideration in a Risk Assessment (including insufficient data on the behaviour of the LMO in environments similar to the PRA area) and factors that may lead to the conclusion that an LMO is not a potential pest (including experience from research trials or experience in other countries).

2.3.3 IPPC Relationship to FAO

The Convention was deposited with the Director-General of the Food and Agriculture Organisation of the United Nations after its adoption in 1951. FAO is the United Nations' lead agency for agriculture, forestry, fisheries and rural development. It works to alleviate poverty and hunger by promoting agricultural development, improved nutrition and food security. FAO's Plant Protection Service is part of its agriculture department.

FAO provides the IPPC Secretariat (through the Plant Protection Service) partial budgetary support, legal advice, support for technical assistance projects, and office space and meeting services. In return, the IPPC Secretariat supports FAO's technical cooperation programmes and FAO-executed projects by working with developing countries to evaluate phytosanitary capacity; assist in strategic planning and strengthening of plant protection infrastructures; update legislation; and develop emergency programmes.

In addition, the IPPC collaborates with other FAO programmes on an "as needed" basis. Examples include a collaboration between the FAO Forestry guidelines and potential collaboration to review and revise FAO treatment manual.

2.4 The Future

The IPPC Secretariat and the CPM have established an important role in the development of international standards and the harmonization of phytosanitary measures, and have fostered a growing involvement of the phytosanitary community in the evolution of phytosanitary systems at an international level (Fig. 2.3).

As the standard-setting process has developed, so have the number of adopted and proposed standards, together with a growing awareness of the importance of collaborative information exchange and the relevance of the Convention to environmental and biosecurity issues.

Member countries have identified a need to increase awareness of ISPMs outside the phytosanitary community, for fuller participation of developing countries in IPPC standard setting and working meetings, and for additional technical assistance programmes to enhance developing countries' phytosanitary capacity. However, at present the IPPC's ability to fully implement its annual programme of work and to expand its mandate is constrained by chronic resource shortages (both staff and budgetary).

Fig. 2.3 Delegates from IPPC member countries at annual meeting in Rome, Italy

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Chapter 3
Domestic Regulatory Framework and Invasive Alien Species in China

Zhi-Hong Li, Shui-Fang Zhu, and Fang-Hao Wan

Editor's Note China has become a significant participant in international agricultural trade during the past 15 years. Regulatory activities in China have not been widely understood by the international community of agricultural trade and travel. Yet China has an important role to play in international Plant Biosecurity. The following chapter outlines some of the significant elements of Chinese Plant Biosecurity as an example of domestic regulatory work within one bureaucratic framework. Each country must construct its own domestic (internal) programme based upon its unique circumstances. Structure and administration of these programmes can vary among countries. Regulatory officials of all domestic programmes should understand and appreciate the complexities of regulatory programmes among their trading partners and work cooperatively to improve management of Invasive Alien Species.

Z.-H. Li (✉)
College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, P. R. China
e-mail: lizh.ento@263.net

S.-F. Zhu
Institute of Animal and Plant Quarantine, Chinese Academy of Inspection and Quarantine, Beijing 100029, P. R. China

F.-H. Wan
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China

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3.1 Official Control: Regulations and Bureaucracy (Zhi-Hong Li)

3.1.1 Introduction

The first reported instance of using legal means to control plant pests occurred in 1660 when the government of Leon, France attempted to eradicate barberry in order to prevent Wheat Stem Rust disease caused by *Puccinia graminis* Pers. (Xia 2002; Li et al. 2004; Xu 2008). The earliest suggestion of plant quarantine in China was proposed during 1916 by Dr. Bingwen Zou, the pioneer and founder of Plant Pathology in China (Chen and Huang 1992). Zou explained the importance of plant quarantine and emphasized four elements of the management of plant diseases (exclusion, eradication, protection and immunization) (Chen and Huang 1992). During 1927–1929, a paper titled "Plant Quarantine" was published in three parts by Dr. Fengmei Zhu (Chen and Huang 1992). The publication emphasized the significance and basic methods of plant quarantine. Zhu advocated import quarantine to prevent plant-pest introduction and protection of domestic agriculture and forestry. He also emphasized that export quarantine would increase overseas customers' confidence and promote international trade. These collective actions were regarded as the scientific foundation of plant quarantine in China. In December 1928, the Ministry of Agriculture and Minerals published 'Regulation for Agricultural Products Inspection' in an attempt to prevent Cotton Bollworm, *Pectinophora gossypiella* (Saunders), from becoming established in China. This is the first official regulation for plant quarantine in China (Chen and Huang 1992). During 1928, Agricultural Products Inspection Services were established in Shanghai, Guangzhou and Tianjin. These services were mainly responsible for the import quarantine of cotton and the export quarantine of plant products to the UK and USA.

"Plant quarantine" is intended to prevent the introduction and/or spread of quarantine pests in China. Plant quarantine and invasive species management activities have been developed in China during the past 80 years. Three Ministries and their branches are responsible for executing plant quarantine regulations (see below).

3.1.2 Legal System

National regulations, international agreements and bilateral/ multilateral agreements form the legal system of plant quarantine in China. Among national regulations, "Law of the People's Republic of China on the Entry and Exit Animal and Plant Quarantine", "Regulations on Plant Quarantine" and related lists of quarantine pests play a very important role in the international and domestic phytosanitary activities.

For international agreements, as a member of FAO and WTO, China conforms to the principles of IPPC, SPS Agreement and Agreement on Technical Barriers to Trade (TBT Agreement) (see Chaps. 2 and 9). During the past 10 years, and following membership in the WTO, China has executed more than 600 bilateral or multilateral agreements related to SPS and TBT measures.

"Law of the People's Republic of China on the Entry and Exit Animal and Plant Quarantine" became effective in 1992. This law now includes eight chapters under the titles "General Provisions", "Entry Quarantine", "Exit Quarantine", "Transit Quarantine", "Quarantine of Materials Carried by Passengers or by Post", "Quarantine of Means of Transport", "Legal Responsibility", and "Supplementary Provisions". In the General Provision, the law was formulated to prevent infectious or parasitic diseases of animals, diseases, insect pests and weeds dangerous to plants, and other harmful organisms from spreading into or out of China. The law also protects crop production, forestry, animal husbandry, fisheries and human health, and promotes foreign economic relations and trade. To execute this law effectively, "Regulations for the Implementation of the Law of the People's Republic of China on the Entry and Exit Animal and Plant Quarantine" came into force during 1997. In these regulations, Import Permits for Entry, Exit and Transit Quarantines, Quarantine of Materials Carried by Passengers/Post, Quarantine Transport, and Quarantine Inspection Supervision were regulated in detail.

"Regulations on Plant Quarantine" was circulated by the State Council in 1983, and amended in 1992. The regulations include 24 Articles and indicate that the agricultural and forestry departments under the State Council are in charge of the plant quarantine work throughout China. The agricultural and forestry departments of Provinces, Autonomous Regions and Municipalities control plant quarantine work in their own domains. The agricultural and forestry departments of Provinces, Autonomous Regions and Municipalities may develop quarantine catalogues for their own regions. Imported seeds, seedlings and other propagating materials must be isolated for trial planting. Plants are released for planting only after they have been observed, tested and shown to be free of quarantine pests.

China maintains three national lists of plant quarantine pests. The lists are periodically revised and used by quarantine officers. All lists are circulated by the government to all members of FAO, WTO and related organisations. The public can read and download all the lists from the relevant website. The newest lists are compared especially with the number of different kinds of quarantine plant pests (Table 3.1).

3.1.3 AQSIQ, MOA, SFA and Branches

China's plant quarantine includes one Ministry and two ministerial administrative organizations under the State Council. General Administration of Quality Supervision, Inspection and Quarantine of the PRC (AQSIQ) and its branches control plant quarantine work at ports throughout the country. The Ministry of Agriculture

Table 3.1 Plant quarantine pest lists of People's Republic of China

PRC list	Year	Pest species	Pathogen species	Insect and snail species	Weed species
Entry quarantine pests	2007	435	242	152	41
Agricultural quarantine pests	2009	29	17	9	3
Forest quarantine pests	2008	21	7	13	1

(MOA), State Forestry Administration (SFA) and their branches control domestic agricultural and forest plant quarantine work respectively.

AQSIQ monitors product quality, standard metrology, entry-exit commodity inspection, entry-exit health quarantine, entry-exit animal and plant quarantine, import-export food safety, certification and accreditation, standardization, as well as administrative law-enforcement (http://english.aqsiq.gov.cn/). AQSIQ maintains 19 Departments including the Department of Supervision on Animal and Plant Quarantine. This department is responsible for: (1) Studying and preparing provisions and regulations involving entry-exit of animals and plants, (2) Studying and preparing lists of prohibited animals and plants, (3) Organizing inspection, quarantine and supervision of entry-exit animals, plants, and animal/plant products, (4) Administering inspection and quarantine of entry-exit genetically modified organisms (GMOs) and their products, (5) Collecting information on animal and plant epidemics outside China and organizing the implementation of Risk Assessment and emergency precaution measures, and (6) Administering registration and approval of entry-exit animal and plant inspection and quarantine. AQSIQ has established 35 Entry-Exit Inspection and Quarantine Bureaus (CIQs) in China's provinces, autonomous regions and municipalities, with approximately 300 branches and more than 200 local offices across the country. AQSIQ has more than 30,000 employees operating in commodity distributing centres at sea ports, land ports and airports. The WTO/TBT and WTO/SPS National Enquiry Points of the People's Republic of China are also located within AQSIQ.

The Ministry of Agriculture (MOA) is responsible for national development of agriculture and the rural economy. MOA includes 11 Departments, 6 Bureaus, and a Permanent Representative Office to the UN FAO (http://english.agri.gov.cn/). The Department of Crop Farming Administration (DCFA) is responsible for domestic agricultural plant quarantine work. Primary functions of DCFA include: (1) Administration of plant quarantine, (2) Organize drafting and implementation of laws, regulations and related standards for plant quarantine, (3) Implementation of IPPC, (4) Draft and negotiate inter-governmental agreements on plant quarantine, and (5) Enact and circulate decrees of prohibited plants. In each Province, Autonomous Region and Municipality, a general station of plant protection and/or quarantine is maintained to resolve agricultural plant quarantine affairs locally.

The State Forestry Administration (SFA) is responsible for national development of forestry and forest economy. SFA includes eight Departments and a Bureau (http://english.forestry.gov.cn/). Department of Afforestation and Greening (Office of the National Afforestation and Greening Committee) is responsible for domestic forest plant quarantine work. The functions of this Department include seven aspects: (1) Quality control of seeds and seedlings, afforestation, management of national seeds and forestry operations, (2) Ensuring the prevention of water loss and soil erosion by biological measures such as planting trees and grasses, (3) Monitoring the cultivation of various types of public welfare forests and commercial forests, (4) Organizing and directing forestry pest control, quarantine and forecast, (5) Directing and supervising nationwide voluntary tree-planting, urban and rural greening activities, (6) Stipulating policies and measures in forestry sector for tackling climate change and supervising their enforcement, and (7) Performing specific tasks assigned by the National Afforestation Committee. In each Province, Autonomous Region and Municipality, a general station of forestry protection operates to regulate forest plant quarantine affairs.

The Ministry of Environmental Protection (MEP) of the PRC administers environment protection including biodiversity and invasive species management (http://english.mep.gov.cn/). MEP guides, coordinates and supervises ecological conservation and biological species activities. This includes organizing and coordinating the conservation of biodiversity. The Department of Nature and Ecology Conservation in MEP is the office of Biodiversity Conservation and the office of National Biosafety Management.

3.1.4 Summary

China has more than 80-years of experience in plant quarantine. The Chinese government has always placed high priority on the development of agriculture since establishment of the PRC. With the development of international trade in horticulture and plant commodities, China is placing more attention to plant biosecurity. This priority is realized in the management of plant quarantine involving invasive species. Based on the three levels of China's legal system, the international collaboration between China and other members of WTO and FAO, and the domestic collaboration among AQSIQ, MOA, SFA, and MEP, the management of plant biosecurity has achieved more transparency and harmonization.

3.2 Technical Support: Academies and Universities (Zhi-Hong Li)

3.2.1 Introduction

Plant quarantine and invasive species management are based on technical support, especially for Pest Risk Assessment, inspection and identification, eradication and treatment, supervision and monitoring. In China, at least three national academies and more than 50 universities are researching techniques and educating students on plant biosecurity. Notable among these institutions are the Chinese Academy of Inspection and Quarantine (CAIQ), Chinese Academy of Agricultural Sciences (CAAS), Chinese Academy of Forestry (CAF), China Agricultural University (CAU), Zhejiang University (ZU), Nanjing Agricultural University (NAU), and Beijing Forestry University (BFU). Considerable contributions of technical support and professional education for plant quarantine and invasive species management are provided by these institutions.

3.2.2 CAIQ, CAAS and CAF

Chinese Academy of Inspection and Quarantine (CAIQ) is a national scientific research body for social benefits. CAIQ was established in 2004 with approval of the State Council. CAIQ was created from two former institutes: The China Import and Export Commodity Inspection Technology Institute and the Animal and Plant Quarantine Institute of General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ). The scope of CAIQ is research mainly on the application of science and technology in inspection and quarantine, as well as relevant basic, high-tech and soft science research. The focus is on solving emergency and basic scientific problems. CAIQ also provides technical support to the policy-making of inspection and quarantine for the national government. Eleven institutes and three centres are affiliated with CAIQ (Table 3.2).

China maintains as least 35 technology centres within CIQs. Among these, the Institute of Plant Quarantine and the Institute of Equipment Technology play an important role in technical support of plant quarantine and invasive species management. The journal 'Plant Quarantine' (established in 1979) is maintained by CAIQ. Papers published in Plant Quarantine include new reports and research developments related to exit-entry plant quarantine, domestic agricultural and forest plant quarantine issues.

CAAS was established in 1957. It is the national agricultural research organisation directly affiliated to the MOA. CAAS has the strategic task of serving nationwide agriculture, rural development and empowering farmers with science and technology (http://www.caas.net.cn/engforcaas/index.htm). CAAS concentrates on strategic and applied research solving scientific problems of national or regional importance. CAAS maintains 38 research institutes located in 17 Provinces, Autonomous Regions and Municipalities. The Institute of Plant Protection (IPP) plays an important role for technical support of invasive species management, especially in recent years (http://www.ippcaas.cn/ippc/ippcaas_e/ippcaas_e.htm). The mission of IPP is to study and seek resolution of theoretically and economically important problems involving plant pests and pesticides. The Chinese Society of Plant Protection is responsible for IPP, in which the Sub-society of Biology Invasion was established

Table 3.2 Research institutes and research centres of CAIQ

Institute/centre	Acronym	Responsibility
Institute of Food Safety	IFS	Hazardous compounds analysis and metabolic mechanism, species identification and genetic modified organism (GMO) safety, food safety information and technical support
Institute of Plant Quarantine	IPQ	Early warning and emergency response of plant pathogenic microorganisms and quarantine pests, and epidemic monitoring
Institute of Animal Quarantine	IAQ	Animal infectious diseases and epidemiology
Institute of Health Quarantine	IHQ	Exotic infectious disease, ports safety, pathogenic microbiology
Institute of Industrial and Consumer Product Safety	IICPS	Safety and quality safeguard for light industrial products, toys and textile products
Institute of Chemicals Safety	ICS	Safety and quality safeguard for daily used chemical product
Institute of Mechanical and Electrical Product Safety	IMEPS	Safety and quality safeguard for electric/electronic products
Institute of Equipment Technology	IET	Quarantine treatment and equipment, on-site rapid inspection and quarantine technology
Institute of Tobacco Safety and Control	ITSC	Safety and quality safeguard for tobacco products
Institute of Food Risk Management and Application	IFRMA	Food safety risk management policy and technical regulation
Institute of Strategy for Inspection and Quarantine	ISIQ	Comprehensive, look-forwarding and basic scientific theories, development strategies and relevant long-term mechanism
Agro-product Safety Research Centre	ASRC	Safety and quality safeguard for agricultural products
Data Centre of Inspection and Quarantine	DC	Information collection and analysis on inspection and quarantine
CAIQ Test Centre	TEST	Third-party inspection and quarantine test according to ISO/IEC17025

in 2009 in response to increasing plant Biosecurity needs. The Biosafety Research Centre of MOA in IPP focuses on research of invasive pests in China.

CAF (established in 1958) is the national forest research organisation directly affiliated with SFA, and is based on the former Central Research Institute of Forestry (established in 1953) (http://www.forestry.ac.cn/). CAF includes 29 research institutes, centres and organisations located across 18 Provinces, Autonomous Regions and Municipalities. Among these Institutes, the Research Institute of Forest Ecology, Environment and Protection (RIFEEP) conducts research on forest plant biosecurity. RIFEEP contributes to the technical support of forest quarantine and in recent years has focused more on invasive species management (http://www.ifeep.cn/).

3.2.3 Higher Education of Plant Protection and Forestry

The higher education of agriculture in China has a history dating from 1905. It is regarded as the basic and significant field of higher education with sustainable development. Plant Protection and Forestry have a close relationship with plant biosecurity for undergraduate and graduate levels. With the development of plant biosecurity and social demands, Plant Quarantine and Invasive Pest Management now receive more attention within higher education in China. Most universities with Plant Protection and Forestry have majors related to Plant Quarantine and Invasive Pest Management for undergraduates and/or postgraduates.

To promote higher education, 'Project 211' and 'Project 985' were implemented by the PRC Ministry of Education (MOE) in 1995. Project 211 is responsible for constructing nearly 100 universities during the twenty-first Century. Its main goal is to establish Higher Institutes and Key Disciplines during the twenty-first Century. Project 211 has improved education quality, scientific research, administrative standards and operational efficiency. Project 985 aims to construct first-class universities, establish world-class research groups, explore new administrative systems and operational mechanisms. Currently, 113 universities are supported by 'Project 211'; 39 distinguished universities are supported by 'Project 985'. This constitutes only 6.6 % and 2.3 % respectively of more than 1,700 universities, colleges and institutes in China.

Table 3.3 compares majors and/or research directions during 2010 related with plant biosecurity in universities supported by 'Project 211'. Three trends are apparent: (1) Plant Protection and Forestry are very popular majors for undergraduates. (2) Plant Pathology, Agricultural Entomology and Insect Pests Control, Pesticide, and Forest Protection are traditional majors for postgraduates. (3) Some universities establish majors or directions of plant quarantine, forestry quarantine and/or invasive species management for undergraduates and postgraduates, e.g. China Agricultural University, Zhejiang University, Nanjing Agricultural University, Southwest University, and Beijing Forestry University among others.

3.2.4 Research Projects

Most research projects related to plant biosecurity in China are supported by the government. During the past 10 years, China has significantly increased funding in support of research on plant quarantine and invasive species management. Projects include the National Basic Research and Development Programme (973) (http://www.973.gov.cn/English/Index.aspx), the National High-Tech Research and Development Programme (863), the National Key Technological Research and Development Programme, the projects of public industry research, the projects of Natural Science Foundation of China (NSFC), the ministerial and provincial

Table 3.3 Degree majors in 2010 related with plant biosecurity at primary universities in China

Universities	Support from 211 project	Support from 985 project	Major for bachelor's degree	Major for master's degree	Major for Ph.D. degree
China Agricultural University	Yes	Yes	Plant Protection	Plant Pathology	Plant Pathology
				Agricultural Entomology and Insect Pest Control	Agricultural Entomology and Insect Pests Control
				Pesticide	Pesticide
				Plant Quarantine and Agricultural Ecology Health (with direction of plant quarantine)	Plant Quarantine and Agricultural Ecology health (with directions of pest risk analysis, quarantine identification and treatment)
Zhejiang University	Yes	Yes	Plant Protection	Plant Pathology	Plant Pathology (with directions of plant quarantine, invasive biology)
				Agricultural Entomology and Insect Pest Control (with direction of Biosecurity and Management)	Agricultural Entomology and Insect Pest Control (with directions of plant quarantine, Invasive Biology)
				Pesticide	Pesticide
Northwest Agricultural and Forest University	Yes	Yes	Plant Protection	Plant Pathology	Plant Pathology
			Forestry	Agricultural Entomology and Insect Pest Control	Agricultural Entomology and Insect Pest Control
				Pesticide	Pesticide
				Plant Protection Resource Utilization	Plant Protection Resource Utilization
				Pest Management Ecological Engineering	Pest Management Ecological Engineering
				Forest Protection	Forest Protection
Sun Yat-Sen University	Yes	Yes	Biology Science	Agricultural Entomology and Insect Pests Control (with direction of Biology Invasion and Control)	Agricultural Entomology and Insect Pests Control
Shanghai Jiaotong University	Yes	Yes	Plant Biology Technology	Plant Pathology	Pending
				Pesticide	

(continued)

Table 3.3 (continued)

Universities	Support from 211 project	Support from 985 project	Major for bachelor's degree	Major for master's degree	Major for Ph.D. degree
Lanzhou University	Yes	Yes	Grass Science	Plant Pathology	Pending
Jilin University	Yes	Yes	Plant Protection	Plant Pathology Agricultural Entomology and Insect Pests Control Pesticide	Pending
Nanjing Agricultural University	Yes	No	Plant Protection	Plant Pathology (with direction of Plant Quarantine and Invasive Biology) Agricultural Entomology and Insect Pest Control (with direction of Invasive Biology and Biology Control) Pesticide	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide Plant Quarantine and Biosecurity (with directions such as Alien Invasive Pathogen Quarantine and Control, Alien Invasive Species Risk Assessment, Alien Insect Pests Invasive Ecology, Alien Insect Pests Control, Alien Pathogen Pests Control, GMO Testing and Security assessment)
Huazhong Agricultural University	Yes	No	Animal and Plant Quarantine	Plant Pathology (with direction of Plant Quarantine) Agricultural Entomology and Insect Pest Control Pesticide	Plant Pathology (with direction of Plant Quarantine) Agricultural Entomology and Insect Pests Control Pesticide
Southwest University	Yes	No	Plant Protection	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide

				Biosecurity (with directions of Biology Invasion, Dangerous Disease Diagnosis Technology, Pests Monitoring and Control, GMO Biology Risk Assessment)	Biosecurity (with directions of Biology Invasion, Dangerous Disease Diagnosis Technology, Pest Monitoring and Control, GMO Biology Risk Assessment)
Hainan University	Yes	No	Plant Protection	Plant Pathology Agricultural Entomology and Insect Pest Control (with directions of Invasive Species Prevention and Control) Pesticide Forest Protection	Molecular Plant Pathology Crop Insect Pest (with direction of Insect Ecology; Invasive Biology)
Guangxi University	Yes	No	Plant Protection (with direction of Plant Quarantine)	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide
Guizhou University	Yes	No	Plant Protection	Plant Pathology Agricultural Entomology and Insect Pest Control	Plant Pathology Agricultural Entomology and Insect Pest Control
Beijing Forestry University	Yes	No	Forestry Forest Resources Conservation and Recreation	Forest Protection (Forest Quarantine)	Forest Protection (Forest Quarantine)
Northeast Forestry University	Yes	No	Forestry Forest Resources Conservation and Recreation	Forest Protection	Forest Protection
Northeast Agricultural University	Yes	No	Plant Protection	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide	Pending

(continued)

Table 3.3 (continued)

Universities	Support from 211 project	Support from 985 project	Major for bachelor's degree	Major for master's degree	Major for Ph.D. degree
Suzhou University	Yes	No	Horticulture	Agricultural Entomology and Insect Pest Control	Pending
Huazhong Normal University	Yes	No	Biology Science	Agricultural Entomology and Insect Pest Control Pesticide	Pending
Sichuan Agricultural University	Yes	No	Plant Protection Forestry Forest Resources Conservation and Recreation	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide Forest Protection	Pending
Ningxia University	Yes	No	Plant Protection	Agricultural Entomology and Insect Pest Control Pesticide	Pending
Shihezi University	Yes	No	Plant Protection	Plant Pathology Agricultural Entomology and Insect Pest Control Pesticide	Pending

research projects and the international collaboration programme. Data on the CAIQ website (2004–2008) show it has undertaken 222 national research projects of high academic level, valued at a total of 160 million RMB.

3.2.5 Summary

Support by CAIQ, CAAS, CAF and many universities (CAU, ZU, NAU, and BFU) for plant quarantine and invasive species management has engaged critical research and higher education in China. With continuing support from government, the study of plant Biosecurity, emphasizing plant quarantine and invasive species management, is acquiring significant biological and ecological information and progress toward exclusion, management and eradication of Invasive Alien Species.

3.3 Quarantine Techniques, Standards and Application (Shui-Fang Zhu)

3.3.1 Introduction

Pest Risk Analysis supports the work of the domestic quarantine system involving entry of plants and plant products (Chap. 9). Detection and identification are daily tasks in the front line of port quarantine (Chaps. 12 and 13). The detection and identification conclusions drawn from the intercepted exotic pests diseases and weeds serve as an important foundation for carrying out Pest Risk Analysis and formulating quarantine policies. Accurate identification of IAS also is critical for quarantine treatment, because under many circumstances only after detecting and identifying the exotic species could the specific quarantine treatment be implemented. Quarantine treatments refer to measures with official license aiming at killing, removing or rendering infertile quarantine pests (Chap. 10). Treatments include various technical procedures adopted towards goods/products/commodities/containers infested with pests. Quarantine actions include disinfection and disinfestations, return of shipment, destruction of material, port transferring, change of use and restriction of utilization. Plant quarantine must monitor and detect new epidemic situations within the country, province or region and promote emergency plans to expeditiously contain or eradicate newly discovered IAS incursions. Quarantine actions should also monitor the distribution trends of quarantine pests present in parts of the country or region, and thus provide a scientific basis for decision-making for entry and exit plant quarantine and prevent specific pests from exiting and re-entering. Facing today's economic globalisation, the frontier quarantine assumes the double responsibilities of preventing exotic pest from entering and exiting as well as guaranteeing the efficient import and export of agricultural trade (Zhu et al. 2004a, b).

3.3.2 Pest Risk Analysis and Pest List

Early in 1981, the former Plant Quarantine Institute of Ministry of Agriculture (the predecessor of Plant Quarantine Inspection Institute of CAIQ) implemented evaluation of quarantine for dangerous pests. Subsequently, the Institute completed topics of 'Study on Quarantine Pest Risk Analysis' and 'Quantitative Risk Assessment of Pests' in succession, and introduced quantitative assessment methods such as agroclimatic analogical distance, ecoclimatic assessment model, geographical information system, Monte-Carlo simulation and risk simulation experiment study, by means of which the Institute analysed the suitable areas in China for important quarantine pests. Some of these quarantine pests include *Ceratitis capitata* (Wiedemann), *Tilletia controversa* Kühn, *Bursaphelenchus xylophilus* (Steiner & Bührer) Nickle, *Erwinia amylovora* (Burrill) Winslow et al. and *Sorghum halepense* (L.). These assessment methods applied to quarantine pests provided the scientific basis for macro decision-making of plant quarantine.

The 'Chinese Entry and Exit Animal and Plant Quarantine Risk Analysis Committee' was established in April 2002. The Committee is comprised of senior experts from relevant departments of the State Council, institutions of higher learning, scientific research institutes, quality inspection system and relevant social groups. The Committee studies and discusses major issues about Risk Analysis of entry and exit animal and plant quarantine in China. The Committee also deliberates on important Risk Analysis reports of entry and exit animal and plant quarantine. The two national standards for risk analysis, 'Technical Requirements for Pest Risk Analysis of Entry and Exit Plants and Plant Products' (GB/T 20879-2007) and 'Work Guidelines for Pest Risk Analysis of Entry and Exit Plants and Plant Products' have been issued and implemented in 2007 and 2008 respectively. The basic principles of these two national standards adopt by equation of the international standards such as ISPM 02, 11, 19 and 21.

Pest Risk Analysis (Chap. 9) is initiated mainly under the following circumstances: (1) Creating or revising quarantine pest lists; (2) Importing or exporting plants and related products; (3) Introducing certain pests for special demands; and (4) Intercepting new exotic organisms. 'Revision of Quarantine Pest List of Entry Plants' was completed in 2006. In 2007 the new 'Regulated Plant Pest List of P. R. China' was issued and the taxa of quarantine pests increased from 87 to 436 (Bulletin MOA 2010; Chen 2009). Currently, Hong Kong and Macao are formulating lists of plant quarantine pests. In 1993 the former Plant Quarantine Institute of Ministry of Agriculture presided over completion of the first Chinese PRA report: 'Risk analysis report of importing American plum'. Subsequently, more than 200 Pest Risk Analysis reports have been completed in China. With the expansion of import and export trade, the number of risk analysis reports initiated has increased yearly. Risk Analysis provides the scientific basis for plant quarantine negotiations with foreign countries and relevant decision making.

3.3.3 Pest Inspection and Testing

The number of exotic pests intercepted by China is increasing yearly (Wan et al. 2009a, b). The prevention and control of exotic pests has become a global public security issue correlated with economic globalisation. In 2005, Chinese ports intercepted more than 2,000 taxa (Genus/Species) of plant pests in more than 100,000 consignments. In 2012, as many as 300,000 consignments and 4,300 taxa (Genus/Species) of invasive alien pests were intercepted. To improve the effectiveness and strengthen the appraisal, evaluation and supervision of port quarantine work, Department of Animal and Plant Quarantine of the State General Administration for Quality Supervision issued the *Assessment Index System of Plant Epidemic Situation Interception*. This included entry pest interception, export violation notification and importance assessment of relevant work which aims at further strengthening the effectiveness of interception work of exotic pests.

The substantial increase of exotic pest interceptions at frontier ports is credited to the improvement and standardization of detection technology and methods. The current identification work mainly involves insects, weed seeds, nematodes and parts of fungi. The work centres upon morphological identification using microscope observation and computer network technologies (remote identification). CAIQ researched and developed a remote pest identification system which integrated various software and hardware resources related to port quarantine such as microscopic image acquisition devices, synthesis module of photos with enhanced depth of focus, remote video conference system, auxiliary pest identification system and records management of identification. This system can realize many practical functions related to pest identification such as remote real-time microscopic video communication, on-line experts' audio/video identification direction, computer auxiliary identification and identification records management and so on; it can also satisfy the business demands of port quarantine tasks from observation, identification and recheck of samples to reporting and filing the intercepted epidemic situation (Chap. 12).

The detection and identification of bacteria, viruses, fungi, insect eggs and larvae, sibling species, subspecies and ecotypes depends on molecular techniques including serological techniques, various PCR technologies, chip technology, gene cloning and sequencing analysis technology (Zhu et al. 2004c; Chen and Zhu 2008; Huang et al. 2011; Zhao et al. 2011; Zhang et al. 2010, 2011) (Chap. 13).

China has formulated and issued more than 300 national and industry standards and operation regulations of 'Entry Plant Quarantine Pests'. In addition, Chinese experts have also actively participated in formulating and revising several ISPM standards.

3.3.4 Pest Treatment

Quarantine treatment plays a vital role in preventing entry or exit of invasive alien species as well as guaranteeing that import/export trade can proceed unimpeded

(Chap. 10). The primary quarantine treatment technologies adopted by China include: fumigation treatment, heating-cooling treatment, radiation treatment, isolated quarantine, detoxification treatment and chemical treatment as well as special equipments, together with pest-free production areas, or with pest-free places of production, or with pest-free production site. Quarantine treatment requirements for most import/export products have been established. Nevertheless, new quarantine treatment standards are always under review and new quarantine treatment technology/methods are under research and development.

3.3.5 Pest Survey, Monitoring and Alert Response

Survey and monitoring are the basis for timely detection of IAS, establishing effective prevention and control measures and reporting epidemiological conditions that help prevent pests from exiting China. Each year the state allocates special funds for entry-exit plant quarantine departments to carry out routine survey and daily monitoring of pests of domestic and international concern. Target insect pests include *Cydia pomonella* (L.), *Liriomyza trifolii* (Burgess), *Leptinotarsa decemlineata* (Say) and Tephritidae. Monitoring occurs near the ports, in distributing centres of imported goods and export facilities. These efforts are the foundation of China's national monitoring system. Monitoring of Tephritidae in China began in 1994, The *State Technical Guide for Tephritidae Monitoring* was formulated subsequently, and has passed through several revisions. Tephritidae monitoring shows that various flies of quarantine concern (such as *Ceratitis capitata* (Wiedemann)) have not become established in China. Survey efforts reveal the distribution of Tephritidae in China and provides the scientific basis for plant quarantine negotiations with other countries and lays foundation for promoting the establishment of Tephritidae-free areas in northern China.

China continually evaluates real-time information of epidemic situations occurring in other countries. This is combined with information on pests intercepted at China's seaports and monitored on the basis of Risk Analysis. This surveillance system enables China to release alert information of epidemic situations in China.

During the past few years, China has strengthened the scientific and technological research in pest monitoring and alert with some notable achievements (Chap. 11).

3.3.6 Summary

The inspection and quarantine departments in China have constructed a framework and technological support system involving Risk Assessment, IAS detection, monitoring, emergency alert and quarantine treatment of exotic pests and diseases. Quarantine Departments have played an important role in preventing the entry/exit of pests and improved the efficiency of import/export trade. Nevertheless, many problems remain unresolved, so investment in science and technology should be increased and the cooperation among trading nations should be strengthened.

3.4 Invasive Alien Species and Research in China (Fang-Hao Wan)

3.4.1 Introduction

The spread of invasive alien species in China is significant and has become a nationwide problem, costing taxpayers hundreds of billions of RMB in environmental degradation, lost agricultural productivity, increased health problems, and expensive prevention and eradication efforts (Wan et al. 2008a). Some IAS have been introduced intentionally and are highly valued by humans (e.g. agriculture, aquaculture and ornamental species). Nevertheless, many other species are introduced as by-products of human activity, especially increasing international trade and travel. Since 2001 when China entered the World Trade Organisation, IAS-related problems have become progressively more acute for humans and entire ecosystems

To establish an effective prevention and control system for IAS, many entomologists and ecologists in China pay more attention to basic and applied scientific research relating to IAS, with strong support of the Chinese government. During the past 10 years scientists have made significant progress on IAS research in China. Here we review the current status and trends of occurrence and scientific research on invasive species.

3.4.2 Invasive Alien Species in China: Current Status and Trends of Occurrence

Species type and habitat distribution: Analysis of literature records and field data to 2012 reveals more than 600 alien species in agriculture, forestry and aquatic ecosystems in China. These IAS include 277 terrestrial plant species (45.9 %), 112 terrestrial invertebrates (18.6 %) as well as microorganisms, aquatic invertebrates, aquatic plants, fishes, mammals, amphibians, reptiles and birds (Fig. 3.1).

Alien species invade almost all ecosystems in China, including farmlands, forests, grasslands, bushes, wetlands, inland waters, oceans and human habitation (Fig. 3.2). Most invasive species are likely to flourish in man-made habitats. About 43.4 % invasive species occur in arable lands and botanical gardens, 34.1 % in seriously disturbed habitats such as buildings and road construction areas, and 17.8 % in orchards and plant nurseries, followed by natural terrestrial ecosystems and aquatic ecosystems.

Our data shows diverse IAS (microorganisms, plants and animals) have unique distribution patterns. Weeds comprise most of the invasive alien plants and typically occur in human-disturbed habitats. Insects top the invasive animals and typically occur in farmlands, forest and orchards.

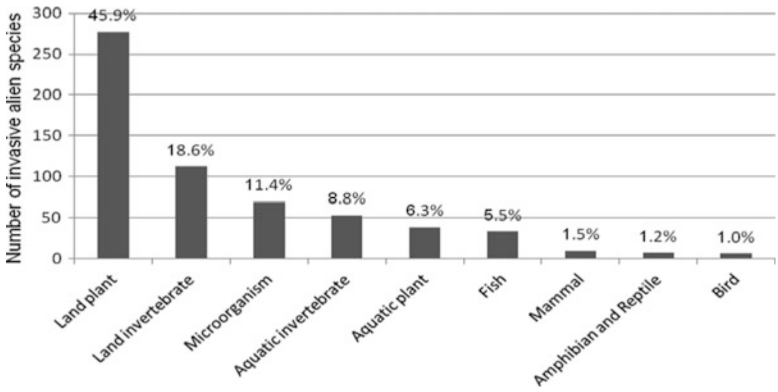

Fig. 3.1 Numbers and percentages of various categories of invasive alien species in China (Wan, unpublished)

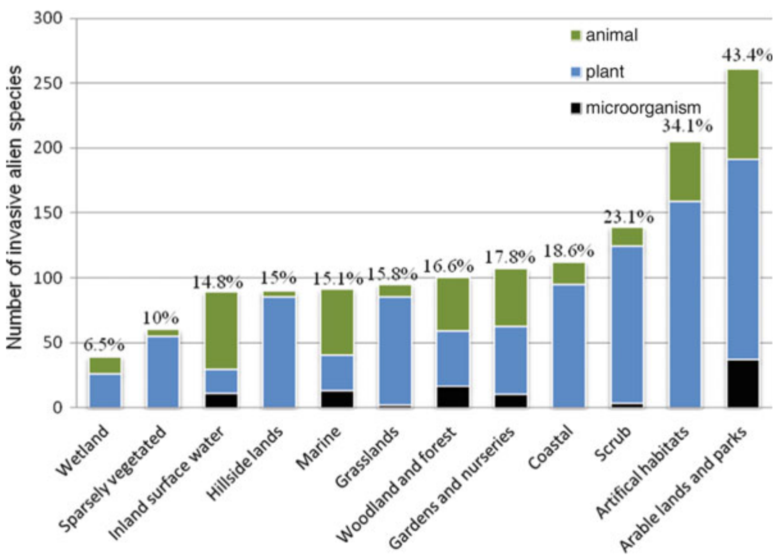

Fig. 3.2 Numbers of introduced invasive microorganisms, plants and animals and percentage of the total number of invasive alien species in different habitat. Total of percentages across habitats is more than 100 % because some species can be assigned to more than one habitat type

3.4.3 Chinese List and Databases of Quarantine Pests and Important Invasive Alien Species

The Chinese Import-Export Plant Quarantine List has been amended six times since 1954, most recently in 2007. The list contains 435 quarantine pests, including 146 species (/genus-level) of insects, 6 species of mollusks, 125 species (/races) of fungi, 58 species (/race) of prokaryotes, 20 species (/genus-level) of nematodes, 39 species of viruses and viroids and 41 weed species (/genus) (See Bulletin 862, Ministry of Agriculture – People' Republic of China). New detection techniques and national/ industry standards of 34 plant diseases in the new quarantine pests list were established, including five bacterial diseases, 12 fungal diseases, 6 nematodes and 11 viral diseases (Table 3.4, Wan et al. 2009a, b).

With IAS research and data becoming more important, a few noteworthy databases have been built by Chinese research institutes and universities. These include: The Chinese Invasive Alien Species Database (www.invasivespecies.org.cn/wzjs/index.asp), Chinese Agricultural Pest Information System (www.agripests.cn), and the Chinese Invasive Alien Plant Information System (www.weed.njau.edu.cn/exowort/exoweeds.htm). The Chinese Invasive Alien Species Database was established by the Institute of Plant Protection (IPP) of the Chinese Academy of Agricultural Sciences (CAAS) and Centre for Management of Invasive Alien Species of Ministry of Agriculture (MOA). The IAS databases are extensive, important and provide detailed information for more than 600 IAS in China. Information includes the Chinese name, Latin name (scientific name), English name (common name), taxonomic status, morphological features, biological characteristics, photos, introduced time and place, introduction pathways, invasion pathways, potential distribution and/or spread regions, prevention methods, control and management methods, etc. The IAS databases have an internet message-board and anyone can remote search and upload or download information after registration.

For IAS management and control efficacy, an IAS list was prepared by IPP, CAAS. Of primary concern is the 'List of the Worst IAS in China' (Table 3.5), identifying species that must be controlled. The list includes the worst IAS in agricultural, forestry, wetland and freshwater ecosystems. The most notable IAS are highlighted in red.

3.4.4 Scientific Research on Invasive Species in China

Scientific research in most fields can be classified into "basic research" (including theory or mechanism researches) and "applied research" (including new methods, technology or regulation). According to different invasive processes (introduction, establishment, lag phase, dispersal and outbreak), research on IAS in China is sorted into five topics:

- Early-warning and invasion pathways,
- Population formation and development,
- Interaction and competition between IAS and host or native species,
- Response mechanisms of ecosystem,
- Technologies and methods for prevention and control of IAS.

Table 3.4 Quarantine diseases with detection technique standards established in China

Disease types	Organism	Code of detection standard
Bacterial	*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*	SN/T1586.1-2005
	Acidovorax avenae subsp. *citrulli*	SN/T1465-2004
	Ralstonia solanacearum race 2	SN/T1390-2004
	Pantoea stewartii pv. *stewartii*	SN/T1375-2004
	Xanthomonas axonopodis pv. *Vasculorum*	SN/T1400-2004
Fungal	*Tilletia controversa* Kuhn, TCK	GB/T 18085-2000
	Tilletia indica	SN/T 1127-2002
	Verticillium albo-atrum	SN/T 1145-2002
	Peronospora hyoscyami f.sp. *tabacina*	GB/T 18086-2000
	Ceratocystis fagacearum	SN/T 1271-2003
	Ophiostoma novo-ulmi	SN/T 1272-2003
	Cephalosporium maydis	SN/T 1900-2007
	Diaporthe phaseolorum var. *Meridionalis*	SN/T 1899-2007
	Monilinia fructicola	SN/T 1871-2007
	Peronosclerospora (Ito)	SN/T 1155-2002
	Mycosphaerella fijiensis	SN/T 1822-2006
	Phytophthora sojae	
Nematode	*Radopholus similis*	SN/T 1505-2005
	Globodera rostochiensis	SN/T 1723.2-2006
	Globodera pallid	SN/T 1723.1-2006
	Bursaphelenchus xylophilus	SN/T 1132-2002
	Heterodera schachtii	SN/T 1140-2002
	Ditylenchus angustus	SN/T 1136-2002
Virus/viroid	*Prunus* necrotic ringspot virus, PNRSV	SN/T 1618-2005
	Cacao swollen shoot virus, CSSV	SN/T 1617-2005
	African cassava mosaic virus, ACMV	SN/T 1616-2005
	Carnation ringspot virus, CRSV	SN/T 1612-2005
	Southern bean mosaic virus, SBMV	SN/T 1611-2005
	Potato mop-top virus, PMTV	SN/T 1135.3-2003
	Arabis mosaic virus, ArMV	SN/T 1150-2002
	Tobacco ringspot virus, TRSV	SN/T 1146-2002
	Potato yellow dwarf Nucleorhabdovirus	SN/T 1135.2-2003
	Coconut lethal yellowing phytoplasma	SN/T 1579-2005
	Coconut cadang-cadang viroid, CCCVd	SN/T 1580-2005

Basic Research. Basic Research includes activities that increase understanding of essential principles. Basic Research is not intended to yield immediate commercial benefit. However, in the long term, it is the source for many commercial products and applied research. Hence, as a novel research domain, Basic Research is necessary on IAS in China. During the past 10 years, the Chinese government has funded many projects and launched many programmes to support the basic research of invasive species. This work has focused on the four key scientific issues, including the relationship of IAS between invasive potential and successful invasion, IAS population expansion and dispersal, IAS ecological adaptation and evolution, response mechanism of IAS on ecosystem. Most of these research

Table 3.5 List of the most significant invasive alien species in China

Latin name	Common name
Insects	
Aleurodicus dispersus Russell	Spiraling Whitefly
Bactrocera cucurbitae (Coquillett)	Melon Fly
Bemisia tabaci (Gennadius)	Tobacco Whitefly
Carpomya vesuviana Costa	Ber Fruit Fly
Cosmopolites sordidus (Germar)	Banana Root Borer
Cylas formicarius (Fabriaus)	Sweet Potato Weevil
Dendroctonus valens LeConte	Red Turpentine Beetle
Frankliniella occidentalis (Pergande)	Western Flower Thrips
Hemiberlesia pitysophila Takagi	Japanese Pine Needle Scale
Leptocybe invasa Fisher & LaSalle	Blue Gum Chalcid
Liriomyza huidobrenisis (Blanchard)	South American Leaf Miner
Liriomyza sativae Blanchard	American Serpentine Leaf Miner
Oracella acuta (Lobdell) Ferris	Loblolly Pine Mealybug
Quadrastichus erythrinae Kim	Erythrina Gall Wasp
Rhabdoscelus lineaticollis (Heller)	Asiatic Palm Weevil
Rhynchophorus ferrugineus (Olivier)	Red Palm Weevil
Trialeurodes vaporariorum Westwood	Greenhouse Whitefly
Other animals	
Achatina fulica Bowdich	Giant African Snail
Pomacea canaliculata (Lamarck)	Golden Apple Snail
Trachemys scripta (Thunberg)	Brazilian Slider
Bacterium	
Clavibacter michiganensis subsp. *sepedonicus* (Spieckermann *et* Kotthoff) Davis et al.	Bacterial Ring Rot of Potato
Fungi	
Cronartium ribicola J. C. Fischer	Soft-pine Stem Blister Rust
Phytophthora infestans (Mont.) de Bary	Soybean Blight
Virus	
Southern rice black-streaked dwarf virus	SRBSDV
Nematode	
Bursaphelenchus xylophilus (Steiner *et* Bührer) Nickle	Pine Wood Nematode

(continued)

Table 3.5 (continued)

Latin name	Common name
Plants	
Aegilops squarrosa L.	Tausch's Goatgrass
Alternanthera philoxeroides (Mart.) Griseb.	Alligator Weed
Ambrosia artemisiifolia L.	Ragweed
Ambrosia trifida L.	Giant Ragweed
Cenchrus pauciflorus Bentham	Field Sandbu
Eichhornia crassipes (Martius) Solms-Laubach	Water Hyacinth
Eupatorium adenophora Spreng.	Crofton Weed
Eupatorium catarium Veldkamp (*Praxelis clematidea* R.M. King)	Praxelis
Eupatorium odoratum L.	Fragrant Eupatorium Herb
Flaveria bidentis (L.) Kuntze	Coastal Plain Yellowtops
Mikania micrantha Humboldt, Bonpland et Kunth	Mile-a-minute Weed
Parthenium hysterophorus L.	Grayule Parthenium
Solanum rostratum Dunal	Buffalo Bur Nightshade
Solidago canadensis L.	Canada Goldenrod
Spartina alterniflora Loiseleur	Smooth Cord-grass
Spartina anglica C. E. Hubbard	Common Cord-grass

programmes were supported by the National Natural Science Foundation of China (NSFC) and the National Basic Research Programme ("973" Programme).

The National Natural Science Foundation of China (NSFC). The China NSFC, established under State Council ratification in 1986, is an increasingly important governmental funding source for sponsoring China natural science research. The NSFC supports research projects usually including Young Scientist (funded to 48,000 USD), General Programme (funds 95,000–130,000 USD) and Key Programme (funds more than 320,000 USD). By 2010, about 300 invasion biology research projects had been sponsored by the NSFC, with accumulative funding exceeding 14 million USD. Analysing NSFC's published data (1999–2010) shows that programmes funded by NSFC dramatically increased basic research of biological invasions. In 2008, 35 projects were active with cumulative expenditure of 1.8 million USD. In 2010, 61 projects were funded with 2.9 million USD (Fig. 3.3).

The National Basic Research and Development Programme ("973" Programme). This Programme is China's on-going national keystone basic research programme. It was approved by the Chinese Government in 1997 and is organized and implemented by MOST. The strategic objectives of the 973 Programme are to strengthen the original innovations and to address the important scientific issues concerning the national economic and social development at a deeper level and wider scope to improve China's capabilities of independent innovations and to provide scientific support for the future development of the country. Generally, every project can obtain support of 3.2–4.8 million USD over 5 years.

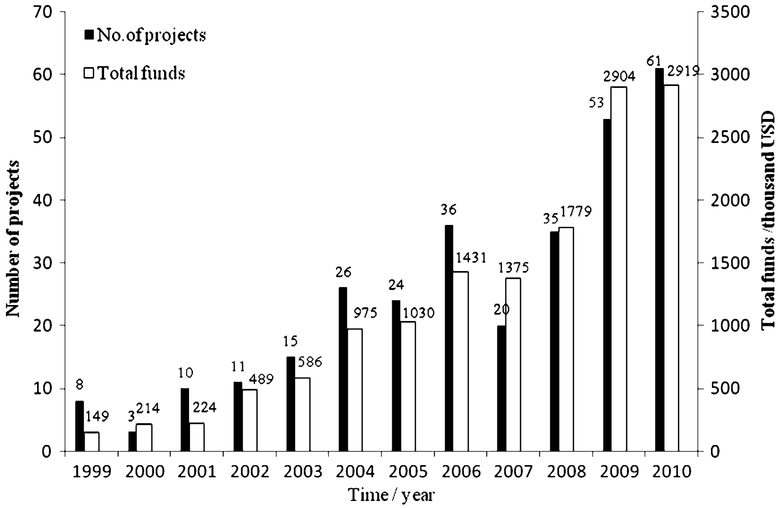

Fig. 3.3 Number of projects funded and expenditure on research into biological invasions in China by the National Natural Science Foundation from 1999 to 2010

Since 2003, several "973 programmes" have conducted basic research on invasion mechanisms of IAS and applied research related to prevention and management techniques involving IAS. The total budget has been over 16 million USD. The most significant work involves a two-phase 10-year research project: "Invasive biology and control strategy of alien species in agriculture and forestry". The second phase "Invasive mechanisms and management of major alien species" involved core scientific issues: (a) Population establishment and expansion of IAS (Doc. State Council 2003; Wan et al. 2002); (b) Ecological adaptation and rapid evolution of IAS (Wan and Guo 2007); (c) Invasion impacts on ecosystem structures and function (Wan et al. 2005, 2009a, b, 2011b, c). This project has engaged a series of "greatest risk" IAS systematically, including: Tobacco Whitefly (biotype B) (Liu et al. 2007; Luan et al. 2008; Lü and Wan 2008; Jiu et al. 2007; Li et al. 2010; Wang et al. 2010; Xu et al. 2010; De Barro et al. 2011), Rice Water Weevil (Jiang et al. 2004; Shi et al. 2007), Oriental Fruit Fly (Shen et al. 2010, 2011), Coconut Leaf Beetle (Lu et al. 2006, 2008), Red Turpentine Beetle (Sun et al. 2004; Yan et al. 2005; Liu et al. 2008), Codling Moth (Yan et al. 1999; Wang et al. 2004), Pine Wood Nematode (Cheng et al. 2008, 2010; Robinet et al. 2009), and Crofton Weed (Wang and Wang 2006; Niu et al. 2007; Feng et al. 2007, 2009; Feng 2008a, b; Li et al. 2008; Gong et al. 2009; Li and Feng 2009; Wang et al. 2011).

Applied Research. Recently, China's government has increased attention to applied research of IAS and initiated a series of programmes focusing on the sustainable management of IAS in China. Examples include the National Key Technologies R & D Programme (China's 11th Five-Year Plan), the National High-Tech Research and Development Programme ("863" Programme), and the Special Fund for Agro-scientific Research in the Public Interest. These programmes mainly investigated or supported new technology of prevention and early warning, detection and monitoring, emergency control, ecological regulation and sustainable management of important IAS in agriculture or forest (Wan et al. 2011c).

Applied research topics can be divided into four areas:

1. **Early warning and risk assessment technology** targeting IAS: Not introduced but with higher potential, introduced but only occurring sporadically, and alien species with higher invisibility and outbreak potential. Until now, we have completed the forecasting of the potential geographical distributions of the 64 worst invasive species.
2. **Rapid detection and network monitoring technology** This topic focuses on invasive species that are difficult to identify from morphological characteristics, or are labour and cost intensive for monitoring. Developing and improving rapid detection methods for inspecting and detecting potentially important IAS would directly prompt the interception techniques of IAS of Custom and Entry-Exit Inspection and Quarantine Bureaus in China's provinces, autonomous regions and municipalities. In addition to introduction of rapid detection methods and field surveillance tools, Wan et al. (2011b) also systematically reviewed the techniques and methods for detection and surveillance of 39 invasive crop pathogens, agricultural and forest IAS.
3. **Effective containment and rapid eradication technology** aims at IAS newly introduced and requiring emergency treatment. To effectively control or manage the IAS, Wan et al. (2008b) introduced theories of classical biological control, the latest technical findings, and summarized the biocontrol achievements for 19 important invasive weeds and insects in agricultural, forest and natural conservation ecosystems.
4. **Area-wide control and sustainable management technology** targets widely distributed IAS that cause serious damage to the environment, agriculture or forest production (Wan et al. 1993). Biological invasion is a novel topic in China and control/ management experience has been limited. Wan et al. (2008a) introduced international IAS management strategies, national regulations and legislation, national prevention and management guidelines in the selected developed and developing countries. Subsequently, Wan et al. (2011a) describe invader characteristics, the relationships between invaders and natives, and how an ecosystem responds to invasion and deals with the invasive potentiality, the population establishment and expansion, eco-adaptation and evolution of invasive species, and resistance and invasive ability of an ecosystem.

Summary. Invasive alien species pose a severe threat throughout China, affecting the ecological environment, national economy, and human welfare. Greater expenditures from governmental public and private funds would be cost-effective to protect the country from on-going and future damages. Increasing losses from IAS brings more pressure upon government to more effectively manage this threat. Therefore, research and management strategies of IAS in China should focus on six elements: (1) Research and management guidelines; (2) Essential work and infrastructure platforms; (3) Basic research and applied innovative technologies; (4) Promotion of invasion biology research and recruitment of talented research teams; (5) Scientific knowledge dissemination, public awareness (outreach) and education; and (6) International and regional cooperation. Under the coordinated guidance of government departments, the research and management of IAS should be gradually implemented.

3.5 Conclusion

With the development of international trades and the requirements of IPPC and SPS Agreement, China places more attention to plant quarantine and IAS management. It has achieved more transparency and harmonization in China, basing on the domestic regulatory framework and technical support. The three levels of China's legal system, the international collaboration between China and other members of WTO and FAO, and the domestic collaboration among AQSIQ, MOA, SFA, MEP, related Academies and Universities, are presenting important contributions in plant quarantine and IAS management. Risk Assessment, IAS detection, monitoring, emergency alert and quarantine treatment of exotic pests constitute the framework of plant quarantine and invasive species management. It is acquiring significant biological and ecological information and progress toward exclusion, management and eradication of IAS with continuing support from government.

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Chapter 4
The Importance of Core Biological Disciplines in Plant Biosecurity

Susan P. Worner, Robert C. Venette, Mark Braithwaite, and Erhard Dobesberger

4.1 Introduction

4.1.1 Overview of Biological Knowledge Needed to Conduct a Pest Risk Analysis

Increased tourism and trade, coupled with a changing climate are resulting in biodiversity loss and undocumented detrimental economic, social and environmental impacts from invasive alien species. Both managed and natural ecosystems are at risk and the economic, social and environmental wellbeing of every nation are threatened. Biosecurity programmes facilitate trade by analysing the risks associated with the movement of plants and plant products and mitigating those risks through various risk management methodologies. Sound biological knowledge is essential to the conduct of a reliable and accurate Pest Risk Analysis (Chap. 9).

Plant biosecurity programmes cannot exclude *all* non-native species because of the large trade volume, the number of species involved and the associated biological diversity. The economic benefits of trade, weighed against the risk posed by invasive alien species, often influences decisions involving importations. Here we highlight themes common to each of the core biological disciplines and explain how a thorough understanding of these disciplines can improve the biosecurity of a nation involved in trade and travel.

S.P. Worner (✉) • M. Braithwaite
Bio-Protection Research Centre, Lincoln University, P.O. Box 85084, Canterbury, New Zealand
e-mail: susan.worner@lincoln.ac.nz

R.C. Venette
Northern Research Station, USDA Forest Service, 1561 Lindig Street, St. Paul, MN 55108, USA

E. Dobesberger
906-1300 Pinecrest Road, Ottawa K2C 3M5, Canada

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Many human activities (airline travel, international mail, commercial trade), have the potential to move undesirable pest species into areas where they are not native, where they become established and cause significant harm to agriculture and natural resources. The negative consequences of some human activities involving trade or travel may far exceed the benefits. With respect to plant biosecurity, some of those activities are more likely to lead to the invasion of species with the potential to cause significant ecological, economic, or social harm. The challenge for biosecurity officials is to permit activities that have net benefits and to prohibit, or strictly regulate, activities that may be detrimental. We mitigate the potential threat and damage from unwanted organisms through Pest Risk Analysis, and begin by identifying species that are "most dangerous."

Accurate evaluation of the threat that species pose requires comprehensive knowledge about species and their responses to management. We must evaluate each species' taxonomy, distribution, biology, environmental requirements, introduction pathways, and potential to impact the target ecosystem. Decision makers within national biosecurity agencies need sound scientific knowledge and reliable advice upon which to inform their decisions, to guide the development of appropriate plant biosecurity policies, to design and implement risk mitigation measures to facilitate trade in a phytosanitary manner and to access the progress related biosecurity activities and to identify information gaps (NZ Biosecurity Science Strategy 2007).

Biosecurity agencies with the responsibility to protect the economic, environmental, social and cultural values of their countries from the detrimental impacts of invasive alien species are required to complete Pest Risk Analyses (Chap. 9). The process and decision-making that comprises the PRA must be informed and guided by suitable biological science.

The full range of plant and pest relationships must be exposed often in an environment of limited data or information, and frequently in languages that are not easily accessed. Despite our best plant biosecurity efforts toward preparedness, prevention is not always possible as evidenced by numerous/frequent incursions (McCullough et al. 2006). If a new non-indigenous invasive species incursion occurs, then a coordinated response programme of surveillance, containment, management or eradication must be designed and implemented.

Determining effective surveillance strategies requires a thorough understanding of pest biology. Surveillance in a target area is essential for early detection of an exotic pest (Chap. 11). The purpose of surveillance is wide-ranging: to determine whether a pest of quarantine significance is present; to estimate population abundance; to monitor seasonal and spatial distribution and to assess how these variables change over time.

Some of the largest surveillance programmes carried out by the US Department of Agriculture, Animal and Plant Health Inspection Service (USDA, APHIS) in the USA have been for invasive species such as Gypsy Moth (*Lymantria dispar* (Linnaeus)), Mediterranean Fruit Fly (*Ceratitis capitata* (Wiedemann)), Khapra Beetle (*Trogoderma granarium* Everts), Golden Nematode (*Globodera rostochiensis* (Wollenweber)), and Soybean Rust (caused by *Phakopsora pachyrhizi* Sydow & Sydow, and *P. meibomiae* Arthur). These species are major global pests and are regulated in many countries. Any survey must take into account at least the life cycle of the target species. Large surveillance programmes also take advantage of our knowledge of the response of the species to certain intra-species or environmental cues.

Insect surveillance tools (light traps. attractive pheromones, etc.) are based on the behavioural characteristics of the species. For plant pathogens, the effectiveness of spore traps to detect fungi, for example, depends, in part, on whether the fungus produces an aerially dispersed spore and the influence of temperature and moisture on the release of spores. Detection tools that allow us to monitor or capture various stages of a plant pest life-cycle are well covered in any good pest management textbook.

4.1.2 Chapter Objectives

Certain biological disciplines, particularly Entomology (including Acarology), Plant Pathology (including Bacteriology, Mycology, Nematology, and Virology), Malacology, and Weed Science, are required knowledge for the study of pests and pest management. We consider these disciplines "core" to various aspects of plant biosecurity and particularly the conduct of a Pest Risk Analysis. Understanding of these disciplines will help biosecurity officials anticipate and conduct biosecurity work, cope with the substantial uncertainty typically associated with the risk assessment process and identify information gaps for future research. This chapter cannot provide all of the necessary background that a biosecurity official might need, but it identifies themes common to each of the core disciplines and explains how a better understanding of these themes can improve a nation's biosecurity. The information provided here may serve as a springboard for students to study one or more of these disciplines. Additionally, it may help scientists with expertise in a core discipline to determine the relevance of their research to plant biosecurity.

4.2 Disciplines Relevant to Plant Biosecurity

Humans compete globally with plant pests for a valuable, sustainable and diverse plant resource in various environments. Economically important plants are traded worldwide by humans as various commodities, comprised of plant parts ranging from seeds, grains, fruits and roots, and parts thereof, to stems or trunks, branches, flowers and even leaves or plant tissue for culture. Shipping containers and pallets for shipping goods and cargo braces (dunnage) are made from lumber or timber, and harbour plant pests (Zahid et al. 2008).

Trade in diverse plant commodities poses significant problems from a plant biosecurity perspective to an importing country. Most plant pests consume or feed in or on plants, use plants as habitat and are adapted to resting in or on plants during

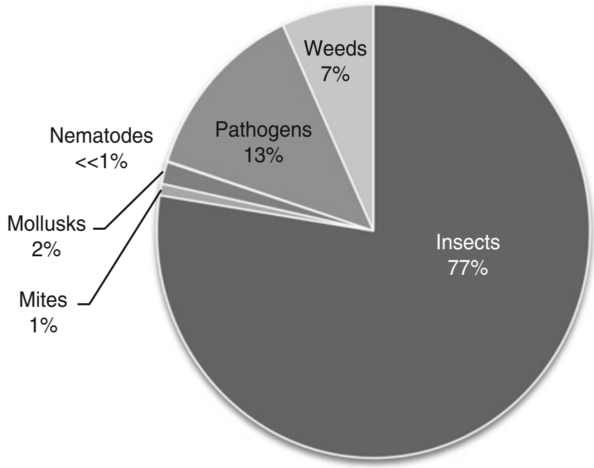

Fig. 4.1 Diversity of organisms intercepted at U.S. ports of entry from 1994 to 2000 (Drawn from data in McCullough et al. 2006)

harsh environmental conditions such as severe cold or drought. Pests also have adapted to utilise specific plant parts such a seeds, flowers, leaves, buds, roots, stem parts or cambial and phloem tissues of trees. Many insects vector numerous plant pathogens. As a result, pests move with plants, plant parts or plant products, particularly in the absence of risk mitigation efforts. Understanding the biology and ecology of pest organisms of various plants (or plant parts) is essential for developing appropriate biosecurity measures that facilitate the movement and trade of plants (Fig. 4.1).

The core biological disciplines Entomology (study of insects), Acarology (study of mites), Plant Pathology (study of pathogens including fungi, bacteria and viruses), Nematology (study of nematodes), Malacology (study of snails), and Weed Science (study of undesired plants in certain environments) are important to understanding the impact a pest organism will have on a plant and its ecosystem. These core disciplines are important even though some are better understood than others. A regulatory officer requires biological knowledge and access to experts in the core biological principles to conduct regulatory work and assure plant biosecurity. Expertise in biological disciplines is essential to reliably scan scientific information sources worldwide and provide foresight to a National Plant Protection Organization (NPPO) from a biosecurity perspective.

Pests intercepted at ports of entry are diverse. In the USA, interceptions of plant pests during 1994–2000 were dominated by insects (>75 %), followed by pathogens (13 %), and weeds (7 %; McCullough et al. 2006). Most (37 %) intercepted insects belonged to the Homoptera, Lepidoptera and Diptera (flies) and represented 21 % of the interceptions; and Coleoptera accounted for 13 % of the interceptions (McCullough et al. 2006). Thus, effective biosecurity programmes need representatives from each of the core biological disciplines.

Understanding the biology of a pest provides knowledge about pest behaviour and activity that enables various aspects of plant biosecurity work to be conducted. This includes determining the level of risk posed by pest organisms and the planning and feasibility of risk mitigation options such as containment, suppression, eradication or exclusion. Specific biological information aids in: (1) design of detection and surveillance tools and programmes, (2) evaluating host vulnerability to attack and evaluating the influence of climate on survival and establishment of pests in new environments, (3) determining the time period of pest activity or quiescence and dormancy, (4) identifying pathways, (5) anticipating the dispersal capacity of the pest organism and (6) design of specific treatments (e.g., heat or fumigation, wood processing) to eliminate the pest. Often information gaps may be identified and appropriate research may be undertaken. Comprehensive reviews of biological disciplines pertinent to plant biosecurity and relevant issues with examples in plant protection and quarantine work can be found in Kahn (1989a, b, c).

4.2.1 Entomology

Entomology is the study of insects, and is an important discipline for plant biosecurity. More than half of the regulatory programmes have involved insects as invasive pests or vectors of invasive plant pathogens. Insects are a significant concern because they frequently cause severe economic and ecological damage after they invade. Damages can accrue for decades or centuries. For example, the accumulated cost to the USA of the cotton Boll Weevil (which arrived from Mexico in the 1890s), exceeds 50 billion $US (Simberloff 1996). Losses to the European Gypsy Moth (a pest that has been invading eastern US forests since 1868) were 764 million $US in 1981 alone (Simberloff 1996). An eradication campaign to eliminate the Asian Gyspy Moth from the Pacific Northwest cost 20 million $US from 1991 to 1996 (Simberloff 1996). Invasive alien insects and mites annually cause about 18 billion $US of losses from damage and control costs in the USA (Pimentel et al. 2000), but these damage estimates do not fully account for impacts to ecosystem services. Charles and Dukes (2007) estimate that *Bemisia tabaci*, a whitefly that feeds on food plants and transmits viruses, will cost Brazil 5 billion $US over 5–6 years in lost ecosystem services which include delivery of food and fibre.

Insects are arthropods (joint-legged animals) related to crayfish, crabs, lobsters, woodlice, centipedes, millipedes, spiders, mites, and ticks. Identifying characteristics of arthropods include: (1) a segmented body, (2) a hard skeleton outside the body and soft parts inside, (3) paired, jointed appendages, (4) a ventral longitudinal nerve cord and, (5) a dorsal heart. Arthropods comprise about 4–6 million species; nearly two million species are named insects. About 70 % of all described animal species are insects. Insects also comprise the bulk of organisms that we call plant pests. Thousands of well-known and newly described species (new to science) are insect plant pests. These species have potential to cause significant economic and environmental damage, and hardship in all regions of the world.

Insects display a diversity of body form and function. Many insect species are pests, while other insects are critical components in our ecosystems providing critical services. For example, insects (such as bees, flies and butterflies) pollinate

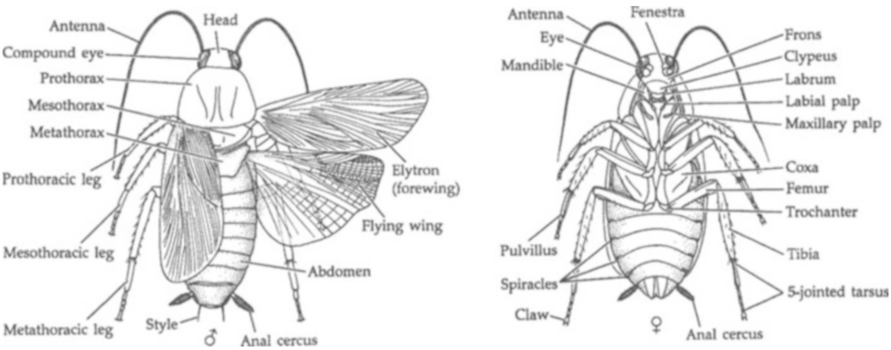

Fig. 4.2 Adult cockroach (Reprinted with permission from Sinauer Associates Inc. from *Invertebrates, Second Edition* by Richard C. Brusca and Gary J. Brusca)

most flowering plants and food crops. Other insect species kill numerous pest species through predation or parasitism, and provide natural control of plant pests (DeBach and Rosen 1991). Many insects vector pathogens of plants and animals (Purcell and Almeida 2005; Lounibos 2002).

Insects are identified by several anatomical features: All insects display three pairs of legs, three body regions (head, thorax and abdomen), one pair of antennae and, most adults possess wings should refer to (Fig. 4.3).

Immature insects grow by a series of moults (ecdyses) in which the external skeleton is shed and a new one is formed. The new skeleton is soft while the body expands, and later hardens. All insects exhibit metamorphosis and change their body form during the life cycle (Fig. 4.2). In fact, the change can be so dramatic that without some prior knowledge there is difficulty recognising the various forms as belonging to the same species. This feature often causes problems for plant biosecurity when the organisms cannot be recognised as a pest. Two types of life cycle are discussed below. Additional information concerning the anatomy and biology of insects can be found in any good textbook of Entomology. The types of damage that insects cause are identified below (Sect. 4.11.11).

4.2.2 Acarology

Acarology is the study of mites. Mites are also arthropods and closely related to spiders. Mites rival insects in terms of their global distribution and the habitats they occupy. Some mite species can be serious pests of agricultural crops, ornamental plants and stored products. Other mites cause sickness and death in animals and humans (Jeppson et al. 1975). Sometimes the economic impact of mites may be greater than more well-known insect pests. Biosecurity authorities regulate many mite species. Mites are small-bodied or minute, easy to transport and difficult to detect. However, their capacity to destroy a crop belies their size. For example, several mites have in their species name the epithet "destructor". *Halotydeus*

Fig. 4.3 Views of a monarch butterfly emerging from its chrysalis (Image courtesy iStockphoto/Cathy Keifer)

destructor (Tucker) (the Redlegged Earth Mite), is a major pest of pastures, crops and vegetables, in regions of Australia, South Africa and New Zealand (Ridsdill-Smith 1997). *Varroa destructor* (Anderson and Trueman 2000) has a large but indirect impact on many plants by killing colonies of bees, their major pollinators. Mites are also efficient vectors of plant pathogens. Some mites are beneficial, though, and can be used to control insect pests.

Mites are assigned to the Class Acarina. People who study mites are called Acarologists. Mites are not insects, but some entomologists (particularly those interested in plant pests) have extensive knowledge of their biology. Mites have four pairs of legs as an adult and have no distinct body divisions (Fig. 4.4).

Curiously, mites have three pairs of legs when they emerge from the egg stage (as larvae). When the larva molts, it acquires a fourth pair or legs and is called a nymph. Many horticulturalists are concerned with leaf-feeding mites; some mites can induce galls or blisters on plants thereby causing aesthetic damage. Various "bud mites" cause plant buds to become enlarged so they do not produce shoots or show only weak growth. If the buds are dissected, large numbers of tiny microscopic elongate mites will be found.

4.2.3 Plant Pathology

Plant Pathology is the study of plant diseases. Plants are diseased when normal physiological functions are altered by abiotic (non-living) or biotic (living) factors. Biotic causes of plant disease, also known as plant pathogens, are a concern to

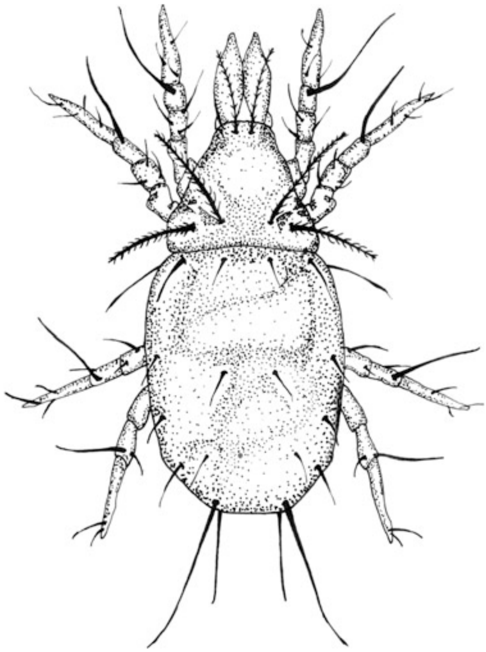

Fig. 4.4 Cereal Mite (*Acarus siro*) adult (© Lincoln University, Natural Sciences Image Library)

biosecurity authorities. Under appropriate conditions, plant pathogens can establish quickly and cause severe damage. Pathogens can be spread quickly by wind, water, farming equipment, insect vectors, plant debris, seeds and planting material, animals and farm workers (Gamliel 2008). Hence, pest risk assessment is important to determine which plant pathogens have high impacts. Typically, high-risk plant pathogens are divided into: (1) pathogens of wheat, corn, rice, or potatoes (the four staples), (2) pathogens of cash and secondary crops, (3) pathogens of non-food crops, and (4) pathogens of wild plants (Anderson et al. 2004). Pimentel et al. (2000) estimate that invasive alien plant pathogens cause crop losses in the USA of about 21 billion $US per year. Invasions of the USA by pathogens that cause Chestnut Blight and Dutch Elm Disease had major effects on the structure and function of forests in the eastern USA (See Sect. 8.2.1). More recent invasions by the pathogen that causes Sudden Oak Death are changing forests in the western USA.

Plant diseases have various effects such as reducing the yield of food crops, downgrading the aesthetic value of ornamental plants and fruits, and producing toxins in human or animal food (Schumann and D'Arcy 2010). Plant diseases are caused by fungi, bacteria, viruses (Chap. 19), nematodes (Chap. 17), and plant-parasitic plants (Brooks 2004). Abiotic factors such as nutrient deficiencies also cause symptoms of plant disease. Plant diseases can result in many different symptoms such as (1) damping-off, (2) foliage, flower and fruit spots and mottles, (3) vascular wilts, (4) cankers, (5) galls, (6) root rots, (7) wood decay and, (8) post-harvest rots. Only a small proportion of all fungi and bacteria are responsible for plant diseases. Most fungi and bacteria have ecological benefits such as contributing to nutrient recycling through decomposition of dead cells. Others have close beneficial associations with plants and animals, for example mycorrhizal fungi that live in a close symbiotic relationship with the roots of plants. Fungi and bacteria have adapted to and colonized virtually all ecosystems and niches on earth. Viruses, however, are much more specialised in that they require a host to survive and reproduce. Many pathogens depend on an insect or mite to serve as a vector (Brunt et al. 2006; Harris and Maramorosch 1980).

4.2.4 Nematology

Nematodes (eelworms or roundworms) are a diverse group of multicellular animals and are probably the most abundant kind of animal on earth (Chap. 17). Nematodes occur from the deepest parts of the oceans to living in beer mats in some bars in Germany (Boucher and Lambshead 2002; Ferris 2009). Nematodes vary in size from <1 mm to several meters in length (Ferris 2009). Species that cause damage to plants are typically minute and require a microscope for study. Indeed, considerable skill is required to identify them. Nematodes can be distinguished from other animals by their elongate shape (at least for part of the lifecycle), bilateral symmetry, undulating movement, and a largely protein-based cuticle. Nematodes are also noteworthy for structures that they lack. Nematodes do not display a coelom (i.e., a body cavity surrounded by muscle), a respiratory system, a circulatory system, and, most importantly, they lack true segmentation. All nematodes are essentially aquatic organisms. Many species occur in freshwater or marine environments while others live in soil occupying water films that surround soil particles and roots. Still other nematodes live as parasites within plant or animal tissues (Ferris 2011).

Some nematodes are harmful to plants or animals while some are beneficial by mineralizing plant nutrients and regulating plant pathogens. Nematodes that feed on plants can impair plant growth and lower yields by damaging plant roots, thus impeding nutrient and water uptake, creating wounds for the entry of plant pathogens, or by vectoring plant viruses. Some nematodes are vectored by insects (Kobayashi et al. 1984). Nematode damage to plants is frequently misattributed to other causes such as nutrient deficiency. Females of some nematode species swell with eggs, die, and harden the cuticle to a tough outer covering (cyst). Cysts are highly resistant to desiccation, and eggs within cysts may remain viable for many years. Epidemics of nematodes develop slowly but many nematode species are of biosecurity significance. Nematodes are small, hard to detect and tend to be spatially aggregated as well as difficult to identify. Many live in the plant or soil and are often associated with imported agricultural commodities so the potential for new incursions is high. Some nematodes, such as the Golden Nematode (*Globodera rostochiensis* (Wollenweber) Behrens) an important pest of potatoes, the Dagger Nematode (*Xiphinema index* Thorne & Allen) a pest of grapes, the Sugarbeet Cyst Nematode (*Heterodera schachtii* A. Schmidt) and Citrus Nematode (*Tylenchulus semipenetrans* Cobb) have a history of invading new areas and have considerable economic impact.

4.2.5 Malacology

Slugs and snails are important pests of plants. Snails are often intercepted in commerce, and invasive alien snails can negatively impact domestic agriculture. For instance, in southern Australia, at least five species of exotic snails have been identified, and are economic pests of wheat, barley, legumes, pastures and vineyards (Baker 1989, 2002, 2004, 2008). Snails often reach extraordinarily high abundance (540 snails/m^2) to clog farm machinery, contaminate harvests and destroy young seedlings (Baker 2002, 2004, 2008). Large numbers of snails in pasture will cause stock to reject the pasture due to slime (Baker 1989, 2008). Snails may also be intermediate hosts for human or animal pathogens (Baker 2008). An APHIS inspection manual cautions personnel to wear gloves when handling snails due to the possibility of disease contamination.

Slugs are pests of field corn in the USA causing severe damage by shredding and stripping young plants. Snails move naturally at a “snail’s pace”, but are associated with humans (synanthropic) movement and have been introduced worldwide, except Antarctica (Grimm et al. 2009). Invasive snail species displace native species often with unknown detrimental impacts on native ecosystems (Grimm et al. 2009). Biological characteristics of invasive snails in cool temperate regions include: (1) A broad host plant food range, (2) a wide habitat tolerance, and (3) efficient dispersal (Grimm et al. 2009).

The USA frequently experiences infestations of the Giant African Snail, *Achatina fulica* (Férussac) (GAS). These snails grow to 8 in. (20 cm) long and chew through plants, plaster and stucco. GAS may carry a parasite that can infect humans with a nonlethal strain of meningitis. The snail is hermaphroditic (each snail with male and female reproductive organs) and can lay 1,200 eggs a year, enabling them to proliferate rapidly. Recently, thousands of GAS have infested at least five separate neighborhoods in the Miami area (Capinera and White 2011). GAS is endemic to east Africa, and now occurs in the Pacific Rim, Hawaii and several Caribbean islands (Raut and Barker 2002). Entry into the USA is prohibited without a permit from the USDA, and permits have not been issued for many years. The origin of outbreaks has not been established, but snails may be smuggled for religious rituals or as pets.

4.2.6 Weed Science

Weed science is the study of plants “out of place” (i.e., in any area where they are not wanted; ex situ). Weeds (Chap. 21) are among the most significant invasive species. Because plants are primary producers, the invasion of a new plant species can dramatically alter the structure and function of ecosystems. The economic impact of invasive alien weeds is at least $34 billion annually in the USA in damages and control costs; most of this impact (23 billion $US) comes from losses caused by crop weeds in agriculture (Pimentel et al. 2000). These damage estimates do not account for adverse impacts to several ecosystem services. Natural ecosystems are particularly vulnerable because weeds can be especially difficult to control in these areas. Drift of herbicides to non-target plants is undesirable. Efforts to intentionally introduce insect herbivores or pathogens to control invasive weeds have led to unintended consequences for native plants, but improved risk assessments for biological control agents have increased environmental safety during biological-control implementation (Cruttwell McFayden 1998).

The discipline focuses on the circumstances under which certain plants may be problematic and on methods to achieve their control or exclusion. Pimentel et al. (2000) report that weeds cause an overall reduction of 12 % in crop yields, representing approximately 32 billion $US in lost production, annually. Figure A shows that 7 % of the interceptions at USA ports of entry are weeds. In general, weeds can interfere with the growth of other valued plant species, impinge upon aesthetics, alter ecosystems, put allergens into the air, serve as alternate hosts for plant pathogens and other pests, or become toxic to livestock. In the USA more than 2,000 plant species have been described as weeds. Several significant weeds, such as "tree of heaven" *Ailanthus altissima* (Mill.) Swingle, were originally introduced as ornamentals, but escaped cultivation (Patterson 1976). Vines such as kudzu, *Pueraria montana* (Lour.) Merr., and oriental bittersweet, *Celastrus orbiculatus* Thunb., were also intentionally introduced but now overtop and smother native vegetation (See Chap. 20).

4.3 Taxonomy and Phylogeny

4.3.1 Overview. The Role of Taxonomy and Systematics in Pest Risk Analysis

The terms "taxonomy" and "systematics" are used interchangeably in some scientific disciplines. Here, taxonomy refers to the naming and identification of organisms, while systematics involves the classification of organisms. Some biologists characterize taxonomy as the "servant" of biology because it provides important fundamental information on identity from which all other information follows (Sivarajan et al. 1991). Accurate identification and correct placement of organisms is important to plant biosecurity because some organisms can be very destructive of plants while closely related taxa may be innocuous. Misidentification of a suspected pest, weed or disease can lead to a failure to undertake biosecurity measures, or reacting when action is not prescribed. An error in decision for either reason can be extremely expensive.

Associated with the correct name of a specific organism, we collect information about its anatomy, biology, ecology and geographical distribution. Thousands of insects, plant pathogens and weeds have potential to impact managed and indigenous ecosystems globally. The unintentional introduction and establishment of a dangerous biosecurity threat can result in substantial costs, including direct costs for eradication, containment or control, and indirect costs. A significant and rapidly realized indirect cost is the loss of bilateral trade involving the affected commodity. If pest species cannot be eradicated, then regulatory agencies also incur ongoing costs of control and pest management. Ongoing control costs can comprise costs associated with the development of new control measures such as new insecticides, biocides, research on new biocontrol agents, research on new control technologies such as pheromones and baits and research on mitigation measures to facilitate commodity movement. The environmental damage and control cost of a single species can be substantial. In the USA, Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) costs 400–600 million $US (Pimentel et al. 2005). In New Zealand, non-indigenous invasive invertebrate pests cause 800 million to 2 billion $NZ of economic impact on pasture and forage production (Goldson et al. 2005). For a country of only four million people that represents a significant portion of GDP.

The misidentification of an alien invasive species can set off a cascade of detrimental consequences that can be extremely expensive for any country. Some biologists believed that misidentification led to the establishment of a serious orchard pest, the Light Brown Apple Moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), in California during 2007 (Suckling and Brockerhoff 2010). This moth became established in California and widespread in the UK causing serious economic loss because it was mistaken for another, innocuous species and not LBAM (Fountain and Cross 2007; Suckling and Brockerhoff 2010).

Mistaken identity caused massive damage in Africa by Cassava Mealybug (*Phenacoccus manihoti* Matile-Ferrero), a pest of cassava (manioc/ tapioca). Cassava is a drought-resistant, staple food crop for over 200 million people in sub-Saharan Africa. In 1973 a mealybug species unknown to science was discovered causing considerable damage in the Congo. By the early 1980s the impact of this pest on cassava production was widespread throughout tropical sub-Saharan Africa. Tens of millions of people were affected. Unfortunately, the mealybug had been misidentified. As a result of mistaken identity, all subsequent attempts at biocontrol were ineffective. This resulted in years of wasted research effort and billions of dollars in crop losses (Zeddies et al. 2001). When the Cassava Mealybug was correctly identified, a highly effective biological control agent was identified and introduced to Africa bringing the Cassava Mealybug under control in 27 countries (Zeddies et al. 2001).

In Nova Scotia, Canada, the Brown Spruce Longhorn Beetle (*Tetropium fuscum* (Fabricius)) also was misidentified as a related congeneric species (*Tetropium cinnamopterum* Kirby), in 1990. It was not officially confirmed until 2000 when significant damage had already been realized (Smith and Hurley 2000). As time has passed, eradication has become no longer feasible and this pest has since spread to neighbouring New Brunswick. Current pest management and control strategies are aimed at slowing or preventing the spread of this pest species. Similarly, the Emerald Ash Borer, *Agrilus planipennis* Fairmaire, remained undetected at low populations probably for at least a decade before its confirmation during 2002 in Detroit, Michigan and adjacent in Windsor, Canada. Correct immediate identification may have led to improvements in the conduct of a pest risk assessment and early development of effective risk mitigation measures. Other examples of why taxonomy matters can be found at: http://www.bionetintl.org/opencms/opencms/caseStudies/.

4.3.2 Binomial Nomenclature and the Linnaean Classification

Correct classification and identification of pests, pathogens, and weeds is important. A nomenclature must allow regulatory officials to communicate clearly about a species without ambiguity. The Linnaean system provides a classification in which each organism has only one correct scientific name, and no two organisms bear exactly the same scientific name. Thus, we have a tool to facilitate risk communication for each pest and disease species (Brusca and Brusca 2003). In 1758, Carolus Linnaeus proposed his system of binomial nomenclature. Based on this naming system, Linnaeus proposed that every organism has a two-part name: A Genus name followed by the species name. The species name distinguishes the individual species belonging to the Genus. For example, the scientific name for the Light Brown Apple Moth is *Epiphyas postvittana* (Walker). Many species are assigned to the genus *Epiphyas,* but only one species has the specific name "*postivittana*" hence the name *Epiphyas postvittana*.

A species name serves two purposes: (1) it provides a way to organise biological information, and (2) it associates a species with a particular group of organisms. So, information gathered about *Epiphyas postvittana* from Melbourne, Australia ought to be relevant to *E. postvittana* from Nelson, New Zealand or California, USA. Second, a species name provides a way to codify our understanding of relatedness of different species. The classification system was developed by Linnaeus and published in the tenth edition of his *Systema Naturae* (1758) in which he provided species names for all animals that he knew and proposed some guidelines for naming animals. Linnaeus was also one of the first taxonomists to base species classifications on species similarity rather than on differences (Brusca and Brusca 2003). He provided the basis for a classification hierarchy that reflected the evolutionary history and relationships between species. Following Linnaeus, the classification system has been modified several times in appropriate disciplines. Currently the system comprises eight major taxonomic categories, for example:

Category	Taxon
Domain	Eukarya
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta

(continued)

Category	Taxon
Order	Lepidoptera
Family	Tortricidae
Genus	*Epiphyas*
Species	*Epiphyas postvittana* (Walker)

Notice the person's name that follows the species name (the genus plus the specific name). That is the name of the person who first described the organism and gave it its name. For animal classification, an author's name is placed in parenthesis, when the species was originally assigned to a different genus. When the author's name is not in parenthesis, it means the species was put in the "correct" genus.

Using this system, insects identified as *E. postvittana* are more similar to each other than to those identified as *Epiphyas pulla* (Turner). The similarity is generally taken to involve physical resemblance. Thus, *Epiphyas postvittana* and *E. pulla* would be more similar to each other than to the moth *Amorbia emigratella* (Busck), which is in another closely related, but different genus. All three of these species belong within the Family Tortricidae (commonly known as the leafrollers or Olethreutine moths), and as such are more similar morphologically and behaviourally to each other than to the silkworm moths (Family: Bombycidae), for example. All moths (Order: Lepidoptera) are more similar to each other than to the beetles (Order: Coleoptera), and so on. All biological disciplines attempt to use this same approach when naming species (though the taxonomy of fungi has some notable exceptions).

Taxonomic relationships among taxa are important because when little information is known about one species, biologists often turn to what is known about closely related species and assume the poorly-studied species is similar in anatomy, behaviour and biology. Such assumptions would be poor if the taxonomy did not accurately reflect the evolutionary history of the group. While exceptions to this generalisation occur, the principle does have value as a working assumption.

4.4 Anatomical Characteristics of Important Insect Plant Pests

The class Insecta includes approximately 25 orders of insects. The word "approximately" is used because taxonomists continually revise their classifications as new taxa are discovered and more biological information becomes available. With respect to important plant pests the most notable species occur in about seven of the larger insect orders. Figure 4.5 shows the anatomical features of a generalised insect that illustrates the common anatomical features upon which the classification of the major orders is based. A simple diagnosis for seven major insect orders that are often of interest in plant biosecurity follows:

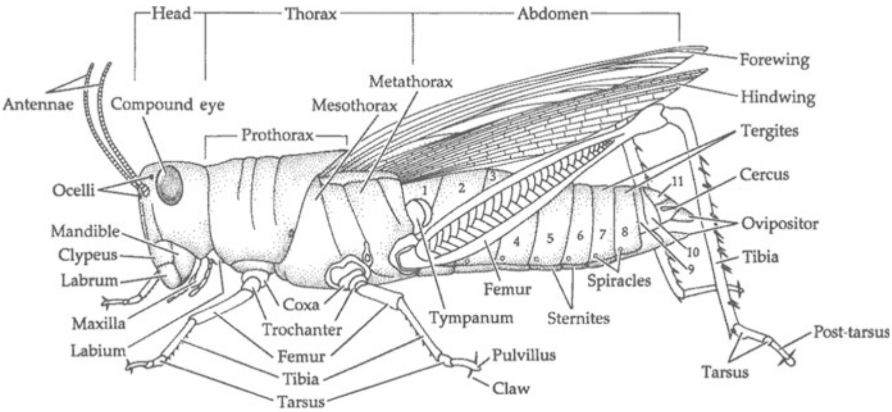

Fig. 4.5 External anatomy of a grasshopper (Order Orthoptera) (Reprinted with permission from Sinauer Associates Inc. from *Invertebrates, Second Edition* by Richard C. Brusca and Gary J. Brusca)

4.4.1 Insect Orders Important to Plant Biosecurity

Order Coleoptera (beetles): Antennae with fewer than 11 segments. Ocelli absent. Mouthparts biting. Prothorax usually large and distinct. Forewings toughened into Elytra (shields) that cover all or part of the Abdomen. Elytra meet along the body midline. Hind wings are membraneous and larger than forewings. Example: Asian Longhorned Beetle, *Anoplophora glabripennis* (Motschulsky).

Order Diptera (flies): Head mobile. Compound eyes large. 2–3 ocelli present. Single pair of membranous forewings. Hind wings reduced to form a pair of small balancing organs called Halteres. Middle segment of thorax enlarged. Example: Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).

Order Hemiptera (true bugs, aphids, cicadas, leafhoppers and scale insects): Mouthparts elongate to form a needle-like Rostrum for piercing and sucking, often projecting backwards under the body when not in use. Two pairs of wings usually present. Forewings thickened with posterior portion or entirely membranous. Example: Glassy Winged Sharpshooter (*Homalodisca vitripennis* (Germar)).

Order Lepidoptera (moths and butterflies): Body and both sides of wings usually covered with minute, overlapping scales or hairs. Compound eyes large. Mouthparts typically form a coiled Proboscis; absent in some species whose adult does not feed. Example: Gypsy Moth, *Lymantria dispar* (Linnaeus).

Order Hymenoptera (wasps, bees, ants): Antennae thread-like, multisegmented. Compound eyes well developed. Three Ocelli typical. Mouthparts biting, adapted in many species for imbibing liquids. Two pairs of membranous wings connected during flight by hook-like structures called Hamuli. Distinctive 'waisted' appearance except in sawflies. Females often with conspicuous saw-like or needle-like ovipositor or sting. Example: Wood wasp, *Sirex noctilio* Fabricius.

Order Orthoptera (grasshoppers, crickets): Body elongate. Compound eyes well developed. Ocelli present or absent. Mouthparts biting, downward-pointing. Pronotum enlarged, saddle or shield shaped. Fore wings toughened, often narrower than hind wings. Hindwings larger often, folded in longitudinal pleats. Some species wingless. Hind legs large, modified for jumping. Abdomen with pair of short cerci. Examples: Migratory Grasshopper *Melanoplus sanguinipes* (Fabricius); Migratory Locust *Locusta migratoria* (Linnaeus).

Order Thysanoptera (thrips): Very small to minute insects. Forewings slender, fringed with setae. Hind wings resemble forewings in size, shape and setal patterns. Short Antennae; rasping-sucking mouthparts. Example: Chili Thrips, *Scirtothrips dorsalis* Hood.

4.4.2 The Insect Life Cycle

Insects undergo considerable physical change as they progress through their life cycle. The life cycle begins with an egg and ends with an egg-laying adult. Between the egg and adult stages, we see immature stages that are the most injurious to plants. Insects have two major types of life cycle called complete (holometabolous) (Fig. 4.6a) and incomplete (hemimetabolous) life cycles (Fig. 4.6b). For the complete life cycle, eggs hatch into larvae that complete 1–8 larval instars. These instars are followed by the pupal stage, which then undergoes the dramatic but familiar metamorphosis from a shapeless pupa to an adult insect such as a beetle or butterfly. Many destructive insects with a "chewing" life stage have this life cycle. Moths, butterflies, beetles, weevils and flies commonly have a complete life cycle.

The incomplete, (hemimetabolous) life cycle starts with an egg that hatches to form a nymph that looks like the adult but without reproductive organs or well developed wings. Several nymphal instars (growth transitions) follow and involve a gradual development to the adult that has fully developed wings and reproductive organs. Species with this metamorphosis include many "true bugs" that have piercing-sucking mouthparts and are serious pests of plants. Hemimetabolous insects include grasshoppers, aphids, thrips, scale insects and plant hoppers.

4.5 Anatomical Characteristics and Life Cycles of Plant Pathogens

4.5.1 Fungi

Nearly all fungi have mycelia (slender filaments) that enable growth and colonization of a host. Fungi can be saprophytic (obtain nutrients from dead organic matter), necrotrophic (kill host cells to obtain nutrients), or biotrophic (obtain nutrients from

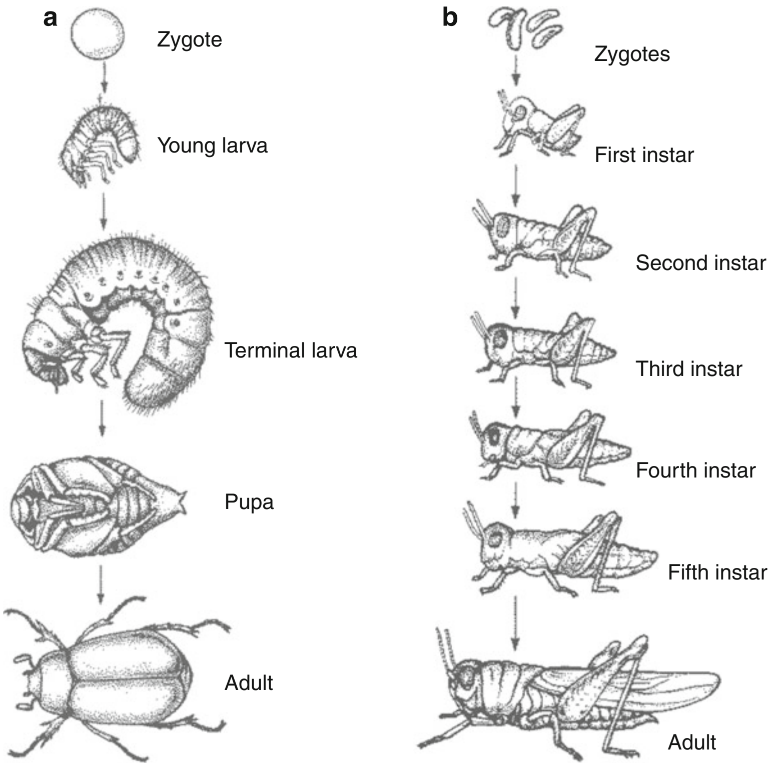

Fig. 4.6 (**a**) Holometabolous life cycle. (**b**) Hemimetabolous life cycle (Reprinted with permission from Sinauer Associates Inc. from *Invertebrates, Second Edition* by Richard C. Brusca and Gary J. Brusca)

their hosts living cells). Fungi mostly exist as mycelia consisting of haploid cells that contain only one copy of each chromosome pair (1N). The simplified sexual life-cycle consists of cell fusion (plasmogamy) to produce a dikaryotic cell which has the two separate nuclei. This is followed by nuclear fusion (karyogamy) to produce a diploid cell (2N) and then meiosis to return to the haploid state.

Fungi continually undergo taxonomic reclassification, often using modern molecular data. Organisms classified as true fungi have now been aligned with other taxonomic groups. For example, Plasmodiophora are now classified as Cercozoa (mostly soil-dwelling Protozoa). An important Plamodiophora plant pathogen of brassicas is *Plasmodiophora brassicae* Woronin, which is responsible for "Club Root." Oomycetes have been grouped with the Stramenopiles (flagellate cells with two flagella) or bear flagellate cells at some stage in their life cycle. Examples include brown algae and diatoms. Oomycota include *Phytophthora* spp. (water moulds), *Peronospora* spp. (downy mildews), and *Albugo* spp. (white rusts) (Fig. 4.7).

Four main groups within the true fungi contain plant pathogens: Basidiomycota, Ascomycota, Zygomycota and Chytridiomycota. Another group (Glomeromycota)

Fig. 4.7 Downy mildew of peas caused by *Peronospora viciae* (*left*) and white blister (rust) of Chinese cabbage caused by *Albugo* sp. (*right*) (Image by M. Braithwaite, courtesy of MPI, New Zealand)

contain the arbuscular mycorrhizal fungi (fungi that grow within the roots of plants and form a symbiotic association providing additional water and nutrients to the plant) and their relatives.

Basidiomycota: About a third of all true fungi fall within this group, including the conspicuous mushrooms, rusts, smut fungi, stinkhorns, earth stars, puffballs, and some yeasts. This group is diverse with no single distinguishing feature. Probably the most common diagnostic feature is the production of basidia (club-shaped cells) that produce haploid sexual basidiospores. Basidiomycetes generally have a long dikaryotic life-cycle phase where mycelial cells contain two non-fused haploid nuclei. Fusion only occurs when basidia are produced. Meiosis quickly follows with the production of the basidiospores. Basidiomycetes can have complex life-cycles with different parts occurring on separate hosts. For example, the significant quarantine pest, Apple Cedar Rust (*Gymnosporangium juniperi-virginianae* Schweinitz) completes its life-cycle on *Juniperus* spp. and *Malus* spp. Both hosts are required for the life cycle to be completed because spores produced on one host can only infect the other host.

Ascomycota: This group accounts for most of the described fungi and contains many plant pathogens. Ascomycots are called “sac fungi” because the ascospores (sexual spores) are produced in a sack-like ascus that defines this group. Ascomycetes also have a second part to their life cycle: The production of asexual spores or conidia and many fungi (formerly Deuteromycetes) that produce conidia and which also lack morphological evidence of sexual reproduction are included in the group. Conidia are often produced during spring and summer and enable a rapid build-up of the fungus. Ascomycetes predominantly exist as haploid mycelia until sexual reproduction occurs. A fruiting structure (ascocarp) protects two types of specialised hyphae (hymenium) that mate to form the diploid state. Meiosis usually occurs quickly to form the haploid ascospores. These fruiting structures can be closed (cleistothecium), have a narrow opening (perithecium) or be cup-shaped (apothecium). An example of an ascomycete pathogen is Chestnut Blight,

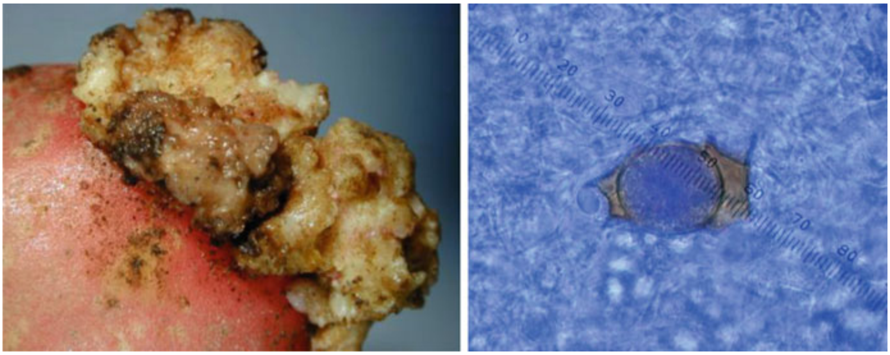

Fig. 4.8 Potato wart caused by *Sychytrium endobitium* showing tuber galling (*left*) and resistant long-lasting oospores (*right*) (Images by M. Braithwaite, courtesy of MPI, New Zealand)

Cryphonectria parasitica (Murrill) Barr, a catastrophically destructive disease of chestnuts in North America (See Sect. 8.2.1).

Zygomycota: A small group of fungi distinguished by sexual production of zygospores through gametangial fusion and asexual production by sporangia. An example is *Rhizopus stolonifer* (Ehrenberg) Vuillemin that is called Black Bread Mould. This fungus can also spoil stored fruit and vegetables.

Chytridiomycota: An ancient fungal lineage, based on its anatomy, Chytridiomycetes are predominantly aquatic. Their gametes display flagella, a feature unique to fungi. Their life cycles are complex and can occur within a single host cell. An example is Potato Wart, *Sychytrium endoboticum* (Schilbersky) Percival, a serious disease and quarantine pest of potatoes, which has highly resistant soil-borne spores (sporangiospores) (Fig. 4.8). When infected potato tissues rot, spores are released into the soil and remain dormant until a potato host is planted. These spores can remain viable for decades; in fact, their life span has not been determined (Hampson 1993).

4.5.2 Bacteria

Bacteria are defined as organisms consisting of a single prokaryotic cell. Their genomic DNA is not contained within a nuclear membrane (that would form a true nucleus). Bacterial cells are encapsulated in a cell wall primarily composed of peptidoglycan (which is not found in fungi). Bacteria reproduce via binary fission not meiosis. Bacteria have various cell shapes. The four basic shapes are: Cocci (spherical), bacilli (rod-shaped), spirochaete (spiral-shaped), and vibrio (comma-shaped). Bacteria occupy a diverse range of ecological niches. The life cycles of plant pathogenic bacteria are strongly tied to their hosts. Bacterial populations build rapidly during spring and summer until disease thresholds are reached. Bacteria can survive on plant debris, alternate hosts, or epiphytically on plant surfaces until

Fig. 4.9 Olive knot caused by *Pseudomonas savastonoi pv. savastonoi* showing galls on stems (Image by M. Braithwaite, courtesy of MPI, New Zealand)

conditions are suitable for infection. For example, *Pseudomonas savastonoi* (Janse) pv. savastanoi causes olive knot and lives as a saprophyte on the surface of leaves, twigs and fruits (Fig. 4.9). These bacterial populations reach their highest numbers during spring when plants are most susceptible to infection. A specialised group of bacteria called phytoplasma lack a cell wall. They are obligate parasites of the phloem tissue of plants and can be vectored by insects. They have become serious quarantine organisms in recent years.

4.5.3 Viruses

Viruses are submicroscopic particles consisting of nucleic acid surrounded by a protein coat. Replication can only take place in the host cell where the virus controls the cell processes for replication. The most common shapes of plant viruses are: Isometric (round, 26 nm in diameter), Rod-shaped (100–300 nm long), Filamentous (to 1,000 nm long), Geminate (two isometric particles joined together), and Bacilliform (short round-ended rods, to 300 nm long). Plant viruses cannot penetrate tough plant cell walls unaided. Consequently, their life cycles include direct transmission of plant sap via wounds (during pruning or grafting) or in association with various vectors. Plant viruses survive in the host, on alternative hosts or in their vectors. The most common vectors include insects, nematodes, mites, and fungi. Plum Pox Virus (Sharka) is a significant plant virus (Filamentous virus) that infects *Prunus* trees and can cause significant yield losses (Chap. 19). PPV is commonly spread on plant material by grafting, and by aphid vectors.

4.6 Anatomical Characteristics and Life Cycle of Nematodes

See Chap. 17.

4.7 Anatomical Structure and Its Relationship to Pest Risk Assessment

4.7.1 Insects

Mouthparts are an important feature of insects that live as plant pests. Mouthparts cause significant harm to plants through mechanical damage, tissue consumption and in the conveyance of disease. Insects that damage plants can generally be divided into three major groups based on the damage characteristics caused by their mouthparts. Also, bacterial, viral and fungal diseases transmitted during feeding can amplify damage caused by some insect mouthparts. Insect plant-pests can be divided into: insects with chewing mouthparts (Fig. 4.10a), insects with

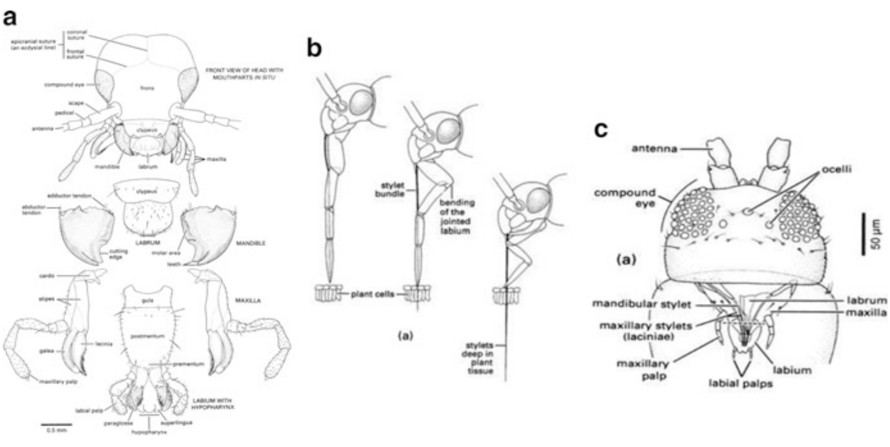

Fig. 4.10 Examples of mouthparts of insects that damage plants: (**a**) Chewing mouthparts of an orthopteran, (**b**) piercing–sucking mouthparts of hemipteran, (**c**) less specialised piercing-sucking mouthparts of a thrips (Reprinted with permission of John Wiley & Sons from *The Insects* by P. J. Gullan and P. S Cranston)

specialised piercing-sucking mouthparts (Fig. 4.10b) and insects with less specialised piercing sucking mouthparts (Fig. 4.10c).

Chewing insects. Chewing insects include grasshoppers, beetles, caterpillars, and earwigs. Chewing pests may be external or internal in their host plant. An example of external injury to plants is the chewing of pierid butterfly caterpillars feeding on brassicas. If left to feed, the caterpillars will consume all leaf tissue except the leaf veins. Significant quarantine pests in this category include beetles and moths whose larval stage causes most damage. Pests causing internal injury to plants by chewing include borers that tunnel into the stems, branches and fruits of plants. Examples include stem borers, fruit-tree borers and borers that damage forest trees. The Steel Blue Sawfly (*Sirex noctilio* Fabricius) is an example of a recent invasive forest pest in the USA. The female lays eggs in the trunks of living trees by making a puncture with her ovipositor. With each puncture, fungal spores are deposited and cause damage to the tree. The boring sawfly larvae spread fungus throughout the tree. The fungus is important to the larvae because it breaks down cellulose, making the wood digestible. Up to 700 *Sirex* larvae per cubic foot of wood have been recorded (Rawlings 1949). The female wasp deposits only one egg with each insertion of the ovipositor. This suggests that many injections of fungus spores occur with each oviposition episode. Other examples include beetles that cause physiological damage and eventually kill their hosts. Examples include the Emerald Ash Borer, *Agrilus planipennis* Fairmaire and the Brown Spruce Longhorn Beetle, *Tetropium fuscum* (Fabricius).

Insects with Specialised Piercing-Sucking Mouthparts. This type of mouthpart is derived from chewing mouthparts. Primitive mouthparts are modified to form a sucking beak that can easily pierce the epidermis of plants to suck sap. Plants injured by these insects appear "unhealthy" and display wilting growth, distortion of plant parts or abnormal growth. Aphids, cicadas, leafhoppers and scale insects possess piercing-sucking mouthparts. To facilitate sap flow and digestion, the insect injects a small amount of saliva into the plant. Some piercing-sucking species also inject viruses and other microorganisms into the plant while feeding. One species, the Glassy-winged Sharpshooter *Homalodisca coagulata* (Say), has been responsible for the destruction of large areas of vineyards in several valleys in California. This sharpshooter can feed on more than 130 different host plants, which makes it particularly dangerous as a pest and vector of a pathogen. Glassy-winged Sharpshooter is responsible for vectoring Pierce's Disease to grapevines. Infected plants can die within 1 or 2 years.

Insects with Less Specialised Piercing-Sucking Mouthparts. Probing-sucking mouthparts are a modification of a primitive form of piercing-sucking mouthparts and are found in thrips (Order Thysanoptera). Thrips use their beak to probe and pierce plant tissues, causing sap to exude. The sap is then imbibed through a hair-like stylet. Thrips damage is distinctive. The entire contents of the ruptured cells are removed. The injured surface displays a characteristic silvered or whitened appearance. If the attack is severe, then the plant will soon appear unhealthy. Thrips species are pests of onions, melons, pears, flowers, cotton, wheat, soybeans, citrus, strawberries and greenhouse plants. The physical injury thrips cause is often more severe in the seedling stage. Further, thrips can also vector viruses. The Chili Thrips, *Scirtothrips dorsalis* (Hood), was first discovered in Hawaii during 1987 and subsequently in Florida during 2005. This thrips attacks over 100 crops, including chili peppers, tea, strawberries, and tomatoes, resulting in defoliation and crop loss.

4.7.2 Mite Damage

Mites have sucking mouthparts and cause damage similar to thrips. However, mites do not cause silvering on damaged foliage. Mites rupture the cells and imbibe the contents that produce bleaching or browning of the foliage. Loss of sap at critical periods can cause severe crop losses, particularly during the fruiting stage. European Red Mite is a global pest that appeared in many countries early in the twentieth century causing then (as today) considerable economic loss in apple, pear, plum, cherry and peach orchards.

4.7.3 Plant Pathogens

Structural adaptations by plant pathogenic fungi include hyphae (mycelia) and spores. Hyphae enable fungi to colonise, penetrate, and infect host plants. Hyphae also form elaborate fruiting structures (mushrooms) and resistant structures such as sclerotia for long-term survival. Spores are structures that allow dispersal and survival of fungi. For instance, many fungi have spores that disperse in air and can travel long distances. Other fungi have spores that disperse by water-splash, insects, on seed, or can be motile in water. Spores highly resistant to adverse environmental conditions enable a pathogen's survival for extended periods.

Plant pathogenic bacteria are single-celled organisms that rapidly increase in numbers by cell division in the presence of the host and when environmental conditions are favourable. Infection can occur through stomata in the leaves or wounds and can be aided by enzymes or toxins that bacteria secrete. Insects vector some plant pathogenic bacteria.

Insects, mites or nematodes vector most plant viruses. Plant viruses are simple, typically consisting of nucleic acids covered by a protein coat. Their small size, association with vectors, and ability to control plant cells for their own replication are features that contribute to their success.

4.7.4 Nematodes

The feeding strategy of soil nematodes is often dictated by the structure of their mouthparts. Bacterial feeding nematodes will often display a collapsed, tubular, or funnel-shaped stoma or mouthpart. Nematodes that feed on fungi or root hairs will use a protrusible, fine needle-like stylet for piercing their food. Nematodes that feed on other plant parts will have a robust, protrusible stylet or spear. Some nematodes with spears feed on soil fauna, including other nematodes. Still other predaceous nematodes may have a broad buccal cavity with one or more large teeth. For an overview of Nematodes in regulatory work, see Chap. 17.

4.8 Risk Assessment, Organism Physiology and Response to Temperature and Moisture

Pest Risk Assessment is used to evaluate the potential of an organism to establish a viable population in a new area. The assessor must understand the response of the species of interest to important environmental factors, particularly temperature and moisture (See Sect. 4.11.4).

Most plant pests and pathogens (excluding higher vertebrates but including weeds) have limited internal control over their body temperature. By definition, the "body" temperature of an ectotherm is determined primarily by the external environment. Enzymes for growth and development function only over a limited range of temperatures. Typically, ectotherm range-of-survival is about 0–50 °C, but exceptions are known. For example, some fly larvae survive at temperatures of 55 °C or higher (Brusca and Brusca 2003). Some bacteria and fungi can survive at even greater temperature extremes.

Every life process of an ectotherm is controlled by temperature. For many species in cool temperature regions, population establishment often depends on the number of individuals able to survive exposure to cold by finding suitable winter habitats that are insulated from the elements (Cloudsley-Thompson 1962). In his review of the effect of microclimates on arthropod distribution, Cloudsley-Thompson (1962) remarks that soil, debris, living bark, and snow are actually good insulators from extreme cold. Clearly, "body" temperature influences an ectotherm's activity, from the physiological to the population level. Important points in the temperature tolerance range should be noted. Such tolerances vary between species and may differ between life stages of the same species. Determination of temperature tolerances of a species can help evaluate whether it is likely to establish in a new region. This is a key evaluation for pest risk assessment.

Figure 4.11 shows a generalised development rate curve for an insect, but the same curve could apply to other ectotherms. The curve passes through several points including the critical thermal maximum in which death is caused by: (1) proteins being denatured, (2) metabolic processes are disturbed and toxic substances accumulate, (3) food reserves become exhausted, and (4) desiccation occurs. At the upper temperature threshold life processes (rates of development and reproduction) are negligible. Next, the optimum temperature range exists within which life processes proceed most favourably or are at a maximum. At the population level, accelerated development means more insect generations per year

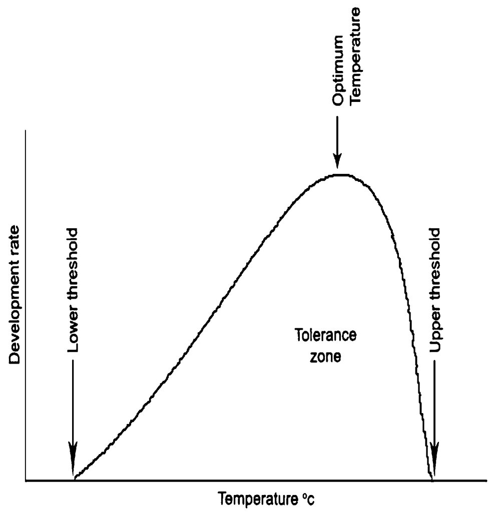

Fig. 4.11 Generalised development rate curve

(season) and subsequently more damage and impact on agricultural commodities. In the lower region of the development rate curve we see one of the most important parameters: the lower temperature threshold for development where development slows to become almost negligible. Then, below that is the lower lethal limit (not shown) where insects die. Death can result from many of the same factors that cause death at high temp lysis of cells as intracellular fluids freeze or dessication of cells as extracellular fluids freeze, or depletion of internal energy stores.

4.9 Water Balance

Moisture is the second-most critical environmental parameter that affects the distribution and activity of many plant pests and diseases. The balance-of-water required to sustain physiological processes versus the amount of water available dictates where an organism can exist. Too much or too little moisture is detrimental. The moisture balance must be maintained within a biologically defined range. We might think of moisture balance with respect to plants in the field. Plants directly provide herbivores with water and elevate the relative humidity of the microclimate through transpiration. We must also consider moisture balance in cargo conveyance (e.g., containers of cargo ships) and storage facilities. These are special habitats in which some insect pests may survive, reproduce and invade areas in which they do not occur.

Insects and many pathogens have a preferred range of humidity as witnessed through their behaviours. Certain fungi may require free water on the surface of a leaf or high humidity (>90 % relative humidity) before spores will germinate and initiate infection. Generally, insects are relatively inactive in their preferred range and more active outside their preferred range of humidities (Gillot 1995). Some insects prefer the very dry part of the range. Xeric-adapted examples include stored grain pests (*Tenebrio* and *Tribolium* beetles). These species live and reproduce under desert-like conditions. They can complete their life cycle in flour that consists only of 15 % water and RH50 %. Beetles exist under these conditions without taking free water. All water comes directly from food or indirectly from oxidative metabolism of protein (Chapman 2003). Many phytophagous insects die when their dietary moisture is low. Other insects prefer the wet part of the humidity range. However, if an insect can replace lost moisture (e.g., by sucking fluid from plants) and thereby maintain its moisture content level, then it can probably withstand extremes in dryness. But there are limits, and mortality at low humidities (even with an ample supply of water) may prevail simply due to the energy needed to maintain water balance. Conversely, death may occur at high humidities because the insect can't eliminate water rapidly. Unfortunately, limits to moisture tolerance are not as clearly defined for many insects or pathogens as they are for temperature.

4.10 Physiology, Life Processes and Risk Assessment

4.10.1 Physiology

In the previous section we noted that temperature and moisture responses are used by risk assessors to judge whether an organism is a potential biosecurity risk and likely to establish in an endangered area. We also discuss how those responses are used to determine habitat suitability and establishment (See Sect. 4.11.4). However, knowledge of both temperature and moisture responses are also needed to evaluate the potential of some sort of quarantine treatment to mitigate organism entry or establishment. Such treatments comprise the application of cold or heat. Knowledge about species responses is also used to assess the probability of surviving transport and storage on traded commodities. One difficulty however, is that the commodity itself (e.g. many fruits) may be adversely affected by such treatments. Careful research is required to develop appropriate protocols. For example, Mediterranean fruit fly is a pest of a wide range of fruit and is regulated by many countries in the world that do not have this species (Chap. 15). Gould (1994) reported that a cold treatment of 16 days at 2.22 °C or below will kill the flies. Moist and dry heated air is also used as quarantine treatments for a wide variety of pest species. For example, sweet potatoes are held at 39.4 °C for 30 h to kill root knot nematodes, *Meliodogyne* species (Hallman and Armstrong 1994). Hot water immersion also can be used as a post-harvest quarantine dip. Almost all of the 300,000 metric tons of mangoes exported to the USA each year are immersed in 46.1 °C water for 65–110 min to control fruit fly larvae (Hallman 2011). However, Hallman (2011) indicates that water immersion is no longer widely used as it is rapidly being replaced by treatments using ionizing radiation.

4.10.2 Life Cycle, Reproduction and Dispersal

Other life processes are important to consider in the pest risk assessment process. These include life cycle constraints, reproductive strategy and reproductive capacity of the organism. Reproductive traits in combination with dispersal can indicate establishment success, how quickly a species can spread and the impact that it might have. Assessment of the method of dispersal and potential to spread over a relevant time frame is always part of a PRA. A species with a high potential for spread is likely to have a high potential for establishment because it can find resources and potential mates quickly.

Insects and plant pathogens have diverse types of reproductive strategies and very high reproductive potential. For example many aphid species not only have high reproductive capacity they have a complex life cycle that often has an asexual and sexual phase. The asexual phase of an aphid's lifecycle involves parthenogenesis in which the mother aphid produces clones of herself. She can produce 50–100 clones. Her offspring already have immature aphids forming inside her body even before the offspring are born (Dixon 1987). Imagine the extraordinary reproductive capacity of aphids as often witnessed in the home garden. Aphids are among the many serious economic pests and biosecurity threats.

Other aspects of reproduction (such as the number of generations per year) can indicate the potential impact of a species in an endangered area and how quickly it can spread. The likelihood of establishment is often based on the number of offspring or propagules as well as the number of generations in an area. For example, the Spotted Winged Drosophila (*Drosophila suzukii* (Matsumura)) can complete 15 generations per year in Japan and as many as 65 adults can emerge from a single cherry (Kanzawa 1939). Along with a wide host range and ability to infest entire intact fruit makes this species an important biosecurity threat for many countries where it is not currently established.

The timing of important stages of the life cycle of the pest in relation to its host species is also a key consideration in assessing risk of exposure to the pest. For example, the fruit fly *Bactrocera dorsalis* (Hendel) is polyphagous and there would be no shortage of host plants available for most of the year in many countries where it might establish. However, the presence of fruit for oviposition may be a limiting factor for the establishment of *B. dorsalis* entering temperate countries in early spring when little mature fruit is available.

Sudden Oak Death (*Phytophora ramorum* Werre et al.) is a disease named after an epidemic involving the sudden death of many tanoak in California during 1995. SOD has an aerial phase in its life cycle, high levels of inoculum production, high virulence and can be transported long distances on alternate nursery-species hosts (Kelly and Meentemeyer 2002). SOD is considered a pathogen of high consequence. *Phytophthora ramorum* produces two propagule types: sporangia (which release zoospores) and chlamydospores (which survive for several years in soil or plant debris) (Chap. 20). Sporangia can be dispersed by water splash, wind or be washed into the soil and infect plant roots. Zoospores are short-lived, motile in water, and directly infect susceptible hosts. Chlamydospores are the dormant stage of this fungus and can survive for several consecutive seasons in the absence of any hosts in soil or plant debris. This dormancy strategy explains the difficulty of growing plants in previously infested soils (Kelly and Meentemeyer 2002).

Containment or eradication of *P. ramorum* is difficult because it has an extensive host range, can rapidly build up inoculum, can survive without hosts for several growing seasons, and is easily transported long distances on alternate nursery stock hosts. The pathogen has no effective control strategies after large host trees are infected.

4.11 Ecology and Epidemiology

4.11.1 Overview

To prevent species from establishing, or to eradicate or manage them if they invade, we require knowledge of their relationship to the environment. Ecology studies the relationships between species and their environment. Ecology may be viewed at several levels of organization. The individual organism is the basic unit of study. For Pest Risk Analysis (Chap. 9) and plant biosecurity activities, we study the population level. For example, an individual organism is rarely a threat. Even if the individual is a vector for a serious disease, the disease typically requires many vectors/individuals (inoculum) to be transmitted throughout the host population and remain viable. Individual insects, plant pathogens and nematodes, for example, may establish in a region but they are unlikely to have impact unless they form a persistent and viable population. Population(s) of a particular species may be detected, delimited, monitored, contained, controlled or eradicated.

The community forms the next level of organization in ecology and comprises coexisting interdependent populations of species (Price 1997). While this level of organization does not seem important in plant biosecurity, questions may centre on how some species can establish in species-rich communities when indigenous ecosystems are considered. Interspecific competition has been a favorite topic of community ecologists. Some invasive species may become serious pests only when out-competing extant, established species. Consider the possibility that competition by native cerambycid beetles such the Eastern Larch Borer (*Tetropium cinnamopterum* Kirby), endemic to North America. This beetle may have delayed the development of high population levels of the Brown Spruce Longhorn Beetle (*Tetropium fuscum* (Fabricius)), in Nova Scotia, Canada (Flaherty et al. 2011). *Tetropium fuscum* was likely to be present at least a decade before its detection in 1999 in Nova Scotia when damage by this species became clearly evident (Smith and Hurley 2000). Thus, complex interactions between an invading species and resident flora and fauna may have important consequences for the course and outcome of an invasion.

4.11.2 Population Ecology, Life Tables, Intrinsic Rates of Increase

Population ecology requires that we know the "life needs" of species of interest. Knowledge of conditions a species requires to establish a viable population and where those conditions are located, gives the plant biosecurity scientist ways of predicting if and where pests may establish before they have a chance to do so. Knowing where a species may find favourable habitat to establish should identify areas for pest surveillance to detect new pest incursions and provide early warning. Regulators must know about the dynamics of populations and what causes populations to grow, develop and decline. To do that, a measure of particular population parameters is required. The parameters that make populations change are those that increase population numbers (births/fecundity/immigration) and decrease population numbers (deaths/mortality/emigration). Immigration and emigration may balance each other; the main birth and death rates that cause change to a population is combined into a single parameter called a per capita rate of increase. Two ways of measuring this rate of increase are: (1) over instantaneous or continuous time where the rate is often referred to as the intrinsic rate of increase (r), and, (2) discrete time (such as in seconds, days, years, etc.) where it is referred to a finite rate of increase (λ). When r is greater than 0 (or $\lambda > 1$), the number of individuals in a population is increasing. When r is equal to 0 (or $\lambda = 1$), the number of individuals remains constant, and when r is less than 0 (or $\lambda < 1$), the number of individuals is declining and the population is on its way to local extinction.

The derivation of such parameters often requires that the species is studied throughout its life or over its life stages by constructing a life table. Examples of life tables can be found in any ecology text. Knowing such parameters allows the plant biosecurity scientist to predict population growth and decline using models of population growth. One of the more simple models that incorporates limits to population growth is the logistic equation that can be expressed by the Ricker Equation:

$$N_{t+1} = N_t e^{r\left(1-\frac{N_t}{K}\right)}$$

Where

N_{t+1} is population size at the next time step
N_t is population size at the current time
r is the intrinsic rate of increase
t is time
e is the base of the natural logarithm
K is the carrying capacity of the environment

Because the finite rate of increase $\lambda = e^r$ we can insert that in to the equation to derive a model more clearly based in discrete time where $N_{t+1} = N_t\lambda^{(1-Nt/K)}$. Species use diverse strategies to maintain populations over time, *i.e.* to keep $r > 0$. Some invest heavily in reproduction, maximizing the number of offspring produced. In extreme cases, species have eliminated the need for males, so that all offspring a female produces are capable of producing offspring themselves. Heavy investments in reproduction are common for species that must exploit habitats that are patchy at the landscape scale. Aphids, for example, live such a "risky" life cycle. Suitable host plants for aphids are often scarce over large spatial scales. When a suitable host plant is found, aphids have high reproductive capacity, and a complex life cycle that often has an asexual and sexual phase. The asexual phase of an aphid lifecycle involves parthenogenesis in which the mother aphid produces clones of herself. She can produce 50–100 clones. As described in Sect. 4.10, her offspring already have immature aphids forming inside their body even before they are born. Similarly, many nematodes and weeds can produce hundreds or thousands of offspring, but only a small fraction will find a host in a suitable environment to perpetuate the population.

Another strategy to maintain a population over time is to increase the number of generations per year. Insects that complete one generation per year are univoltine; insects that complete two generations per year are bivoltine, and insects that complete several generations per year are multivoltine. The LBAM can be multivoltine with up to four generations per year, each generation being completed in a few months (Suckling and Brockerhoff 2010). The invasive Mediterranean Fruit Fly can complete a generation in 32 days (Vargas et al. 1984). The bacterium *Erwinia amylovora*, the causal agent of fireblight, can double its population size in as little as 1 h when conditions are appropriate (Billing 1974).

A third strategy species use to maintain a positive growth rate is to increase survivorship of offspring. Species with greater survivorship typically have slower development and produce fewer, but larger, offspring.

As the population growth rate increases, so does its dispersal potential. A high dispersal capacity can contribute to successful invasion potential because it increases the chances that an invading species will locate suitable food, microclimate, and mates. A species with a high dispersal potential is also more likely to cause widespread impacts than a species with a low dispersal potential.

The intrinsic rate of increase is not always a meaningful indicator of the impact an invader will have. A more useful indicator may be the number of affected plants over time, reflected by a disease progress curve. Analysis of the area under the disease progress curve provides a measure of the "growth" of affected plants over time. Specifically, the rate of increase can be calculated as:

$$r = \frac{\ln\left(\frac{y_t}{1-y_t}\right) - \ln\left(\frac{y_0}{1-y_0}\right)}{t}$$

where r is the rate of increase, y_t is the proportion of affected plants at time t, and y_0 is the initial proportion of affected plants. The expected proportion of affected plants at any point in time is:

$$y_t = \frac{1}{1 + Ae^{-rt}}$$

where A is $(1 - y_0)/y_0$.

The area under the disease progress curve (AUDPC) can be often conveniently approximated from empirical observations as:

$$AUDPC = \sum_{i=0}^{n-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

where n is the total number of observations y_i is the proportion of affected plants on observation i, and t_i is the time (often measured as hours, days, weeks, or years) at which observation i was recorded (Madden et al. 2007). The first observation is counted as observation 0. AUDPC has been used most extensively for pathogens.

4.11.3 Establishment Potential

Establishment potential of a non-indigenous invasive species requires assessment of host availability, the species response to climatic conditions, its environmental tolerance, its potential to adapt to current conditions in the target area, its method of overwintering survival or dormancy, its previous history of establishment, how abundant it might be in the PRA area, its distribution in its native range, its potential to spread in the target area and available methods of control. Again, much biological and ecological knowledge is required to assess these criteria. For example, we must consider the lifecycle and biology of the pest species to fully assess its potential for movement with commodities and conveyances. Some pest species are clearly associated with certain commodities; a particular aspect of the lifecycle of other species may mean they are more likely to become associated with other types of commodities. Such species are called "hitchhikers." For example, the Asian Gypsy Moth in Japan is attracted to lights around ports and ships moored near their habitat when they oviposit. Eggs are often laid on vehicles, and heavy equipment that are shipped overseas. Because New Zealand imports many Japanese cars, the Asian Gypsy Moth is a serious threat to New Zealand where this polyphagous species is considered to have the potential to cause many millions of dollars of damage if it establishes there.

Some plant pathogens are highly host specific whereas others infect a wide range of hosts. For example, Guava or Eucalyptus Rust, *Puccinia psidii* (Winter) is of particular concern to Australia and New Zealand. A native to South America this rust has a remarkable host range within the Myrtaceae (over 70 known species), which includes *Eucalyptus* and *Metrosideros*, respectively natives of Australia and New Zealand.

The species response to climatic conditions, its environmental tolerance, its potential to adapt to current conditions in the target area, its method of overwintering survival are all related to the assessment of habitat suitability.

4.11.4 Habitat Suitability

Considerable information is required to determine suitability of the environment for establishment of target species. A key component of the risk assessment process is to evaluate the known temperature and moisture tolerances of the target species. If a species is judged not able to sustain a viable population in the PRA area, then it may be ignored. The information for such evaluations can be based on laboratory studies. If such studies are not available, then alternative methods based on habitat and climate matching require the collection of large amounts of data.

Insects and fungi are adapted to a relatively narrow range of optimum temperature and moisture conditions but they may develop over a wide range of temperatures. Knowledge about the species' lower threshold for development, its lower lethal limits and its optimum range for development allows the assessor to estimate how many generations, if any, can be completed at a particular site. For insects, much more is known about their response to temperature than their response to moisture conditions. For both responses, the data may take many years of laboratory study.

4.11.5 Temperature

Entomologists agree that temperature is a dominant environmental parameter affecting insects. A pest risk assessor would need, at the very least, the development rate curve for the species of insect (Fig. 4.11). Using the quantitative relationship that describes the association between insect development and temperature, we can determine the developmental requirements of the species in terms of physiological time, often referred to as "degree-days". The calculation of the number of degree days required for development of particular life stages allows the number of degree days needed to be accumulated by the species for complete development. From this data we can determine the adequacy for development of the pest in the climate associated with the endangered PRA area.

When detailed data about the species response to environmental factors is not available we can infer its habitat requirements from its current distribution. In other words, we can use analytical techniques or models to characterize the range of conditions where a species is known to occur. The inference is that the range of conditions represents those conditions to which they are adapted and therefore

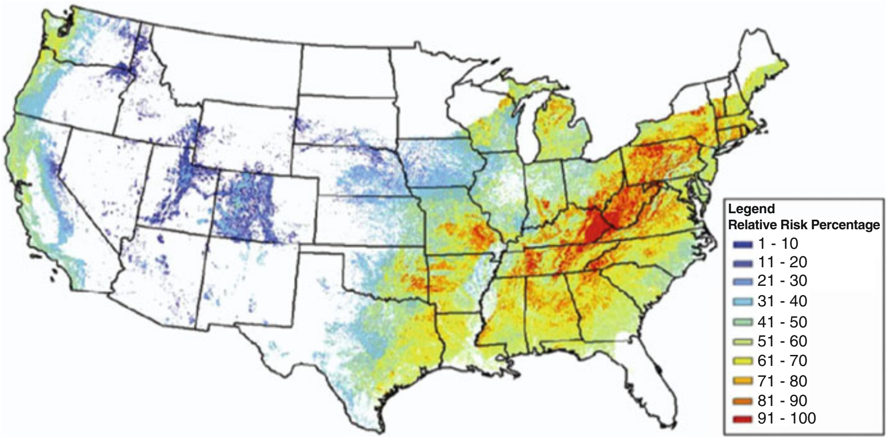

Fig. 4.12 Relative risk of *Phytophora ramorum* establishment based on infection frequency, extreme cold temperatures, and host range. Maps are based on 10 years of weather data (Reprinted from Magarey et al. (2007) with permission from the American Phytopathological Society)

represents suitable habitat for the species. Several quantitative methods can project that data to other areas where the species is not currently established. The methods are called species distribution models and the resulting spatial maps are called pest risk maps (Elith and Leathwick 2009; Venette et al. 2010). A typical species distribution map is shown in Fig. 4.12.

Some plant pathogens may grow and develop over a wide temperature range whereas other pathogens have specific requirements. For example, the teliospores of *Tilletia controversa* J.G. Kühn (Dwarf Bunt of Wheat) require a preconditioning exposure to light and at least 3–5 weeks at about 5 °C for germination (conditions typically found under persistent snow cover). Temperatures of 0–8 °C (maximum 10–12 °C) are optimal for infection to occur whereas temperatures of 15 °C strongly inhibit spore germination (Hoffmann 1982).

4.11.6 Moisture

Unfortunately, the limits to moisture tolerance are not as clearly defined for many insects as they are for temperature. Environmental extremes of moisture become the dominant factors affecting insects when temperatures are favourable. When temperatures are favourable, environmental moisture content directly influences many of the activities of insects. For example, some Lepidoptera larvae stop feeding when the air becomes saturated with water (100 % humidity). Moisture therefore can effect insect development when periods of non-feeding slow development more than expected for the particular temperature.

For most insects low humidity (under about 40 % RH) generally inhibits the rate of oviposition. For example, newly emerged adult migratory locusts that are

Fig. 4.13 Apple Black Spot or scab caused by *Venturia inaequalis* on leaves (*left*) and fruit (*right*) (Image by M. Braithwaite, courtesy of MPI, New Zealand)

severe pests in many parts of the world do not produce eggs under about 40 % RH. Also, the level of humidity can facilitate susceptibility to disease or parasitic attack. Insects succumb more readily to viruses when the weather is warm and the humidity is high. Heavy rainfall may cause heavy mortality by drowning, especially over-wintering pupae in the soil. Rainfall may affect dispersal by inhibiting migration or may increase the effect of winter cold or influence the moisture content of the soil, thereby limiting the distribution of soil dwelling insects. By influencing the moisture content of the soil, rainfall can affect successful pupation or adult emergence. Its effect on host-plant quality will also influence insect distribution.

Drought directly kills insects and natural enemies through loss of body water. However, the effect of drought is probably more indirect. Drought also clearly effects host plant quality. Under water stress, antibiotic activity of plants increases due to increased levels of tannins in the leaves that affect phytophagous insects by decreasing survivorship, growth rates and deterring grazing (Mochida et al. 1987). But in the opposite direction, drought often leads to mass attack by bark beetles which respond well to the effects of drought on the tree allowing them to overcome the natural defenses of the host during such a period (Price 1997). Such knowledge is clearly important for assessing habitat suitability of a species.

Moisture in the form of leaf wetness can be critical during the infection process of some fungi. For example with Apple Black Spot, *Venturia inaequalis* (Cooke) Wint., the duration of leaf wetness determines the amount of infection and subsequent disease severity (Fig. 4.13). Thresholds for infection have been determined at different temperatures. At 1 °C, 10 °C, and 25 °C the time required for infection of Apple Black Spot to occur are 48, 11, and 8 h respectively (Gadoury and Seem 1997). Other pathogens can react differently to free water. For example, high relative humidity is necessary for Apple Powdery Mildew, *Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon infection, but free water stops spore germination and rainfall can destroy the chains of spores and wash the spores from the leaf surface (Sivapalan 1993).

4.11.7 Seasonal Activity

To establish in a new area, the species must avoid unsuitable or harsh conditions. Most insects diapause to over-winter or aestivate to avoid harsh summer conditions. Diapause and emergence are often controlled by photoperiod (Tauber and Tauber 1976). This has an adaptive advantage, ensuring that the insect emerges when favourable conditions are more probable and helps them to avoid unfavourable conditions. The influence of photoperiod on diapause however, is difficult to measure for some species, as it usually acts in conjunction with temperature. Clearly, temperature is the second most important factor in diapause induction (Tauber and Tauber 1976). In temperate regions, high temperature (when combined with short photoperiods) generally suppresses diapause, whereas low temperatures enhance the tendency. However, this response varies greatly between species. In general, the life cycles of species are usually synchronised with seasonal changes in the particular habitat in which they live and climatic conditions vary geographically, such that there is usually some variation in physiological traits involved in the seasonal adaptation system. Variation in diapause geographically, is often closely correlated with a geographic gradient in climatic conditions. For example, European Gypsy Moth, *Lymantria dispar* is unlikely to establish in southern Florida and Central America where warm temperatures do not provide adequate chilling requirements for development of the Gypsy Moth life cycle (Allen et al. 1993). Thus, a shipment of *Abies balsamifera* (L.) Mill. Christmas trees to tropical areas to Central America poses little risk of introducing Gypsy Moth into these countries by this pathway.

Plant pathogens have adapted to survive adverse conditions through mechanisms such as seasonal complexities in their life cycle, highly resistant survival structures, alternate hosts and inactivity in plant debris. For example, fungi in the Ascomycetes often have the sexual phase of their life cycles synchronized with the seasonal growth patterns of their host. For example, consider *Monilinia* spp. (*M. fructigena* Honey, Apple Brown Rot and *M. fructicola* (G. Winter) Honey, Peach Brown Rot). These fungi overwinter on fallen fruit mummies in the soil. During spring, they produce apothecia and ascospores that are released under moist conditions to coincide with newly emerging young shoots and flowers of their host.

4.11.8 Distribution and Spread

To predict or delineate the potential distribution of a pest species in an endangered area requires all the information discussed above to model habitat suitability. Previously we indicated there are several quantitative methods to project our knowledge of habitat suitability to other areas where the species is not established. These methods have many names in the literature. For our purposes we refer to them as "bioclimatic assessment methods" and sometimes as "species distribution models." Often, spatial maps of projected species distribution are produced. We refer to the resulting spatial maps as pest risk maps. When we have some idea of habitat suitability and where a species could potentially establish, then we can model its potential spread.

After establishment, an invasive pest may be observed to increase its population almost exponentially and start to spread. As population numbers and density increase in a local area, the invasion becomes more visible to environmental managers. The changing spatial pattern of the species distribution that leads to an increase in the area occupied can often be partitioned into three phases: Pioneering, expansion and saturation (Shigesada et al. 1995). The pioneering period is characterized by the establishment of new sub-populations followed by a middle period in which rapid increase occurs as the population expands into new habitat. Finally saturation or fill-in occurs when new available habitat and resources become scarce. Clearly, some prediction of where a species might spread will enable focused monitoring and surveillance programmes. If the species is to be eradicated or contained, then some prediction of suitable habitat at high resolution and whether the species can reach those areas in the time available, would allow effective response programmes. Indeed, that same information would also inform more accurate impact prediction. Many theoretical models of insect spread have been published, but few detailed models show insect spread over the heterogeneous landscape. For the latter, we must also relate spread to habitat suitability and population growth.

Besides information on habitat suitability derived from bioclimatic models or species distribution models, essential information to predict spread, comprises the species normal means of dispersal and how far it moves over some time unit (metres/day, kilometres/year). Many studies on insect movement exist and recent studies use micro-radio antennae to monitor insect dispersal. But dispersal distances are rarely recorded and this is why few specific models exist showing the dispersal of pest species.

The winged adult is the common dispersal stage for most insects. Some insects can only move short distances during a day, often less than a few meters (Speight et al. 1999). Other insects migrate long distances. Locust swarms in Australia can disperse more than 20 km during 1 day. In response to favourable environmental conditions, the Australian locust species can outbreak and devastate crops over vast areas. Plague locust swarms in Africa can even utilise large synoptic weather events to move even larger distances between countries or across oceans (Gillot 1995).

Wind assisted immigration can be a biosecurity problem especially when insects can be carried over large distances. Records show aphids being carried over

Fig. 4.14 Citrus Canker caused *Xanthomonas axonopodis* pv. *citri* showing lesions on fruit (*left*) and leaves (*right*) (Photographs by Dr. Cherie Gambley, DEEDI, Queensland, Australia)

1,000 km (Irwin and Thresh 1988). Rare mass immigrations in England of a small Willow Moth (*Laphygyma exiguia* Hubner), whose larvae are agricultural pests in northern and central Africa, are found in small numbers in Britain each year. Periodically however, large damaging numbers can appear. Investigation has shown migration is correlated with favourable synoptic weather events: An unusually prevalent south-south-westerly airstream can indicate indicate exactly when the moths leave North Africa. Another example is the Tropical Grass Webworm, *Herpetogramma licarsisalis (Walker)*. In sub-tropical northern parts of New Zealand, larvae of this pasture pest can completely destroy 5-ha paddocks within 48 h. The species is found in tropical parts of Australia, Asia and some Pacific countries and is a serious pest in Hawaii. It is thought to have arrived, and possibly continues to do so, by travelling on trans-Tasman jet streams from Australia. The Golden Twin Spot (*Chryosodeixis chalcites* (Esper)) and the Green Semi-looper (*Chrysodeixis eriosoma* (Doubleday)) are also capable of long distance mass migration from northern Africa to Europe and North America. They can invade greenhouse environments even though they cannot successfully overwinter in most of northern Europe. These species are also difficult to identify and are a taxonomic challenge.

Long distance spread of plant pathogens commonly occurs by airborne spores or on plant material such as seed, nursery stock or produce. There is also a risk of spread in soil associated with plant parts or movement of equipment and containers. Spread over short distances is usually by mechanisms such as wind, water-splash, farm machinery and insect vectors. Stripe or Yellow Rust, *Puccinia striiformis* Westend, was introduced into New Zealand from Australia during 1980. The rust was carried from Australia to New Zealand on the prevailing westerly wind currents from an introduction in 1979. Conditions conducive for such spore dispersal are only thought to occur about six times in any 1 year (Viljanen-Rollinson and Cromey 2002).

Xanthomonas axonopodis pv. *Citri* (Citrus Canker), an important quarantine pest of citrus, is spread short distances by wind-driven rain or overhead irrigation (Fig. 4.14; Chap. 18). Long distance spread is by movement of infected plant material (Crop Protection Compendium 2010). A serious bacterial plant pathogen of grapes, Pierce's Disease (caused by *Xylella fastidiosa* Wells et al.) is spread by xylem-feeding insect vectors (Homoptera) that includes the Glassy-winged Sharpshooter, *Homalodisca coagulata* (Purcell and Hopkins 1996). Distribution and spread is strongly correlated with movement of the vector, either short distances by flying or long distances by association with the transport of plant material.

4.11.9 Impact and Yield Loss

Pests negatively impact the growth and development of agricultural, forest, urban and horticultural crop plants in diverse ways. A detailed understanding of the biology of a particular pest species may provide insight into its potential invasiveness and may offer and may offer an opportunity to design risk mitigation measures so that plant products can be traded in a phytosanitary manner. The method and pattern of pest attack influences or determines the quantity of biomass loss, usually assessed as yield loss, or the reduction in market value of a plant product as a result of cosmetic damage, either directly or indirectly.

Discussion by pest management specialists involves determining economic damage from a potential crop pest. Horticultural crops, arable crops and livestock provide product that is of value to the grower. The amount of useful product is referred to as "yield." For example, weight of grain, fruit, tubers per unit of land, volume of timber, growth increment loss, weight of plants, animal weight, fleece weight or percentage of undamaged product. The actual yield of the crop will depend on the type and level of inputs available to the grower and the interaction of those inputs with the weather and pests. In other words, there will be a difference between the actual yield and the potential yield due to the interaction of all these factors.

Infection by plant pathogens can affect yield in different ways. For example, yield loss in cereals has been extensively studied and diseases principally reduce dry matter production leading to reduced grain size and quality. In an artificially infected trial, Jordan et al. (1985) showed that Net Blotch of Barley (*Pyrenophora teres* Drechs.), reduced multiple parameters of yield including; ear number by 15 %, grains per year by 20 %, and grain weight by 48 %.

4.11.10 Crop or Plant Susceptibility to Injury

When considering the types of plant pests that cause injury, we must consider the way plants respond to injury. Generally, herbivores cause "detrimental" damage to the host plant. However, many plants can compensate for injury or the plant may even benefit in other ways in the short run (Delaney and Macedo 2001). These considerations will be critical to any impact assessment. For biosecurity purposes, two major factors must be considered in the injury/plant-response relationship: the time of injury and the plant-part injured. Time of injury is an obvious factor. Seedlings are most susceptible, whereas older plants can tolerate or compensate for injury. Also plants are very susceptible when yield-producing organs are forming such that disease or feeding damage on young fruit or young leaves can be very damaging. With respect to the plant-part injured, usually a distinction is made between direct injury and indirect injury. Injury to yield forming, storage and reproductive organs is direct injury. Indirect injury pertains to effects such as distortions of plant architecture or growth and development, quality losses or aesthetics, and transmission of disease organisms by mechanical transmission or insect vector. Hill (2008) reviews various forms of direct and indirect plant injury.

In a biosecurity setting, quantitative yield loss may not be easily determined and damage assessments are often based on qualitative criteria. The following section outlines the biological basis for damage assessment that is key to the conduct of a proper pest risk assessment.

4.11.11 Injury and Damage Caused by Insects

Plant pests may be placed in six categories based upon the manner in which they cause yield reduction (Pedigo and Rice 2006; Hill 2008; Peterson and Higley 2001).

1. **Stand reducers** – Many Lepidoptera species (cutworms that cut through young seedlings) cause sudden loss in biomass and as a result, stand reduction.
2. **Leaf-mass consumers** – Insects that consume leaves directly affect the total photosynthesis of the plant canopy. Many plant pests fit into this category.
3. **Assimilate sappers** – Piercing, sucking and rasping insects remove plant carbohydrates and nutrients before they are converted into plant tissue. Some pest species can increase the level of injury by injecting toxic substances into the plant while feeding. An example is the Tarnished Plant Bug feeding on peaches.
4. **Turgor reducers** – Soil insects that feed on plant roots and stem feeders influence plant water and nutrient balances at their feeding sites. For example, Corn Rootworm species (*Diabrotica spp*.) prune corn roots reducing their number and size. Other insects can girdle the stems of plants thus destroying their means of conveying water to the leaves, stems and fruits that may not expand fully. Photosynthesis may also be reduced. The Emerald Ash Borer is a pest that causes physiological damage as described above.
5. **Fruit feeders** – Can directly damage the fruit, causing reduced quality, in terms of appearance, yield or both. A good example is Mediterranean Fruit Fly. The Blueberry Maggot (*Rhagoletis mendax* Curran) affects both fruit yield and quality.
6. **Architecture modifiers** – Insects such as stem borers can change plant shape and anatomy to reduce yield. Most major defoliators cause such damage over successive years of defoliation (e.g., *Lymantria monacha* (Linnaeus), *Ditula angustiorana* (Haworth) and many others). Other species can cause loss of the growing tip and cause tillering and low yielding or barren plants.

4.11.12 Injury and Damage Caused by Plant Pathogens

The effect of plant pathogens on yield can also be categorised into various mechanisms such as stand reducers, photosynthetic rate reducers, senescence accelerators, light stealers, assimilate sappers, tissue consumers, and turgor reducers (Gaunt 1995). However, these categories generally relate to the major effect of reducing the amount of sunlight received by the plant and the efficiency by which the plant can utilise this energy. For instance leaf blights such as Late Blight of Potato, *Phytophthora infestans* (Mont.) de Bary, effectively reduces the amount of photosynthetic leaf area available by increasing the amount of dead or necrotic leaf tissue. In contrast, pathogens like bacterial wilt, *Ralstonia solanacearum* (Smith) disrupt the plants vascular tissues severely limiting nutrient and water uptake which stresses the plant and reduces the efficiency of energy conversion. Plant viruses, such as Tobacco Mosaic Virus, disrupt the normal functioning of a plant cell by directly competing for cellular substances and nutrients the plant would normally use. This leads to the array of symptoms that viruses cause, such as mosaic, chlorosis, ring spots and necrosis.

4.11.13 Injury and Damage Caused by Nematodes

The feeding habits of plant parasitic nematodes are often classified based on where they feed with respect to plant tissues, which tissues they feed upon, and their tendency to remain migratory or sedentary (Ferris 2011). Endoparasites completely enter the plant tissue. There are endoparasitic nematodes that affect leaves, seeds, bulbs and stems, and roots, respectively. Migratory endoparasites of roots feed on plant cells as they move through a root and remain mobile for their entire life. Sedentary endoparasites trigger the formation of highly modified plant cells that nurse the developing nematodes; as these nematodes develop, they lose the ability to move. Only the anterior portion of semi-endoparasite will enter a root. Ectoparasites remain outside plant tissue when feeding.

Migratory ectoparasites retain the ability to move throughout their lives and feed on cortical, or slightly deeper, root cells. Sedentary ectoparasites remain outside the root for their entire lives, but lose the ability to move as they develop. The effects of these different feeding strategies on yield or other plant functions are not easily divided into distinct categories because the same nematode species may cause more than one type of damage. Nematode feeding can cause three kinds of symptoms on roots (Ruehle 1973). Necrotic damage results from the death of plant cells on the surface of a root. Necrotic damage may progress into root splitting and blackening of the entire root. *Xiphinema americanum* Cobb 1913, a migratory ectoparasite, causes such damage as it feeds on laurel oak. Hyperplastic symptoms develop when nematodes cause roots to form galls. The galls triggered by feeding of Root Knot

Nematodes (*Meloidogyne* spp), sedentary endoparasites of roots, are a classic example. Hypoblasitc symptoms emerge as nematodes devitalize root tips or inhibit apical growth. For example, *Paratrichodorus minor* (Colbran), a migratory ectoparasite, feeds on root tips and causes an underdeveloped root system in a number of pine species (*Pinus* spp). All three types of damage interfere with the translocation of water and nutrients in a plant. Nematode damage can easily be confused with symptoms of nutrient deficiency, drought, or other root pathogens.

4.12 Conclusions

A comprehensive understanding of the core biological principles is essential for the development of plant protection policies and adds foresight to assure the plant biosecurity of any nation. Fundamental biological knowledge contributes to the conduct of a reliable Pest Risk Analysis and minimises uncertainty. Insight into various biological interactions may provide information with which to anticipate, identify and characterize the potential invasiveness of a particular pest species. Biology in conjunction with various other related disciplines provides essential information throughout most aspects of the Pest Risk Analysis process. Often development of biological knowledge is dynamic and requires improved research to address information gaps, immediate concerns and uncertainties.

Improvements are required in risk mitigation methodologies based on biology to facilitate movement of plants or plant products in a phytosanitary manner. Improvements in research to characterize the potential invasiveness of a plant pest are also required. In many cases, the virility or voracity of any pest/disease activity remains unknown until severe damage is realized. Enhancements in surveillance and detection methodologies may provide improved early warning. Better predictive models are required as the Pest Risk Analysis process often is based on minimal information and may benefit from investment in development of a multitude of modeling methodologies that can explore scenarios or attempt to answer what-if questions. Again, those models must be based on sound biological knowledge.

Enhanced research is required to address the plant-herbivore interaction at the population level of both pest and plant (Peterson and Higley 2001). Also, the nature of impacts of pests on plants, particularly perennial plants, and the successional damage patterns and impacts caused by multiple pests and diseases and their interactions should be explained at the individual plant level, and population level. Both natural and managed ecosystems should be examined, and that of course, presents research challenges for long living perennial plants such as those that occur in forestry.

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Chapter 5
The Biosecurity Continuum and Trade: Pre-border Operations

Ron Sequeira and Robert Griffin

5.1 Introduction

Pre-border organisational structures and activities have historically focused on pest exclusion programmes and policies. The underlying philosophy for national plant protection programmes was based on two strongly held beliefs: (1) the border is the primary line of defense, and (2) preventing pest entry is a much more effective strategy than reacting to pest establishment.

Given the potentially catastrophic consequences from the introduction of harmful pests, a significant investment in exclusion has always been easily justified and resulted in many decades of increasing investment in regulations and programmes focused on port of entry actions as the central theme. The key role of inspection programmes as a prevention measure continues, but in a more thoughtful and deliberate way. One reason for this is that resources for this work are generally flat or decreasing. However, the SPS Agreement coming into force has also had a major effect on underlying concepts. The prevailing philosophy of "when in doubt, keep it out", has been replaced with the SPS philosophy of "apply phytosanitary measures consistent with the risk". The result is much more emphasis on risk analysis and the technical justification for the strength of measures in policies and operations. At the same time, inspection processes are seen not only as a means for risk mitigation, but also an important source of data for better analysis to support decision-making.

As pre-border systems continue to evolve and adapt in the post-SPS environment, a deeper understanding of the relationship of policies and operations to risk is

R. Sequeira
Center for Plant Health Science and Technology, Plant Protection and Quarantine, APHIS, USDA, Raleigh, NC 27606, USA

R. Griffin (✉)
Pest Epidemiology and Risk Assessment Laboratory, Plant Protection and Quarantine, APHIS, USDA, Raleigh, NC 27606, USA
e-mail: Robert.L.Griffin@aphis.usda.gov

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quickly developing. Decision makers are becoming more aware of the roles that evidence and uncertainty play in deciding risk-based policies and designing risk-based programmes for safe trade as opposed to simply focusing on exclusion.

This chapter discusses pre-border systems in the biosecurity continuum from the standpoint of transitioning to a post-SPS world that integrates the historical concept of exclusion with the contemporary concept of a technical justification for the strength of measures.

5.2 Trade and Plant Protection

From its inception after World War II, the overarching purpose of the General Agreements on Tariffs and Trade (GATT) was to reduce political friction by increasing economic prosperity through a voluntary rules-based system for globalized trade. Agriculture negotiations over the decades that followed evolved through three stages; beginning with free trade (reducing and removing tariffs), then fair trade (reducing and removing technical barriers), and finally safe trade which was addressed by the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement). The SPS Agreement has been the dominant feature in the evolution of plant protection systems since it came into force in 1995 with the adoption of the results of the Uruguay Round negotiations of GATT, including the formation of the World Trade Organisation (WTO) with a binding dispute settlement system.

The most significant adjustments needed by the International Plant Protection Convention (IPPC) to complement the SPS Agreement were the establishment of a standard-setting mechanism and a shift in focus from collaboration on plant protection programmes through the Food and Agriculture Organisation of the United Nations (FAO) to direct collaboration by National Plant Protection Organisations (NPPOs) on trade concerns. This shift was not entirely consistent with the design of the IPPC, which was broader in scope than its application to trade.

The IPPC applies to cooperation in protecting plant resources generally, whether or not associated with trade. The emphasis on trade can therefore cause some discomfort because only justifying measures that restrict trade could be interpreted to be sacrificing protection for the sake of facilitating trade. The intent of the SPS Agreement however is not to promote trade at the peril of protection but rather to advance 'safe trade' as a single concept supported by scientific principles and evidence. Taken in this context, the revision of the IPPC was not a compromise but a complement to the concept of safe trade.

Safe trade is a two-way commitment. Both the SPS Agreement and the IPPC anticipate that all trading partners adopt the philosophy of shared responsibility for the safety of the products they trade and also share the burden of proof for the technical justification upon which restrictive measures are based. The 'give and take' on which the SPS Agreement is based is sometimes perceived to undermine protection because measures that may be comfortable but have no scientific basis can be challenged and removed.

Every trade issue faced by an NPPO, whether existing or new is associated, to some degree, with past relationships, policies, and perceptions. In addition, there is always background pressure from stakeholders in the private sector who press their NPPOs for a commercial advantage. Addressing these issues in the framework of the SPS-IPPC means that NPPOs are working both backward and forward through their trade policy decisions; addressing past and present trade irritants which may be inconsistent with the SPS-IPPC framework, while also trying to avoid the tendency to let past policy adversely affect new and future decisions and to ameliorate stakeholder concerns. Positive movement of national policies toward the SPS-IPPC framework often results in more measures being reduced or eliminated than strengthened or added; hence the general impression by stakeholders and politicians that protection is lost.

Under ideal circumstances, the influences of distrust, precedent, and precaution would be minimised in favor of transparency and cooperation toward the most workable approach for managing pest risk. The resulting trade would have the greatest chance of being successful based on the best efforts of both trading partners. Successful programmes increase economic benefits, reduce trade friction and promote better relations. In contrast, trade that may occur under less desirable circumstances is more likely to be either overly restrictive or riskier than it should be. Both conditions encourage further distrust, increase unjustified precedents, and promote precautionary measures.

Understanding this balance is crucial for policy makers. By deliberately withholding information or failing to work with the importing country in an open technical dialogue, the exporting country may experience short-term success by gaining market access, but the long-term credibility and relationship of both countries is damaged when programmes fail due to unanticipated and unmitigated pests. The result is weakening rather than strengthening the biosecurity continuum.

5.3 Legislation and Regulations

Regulatory authority is a basic feature of the phytosanitary system in every country. Since the coming into force of the SPS Agreement in 1995, countries have become more concerned about ensuring that their legislation and regulations are consistent with their international obligations. The revision of the IPPC adopted in 1997 heightened this concern as regards phytosanitary systems because the legal underpinnings of a phytosanitary structure that is incomplete or does not conform to the SPS-IPPC framework is more likely to be ineffective or challenged.

At the same time, countries are also looking carefully at their phytosanitary systems to evaluate their effectiveness, identify opportunities to improve efficiency, explore alternative funding mechanisms, and in many cases to structurally reorganise with the objective of improving decision-making and strengthening

Table 5.1 Hierarchy of phytosanitary authority

International agreements WTO-SPS IPPC ISPMs[a]	Statutory authority	Acts, laws, statutes, promulgating legislation, enabling legislation	Enacted by the national government; establishing general charges, granting authority to promulgate and enforce regulations
	Regulatory authority	Regulations, directives	Legal instruments promulgated by the NPPO[c]; describe requirements and the framework for enforcement; published through an administrative procedure that may include public comment
	Policy authority	Policies, manuals, work plans, compliance agreements, MOUs, BQAs,[b] permits, pest lists	Written requirements, decisions, or other interpretations of regulations providing detail or addressing specific situations
	Discretionary authority	Actions (routine and emergency)	Decisions made in the course of daily activities, consistent with the interpretation of current authority, believed to be legally supportable and based on sound quarantine judgment

[a]SPS is the World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures; IPPC is the International Plant Protection Convention; ISPMs are International Standards for Phytosanitary Measures adopted under the IPPC
[b]MOU is a Memorandum of Understanding. BQA is a Bilateral Quarantine Agreement
[c]NPPO is National Plant Protection Organisation, referring to the office or agency holding official national authority for plant protection as identified to the IPPC

collaboration across related disciplines and sectors. The trend toward combining animal and plant health and food safety regulatory agencies to signal a stronger focus on consumer issues is indicative of this new environment which can also involve extensive statutory and regulatory changes.

The SPS Agreement and the IPPC provide an international framework for statutory and regulatory designs. These are consistent with the obligations that national governments have agreed are desirable to observe. The international obligations arising from these treaties have no legal status within the country except to the extent that the national government has incorporated the same concepts and requirements into national legislation and regulations that then translates into requirements, policies, and actions at the working level. This hierarchical relationship is summarized in Table 5.1.

Table 5.2 Levels of authority for import permit processes

International agreements:	SPS Agreement: Control, inspection and approval procedure (Annex C) IPPC: Requirements in relation to imports (Article VII) ISPMs: Guidelines for a phytosanitary import regulatory system (ISPM 20)
Legislation:	Provides the authority for the NPPO to designate articles that will be regulated and the processes and conditions for authorizing imports with the objective of ensuring an adequate level of phytosanitary security
Regulations:	Identifies regulated articles and describes the process for authorizing imports under permits, including for instance, required forms or information
Policies:	Working decisions regarding the format of forms, the duration of validity for permits, the nature, location, staffing, and priorities of offices for processing, etc.

Strategies for updating regulatory authority begin with understanding the key elements and responsibilities of a National Plant Protection Organisation. Articles IV and VII of the IPPC, and ISPM 20, *Guidelines for a phytosanitary import regulatory system*, provide useful guidance. By identifying the strengths and weaknesses in existing regulatory authority within the context of the national legal system, governments can begin to address their current and future needs for regulatory reform beginning with updated legislation.

Legislation is the enabling authority provided by the national government (Parliament or a similar body). As enabling authority, legislation does not typically include programmatic details, especially concerning issues that are dynamic. Legislation is designed to provide the powers and provisions for regulatory authority that follows. Regulations then provide the details for actions and enforcement derived from the legislation.

Policies are ideally linked directly to the implementation of regulations but provide a level of working flexibility that is not possible with regulations because regulations cannot contain all the details for every possible operation and situation, and must go through a legal process to be updated. The key characteristics of policies, regulations, legislation, and international treaties are described in Table 5.2 using the import permit processes as an example.

The level of detail found in national regulations varies greatly depending on the range of powers, extent of programmes, and political priorities of the government. As a general rule, countries should strive for a high level of transparency in phytosanitary regulations consistent with their SPS and the IPPC obligations, but also for regulations to be effective communication and enforcement tools for trading partners, stakeholders, and the workforce responsible for their implementation.

A very high level of transparency creates two challenges: First, the legal and administrative cumbersome. Second, the flexibility needed for effective programmes can be limited by the need for constantly updating regulations. Striking the best balance between transparency and flexibility requires a thoughtful analysis of the resources and level of authority where decision-making should occur in each country's circumstances.

A key point to emphasize is the relationship of regulations and policies in the context of Approval Procedures (Annex C of the SPS Agreement) and Import Authorization (Sect. 4.2.2 of ISPM 20). Where regulations can provide general authorizations, there should be no need for specific authorizations (although Customs may require such for other reasons). By limiting permits to specific needs and dealing with routine imports as general authorizations, NPPOs avoid unnecessary bureaucratic requirements that do little to increase phytosanitary security and could be challenged as unjustified administrative barriers.

The entire regulatory authority, from legislation to regulations and policies, should be seen as a continual work in progress. The overall objective is to make changes as needed for effective phytosanitary programmes, but focus on the lowest possible level that still provides the necessary authority, transparency, and enforceability.

In many countries, the system for establishing national regulations includes a process of national consultation. WTO members are also expected to report new measures to the WTO through their official Enquiry Points (Annex B, paragraph 5). Such efforts contribute significantly toward transparency. Feedback from trading partners, industry and experts can also help to improve regulatory changes and provide useful insight into potential problems with implementation.

Another point, sometimes overlooked, concerns the authority for NPPOs to enter into bilateral agreements and to make commitments on behalf of their government in the standard-setting activities of RPPOs and the IPPC. This authority places phytosanitary officials in a better position to make decisions that are consistent with contemporary phytosanitary concepts and also raises the need for the same officials to be keenly aware of their government's broader political strategies in other forums to avoid internal conflicts and confusion.

5.4 Cooperation and Communication

The ability to prevent harmful plant pests from moving in trade is strongly linked to the capability of trading partners to effectively manage pests and their pathways. When pests become established in a new area, they pose a threat to the entire region surrounding it, as well as to other trading partners. No country can succeed in addressing its pest problems without some level of national and international cooperation and communication.

Linkages to stakeholders, other agencies, and collaborating institutions (such as universities and sub-national regulatory authorities) are crucial for developing and implementing effective national programmes. The nature of these relations is strongly influenced by the organisational structure, authority, and resources provided to the NPPO by the national government. These relationships vary from country to country, but are relatively stable within a country, changing primarily in response to the political environment.

International cooperation and communication is also very susceptible to political changes, especially as regards trade relationships. Countries typically adopt a dual approach to these relations; supporting multilateral harmonization as one objective, but also continuing to rely heavily on bilateral arrangements.

The importance of multilateral agreements has increased in recent years due mainly to the establishment of the SPS Agreement with its strong implications for trade and the formation of the WTO with its binding dispute settlement process. Likewise, the participation of countries in international and regional institutions has grown as countries recognise the advantages of being active participants in harmonization. International cooperation and communication associated with these arrangements are relatively formal and stable, with a long-term view for generally better relations resulting from a baseline commitment by all parties to observe and support harmonization.

Harmonization makes trade more predictable depending on the quality and quantity of harmonization and the commitment of trading partners to consistently embraces it. Countries with widely variable political climates or weak commitments to multilateral cooperation and communication tend to be less predictable and benefit less from such arrangements except to the extent they are able to make a case for technical assistance to address institutional shortcomings.

Although harmonization and predictability are expected to be beneficial for all countries over the long-term, effective short-term solutions to most trade issues result from bilateral arrangements. The bilateral process does not have harmonization as its primary objective but rather develops from the history, geography, political and economic affiliations, and the short-term interests of trading partners. Bilateral arrangements are therefore informal and dynamic, driven by the need for flexibility and situational decision-making; usually with strong political overtones.

Unfortunately, bilateral relationships tend to be focused on specific needs and short-term results. These relations can vary significantly over time and among trading partners depending on the needs of each party and the panorama of political, economic, and technical issues facing the countries in question at a particular time. As a result, the type and quality of bilateral cooperation and communication can begin on a negative note and include elements of quid pro quo.

The dynamic and variable nature of bilateral arrangements makes them unpredictable and difficult to characterize more specifically except to say that most anything is possible. The structure and stability of multilateral relationships however has growing importance and influence in cooperation and communication as countries are increasingly driven to predictability in commerce by private sector forces eager to leverage globalisation for economic success. From this standpoint, cooperation and communication in the phytosanitary world has experienced a tremendous evolution linked to two key events: The coming into force of the SPS Agreement in 1994 and the revision of the IPPC in 1997.

The final text of the SPS Agreement struck a balance between obligations of the importing and exporting countries, in particular as regards their responsibilities for cooperation and communication. The centrepiece of the Agreement is ensuring that importing countries are able to justify their SPS measures based on international standards or risk assessment (Articles 3 and 5). The SPS Agreement explicitly identifies three organisations responsible for standard setting: The Codex

Alimentarius Commission for food safety, the World Organisation for Animal Health (the OIE), and the International Plant Protection Convention (IPPC) for plant health.

At the time the SPS Agreement came into force, the IPPC had very little background or structure for international harmonization. The basic framework of the Convention offered some structure for key concepts and provisions related to trade, mainly focused on phytosanitary certification. The objective of harmonization and broader mechanisms for harmonization were vague and tied mainly to the Food and Agriculture Organisation of the United Nations (FAO) where the Convention had been deposited since 1952. A revision to the Convention was completed in 1997 to establish a mechanism for standard setting and to shift from the historical reliance on FAO for cooperation and communication to a stronger commitment by contracting parties to cooperate and communicate directly with each other on matters related to trade.

Key points in the 1997 revision regarding *cooperation* are:

- Art I.1 . . .With the purpose of securing common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control. . .
- Art VI.2i . . . Establish and update lists of regulated pests, . . . and make such lists available to the Secretary, to regional plant protection organisations of which they are members and, on request, to other contracting parties.
- Art VIII (International Cooperation). . .The contracting parties shall cooperate with one another to the fullest practicable extent in achieving the aims of this Convention, and shall in particular:

  (a) Cooperate in the exchange of information on plant pests, particularly the reporting of the occurrence, outbreak or spread of pests that may be of immediate or potential danger, in accordance with such procedures as may be established by the Commission;
  (b) Participate, in so far as is practicable, in any special campaigns for combatting pests that may seriously threaten crop production and need international action to meet the emergencies; and
  (c) Cooperate, to the extent practicable, in providing technical and biological information necessary for pest risk analysis.

Each contracting party shall designate a contact point for the exchange of information connected with the implementation of this Convention.

- Art IX.1. . .The contracting parties undertake to cooperate with one another in establishing regional plant protection organizations in appropriate areas.
- Art X.1. . .1. The contracting parties agree to cooperate in the development of international standards in accordance with the procedures adopted by the Commission.

- Art XI. . .Contracting parties agree to establish the Commission on Phytosanitary Measures within the framework of the Food and Agriculture Organization of the United Nations (FAO).
- Art XX (Technical Assistance). . . The contracting parties agree to promote the provision of technical assistance to contracting parties, especially those that are developing contracting parties, either bilaterally or through the appropriate international organizations, with the objective of facilitating the implementation of this Convention.

Note that the IPPC requires contracting parties to identify a contact point for official communications both between contracting parties and with the IPPC Secretariat. The SPS Agreement requires WTO members to identify Enquiry points for the same purpose but with a broader scope because enquiry points are also used for official communications on food safety and animal health. The point of contact (IPPC) and the enquiry point (SPS) can be the same person or office or they may be different. The agreements do not make a distinction, but countries normally consolidate the function for convenience, consistency, and to reduce confusion.

Key points in the 1997 revision regarding *communication* are:

- Art III.2b . . . reporting the occurrence, outbreak, and spread of pests. . .
- Art III.3a . . . distribution of information within the territory . . . regarding regulated pests and the means of their prevention and control. . .
- Art VI.2b . . . publish and transmit phytosanitary requirements, restrictions, and prohibitions. . .
- Art VI.2f . . . inform the exporting contracting party . . . of significant instances of non-compliance with phytosanitary certification . . . on request, report the result of its investigation. . .
- Art VI.2j . . . develop and maintain adequate information on pest status in order to support categorization of pests . . . information shall be made available to contracting parties, on request.
- Art VIII.1a . . . exchange of information on plant pests, particularly the reporting of the occurrence, outbreak, or spread of pests that may be of immediate or potential danger. . .
- Art VIII.1c . . . providing technical and biological information necessary for pest risk analysis. . .

The overall aim of the communication provisions is twofold: First to establish the baseline expectations for information needed by National Plant Protection Organisations to be effective; and second, to encourage the exchange of information necessary for trading partners to engage in a transparent and meaningful dialogue on trade questions – in other words, to reduce the uncertainty associated with negotiating market issues.

We should note that the IPPC definition of Pest Risk Analysis refers to *available* scientific information in contrast to the SPS Agreement that refers to *scientific principles* and *evidence*. The difference is subtle, but a key distinction intended by the IPPC is to acknowledge that the availability of information and the capability of contracting parties to obtain information will vary, and also that information will be dynamic. The IPPC therefore recognises that uncertainty due to information gaps is a normal and expected condition that is always subject to change. The IPPC also does not assume that all countries have the same access to information, so uncertainty on any given point is both natural and situational. This latter point emphasizes the need for technical dialogue between trading partners to understand the nature of information that is available and the types of uncertainty associated with it.

The IPPC and SPS Agreement also have complementary provisions for Technical Assistance (Article XX in the IPPC and Article 9 in the SPS Agreement). The main difference is that the SPS Agreement focuses on developing country members where the IPPC is not explicit in this regard. This was a deliberate design to encourage international collaboration on plant pest issues regardless of the economic status of the countries in question.

Technical assistance or capacity building may be done in the context of Bilateral relationships, Multilateral initiatives or Multilateral organisations.

In the case of bilateral relationships, the sharing of information, experience, or resources may be in direct relation to a specific trade issue (e.g., providing diagnostic training for a pest of concern) or for more altruistic purposes and general relationship building. This type of capacity building usually results from government-to-government commitments, but can also involve private sector resources as either the donor or recipient. For example, pest risk management specialists from the USA may go to an exporting country to assist growers with pest management strategies that facilitate exports to the USA and other countries, or the USA may contract with a private consultant or university to provide training on good agricultural practices.

Multilateral initiatives are less common. The ‘Quads’ (a cooperative framework for plant and animal health including Canada, New Zealand, Australia, and the USA) is an example of multilateral cooperation and communication. The objective is not necessarily to harmonize, or even to share resources, but rather to share common problems and explore common solutions.

The primary mechanism for multilateral technical assistance and capacity building is through international and regional organisations, including for instance:

- FAO – IPPC Trust Fund and FAO Capacity Development Programmes
- WTO – Standards and Trade Development Facility
- World Bank – Country Assistance Strategies
- IICA (Inter-American Institute for Cooperation on Agriculture) – Cooperative Programmes
- GIZ (Deutsche Gesellschaft für Internationale Zusammenarbeit) – private-government programme

The above is only a sample of the numerous multilateral organisations either dedicated to technical assistance and capacity building or including such services in their portfolio. GIZ is included her as an example of a national government (Germany) that partners closely with private organizations to address a broad range of projects in the developing world generally aimed at overcoming poverty. The relationship of NPPOs to these organisations usually begins with membership by their government and then depends on the role of the country as either a donor or recipient. In recent years, an increasing number of emerging economies (e.g., Brazil) have had both roles in such organisations.

The key to the effectiveness of multilateral organisations is matching identified needs to the interests and resources of donors. Each organisation has its own scope, objectives, priorities, procedures, and criteria for deciding on projects. As a general rule, the process begins with a proposal and invitation from the recipient country. NPPOs do well to understand these differences and work strategically with a range of organisations toward their national objectives by identifying and prioritizing projects with well-defined objectives and mechanisms for demonstrating effectiveness.

5.5 Pest Risk Analysis

Pest Risk Analysis (PRA) is the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it (IPPC, Art. II) (See Chap. 9). PRA thus provides a mechanism for characterizing risk as a key component of deciding whether regulatory actions are justified and the extent to which they should be applied. In the phytosanitary world, the hazard that NPPOs usually want to avoid, reduce, or eliminate is the *introduction* (entry and establishment) of harmful plant pests. Mitigating the risk of pest introduction is a primary objective of pre-border regulatory programmes.

The risk of pest introduction has both likelihood and consequences, and some level of uncertainty surrounds both elements. The likelihood of pest introduction depends on the pathway, or series of events leading to establishment. Each of the events in the pathway has some probability. If any of the necessary events does not occur, establishment is not possible. If there are any adverse consequences however, there will be some consequences. Consequences are commonly, but not always, expressed in economic terms even though they may be primarily non-market in nature such as aesthetic, social, environmental, or other impacts that are not usually measured in direct market effects. The IPPC substantially affects our understanding of this component of risk in the phytosanitary context. This is because the IPPC requires all consequences to be expressed in economic terms. The IPPC also refers to various analytical methods for expressing non-market consequences in economic terms (ISPM 11).

Another important difference between the likelihood and consequences of pest introduction is that the prediction of potential consequences will usually be based on the full impact of the hazard (the worst case scenario) that assumes maximum spread and impact of the pest in question. In most cases, this does not include a discussion of the most likely scenario and is not balanced by whatever benefit there may be from taking the risk. This is another point where the role of the SPS Agreement becomes an important factor in shaping the view of risk in the phytosanitary world. A key assumption behind the Agreement is that all WTO Members benefit from fairly applying the disciplines of the Agreement that includes, but is not limited to, specific consequences which should be considered but not specific benefits.

Some uncertainty, and often great uncertainty, always is associated with both the likelihood and the consequences. Uncertainty may take several forms but most often involves incomplete or conflicting information, linguistic imprecision, bias, inappropriate methodologies, and incorrect assumptions. Regulatory decisions in the face of uncertainty are uncomfortable; the greater the uncertainty, the less comfortable the decision and the greater the natural tendency to be precautionary. Decision makers are most comfortable with routine situations that have a history of acceptable outcomes, in contrast to the many unique challenges associated with managing new pest risks or taking new approaches across hundreds of commodities and thousands of potential pest threats. In this light, risk analysis can be as important for understanding uncertainty as it is for understanding the evidence, and policy making is often more focused on uncertainty than on evidence.

Because the SPS Agreement requires measures based on scientific principles and evidence, it is crucial for a PRA to link conclusions to scientific principles and evidence while also distinguishing uncertainty for policy interpretation and the discipline of consistency. Depending on the urgency of the decision and how comfortable decision makers are with the uncertainty, pest introduction may be found to be acceptable if it can be easily managed or there is no practical way to manage it and it must be accepted. On the other hand, if the risk is judged to be unacceptable, an analysis of risk management options is needed to identify ways to reduce, avoid or eliminate the risk of pest introduction.

We must recall that the risk analysis process includes judgments regarding both the evidence and the uncertainty that can change over time with different conditions and perspectives. The completion of a risk analysis therefore represents a snapshot in time of the information, judgments, analysis, and conclusions associated with the question being addressed. We should therefore expect that analyses and their results will change based on more or better information as well as changing conditions.

Another point that deserves attention is the common misconception that quantitative analyses require more data and are more precise than qualitative analyses. In fact, no risk analysis is either purely quantitative or purely qualitative (Devorshak 2012). The assessment of consequences is almost always quantitative to the extent that it is cast in economic terms, but ultimately a qualitative judgment will be made regarding the acceptability of the consequences. More often, quantitative analysis refers to a probabilistic assessment of the likelihood. This involves assigning probabilities to events in a scenario leading to the hazard. Expert opinion can be very important for probabilistic scenario analysis. Numerous other qualitative inputs may also be associated with the background data. One great advantage of these types of analyses is that the uncertainty around estimates is explicit where probability curves represent the data and assumptions.

A common misconception is that a certain quantity and quality of data is required for a good analysis. Some observers argue that a risk analysis cannot be undertaken until a (usually undefined) threshold level of data is available. In fact, the amount and type of information available for the analysis has no relation whatsoever to the quality of the analysis, but everything to do with the uncertainty associated with the results. An excellent analysis can be done on poor data, just as a poor analysis may be done on excellent data. The key criteria of transparency and consistency, especially as regards the treatment of uncertainty, make the difference.

Ample opportunity exists for the application of risk analysis beyond the pest risks associated with trade. Domestic pest management programmes, regulatory policy formulation and modification, prioritization for surveillance, and practically every other facet of national plant protection programmes can benefit from risk analysis or aspects of risk analysis. Risk analysis can be an especially powerful tool for evaluating and prioritizing programmes, allocating resources, identifying research priorities, and focusing on technical points of difference with trading partners as well as stakeholders.

Perhaps the most important benefit of risk analysis is the linkages that are created and strengthened as a result of engaging in a scientific exchange and dialogue. Policy makers become more aware of the importance of research, and regulators become more effective through greater coordination with the research community. Trading partners, stakeholders, and civil society all benefit from the focus on science rather than politics to meet legitimate protection needs.

5.6 Risk-Based Decision Making

Risk management involves NPPOs deciding what to do about a pest risk by applying one or more measures to mitigate the risk (Sect. 9.3). The concept of risk management is not strictly analytical but also is broadly associated with policy-making and the operational aspects of implementing programmes. Sometimes risk management also includes the evaluation of measures that are in place to determine their appropriateness and needed adjustments. This monitoring or feedback process, whether or not it is technically part of the analytical process of risk management in PRA, is crucial for connecting the analytical process to information from the real-world for validating and improving PRAs.

The two principles that most discipline risk management are *transparency* and *consistency*. The principle of transparency is crucial for communicating the results of risk management analysis to relevant parties. The principle of consistency is applied alongside other principles in risk management for the selection and implementation of measures. Other key principles begin with the principle of *necessity*; recognizing that countries have the sovereign right to protect themselves but they must first demonstrate that a potential hazard exists which justifies the need for protection. At the same time, they must realize that zero risk is not a realistic objective, so a policy of *managed risk* must be adopted based on an appropriate level of protection (or acceptable level of risk). Measures resulting from these policies should aim to have *minimal impact*; representing the least restrictive measures available and resulting in the minimum impediment to trade. The measures should *not discriminate* between trading partners or between trading partners and domestic producers with similar risks (*national treatment*). A proper PRA, based on scientific principles and evidence and consistent with international standards, provides the *technical justification* for measures if the measures are not based on international standards or deviate from international standards. PRAs should be *modified* and measures adjusted without undue delay when new or better information indicates the need. Measures should not be changed without a technical justification and appropriate notifications.

Equivalence also has a critical role in risk management. The concept as described by both the SPS and the IPPC is designed to avoid unjustified prescriptive measures by considering mitigation options that have equivalent or better efficacy and are also feasible. One common problem with applying the principle of equivalence in practice is assuming that existing measures represent the appropriate strength of measures when many of the requirements countries have in place have been established prior to the coming into force of the SPS Agreement or are based on bilateral agreements that did not directly link the strength of the measures to a defined level of pest risk. ISPM 24 (Guidelines for the determination and recognition of equivalence of phytosanitary measures) provides additional clarification on this principle and its application in practice.

Another important concept that might be described as a principle (although it is not identified as such in the IPPC or SPS Agreement) is the concept of *rational relationship* (Sect. 9.1). This concept, which has developed as a central issue in SPS jurisprudence, requires that the measure in question has an effect on mitigating risk and also that the strength of measures is proportional to the risk (Devorshak 2012). The strength of measures for any situation depends on the level of risk and type of measure. The level of risk can be visualized as a sliding scale where stronger measures correspond with higher risk and vice versa except where the measures are emergency or provisional. This distinction makes it important to understand the difference between established measures, provisional measures and emergency measures.

Established measures define the appropriate level of protection by virtue of the range of risks and the strength of measures they represent. They also offer reference points for equivalency where the measures are linked to the acceptable level of the risk. Established measures should be based on international standards or a PRA.

Provisional measures are taken when there is insufficient scientific evidence to permit a final decision on the safety of a product or process (may or may not be an emergency). Provisional measures are designed to facilitate trade by making it possible to put in place what are probably overly restrictive requirements that will be adjusted later when new or better information is available. We should note that the country imposing a provisional measure must actively pursue the information required for a more objective assessment of the risk and review of the measure within a reasonable period of time. This is one of the few situations under the SPS

Agreement where the burden of proof is completely one-sided. In nearly all other circumstances, both the importing and exporting country share the responsibility for providing information necessary to evaluate and agree on appropriate measures.

Emergency measures are taken when a new or unexpected situation arises (may or may not be provisional). Although not explicit in the SPS Agreement, emergency measures extend from Annex B (urgent problems) and the resulting Emergency Notification format adopted by the SPS Committee (G/SPS/7 Rev 1). The IPPC (the Convention) is explicit about emergency action based on the detection of a pest, indicating that such action should be evaluated as soon as possible to ensure that it is justified (Article VII.6). ISPM 1 (Sect. 2.11) refers to emergency actions for new or unexpected phytosanitary situations based on a preliminary PRA and indicating that such measures are temporary and the subject of a detailed PRA as soon as possible.

Provisional measures need not be emergency measures, i.e., not necessarily in response to an immediate threat. Likewise, emergency measures need not be provisional, i.e., require additional information and reconsideration.

Precautionary measures are not identified by either the SPS Agreement or the IPPC. The term is variously understood and usually linked to the application of the 'precautionary approach'. It may be argued however that phytosanitary measures are by their nature more or less precautionary depending on the influence of uncertainty in the judgment regarding acceptable risk. Questions in this regard have surfaced in SPS disputes associated with the interpretation and application of the concept of provisional measures. Deliberations in this context have resulted in statements from the WTO Dispute Settlement Body that clarify that provisional measures have a precautionary aspect to them but are not intended to be precautionary measures.

Phytosanitary measures that have a direct effect on pests by reducing their prevalence or survivability are often called *mitigations* (e.g., a treatment). Measures that have no effect on pest prevalence or survival but promote phytosanitary security or shipment integrity are *safeguards* (e.g., pest proof packages). Most other measures are *procedures* that have no effect on pest prevalence or survival and do not contribute to phytosanitary security or integrity but support or enhance the effectiveness of risk management (e.g., inspection, certification).

Mitigation options can cover a range of possibilities and different levels of efficacy. Clarifying the role and effect of industry practices and identifying the primary mitigations used to reduce risk is necessary to begin evaluating the efficacy of measures. The three most common measures used for risk management are *prohibition, inspection,* and *treatment*. A closer look at these measures provides useful insight into the primary points associated with risk management.

Prohibition. The SPS Agreement makes a critical distinction between "prohibited" (which is a phytosanitary measure) and "not authorized" (which invokes a process). Because prohibition is a measure, it must be based on an international standard or a PRA according to the SPS. But the Agreement also recognises the need for administrative approval processes with provisions found in Annex C. This provides the basis for the "not authorized" category, which is for those articles that must be evaluated for their measures to be decided (i.e. for a PRA to be completed). Although the end result is the same (the article cannot be imported), the rationale and authority behind the condition is extremely important for trading partners to determine the actual status.

Prohibition is generally regarded as a highly effective measure. This is a common misunderstanding. Prohibition is generally assumed to close a pathway, but there are at least two situations where this is not the case. One situation involves natural spread as a viable pathway. Any regulatory strategy should be weighed against the likelihood of natural introduction (e.g. insects migrating across a border). The other situation involves a strong motivation for the pathway to exist. In such cases, prohibition will not result in absolute phytosanitary security, and in some cases, prohibition may actually contribute to increasing the risk because it increases the probability for smuggling. Sometimes regulators should authorize articles or activity with an uncomfortable level of risk in order to increase the potential to manage the pathway.

Treatment Phytosanitary treatments are mitigations that reduce the prevalence or viability of pests by exposing them to conditions and agents that have a detrimental effect. The primary aim of treatments is to ensure a specific effect on pests while minimizing harmful effects on the articles being treated. The historical model for treatment is a single, high-mortality treatment prior to export or immediately upon entry. The best known and perhaps most widely used post-harvest phytosanitary treatment is fumigation with Methyl Bromide, but the possibilities for greater flexibility and creativity in treatment technology is increasing as countries continue to explore alternatives to Methyl Bromide and translate SPS principles into practice. Combination treatments, low-dose treatments, non-mortality treatments, and treatments as part of systems approaches are becoming more common, dramatically increasing the options which can be considered for mitigation.

The process of identifying, evaluating, and selecting treatment options must consider both efficacy and feasibility. Efficacy is characterized by the *required response*, a concept that is described in ISPM 18 (Guidelines for the use of irradiation as a phytosanitary treatment). The required response has two elements: (1) A precise description of required response (mortality, sterility, etc.); and (2) the statistical level of response (metric and methodology). It is not sufficient to only specify a response without also describing how it is measured. 'Probit 9 mortality' as an example where mortality describes the response and Probit 9 is used to identify the level of response and the type of statistical analysis that will be applied.

Some treatments (e.g., irradiation) offer the possibility for a range of responses other than mortality (e.g., sterility). The selection of a treatment and specification of a required response begins with a clear understanding of the risk that is being addressed, the biology of the organism, the tolerance of the commodity being treated and the operational realities of implementation. Factors to consider regarding the feasibility of treatments include phytotoxicity, the availability of facilities/equipment, and use or label limitations.

High-mortality treatments are typically applied to cases where both the risk and the infestation rate are potentially high. Probit 9 level treatments (99.9968 % required response) are often considered synonymous with high-risk situations. This level of treatment in most instances is employed without the need for detailed or time-consuming data collection on the prevalence of the pest, the level of infestation, or the likelihood of establishment. Probit 9 level treatments are an attractive option for pest risk management because they provide a conservative level of quarantine security, although they may not always provide the desired level of consistency in all situations (Liquido et al. 1997).

Probit 9 level treatments are convenient mainly due to the relative speed in which they can be developed, tested, and implemented for tolerant hosts (Liquido et al. 1997). As a result, a number of precedent-setting treatments have been established that come to be viewed as risk management standards when they are actually based on bilateral agreement for a measure that is believed to result in substantial "overkill" in order to facilitate trade. Such treatments are difficult to link to consistent policies for a threshold level of risk or arguments of equivalency, and generally violate the principle of "least restrictive measure". The legitimacy of such treatments comes from the fact that they are bilaterally agreed, not that they are technically justified.

From a risk standpoint, mortality is not important; survivorship is important. Linking the efficacy of the treatment to some level of risk, even if it is only a threshold, requires an estimate of the infestation level in order to predict survivorship. Beyond this, there are biological and other important variables to consider for estimating the likelihood of establishment if the treatment is to have a rational relationship to the risk.

The use of Probit 9 level treatments for phytosanitary security is not based on any scientific studies yet it remains an effective tool in treatments. The application of Probit 9 level treatments in the future should be justified through treatment models linked to PRAs, and not exercised in all quarantine situations as a de facto standard.

Inspection Inspection is the most widely applied phytosanitary measure and has historically served as a fundamental component of most all risk management strategies. Hundreds of decisions are made by phytosanitary officials each day based on inspection; however, the technical meaning of this procedure as a risk management measure is frequently misunderstood.

Inspection can be broadly interpreted to include a wide range of activities, processes, and methods used for various reasons. The verification of documentation is an activity commonly associated with a phytosanitary inspection, as is the examination of a site or facility for compliance or suitability under phytosanitary requirements. Inspection may also be used to gather information or to monitor or audit phytosanitary programmes. Actions taken as a result of inspection determine how risk is changed when inspection is used as a phytosanitary measure for risk management. Operational decisions will be acceptance (no action), rejection, or the application of other measures (e.g., treatment). Risk-based decisions will link inspection to the acceptable level of detection and most appropriate action based on the characteristics of the pest, pathway, and situation. ISPM 23 (Guidelines for Inspection) provides substantial background on the role and applications of inspection in both import and export systems.

Risk-based inspection designs begin by understanding that the pests of concern must be detectable. The organism or its signs/symptoms must be visually discernible and distinct enough to minimise the potential for confusion with non-pest organisms or other conditions. Inspection is not an appropriate option for pests that are difficult to detect, the commodity is difficult or unsafe to inspect; or the pest of concern is high risk and establishes easily with a few individuals.

Under normal circumstances, an inspection is not done on 100 % of regulated articles, and inspection is not 100 % efficient. Thus, two key assumptions associated with inspection are: (1) that the result will be based on a sample, and (2) that a certain amount of risk and uncertainty is acceptable. There will always be some probability that pests will escape detection. The level of pest prevalence that is likely to be detected may be described as a detection level or tolerance. For example, finding that two boxes of fruit from a total of ten are free of pests does not provide absolute assurance that all ten boxes are free of pests. There is some probability that pests occur in the remaining boxes and there is a degree of uncertainty associated with the two boxes that were inspected. The level of detection and tolerance can be calculated for the two-box sample based on some defined level of confidence (usually 95 %). Because a tolerance is inherent in the procedure, it is not appropriate to use inspection if the objective is pest freedom.

Risk-based inspection relies on a*cceptance sampling*. This concept is applied in risk management to help determine whether inspection is the most appropriate phytosanitary procedure to use for managing pest risk and the characteristics of a proper inspection design. A risk-based inspection is one that has as its objective a defined level of pest prevalence to be detected and a desired level of confidence.

Typically, phytosanitary inspections are based on a percentage of a lot (e.g., 2 % of a consignment). This action is based on the mistaken idea that the detection level is constant if the ratio of sample size to lot size is constant. However, the laws of probability argue differently. We must understand this mathematical relationship in order to identify the most statistically sound design for risk-based inspection. ISPM 31 (Methodologies for sampling consignments) provides detailed guidance on designing and selecting appropriate inspection methods and describes relevant statistical and operational parameters.

The development and adoption of risk-based inspection programmes enhances the ability of officials to establish priorities for their inspection resources and to design inspection programmes that are transparent and predictable for trading partners and the private sector. By establishing reference points (risk-based inspection objectives) and a way to measure the results, it becomes possible to identify the areas where inspection resources are most needed and the level of resources required. These determinations then correspond with the acceptable level of risk and the strength of measures to be applied.

Systems approaches A systems approach is the combination of distinctly different pest mitigation measures, procedures and phytosanitary safeguards which cumulatively achieve the desired level of phytosanitary security. Although often complex and more difficult to implement than single-mitigation approaches, systems approaches hugely increase risk management possibilities and provide greater

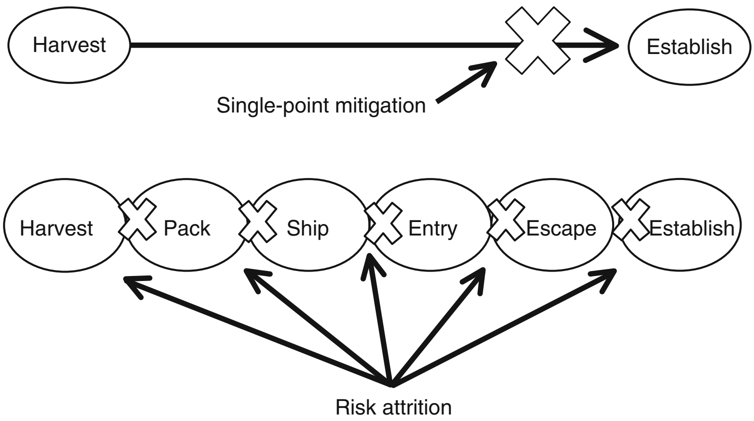

Fig. 5.1 Single-point mitigation contrasted with risk attrition through a systems approach

flexibility to adjust the strength of measures by combining measures with defined efficacy in a specific way.

A great advantage of systems approaches is that risk mitigation can include a full range of measures – both existing and prescribed that adds to phytosanitary security – in the growing area, at the packinghouse, or during shipment and distribution of the commodity. Cultural practices, field treatments, post-harvest disinfestation, inspection, and any procedure or event that can be identified as a mitigation or safeguard which supports pest attrition has the potential to be a component in a systems approach (see Fig. 5.1). Processes such as pest survey, trapping and sampling can also be components of a systems approach. Safeguards such as maintaining the integrity of lots, designated harvest or shipping periods, restrictions on maturity, the use of resistant hosts, and limited distribution at the destination can be key elements of a systems approach. This opens many opportunities for growers, packers and others to work with phytosanitary officials to identify the points and controls needed to develop and support a feasible programme. Likewise, it provides substantial flexibility and extensive possibilities for achieving equivalency where alternatives are sought.

Systems approaches, however, are more difficult to evaluate and manage than point mitigation (such as disinfestation treatments) because they are usually more complex and require greater effort to develop and implement (Fig. 5.1). In particular, systems approaches require a relatively high level of knowledge and confidence concerning the pest-host relationship and the ability to manage diverse elements of cultivation, harvest, packing and distribution systems. The primary limiting factors to the use of a systems approach is the availability of data on the efficacy of measures and practicality of implementation under specific conditions.

Phytosanitary treatments, such as Methyl Bromide fumigation at a rate sufficient to kill 99.9968 % of treated individuals (i.e., Probit 9 mortality) provide a familiar framework for determining phytosanitary security. However, when a systems approach that does not include a phytosanitary treatment is used as a phytosanitary measure, this framework is lost and alternative methods for evaluating the efficacy of measures are needed. Systems approaches that involve two or more diverse components can also present problems when trying to express the degree to which these various components mitigate the pest risk and the sum of their combined actions in common terms.

The efficacy of the individual mitigation measures can seldom be expressed in common terms, and seldom in the same terms as a treatment. For example, a disinfestation treatment provides a demonstrated level of mortality whereas pesticide treatments in the growing area reduce the incidence of pests as evidenced by reduced trap captures or fruit cutting. Culling at the packing facility may remove almost 100 % of the pests that are visually detectable but only some of the pests that are not detectable. Further, some components of a systems approach are not mitigation measures at all, but instead serve as a way to monitor and verify the prevalence of pests (e.g., trapping surveys, biometrically based inspections). In sum, it is clear that systems approaches require more complex methods for evaluating phytosanitary security.

In order to express the overall efficacy of a systems approach, a "common currency" (i.e., term of expression) is needed. The chosen endpoint affects how phytosanitary security will be expressed. Examples of endpoints include:

- Prevalence of pests in a consignment or proportion of pests removed;
- Frequency of entry (i.e., number of pests entering per unit time);
- Probability of entry (e.g., probability of pest entry per unit of commodity imported);
- Frequency or probability of establishment; or
- Frequency or probability of pest outbreaks.

Regardless of endpoint, the evaluation of measures should be done in the context of a Pest Risk Analysis and should always be directed toward consistency with the endpoint. This is done by breaking the system into its individual components and evaluating the contribution of each component to achieving phytosanitary security. Qualitative, quantitative, or mixed analyses may be used to develop estimates and identify strengths, weaknesses, and redundancy.

The key to the acceptance of systems approaches is an objective, analytical view toward pest risk management. Traditional assumptions and benchmarks for measuring efficacy are not always the most appropriate. Likewise, the principle of equivalence asks to define the level of phytosanitary security required and to consider those that are feasible and demonstrated to achieve this level. As this principle gains recognition in practice, the possibilities for non-traditional alternatives will increase. Foremost among these alternatives will be systems approaches because of the flexibility and range of options.

Redundancy Somewhere between no measures and the most restrictive measures is the best balance of the strength of measures with the risk, which achieves the appropriate level of protection. In any risk management process however, there will be a degree of uncertainty that will generate concern for the need to err on the side of caution by relying on conservative assumptions and worst-case data to determine the strength of measures. In most instances this will result in risk management strategies designed for when measures are failing or operating at the lowest end of their efficacy at the same time pest challenges are maximized. Adding measures or extra strength to measures as a means to compensate for uncertainty is sometimes referred to as redundancy. ISPM 14 states:

> A systems approach may include measures that are added or strengthened to compensate for uncertainty due to data gaps, variability, or lack of experience in the application of procedures.

This reference to uncertainty does not explain the concept, but it is reasonable to assume that uncertainty includes both *variability* (which cannot be improved) and *error* (which can be improved) as related to measuring the *efficacy* and *consistency* of phytosanitary measures. The implication is that each phytosanitary measure has some specific level of uncertainty associated with its efficacy. In the case of a systems approach, the accumulated uncertainties for each measure result in a higher level of uncertainty for the entire system.

ISPM 14 gives no guidance on the criteria used to determine the degree to which the strength of measures can or should be increased. Presumably, it is at least justified to increase the strength of measures to meet the appropriate level of protection under conditions of minimum efficacy (i.e., each measure is operating at minimum efficacy and at the upper limit of its uncertainty). Difficulties will arise when the strength of measures is boosted for additional redundancy to cover undefined contingencies and unknown or unanticipated uncertainties with no evidence that they actually exist (i.e., possibilities, not probabilities). This additional redundancy will not have a clear technical or operational justification and no basis in the standard. Such redundancy occurs frequently but is often not challenged unless severely limiting trade.

A problem with systems approaches is the general perception that uncertainty is higher and less easily controlled and therefore greater redundancy is justified. This is partly true because the probability of error and failure generally increases with the complexity of the system. In practice however, phytosanitary systems are not very complex and systems approaches are often more precisely measured and monitored for efficacy so that the uncertainty may actually be significantly less than single-measure strategies.

Another way to consider redundancy in a systems approach is to take a holistic view of the entire pathway, beginning with officially prescribed measures that are the core of the systems approach and also including other conditions or procedures that have a pest mitigation effect.

Assume for instance that the export of a certain fresh fruit requires: (A) Use of a specified non-preferred host; (B) field surveillance and treatment to ensure a defined level of low prevalence; and (C) cold storage under specific conditions. Each of these measures is independent and has a known level of efficacy and uncertainty. Also, fruit for export will be culled, graded, washed and waxed when packed. This packing procedure significantly reduces the prevalence of pests of concern, but the procedure is not officially considered part of the systems approach.

Other factors may also contribute to reducing pest prevalence or viability which are likewise outside the official systems approach but add to the 'comfort-level' associated with the entire system.

In this case, the non-official elements of the system are loosely viewed as adding redundancy that may partially or fully compensate for the uncertainty associated with the official measures. This possibility is recognised and thought to be legitimate by some experts but complications arise from the philosophical difference regarding whether non-official elements are legitimately considered or not. Some experts consider that redundancy should be limited to incremental increases in officially prescribed measures while other experts believe there is value in a holistic view that recognises non-official elements contributing to overall efficacy. ISPM 14 supports the former but not the latter. A systems approach designed with a holistic view could not be based on the standard and would therefore require a clear bilateral understanding of non-official risk mitigation factors.

5.7 Import-Export Programmes

The discussions above address many of the fundamental concepts underlying effective pre-border decision-making. ISPMs provide important guidance and future efforts toward additional harmonization will further promote common approaches to safe trade. Although internal arrangements for pre-border biosecurity systems will vary considerably from country to country depending on legal and government structures, resources, and priorities, a key point that historically links the import and export programmes of all NPPOs is phytosanitary certification.

Phytosanitary certification is a uniquely IPPC concept. It was established as a central tenet of the original Convention in 1952 and has endured as the single most globally recognised point of harmonization where the plant protection community intersects with trade. Both the process and the document were recognised and accepted by countries for decades before the SPS Agreement existed.

Phytosanitary certification is both an import and an export process requiring infrastructure and support systems directly controlled by the NPPO and consistent with the SPS-IPPC framework discussed above. Import systems are designed to establish requirements and verify that imports meet the requirements. A policy and operational infrastructure is needed for routine authorizations as well as emergency actions for unanticipated hazards. Export systems are designed around the requirements of trading partners and depend on an infrastructure linked to producers and sub-national authorities to assure a credible system for certifying conformity with the requirements of trading partners and overall integrity of exports generally, including non-agricultural products.

Although phytosanitary certificates are not always required, especially when articles in trade are not agricultural (e.g., empty containers) or there are no special requirements (subject only to inspection upon entry), the policy and operational components of import/exports systems remains the same. From this standpoint, it is convenient to think of phytosanitary certification processes whether or not an actual certificate is involved. As the certification process becomes increasingly more automated and electronic, the physical document becomes less important than the processes and infrastructure that provide the assurances that are the basis for the document.

With the exception of processes that are directly linked to issuing phytosanitary certificates, the basic infrastructure requirements for export are also necessary for import. These include mechanisms for:

- Regulations and policies;
- Enforcement;
- Risk analysis and scientific information;
- Pest identification;
- Inspection and treatment;
- Surveillance;
- Stakeholder and international relations.

Aspects of this infrastructure are the sole responsibility of the NPPO. Other parts can be addressed through collaboration with other agencies and institutions. For instance, only the NPPO has the authority to create official regulations and policies, but the NPPO may collaborate with universities for analyses, or work with extension on surveillance, or work with Customs on enforcement. Article IV of the IPPC describes basic organisational arrangements for an NPPO and Article VII outlines specific requirements in relation to imports, but only general guidance for a framework can be provided because each country has unique conditions. The following discussions are intended to provide additional insight as regards key points regarding each function as well as its relationship to other functions and the discussions above.

5.7.1 Regulations and Policies

A primary challenge with regulations and policies involves striking a balance between providing the appropriate level of detail for transparency and enforcement without handicapping the NPPO's ability to be responsive and flexible for changing and unanticipated conditions. For example, including a list of regulated plant pests in regulations implies that all or most of the pests of concern are known and anticipated. Emergency action is required for new and unanticipated pests which, if consistently found in the future, should be added to the pest list via a change in the regulations. Regulatory updates require time, effort and resources for the administrative and legal processes that would not be necessary if the pest list were maintained instead in a more flexible and responsive policy format. A policy format is therefore preferable if it can be equivalent (publicly available and legally defendable).

Functions having strong linkages to regulations and policies include enforcement, risk analysis, international relations, and stakeholder relations. Aside from the overall authority for emergency actions, regulations need to provide the appropriate level of enforcement authority depending on the legal structures of the country in question. This varies considerably across different countries and may be shared or delegated. For instance, the authority for some or all actions at ports of entry may be with Customs. Certain domestic actions may be the responsibility of local authorities.

Ensuring that regulations and policies are legally defendable generally requires legal counsel either within the NPPO or available to the NPPO for consultation and support as needed for two distinct types of legal questions. The first is consistency with the national legal framework (enabling legislation) and to ensure enforceability. Another level of legal consultation regards international obligations, including in particular the WTO-SPS Agreement and the IPPC with associated ISPMs. The primary objective at this level is to avoid challenges that could result in disputes, but also to help identify weaknesses and priorities in the national regulatory framework that require attention. Although the WTO and IPPC are strongly relevant to NPPOs from a legal standpoint, governments will also be involved with numerous other international commitments including other treaties, trade agreements, and defense policies which legal counsel needs to be tracking and anticipating impacts to national biosecurity systems. One example is the Montreal Protocol on Substances that Deplete the Ozone Layer, which has implications for the availability of Methyl Bromide as a phytosanitary treatment.

Ensuring that regulations and policies are technically and scientifically defendable is where the regulatory function links to risk analysis and information management. This relationship is relatively simple to the extent that there is the political will to consistently provide a scientific justification for regulatory actions. Difficulties arise when analyses are not used or are used inconsistently, and especially when analysis is policy-driven for pre-determined results. Another serious difficulty is avoiding analyses completely under the auspices of a precautionary approach due to "insufficient evidence". The weakness in this situation is the lack of clarity regarding the type, quantity and quality of evidence (scientific or otherwise) that is required to overcome the uncertainties that are the cause(s) for concern. In other words, an arbitrary and non-transparent threshold for reasonable certainty is established by adopting a precautionary approach. An important outcome of a proper risk analysis is recognizing and characterizing the uncertainty. The absence of such an analysis makes it impossible to understand a strategy for overcoming uncertainty.

Regulations and policies link to international relations at two levels. First is the direct NPPO to NPPO link that forms the basis for bilateral relationships that are important for negotiation and notification. Mechanisms for the exchange of official information in the development or debate leading to new regulations and a means to ensure trading partners are aware of non-conformities are fundamental. The other type of linkage is associated with multilateral relationships, especially the IPPC and standard setting, but also other international and regional organisations and initiatives relevant to biosecurity regulations. One important example is notifying regulatory changes to the WTO for a period of comment from WTO members.

The degree to which stakeholder relations are formalized depends greatly on the regulatory system and culture. Many countries are extremely transparent with multiple levels and types of consultation with stakeholder in their regulatory process. Other countries have relatively simple mechanisms or none at all. The key point is that plant biosecurity authorities are aware of stakeholder concerns and information that may be available in the public and private sectors, which inform or impact the development or maintenance of regulations and policies. In countries such as the USA where public consultation on regulatory changes is a national legal requirement, a process for notice and comment is established with both printed and online systems for public use.

5.7.2 Enforcement

As noted above, enforcement is most strongly linked to regulations and policies, but may not be completely administered by the NPPO. It is not unusual for NPPOs to share enforcement with, or delegate to, other agencies – most notably Customs.

As a general rule, enforcement is focused on port-of-entry violations and post-entry (domestic) violations. Aside from the broad authority needed for emergency and routine actions to enter, search, seize, and safeguard personal property, authority must also extend to punitive actions such as penalties. Enforcement also links to both international and stakeholder relations in the process of notifying non-conformities. For example, an importer may have a shipment rejected that has material which is prohibited. The importer may be penalized (e.g., fined), but a notification to the NPPO of the exporting should also occur, especially if a phytosanitary certificate was associated with the shipment indicating that the NPPO of the exporting country authorized the consignment.

Because international trade involves multiple agents, including at least an exporter, a shipper, and an importer, it is important to have regulations and policies that clearly identify a private party which will be responsible and therefore subject to punitive actions. At the same time, NPPOs also need clear and consistent policies on actions that are taken against broader authority. This is to distinguish the enforcement action on a shipment from entirely withdrawing the authorization to import the commodity. The latter is not strictly an enforcement action but has legal repercussions, especially if not technically justified. To demonstrate this concept, imagine that a particular type of import from a single supplier is rejected for pests that certification indicates are not present. The result is likely to be rejection or treatment. Now imagine that the same commodity is imported from the same country but multiple suppliers and multiple shipments are rejected over an extended period. In addition to rejecting shipments, and in the absence of any changes by the exporting country in response to notifications of non-compliance, the importing country may withdraw the authorization to import.

5.7.3 Risk Analysis and Scientific Information

The role of risk analysis is cemented firmly into import and export systems by the SPS Agreement and reinforced by the IPPC and associated standards, including several specific standards for risk analysis. The ability to collect and use scientific information for the range of pest risk-related issues associated with biosecurity in trade is crucial to the effectiveness of an NPPO. The linkage of risk analysis to regulations is discussed above and forms one of the most critical relationships for demonstrating consistency with the objectives of the SPS Agreement. An unfortunate repercussion of the emphasis placed on the importance of scientific evidence is the lack of attention given to dealing with uncertainty. In many instances, it is not the evidence that concerns decision makers so much as the uncertainty, and little guidance has been developed regarding methods for responding to uncertainty.

NPPOs must understand the importance of investing in information management systems. Scientific information, including operational data such as surveillance results, is the raw material for the analyses that support regulations, policies and programmes. Sometimes we see a tendency to separate information used for imports from information used for exports. Although there are differences, the base need for both is information on pest biology and distribution. For example, accurate identification and reliable surveillance data is needed on pests that occur domestically so that an accurate determination can be made by the NPPO on the quarantine status of an exotic pest. Once that determination has been made and an analysis has been completed, the ability to effectively collect, archive, search, and distribute the analyses and supporting evidence becomes a practical challenge that is facilitated by having dedicated processes for efficient document management rather than relying on ad hoc archives and institutional memory.

5.7.4 Pest Identification

Pest identification capabilities are linked directly to the inspection and treatment function and also with surveillance. The main challenge is providing identification services as accurately and quickly as possible, especially when the pest is associated with perishable cargo (either import or export). Identification for surveillance also requires a high level of accuracy but may or may not have the same urgency depending on the nature of the programme. For instance, new pest detections and the identifications associated with delimiting or monitoring survey in control programmes are more likely to require urgent processing whereas general surveys and those associated with confirming presence or absence in an area for export certification purposes will be a lower priority.

A key point to understand with identification is that NPPOs frequently rely on expertise outside their organisation, including experts in universities, research institutions, and even the "general" public or in another country – wherever credible expertise may be available. Associated with this however, are legal issues associated with the “chain of custody” and voucher specimens. Policies are needed to ensure that the NPPO minimises the potential for challenge by shippers, exporters, and the exporting country on these issues.

An important and rapidly evolving area of identification involves the use of molecular diagnostic techniques (Chap. 13). Molecular techniques are essential tools for identifying organisms that cannot be identified using traditional techniques, and for distinguishing organisms that are morphologically similar, especially pathogens. Notably, no internationally agreed guidelines have been formulated to help NPPOs determine the extent to which molecular characteristics are used to designate new species for regulatory purposes. Genetic and behavioural characteristics are both important for distinguishing pests, but the criteria that should be applied for regulatory purposes is not yet clear and is likely to be a key area for harmonization in the future due to the potential this technology holds for affecting established policies.

5.7.5 Inspection and Treatment

As noted at the beginning of this chapter, inspection is a primary component of import and export systems for plant biosecurity. Historically, import inspection has assured conformity with phytosanitary certification requirements in terms of accurate documentation and pest freedom. The applications for inspection in export are operationally similar to those for import except they are designed to meet the certification requirements of the importing country. In some countries, there are also national requirements for exports (e.g., Chile). Such requirements are usually focused on commodity quality but sometimes also address quarantine issues.

Import inspections are also a mechanism for detecting unanticipated pests that may be of concern and prohibited items, including contaminants (e.g., soil). Import inspection also applies to travelers and their belongings, but the objective in this case is more focused on prohibited articles than pests. Conveyances and other articles may be subject to inspection as well. ISPM 23, Guidelines for inspection (2011) and ISPM 31, Methodologies for sampling consignments (2011) provide important conceptual and technical guidance on inspection.

The typical design of an import inspection for commodities is the visual examination of a convenient sample upon entry; however, variations on this theme are diverse. Statistically designed inspections that use a calculated sample-size to achieve a designated detection level are an example of a more technically rigorous approach than what is typically used. Selecting samples for lab testing is another variation.

Perhaps the most widely recognised and frequently used variation on typical port-of-entry inspection for commodities is preclearance (also known as pre-inspection) at origin. The concept involves trading partners realizing some advantage by applying inspections for agricultural clearance at the origin. (Note that this process does not imply Customs clearance.) Preclearance can also provide the opportunity to implement a programme which might otherwise be unfeasible, and it can result in capacity building upon which other programmes can be built and credibility can be enhanced. RSPM 2, Guidelines for Pre-clearance programmes provides useful guidance regarding the concept and application of preclearance (NAPPO 2008).

The term "preclearance" is applied to the type of clearance that results from inspection as well as clearance based on prescribed treatments. Criteria that argue for the establishment of a preclearance programme include:

- High pest risk;
- High volume commodity;
- Inspection on entry is less feasible;
- Treatment on entry is less feasible; and
- Lack of capacity for inspection at the origin.

Arrangements for preclearance programmes, including funding questions, are bilaterally agreed. One of the biggest barriers to the implementation of preclearance programmes is the cost associated with training inspectors and covering their travel expenses that can be for extended periods.

Treatment is one of the three primary measures used by NPPOs for risk management (prohibition, treatment, and inspection). NPPOs have two basic approaches to treatment: (1) Treatment as a condition of entry, and (2) Treatment as an emergency action.

When treatment is prescribed as a condition of entry, the treatment may be done at origin, in transit, or upon arrival. Each approach has its own set of conditions, which must be met to assure efficacy. In the case of treatments for exports, certification of the treatment can be provided on the phytosanitary certificate or an official treatment certificate accompanying the consignment. Treatments performed for imports will either be associated with an emergency action that is linked to non-compliance, or an established treatment requirement.

Treatment at the origin often involves preclearance. Treatment in transit requires approved equipment and mechanisms to verify conformity with the treatment parameters. Treatment on arrival may be done by the NPPO or contracted to NPPO-approved specialists. In the case of chemical treatments, there are also labeling and residue requirements that need to be considered, and these may vary between countries.

5.7.6 Surveillance

ISPM 6, Guidelines for surveillance (IPPC 1997) describe two types of survey: General and specific. Both types of survey provide basic information about pest presence and distribution, which is crucial for both import and export decision-making, and link with every other function in direct and indirect ways.

Art IV.2.b of the IPPC states... *The responsibilities of an official national plant protection organization shall include . . . the surveillance of growing plants, including both areas under cultivation (inter alia fields, plantations, nurseries, gardens, greenhouses and laboratories) and wild flora, and of plants and plant products* *in storage or in transportation, particularly with the object of reporting the occurrence, outbreak and spread of pests, and of controlling those pests, including the reporting referred to under Article VIII paragraph 1(a);*

Art VII.2.j states... *Contracting parties shall, to the best of their ability, conduct surveillance for pests and develop and maintain adequate information on pest status in order to support categorization of pests, and for the development of appropriate phytosanitary measures. This information shall be made available to contracting parties, on request.*

These provisions of the Convention highlight the importance of surveillance as a baseline function for every NPPO. From a programmatic standpoint, surveillance is used to:

- Aid official pest status determinations (presence/absence);
- Support NPPO declarations of pest freedom or low prevalence;
- Aid the early detection of new pests;
- Delimit or monitor the spread of regulated pests;
- Establish host and commodity relationships;
- Decide pests which meet the defining criteria for a quarantine pest;
- Create and update pest distribution records.

When performing survey or collecting survey data from others or the literature, NPPOs require mechanisms to hold and analyse the data. This also can be through collaboration with other agencies, institutions, or organisations, but notification of information such as new pest outbreaks is a responsibility of the NPPO.

5.7.7 Stakeholder and International Relations

An earlier section of this chapter discussed cooperation and communication with reference to the importance of relationships with stakeholders, trading partners, and relevant international organisations. From a trade perspective, the relationship with stakeholders is crucial to understanding priorities and the feasibility of risk management options. In many cases, stakeholders in the private sector also conduct research or are aware of scientific information that is not widely available. Likewise, stakeholders may not be aware of the information and rationale used by the NPPO for decision-making. Regular, two-way communication is therefore important for strong relations with stakeholders.

International relations associated with import/export are different than those associated with harmonization (standard-setting) and technical capacity building. Trade relations always have an element of tension associated with them and can be very dynamic, often subject to changes depending on political and economic factors outside the NPPO's control. A key point to emphasize here is that continuity of policy is important for strengthening these relations and avoiding unnecessary friction. One of the best ways to ensure continuity is to have dedicated individuals or offices devoted to specific areas of trade such as a specific country, region, or commodity.

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Chapter 6
The Biosecurity Continuum and Trade: Border Operations

Mark Whattam, Gerard Clover, Michael Firko, and Tom Kalaris

6.1 Introduction

With the advent of rapid transport systems, regulatory officials have seen a significant expansion in the international movement of plants and plant products from their centres of origin. The world's human population is predicted to rise to nine billion people by 2050 (Anon 2009a). Almost twice the current amount of food will be required to feed the world's population. Since many countries cannot support their current populations, international exchange of plants and plant products will inevitably increase substantially. The large-scale international transfer of plants and plant products has provided pathways for rapid, long-distance movement for many plant pests including invertebrates, weeds, and microorganisms (bacteria, fungi, and viruses). When new pests are introduced into areas where they did not previously exist, we see a significant potential for severe negative ecological, economic and aesthetic/social impacts. Introduced pests may arrive without antagonistic factors; competitive species that kept a pest in check in its original environment (or a pest relatively unimportant in its original habitat) may find another country's environment and flora more suitable and flourish to pest proportions. Endemic host species that have evolved in the absence of the introduced pest typically do not have the

M. Whattam (✉)
Department of Agriculture, PMB 19, Ferntree Gully
Delivery Centre, Melbourne, VIC 3156, Victoria, Australia
e-mail: mark.whattam@daff.gov.au

G. Clover
Ministry of Agriculture and Forestry, PO Box 2095, Auckland 1140, New Zealand

M. Firko
Animal and Plant Health Inspection Service, 4700 River Road, Riverdale,
MD 20737, USA

T. Kalaris
Center for Plant Health Science and Technology, 2301 Research Blvd, Fort Collins,
CO 80526, USA

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opportunity to develop resistance against the pest making them more vulnerable to attack. The converse is also true: Endemic pests can infect and damage newly introduced crops. Modern agricultural systems essentially evolved from 8 to 9 world centres of genetic sources (Harlan 1971). Modern (necessary and unnatural) intensive cultivation of genetically uniform monocultures frequently promotes damaging pest epidemics (Jones 2009).

Plant pests have played an important role in human history with some of the worst plant pest epidemics occurring as a result of entry and establishment of an exotic pest. Ancient Greeks and Romans had unpleasant experiences with plant diseases, particularly red rust of wheat. The Romans held an annual feast (the Robigalia) during which they would offer wine, burn incense and sacrifice a red dog to avert danger to the crops. The mass hallucinations in the Middle Ages, Joan of Arc's visions and the Salem witch-hunts may have been caused by the Ergot fungus (*Claviceps purpurea* (Fr.) Tul.) that attacked cereals such as rye and contaminated bread with LSD-like substances (Agrios 2005). The Irish potato famine of the 1840s was caused by the fungus *Phytophthora infestans* (Mont.) de Bary. The fungus found the mild, moist climate of Ireland more conducive than the cold arid mountain peaks of its homeland in South America. The fungus led to the starvation and death of more than 1.5 million Irish and caused the emigration of about the same number to the USA (Agrios 2005). Today about $1 billion per year is spent on fungicides to control the disease in the USA, Europe and developing countries (Forbes and Lizarraga 2010). The English are renowned tea drinkers, largely as a result of a plant disease, Coffee Rust (*Hemilia vastatrix* Berk. & Broome), which destroyed the colonial coffee plantations in Ceylon (Sri Lanka) in 1875. Coffee Rust forced the replacement of coffee with resistant tea plantations. The introduction of the Chestnut Blight fungus (*Cryphonectria parasitica* (Murrill) Barr) on imported lumber or live planting material from Asia in 1904 had a momentous impact on the natural flora of North America. Within 35 years the fungus destroyed 3.4 billion chestnut trees and by 1940, mature American Chestnut trees were virtually eliminated by this disease (Sect. 8.2.1; Agrios 2005).

An example of the introduction of an extremely damaging plant pest is the Gypsy Moth *Lymantria dispar* (Linnaeus). The USDA spends about US $12 million annually trying to slow its spread (Lodge et al. 2009). Gypsy Moth (GM) is an exceptionally damaging pest of hardwood forests and ornamental shade trees. Amateur Entomologist Leopold Trouvelot introduced it from France into Massachusetts in 1868/1869. Trouvelot was attempting to mate the moths with silkworms but they escaped from his house. Despite efforts to control GM, caterpillars have defoliated an estimated $>34 \times 10^6$ ha of hardwood forests in the USA since 1924.

Gypsy Moth continues to expand its range south and westward, predominantly by larval dispersal on wind currents and human-assisted movement of life stages such as egg masses on shipping vessels and cargo. Still, management costs would be far greater if the Asian biotype of *L. dispar* (AGM) became established in North America. European Gypsy Moth (EGM) females are flightless; AGM females can sustain flight up to 100 km (Rozkhov and Vasilyeva 1982). Also, AGM has a broader host range, with larvae feeding on over 600 species of plants (Baranchikov 1989). Multiple introductions of AGM into North America have occurred over recent decades with egg masses on ships and cargo arriving from ports in the

Russian Far East and Japan. All of these were subjected to aggressive eradication programmes and AGM has been prevented from establishing. Many countries maintain strict phytosanitary measures to minimise the risk of AGM entry including inspection and certification of vessels that visited high risk ports during the moth's flight season and inspection and trapping arrangements to monitor port areas.

Another notable introduction into the USA has been the Asian Longhorn Beetle (*Anoplophora glabripennis* (Motschulsky)), a polyphagous pest of healthy hardwood trees (Chap. 16). The beetle was first discovered in Brooklyn, New York in 1996 damaging Norway Maple trees and was probably introduced with solid wood packing material from China (Smith et al. 2001). Nowak et al. (2001) estimate the maximum potential urban impact of this pest in the USA is a loss of nearly 35 % of total canopy cover including 30 % tree mortality (1.2 billion trees) with a value loss of $669 billion.

The global ecological and economic impact of exotic plant pests on plant health is staggering. Today, conservative estimates suggest that plant pests (including pathogens, invertebrates and weeds) together annually destroy or impact 31–42 % of all crops produced worldwide (Agrios 2005). The total annual worldwide crop loss from plant diseases is about $220 billion. Roughly $38 billion is spent annually on pesticides (including fungicides, insecticides and herbicides) in Europe and the USA to control plant pests. Despite these control measures in the USA each year, crops worth $9.1 billion are lost to diseases, $7.7 billion to invertebrates and $6.2 billion to weeds (Agrios 2005). An additional 6–12 % of crop production is lost to post-harvest diseases. The losses are usually highest in developing countries; typically in areas where people are most in need of food. Consequently, measures that prevent or minimise the entry of plant pests are extremely important for the protection of a country's economic and social well-being.

An effective biosecurity management system is essential to support and protect primary agricultural producers and natural ecosystems from the entry of regulated pests[1]. Many plant pests capable of causing significant damage have not yet established around the globe. A robust border operation system is a primary component of a successful biosecurity strategy.

In Australia, New Zealand and USA, border operations provide two principal functions as part of the biosecurity continuum, viz. import regulations and export certification. Both functions are intimately linked as they rely on each other to achieve interdependent and synergistic outcomes. This chapter summarizes various phytosanitary risks associated with trade in plants and plant products and reviews the pathways and risk mitigation systems employed by border agencies in managing these risks. The chapter concludes with a brief discussion on the involvement of border agencies regarding exporting plants and plant products.

[1] The International Plant Protection Convention (IPPC) defines a regulated pest as a quarantine pest or a regulated non-quarantine pest. The term '*quarantine pest*' incorporates the threat posed by insect pests, plant diseases and weeds as a '*pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled*' (ISPM 5 2009). A non-regulated pest is a pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (ISPM 5 2009).

6.2 Brief History of Plant Quarantine

The term "quarantine" is derived from the Latin word 'quarantum' (Italian 'quarantina') meaning "forty." Quarantine originally referred to the period of detention for ships arriving from countries after the *Black Death* reached Europe in 1347 (Morschel 1971). An isolation period of 40 days was applied to ships and their travellers to allow latent cases of the disease to develop before anyone was permitted to land. In many ways, the same concept still applies for imports of animals and plants today. During the early development of phytosanitary systems, the focus was on protection of human health from Bubonic Plague, Cholera and Small Pox. During the early and mid-twentieth century, focus extended to domestic animals and plants. Historically, we see a tendency to have a stronger focus on animal quarantine issues. This has been due to the small number of economically important domesticated-animal species; the dramatic impact exotic diseases can have on these animal host and the emotive response from the community compared with plant diseases. Most countries with advanced agricultural industries now have well-developed border operations in place. These operations are supported by appropriate legislative powers, which enable phytosanitary regulators to manage the risks posed by imported plants and plant products.

Today, the terms 'phytosanitary', 'plant quarantine' or 'plant protection' cover legislative and regulatory measures and associated activities designed to minimise the entry and spread of phytosanitary pests. One of the earliest plant phytosanitary laws was passed in 1873 in Germany with the prohibition of plants and plant products from the USA to prevent the introduction of Colorado Potato Beetle (*Leptinotarsa decemlineata* Say). In the USA, the first plant phytosanitary measure was introduced in 1891 when California established an inspection depot at the seaport of San Pedro (Mathys and Baker 1980). The Federal Plant Quarantine legislation was enacted during 1912. The National Plant Protection Organisation (NPPO) for the USA is the Plant Protection and Quarantine (PPQ) programme within the USDA Animal and Plant Health Inspection Service (APHIS). In Australia, plant phytosanitary regulations first came into operation on 1 July 1909 following introduction of the Quarantine Act during 1908. The Act still forms the basis of current phytosanitary regulations.

In New Zealand, the Biosecurity Act (1993) currently provides the legal basis for excluding, eradicating and managing regulated pests. However, there has been a long history of phytosanitary regulation. In 1884, the Codling Moth Act was passed after an outbreak of the moth threatened fruit production. This was followed by the Orchard and Garden Pests Act (1896) and in 1897 the government prohibited importation of plants and fruit infested by Codling Moth, scale insects or Queensland Fruit Fly and grapevine cuttings infected by *Phylloxera* sp. The Department of Agriculture administered the 1896 Act and began inspecting imported fruit and plants at principal ports in the 1890s. As well as complying with regulatory requirements of the importing country, other international agreements (including the Convention for International Trade in Endangered Species of Wild Fauna and Flora, CITES) must be complied with to protect species threatened by excessive commercial exploitation. Additional information on CITES can be obtained at: http://www.cites.org.

6.3 Objective and Principles of a Phytosanitary Regulatory System

The objective of a phytosanitary import regulatory system is to implement appropriate regulations to facilitate trade in plants and plant products in the least "trade restrictive" manner while minimising the introduction of regulated pests (Chap. 2). Phytosanitary systems consist of two components: (1) A legal framework covering the legislation, regulations and procedures; and (2) An official organisation responsible for delivery of services in compliance with international obligations (ISPM 20 2004). Some legislative powers required by regulatory agencies include: (1) Authority to enter premises where imported commodities or regulated pests may be present. (2) Power to detain, inspect, treat or test regulated articles. (3) Authority to destroy or re-export regulated goods. Typically, border agencies employ their own officers to operate the import regulatory system. Also, other government organisations, industry groups or individuals may be authorized to carry out defined functions on its behalf and under its control.

To be fully effective, a country's border programme must be coordinated on a national level and developed with consistency, transparency and scientifically justifiable policies that meet national and international regulations. Countries manage phytosanitary risks associated with plants and plant products in different ways, but the principles (as enumerated by Morschel 1971) are more-or-less the same:

1. Phytosanitary pest risks associated with imported goods and pathways are identified based on sound and transparent scientific analysis;
2. Risk mitigation strategies that minimise the entry and establishment of pests are least trade-restrictive and consistent with international agreements;
3. Appropriate legislation and regulations are developed and passed by the appropriate governing authority, regulations and usually promulgated by the National Plant Protection Organisation (NPPO); and
4. Regulations are reviewed and modified in response to changed pest status or extended to include other risk commodities or new hosts.

The Food and Agriculture Organisation (FAO) has developed a detailed explanation of the principles associated with the application of phytosanitary measures for international trade (ISPM 1 2006).

The principle agreement governing international movement of plant pests is the International Plant Protection Convention (IPPC) (see Sect. 2.2.3). The agreement prevents international movement and introduction of invasive pests and promotes appropriate measures for their control. The scope of the IPPC extends to protection of natural flora and covers direct and indirect damage by pests.

Countries cannot implement a “no risk” phytosanitary policy because the only no risk policy is a “no trade/tourist” policy, which is indefensible. Also, natural risk pathways (which cannot be managed) present a route for entry of exotic pests. The best a country can do regarding phytosanitary measures is to implement a “risk managed” approach and implement controls at pathways designed to reduce risks to an ‘appropriate level of protection’ (ALOP) also referred to as “the acceptable level of risk”. The concept of ALOP for phytosanitary purposes is the level of protection a country decides is necessary to protect its plant health against the harmful effects of exotic pests. Where the risks of pests are above the ALOP, importing countries may require the application of measures to reduce the risks to specified and acceptable levels. Measures may include treatments, inspection and other procedures intended to reduce the pest risks (Sect. 2.2.5). In choosing and applying measures, a country must follow the IPPC principles of necessity, equivalence and harmonization. Although prohibition or total ban of trade will reduce the pest risks, this action may encourage people to deliberately bypass quarantine or smuggle products into the country thus bypassing managed systems and potentially creating a greater biosecurity risk. Further, prohibition of trade goes against international efforts to liberalise trade using the least trade restrictive measures. Restriction could result in retaliatory action by trading partners. As a consequence, international efforts established the World Trade Organisation (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) to ensure that exchange in agriculture and food products is not impeded by trade barriers disguised as phytosanitary measures (WTO 1994; Sect. 2.2).

6.4 Role of a Phytosanitary Inspector

Phytosanitary inspectors examine and clear imported goods including plants and plant products, vessels, international mail, passengers and their baggage. Phytosanitary inspectors may be located at the main border entry points including airports, seaports, border road crossings, mail centres, cargo depots and post-entry plant quarantine facilities. Phytosanitary inspectors have a considerable responsibility as their decisions can influence the potential entry of pests and diseases. On a daily basis, inspectors decide whether a consignment or a passenger’s goods meet the phytosanitary regulations of the country or whether further intervention and treatment is required. To carry out this task effectively, a phytosanitary inspector must have the ability to recognise diverse regulated articles, a basic knowledge of plant health and the main regulated pests of concern.

Inspectors must be familiar with the inspection techniques and the seizure, release and treatment of goods in the event a regulated pest is detected. Phytosanitary inspectors must have a comprehensive knowledge of the relevant national phytosanitary regulations and policies and understand their powers and limitations under the legislation. A high level of integrity and well-developed customer service skills are other desirable attributes; inspectors must be friendly and polite to members of the public while enforcing appropriate regulations. The regulations can sometimes be confusing to people, particularly for tired passengers that have travelled long distances from foreign countries and are unfamiliar with phytosanitary procedures.

Another role of a phytosanitary inspector involves issuing certificates for exported plant products. Officers should have a sound knowledge of local pests that may be of concern for the importing country.

6.5 Phytosanitary Risk Products

Imported plants and plant products (including live plants, seeds, plant produce, timber and soil) along with their associated packaging present a phytosanitary risk because they can be infected with or have the capacity to transmit or carry exotic plant pests.

Invertebrate pests have potential to directly damage plants and vector significant plant pathogens, particularly plant viruses. Insect vectors that transmit pathogens for a short time (hours) pose a lower biosecurity risk compared with vectors that persistently transmit pathogens for weeks or longer (Purcell and Almeida 2005). A pest's reproductive cycle can also influence invasion risk. For instance, parthenogenic (asexual) female insects can produce progeny without a male partner. Thus if a single female is introduced, then she could potentially generate a large pest population. Vectors (nematodes, aphids, thrips, mites, leaf hoppers, white flies, mealybugs and beetles) are targeted by border agencies even when these organisms are present in the importing country due to the risk of transmission of phytosanitary disease agents. A brief description of phytosanitary risk groups is provided below.

6.5.1 Live Plants

Imported live plants, often referred to as 'nursery stock' by phytosanitary agencies, consist of entire plants or parts of plants imported for growing purposes. Nursery stock includes cuttings, budwood, roots, bulbs, corms, rhizomes and tissue culture plantlets. (Seeds are considered separately below.) Live plants present the highest plant phytosanitary risk because they are an ideal vehicle for introducing regulated pests as well as being a potential weed threat. Because propagation is the primary objective of exchanging live plants, the chances of establishment and distribution of a pest is more likely given the availability of a suitable host to complete their lifecycle.

In addition to posing a risk of introducing exotic plant pests, live plants are now recognised as potential phytosanitary pests (i.e. weeds) in their own right. Introduced plants can quickly colonise a new environment. Without natural competitive forces, introduced plants can displace native plants, clog waterways and compete with cultivated crops for nutrients, water and light. Worldwide, weeds are estimated to cause crop losses worth nearly $150 billion annually (Agrios 2005; Chap. 20). An example of the potential damage an introduced plant can have on a natural ecosystem is demonstrated by the Prickly Pear Cactus (*Optunia inermis* de Candolle), introduced into Australia from South America in 1839. The cactus readily adapted to the arid conditions of Australia and rapidly displaced more than 24 million ha of native vegetation. The introduced weed was expanding at a rate of 400,000 ha per year until the moth *Cactoblastis cactorum* (Berg) was introduced in 1925, initiating one of the world's most successful biological control programmes (Kwong 2004). Many National Plant Protection Organisations (NPPOs, Regulatory Agencies) now require comprehensive Weed Risk Assessments (WRA) as part of the import conditions for new plant species to ensure the introduced plant does not itself become a phytosanitary pest. Detailed information about the WRA system used by Australia's border agency is available at: www.daff.gov.au/ba/reviews/weeds.

6.5.2 Seed and Grain

Due to their hardiness and durability, seed and grain have been internationally exchanged across borders for thousands of years (Chap. 5). The phytosanitary risks of seed and grain vary depending on the species, country of import and type of introduction. The end use is an additional factor considered by the NPPO; seed and grain imported for sowing and propagation present a higher biosecurity risk compared with seed imported for processing and consumption because typically the product will be processed or treated in such a way that reduces the viability of the seed and/or removes pests that may be present. Seeds of numerous species, including many flower and vegetable seeds, present a low phytosanitary risk because they are not hosts of significant phytosanitary pests and can be safely imported with no or only minimal phytosanitary intervention. This is expected given the long history and established trade in seed and grain between many trading partners. Long-established trade has presented numerous opportunities for pests to establish. Nevertheless, many serious pest risks are associated with seed and grain that have limited worldwide distribution. Examples include Khapra Beetle (*Trogoderma granarium* Everts), Karnal Bunt (*Tilletia indica* Mitra), Greater Grain Borer (*Prostephanus truncatus* (Horn)) and Sunflower Downy Mildew (*Plasmopara halstedii* (Farlow) Berlese & de Toni). Hosts of these species are restricted by some border agencies and require strict phytosanitary intervention measures.

6.5.3 Timber and Wooden Products

Timber and wooden products including solid wood packaging material (skids, flooring, pallets and dunnage), logs, woodchips, sawn timber and manufactured wooden products present a phytosanitary risk as they may be infested with a range of significant pests. Decayed and damaged timber is often used in dunnage and solid wood packaging; this material presents an ideal environment for pest infestation. Excessive bark on timber and wooden packaging further increases the potential risks because many insects and fungal pathogens readily inhabit the microclimate created by the bark. Many timber pests feed internally, revealing little or no evidence of their presence until they mature and emerge from their host many months or years later.

Many timber insects are cryptic and many timber and wood-inhabiting insects have been inadvertently transported around the globe in solid wood packaging. Mattson et al. (1994) estimated that more than 368 alien phytophagous insect species occur in American forest systems. Many of these invasive pests may have entered on wooden packaging material although the exact mode of entry is unknown (Haack et al. 2007). In the USA, the Asian Longhorn Beetle (*Anoplophora glabripennis* (Motschulksy)) (Chap. 16) and Emerald Ash Borer (*Agrilus planipennis* Fairmaire) are both suspected to have been introduced on wood packaging accompanying goods imported from Asia (http://www.asian-longhorned-beetle.com/ and http://www.emeraldashborer.info/). Both pests damage forest trees in the same way with their larvae feeding on the inner bark of the tree, thereby disrupting the tree's ability to transport water and nutrients. Asian Longhorned Beetle poses a serious threat to many species of deciduous hardwood trees and phytosanitary controls have been established around infested areas in the USA resulting in the removal of many thousands of infested trees. Emerald Ash Borer has killed more than 50 million ash trees and threatens most of the ash trees throughout North America (Kovacs et al. 2010).

Many plant pathogens can be transported in timber either directly or associated with insect vectors. Some of the more important plant pathogen risks include Chestnut Blight (*Cryphonectria parasitica* (Murrill) Barr), Sudden Oak Death (*Phytophthora ramorum* Werres et al.) and Pine Wilt Nematode (*Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle). During 2004 in Australia, spores of Guava Rust (*Puccinia psidii* Winter) were found associated with a shipment of eucalyptus timber imported from South America. This interception resulted in the suspension of all timber imports from countries where Guava Rust is present (Lawson 2007).

Newly manufactured plywood, veneer or reconstituted wood products such as particleboard, chipboard, medium and high-density fibreboard generally present minimal phytosanitary concerns. These products are highly processed and unlikely to be hosts of phytosanitary pests or diseases.

6.5.4 Fresh and Dried Plant Products

Fresh plant products may include fresh fruit, vegetables, cut flowers, herbs and spices intended for consumption but not for planting or growing. Given the perishable nature of fresh plant products, they generally pose a lower phytosanitary risk compared with live plants and seeds. Significant phytosanitary risks still may be associated with these products particularly invertebrate pests (e.g. fruit fly, thrips and aphids) and plant diseases such as Citrus Canker (caused by *Xanthomonas axonopodis* pathovar *citri* (Hasse) Vauterin et al.) (See Sects. 18.3 and 18.4). Fresh plant products that carry seeds or are capable of being propagated may pose a higher biosecurity risk and typically require additional risk assessment and mitigation.

Dried and durable plant products (excluding seed, grain and wooden articles) include: (1) Dried food of plant origin, flowers, foliage, herbs and spices; (2) souvenirs and handicrafts including mats, bags and baskets made from plant material and non-propagative stems such as bamboo, rattan, reed, cane and willow; (3) Christmas decorations, wreaths and ornaments including pine cones; and (4) Plant-based stockfeed.

Dried plant products generally pose a lower phytosanitary risk because most plant pathogens require a living host to remain viable. However, dried plant products may be infected or contaminated with the resting stages of plant pathogens, soil or seeds and infested with live insects. Dried plant packaging materials including straw, coconut fibre, rice hulls or similar plant material can carry exotic insect pests, pathogens and weed seeds and should not be used as packaging material for the international transfer of goods.

6.5.5 Highly Processed Plant Products

The method and degree of processing and the intended end-use of a commodity significantly influences the phytosanitary risk presented by plant products (ISPM 32 2009). Plant products that have been "highly" processed normally present fewer phytosanitary risks because the treatments typically remove the biosecurity viability of the pest. As such, importing requirements for commercially processed, packaged and labelled plant products including frozen, milled, pasteurised, fermented, cooked, pureed, pickled or suitably preserved (e.g. crystallised, jellied, salted) products are typically released based on verification of documentation to ensure compliance.

6.5.6 Soil

Soil deserves special mention because it can readily act as a medium for transporting many plant pests including invertebrates in their various stages of development, fungal sclerotes, bacterial spores, nematodes in a resting state and weed seeds of phytosanitary importance. For example, cysts of the Potato Cyst Nematode (*Globodera rostochiensis* (Wollenweber) Behrens, and *G. pallida* (Stone) Behrens) may remain viable in soil for 30+ years (Eyres et al. 2005). Soil can be readily moved in rooted plant material or as contamination on the outside of cargo containers and transport vehicles including used cars, farm and military machinery and earth moving equipment. Soil can also be a contaminant on passengers' personal goods including boots, tents, bikes, etc. Most NPPO's prohibit the movement of large quantities of soil that has not been treated to reduce the pest risk to an acceptable level (Anon 2011).

Peat is often imported for agricultural purposes or is used as a packaging material for plant bulbs. In theory, peat presents a minimal phytosanitary risk because it consists of composted vegetable matter and typically is acidic (presenting a harsh environment for pest survival). However, when collecting peat for international exchange we must ensure that it is free of soil and other phytosanitary risk material.

6.6 Phytosanitary Risk Pathways

A "zero risk" phytosanitary policy is impossible to implement because natural pathways including migratory birds, trade winds and ocean currents continually pose a route for entry of exotic pests. This is particularly true for windborne fungal diseases including rusts and smuts. Windborne plant pathogens, as evidenced by the presence of new diseases, are commonly discovered in New Zealand soon after they arrive in Australia (Sheridan 1989). This phenomenon is believed to be a result of the predominant direction of trade winds blowing west to east across the Tasman Sea between the two countries. In New Zealand, Poplar Leaf Rusts caused by *Melampsora medusa* Thümen and *M. larici-populina* Klebahn were recorded in 1973 (Dingley 1977), Oxalis Rust (*Puccinia oxalidis* Dietel & Ellis) in 1977 (Versluys 1977) and Stripe Rust of wheat (*Puccinia striiformis* Westendorp) in 1980 (Harvey and Beresford 1982). All were identified about 1 year earlier in Australia. Long-distance dispersal of rust spores across the Tasman Sea by wind currents is the commonly accepted explanation for many of these occurrences (Close et al. 1978).

Some countries have a natural geographic defence against the entry of many plant pests due to deserts, mountains or oceans but the advent of modern shipping and air freight combined with the liberalization of international trade has significantly increased the global movement of plants and plant products and this has reduced the effectiveness of these natural barriers.

The main risk pathways for movement of serious phytosanitary pests crossing international borders are due to: (1) Imported cargo involving trade in plants and plant products including potentially contaminated goods such as used agricultural, military and earthmoving equipment; (2) Passenger movement at airports and seaports including personal effects and the vessel itself; (3) International mail exchange; and (4) Unregulated movement across inter-country borders.

6.6.1 Imported Cargo

The international transport of commercial and non-commercial cargo in containers, shipping vessels and aircraft between countries is a cost effective and well-established practice. The increase in the number of sea containers transported around the world shows trade more than doubling during the past decade. In 2004, global merchandise trade was valued at US$ 8.9 trillion, compared with less than half that 10 years earlier and a mere US$58 billion in 1948 (Anon 2006). More than 80 % of international trade in goods is carried by sea transport. In 2008, world seaborne trade (goods loaded) increased by 3.6 % to surpass a record 8 billion tons (Anon 2009b). Border agencies must manage the risk posed by the imported cargo being transported, the packaging of goods (particularly regarding wood packaging) and the external and internal surfaces of containers. Cargo, packaging and shipping container can present potential pathways for the transport of many significant pests including Khapra Beetle, Asian Gypsy Moth, Giant African Snail and other hitchhiking (contaminating) pests.

Shipping containers can present a risk pathway particularly regarding soil contamination and other plant phytosanitary risk material adhering to the outside of the containers. Often, imported containers are moved between the port of entry and metropolitan areas, and they normally stand on hard surfaces with limited availability of host plants thereby reducing the risk of pest establishment. Empty containers are then reloaded and returned to the port for export. However some containers are destined for rural depots and soil and other contamination could dislodge during the transport process and potentially present a risk pathway for certain pests given the increased likelihood of suitable host plants being available. Stanaway et al. (2001) undertook a survey of the floors of 3,000+ empty sea cargo containers to estimate the quarantine risk of importing exotic insect pests into Australia. More than 7,400 live and dead insects were collected from 1,174 containers. No live infestations of timber-feeding insects were recorded but the collection of dead insects demonstrates that containers are regularly exposed to economically important regulated insects including timber pests, agricultural pests and nuisance pests. Stored product pests were found in more than 10 % of containers.

International aircraft and vessels including cruise ships, itinerant yachts and cargo ships present a risk pathway as they can carry food wastes, refuse in holds and galleys, imported cargo of bulk grain, timber products, stock feed and may also have live plants on board. In addition, the vessel itself has the potential to introduce hitchhiking pests. Two serious forestry pests, the Asian Gypsy Moth and the Burnt Pine Longicorn Beetle (*Arhopalus ferus* (Mulsant)) are regularly detected on international vessels that have visited high-risk ports during the insect's respective flight seasons. Female Asian Gypsy Moths are active flyers during July-September in the Russian Far East, China, Korea and Japan. Female moths are attracted to lights on shipping vessels and lay egg masses on the infrastructure (Walsh 1993). A similar situation occurs with adult Burnt Pine Longicorn Beetles contaminating vessels and hitching rides, normally in summer, on imported timber and cargo during the adult beetle's flight period (http://www.daff.gov.au/aqis/quarantine/pests-diseases/forests-timber/burnt-pine-longicorn).

Similarly, aircraft can be a pathway for the transport of pests around the world (http://www.pnas.org/content/103/16/6242.full). Insects can readily gain access to aircraft while the holds are open during loading operations or be transported in freight or carried on-board unwittingly by passengers in their luggage or on their person. Factors such as season, cargo type and time of departure (night or day) influence the risk of contamination in aircraft. In a study of hitchhiking insect pests on international cargo in aircraft at Miami International Airport from 1998 to 1999, Caton et al. (2006) found that contamination rates on flights were greatest during the wet season departing at night from the country of origin.

Global changes in trade and population migration are causing demands for agricultural products with new plants and plant products being imported from new global trading partners, often with limited biological knowledge of the associated pest risks. For example, international trade in cut flowers has grown significantly in volume during the last decade and so too has the number of countries exporting these goods. A decade ago most of this trade originated from European countries; currently, newer trading partners are entering this market. African countries are becoming a significant supplier, presently accounting for about 8 % of world exports of cut flowers. This trade in cut flowers is expected to grow (Areal et al. 2008). This spike in African exports is likely to bring a range of new phytosanitary pest risks associated with cargo pathways for pests unknown to science may arise. A recent example of this occurred in Australia with the detection of a Genus of thrips previously unknown to science being intercepted on plant produce imported from Kenya and Ethiopia (Mound 2009).

6.6.2 *Passenger Movements at Airports and Seaports*

As international air and sea travel has become more affordable with ever increasing exotic destinations being visited, phytosanitary risks are escalating. According to the Airports Council International, in 2010 more than 4.8 billion passengers travelled to 1,633 international airports in 179 countries and territories (Anon 2010b). Airport and seaport passengers pose a phytosanitary risk pathway often due to ignorance of the potential risks associated with an unusual wooden artefact they have purchased at a market place or seeds of some exotic plant they have collected.

Passengers can inadvertently transfer phytosanitary risks on their clothing, shoes and personal goods such as tents and bicycles. For example, over the last few years live Black-spined Toads (*Bufo melanostictus* (Schneider)) have been detected frequently by Australia's national plant protection agency concealed in empty shoes in the bags of passengers arriving from South East Asia (Anon 2008). Baker (1966) cultured fungi from shoes of air travellers arriving in Honolulu International Airport and identified 65 different fungal species. Likewise Gadgil and Flint (1983) examined 45 tents accompanying incoming passengers at Auckland International Airport and found a range of pathogenic fungi along with several live insects. Sheridan (1989) found 35 fungal genera mainly consisting of rust uredinospores and smut teliospores in a survey carried out on passengers clothing arriving at Wellington International Airport from Australia. About 10 % of the spores were viable with higher numbers of fungal spores recovered from passengers originating from a farm or recreational area compared with an urban area. While this pathway is difficult to regulate, travellers, particularly plant scientists, horticulturalists, farmers and other passengers who visit rural areas should at least be aware of the potential for this risk and take appropriate precautions including laundering of clothing and cleaning of shoes and equipment.

6.6.3 International Exchange of Mail

Rapid expansion of the Internet and international exchange of goods purchased through e-commerce mail order sites presents a major risk pathway for plant goods sent in the mail. This can be a high-risk pathway because the goods may carry foreign pests or seed and other propagating plant material that can pose a significant phytosanitary risk for countries. For instance, more than 137 million items of international mail are sent to Australia each year with over 400,000 items of quarantine interest being detected with mail order and Internet purchases making up a significant portion of the seized items (Anon 2010c).

6.6.4 Inter-country Borders (Regulated and Non-regulated People Movement)

Some countries have a natural geographical, seasonal and historical advantage in regard to phytosanitary border controls. Being surrounded by oceans, the international movement of people and goods through designated ports into Australia and New Zealand is easier to regulate compared with countries that share borders where controlled movement of people and plant products across borders is more difficult. Although commercial tourist traffic may be regulated, illegal border crossings by people bringing with them unregulated plants and plant goods can present a significant phytosanitary risk pathway.

Given the different climatic and growing seasons between the Northern and Southern Hemispheres, the likelihood of phytosanitary pests establishing on susceptible hosts under suitable environmental conditions is significantly influenced. Consider fresh fruit harvested in one hemisphere and exported to another country (and another hemisphere). The host material may be in a dormant state that will typically reduce the likelihood of the phytosanitary risk pathway. In addition, given the relatively recent international exchange of goods into Australia and New Zealand, pathways and establishment of many significant phytosanitary pests has been historically lower compared with Asian and European countries where trade has been occurring for many centuries.

6.6.5 Climate Change

Climatic conditions significantly impact crop production, population dynamics and pest risk distribution thereby influencing existing pest risk pathways (see Chap. 21; Scherm and Coakley 2003; Coakley et al. 1999). The earth is in a warming phase with 2000–2010 being the warmest decade since record keeping began in 1860 (Norse and Gommes 2003). Climate change will have significant implications for pest movements particularly for plant products imported from pest-free areas. Increased levels of greenhouse gases CO_2, (carbon dioxide) CH_4 (methane), NO_2 (nitrous oxide) and O_3 (ozone), combined with changes in temperature and rainfall, will affect the distribution of crops and pests (Chap. 21). Cooler areas will become more conducive for plant growth; drier areas will become less suitable for plant growth. Pest lifecycles are forecast to change with the potential for more generations per year (multivoltine) and different seasonal population peaks. This is likely to result in more movement of pathogens, particularly for plant virus transmission, due to increased vector activity associated with aphids, nematodes, thrips, mites and whitefly species (Jones 2009).

Climate change is predicted to increase the frequency and severity of extreme weather events and this may increase the likelihood of incursions at national and domestic borders and may impact on area freedom compliance. For instance, increased cyclonic activity in areas where Citrus Canker is present has expanded the distribution of this important citrus disease (Chap. 18). Emergency responses to natural crises (e.g. tsunami, flooding, famine), particularly in developing countries, typically result in a rapid food-aid response. This presents possible pathways for movement of exotic pests into countries that have more pressing issues compared with phytosanitary compliance.

6.6.6 Other Considerations

Several risk pathways must be managed by border agencies, including: (1) Removal of long-established pest control practices (pesticides and fumigants) may increase the presence of pests on plants and plant products exchanged internationally; (2) Economic challenges (loss of skilled staff and discontinued programme funding for pathogen testing) reduce availability of high-health planting stock; (3) Bioterrorism threats that involve deliberate introduction of exotic pests; (4) Escalating costs of managing new plant pest outbreaks with decreasing budgets will lead to acceptance of more pest incursions, and; (5) Military conflicts may cause breakdowns in biosecurity systems.

6.7 Risk Mitigation: Managing Plant Phytosanitary Risks

NPPO's rely on a range of mitigation processes to manage risks posed by the international trade of plants and plant produce. Risk mitigation strategies can be applied to goods before entry, at the border or post entry. Management systems may be based on one approach or involve a "systems approach" process integrating two or more processes. The systems approach reduces risk to meet the appropriate level of phytosanitary protection. Alternatively, processes are independent so if one system fails then a "backup" exists to offer added levels of protection that reduce risk to an acceptable level.

Regulatory measures commonly used for managing risk pathways associated with imported plants and plant products generally fall into one of several areas: Documentation and information management; Pest-free areas and pre-clearance; Inspection and detection systems; Treatment options; Re-export and/or commodity destruction; Post-entry plant quarantine; Stakeholder awareness and engagement; Enforcement and compliance; and "Other" (sundry) measures (Sect. 5.6).

6.7.1 Documentation and Information Management: Important Components for PPOs Mitigating Phytosanitary Risks

Documentation requirements and information management vary depending on the commodity and country and may include: (1) Import permits and phytosanitary certificates; (2) Incoming passenger cards; and (3) Electronic data analysis and risk profiling.

Import permits and phytosanitary certificates (Sect. 5.7) are documents commonly required by border agencies for the importation of plants and plant products (ISPM 12 2001). An import permit is a legal document that stipulates specified import requirements for a commodity that are legally binding. Import permits are issued by the importing country's NPPO. A phytosanitary certificate is an official government-to-government document stating that goods have been inspected according to appropriate procedures and are certified to be practically free from phytosanitary pests. Specific additional declarations may be required on the phytosanitary certificate to provide assurance that the imported products have been officially inspected, tested, treated or sourced from pest-free areas and that the goods conform to the phytosanitary regulations of the importing country.

Managing the risks of phytosanitary items entering on passengers or with their baggage at international airports and seaports is complex and difficult. Many NPPOs have designated "first ports of entry" where border officials clear international aircraft and shipping vessels including their goods, baggage, crew and passengers. Before landing, international aircraft may play an in-flight quarantine announcement alerting passengers of the importance of quarantine and the process to follow to comply with regulations. In some countries, disembarking crew and passengers are required to complete an incoming passenger card or similar legal document to declare items of phytosanitary concern including food, plants, parts of plants, traditional medicines and herbs, seeds, wooden articles, soil or articles with soil attached e.g. sporting equipment, and shoes. Passengers also must declare whether they have visited high-risk areas such as farms where they may have picked up phytosanitary risk items. In this way, a risk assessment of items being carried by passengers can be made by phytosanitary inspectors and directed for further assessment or treatment (Chap. 9).

Some border agencies employ specialist risk assessment officers at international airports to facilitate efficient passenger clearance. These officers assess risks posed by different pathways and flights so flights/passengers representing a lower risk can be cleared without further intervention. This allows border agencies to more effectively utilise limited resources to target risks. This includes clearing passengers who may be moving high-risk goods or have incorrectly declared goods and direct them to baggage examination or x-ray. The risk assessment officer plays a role in educating passengers on the importance of compliance with phytosanitary regulations and implications of false declarations that may result in issuance of an infringement notice, financial fine or court prosecution.

Phytosanitary activities at the border generate an enormous volume of data. Sophisticated computer technology enables regulatory authorities to utilise modern electronic systems that support risk mitigation associated with importation of plants and plant products. NPPOs typically have electronic databases that contain phytosanitary information relevant to the trade of plants and plant products. These databases allow prospective importers/exporters to determine what import conditions are required and whether a need exists for import permits, phytosanitary certificates, treatments and any other relevant information relating to the importing country's requirements. Australia's border agency maintains an import conditions (ICON) database that contains data on the import requirements for thousands of commodities (www.daff.gov.au/icon). New Zealand's plant import requirements are documented in a series of import plant health standards (http://www.biosecurity.govt.nz/enter/plants) and an electronic database (Plants Biosecurity Index) which details import specifications for seed and nursery stock of plants by genus/species (http://www1.maf.govt.nz/cgi-bin/bioindex/bioindex.pl). The USA maintains import/export conditions for plants and plant products (http://www.aphis.usda.gov/favir/ and http://www.aphis.usda.gov/import_export/plants/plant_imports/plant_inspection_stations.shtml).

Computer systems support detailed records of pest interceptions on imported goods; data can be analysed to identify risk pathways and risk profiles. This information enables border agencies to incorporate flexible intervention strategies to use border resources to target areas of greatest biosecurity risk so fewer resources are directed to low-risk activities. Data collection and risk modelling enables agencies to develop phytosanitary risk profiles.

Profiling is typically used at international airports, seaports and mail centres as a method of identifying high-risk passengers, goods, pathways, suppliers and importers. A profile is a set of characteristics created by analysing historical data on the incidence of phytosanitary risk material and/or compliance with regulations. For example, seasonal profiles can be used to target specific seasonal events (e.g. Asian Gypsy Moth peak flight-cycles) and cultural events known to the NPPO. Annual events such as the start of university terms (students returning from overseas with food items or other risk goods), religious or cultural events (Christmas, Chinese New Year, Easter, Ramadan and Sukkoth) and "one–off" events (sporting activities or conferences) are typical profile targets.

Other profiling models may include targeting a particular group of passengers who infrequently travel and may not be aware of phytosanitary regulations. This can be particularly effective in targeting groups of passengers who have a historical tendency of non-compliance (non-declaration) of phytosanitary risk items. Likewise, commercial importers with a poor compliance history may be targeted with a higher level of phytosanitary intervention including increased inspection or mandatory treatment. In the same way, risk profiling can be used to reduce intervention for specific types of imported cargo that historically present a lower phytosanitary risk. Profiles must be regularly analysed and reviewed to ensure they remain effective in light of new information that may alter the risk status.

6.7.2 Pest-Free Areas and Pre-clearance

Phytosanitary agencies recognise pest-free areas, and areas of low pest prevalence. A pest-free area is an area in which a specific pest does not occur as demonstrated by scientific evidence. The PFA is officially maintained by checks to verify freedom and/or phytosanitary measures to maintain freedom (ISPM 4 1996). This allows specified plant products to be exported with a lower level of phytosanitary intervention because goods can be certified free of specific regulatory pests and cleared more efficiently by the importing country. The term 'pest free area' can encompass all of a country (country freedom) or parts of a country (area freedom). The requirements for establishing "pest free areas" or "pest free places of production" are defined in ISPM 4 (1996) and ISPM 10 (1999). More detailed information on these standards is available from the IPPC website.

Pre-clearance involves performing pest inspections, testing and treatments in the country before export. Pre-clearance was first proposed in 1914 at the International Phytopathological Conference held in Rome (Morschel 1971) and is now used by border agencies around the world. The exporting country carries out Phytosanitary pre-clearance inspections and testing under an approved and auditable system or by officers from the importing country's phytosanitary service. Field inspections of the growing crop in the country of origin are a particularly effective tool in managing disease risks in plants that may be dormant or where the disease is latent. For example, the USDA (in co-operation with NPPO of many countries) has been performing pre-clearance of ornamental flower bulbs since 1951 (Santacroche 2008). In Australia, a pre-clearance scheme has been established with several trading countries for products that are treated with Methyl Bromide (Sect. 10.3.2). Under the Australian Fumigation Accreditation Scheme (AFAS), overseas fumigation companies are trained and accredited to perform Methyl Bromide fumigations. This has contributed to the effectiveness of such treatments.

Another pre-clearance arrangement, widely adopted internationally, manages the movement of wood packaging material. (ISPM 15 2002: *Guidelines for Regulating Wood Packaging Material in International Trade*). ISPM 15 was an important step in minimising the importation of timber pests associated with wood packing material in international trade. Some countries (including Australia and New Zealand) had an additional requirement that wood packaging be essentially "bark free" given the potential of bark to shelter numerous pests of phytosanitary concern and provide a site for re-infestation after fumigation treatment. Haack et al. (2007) examined the risk posed by residual bark and concluded that bark pieces larger than a "credit card" in size could enable bark beetle species to complete their lifecycles. Consequently, the ISPM 15 standard was revised in 2009 to define "bark–free" wood as 'wood from which all bark, except in grown bark around knots and bark pockets between rings of annual growth has been removed'. This standard accepts that vestigial bark may remain after the debarking process and sets out acceptable tolerance levels.

Pest-free areas and pre-clearance arrangements are mutually beneficial for importing and exporting countries. Importing countries benefit by early detection and elimination of plant pests, thereby reducing the chances of phytosanitary risks entering at the border. Exporters benefit because products that do not meet the phytosanitary requirements of the importing country can be removed during the early stages of the export process rather than at a later stage after much cost has been added to the product. Pre-clearance arrangements typically reduce the need for time-consuming and expensive on-arrival inspection at the port of entry, thereby making the process at the border more efficient.

6.7.3 Inspection and Detection Systems

Inspection of regulated goods is the most frequently used phytosanitary procedure employed worldwide to determine compliance with import requirements and detection of pests (ISPM 23 2005). Inspection of consignments confirms compliance with import or export requirements relating to regulated pests. Import inspections verify compliance with the importing country's phytosanitary requirements and detect pests of phytosanitary importance. An export inspection ensures the consignment meets regulatory requirements of the importing country at the time of inspection and may result in the issuance of a phytosanitary certificate. Inspections of imported/exported products involve three distinct processes (ISPM 23 2005): (1) Examination of documents accompanying the consignment; (2) Verification of the identity and integrity of the consignment; and (3) Examination for pests and other phytosanitary risk material.

Documents accompanying a consignment may include phytosanitary certificates, import permits, treatment certificates, manifests, airway bills, field inspection reports and other country-specific reports. The inspector examines and verifies that the documents and goods are clearly identifiable and the integrity of packaging is intact and consistent with phytosanitary requirements. An appropriate sample is taken from the consignment and examined for regulatory pests and other phytosanitary risk material. ISPM 31 (2008) provides guidance to NPPOs in selecting the most appropriate sampling method for inspection or testing of consignments to verify compliance with phytosanitary requirements. The inspector may use microscopic devices to detect microorganisms and small invertebrate pests and their life stages (e.g. mites and insect eggs). Some imported plant products (including cut flowers and foliage) are difficult to inspect under the microscope and other inspection techniques (including tapping or shaking produce over a sheet of white paper) are used to dislodge invertebrate pests. If regulatory requirements are met then consignments may be released or a phytosanitary certificate issued for exported goods. If phytosanitary requirements are not met then further risk-mitigation processes may be required depending on the nature of non-compliance. If regulated pests are detected then the inspector may seek advice from specialists (including entomologists and plant pathologists) or direct the consignment for appropriate treatment and other phytosanitary risk-mitigation measures consistent with import requirements.

Visual Inspections. Visual inspection and physical examination are commonly employed by border agencies for managing entry of phytosanitary risk items associated with passengers, international mail and imported cargo. Inspections can be simple (reviewing a passenger's written declaration and assessing compliance with regulations) or more detailed (examining a passenger's baggage). International mail is subject to border inspections depending on the level of risk taking into account seasonal factors, declarations on the package, country of origin and likelihood plants and plant products are present in the package. If goods are found contaminated with pests, soil or other phytosanitary risk material, then they are directed for further treatment or other risk mitigation including destruction, re-export and/or prosecution.

Inspectors at international cargo depots visually assess imported goods, equipment, timber and other traded products for signs of phytosanitary risks. Sea containers are transported around the world. Some NPPOs have developed requirements for imported containers and cargo, especially regarding container cleanliness and whether it is carrying wood packaging material, which can harbour wood-boring insects or fungi. The contents and external surfaces of imported containers may be subject to inspection for phytosanitary risk material including hitchhiking pests (e.g. snails), seeds and unacceptable levels of contamination (such as soil). If present this may result in delays because the container may require cleaning and re-inspection before it can be released.

Visual inspection of an entire consignment is impractical for large consignments of plants and plant products. A representative sample typically is drawn for examination. Sampling detects regulated pests and provides assurance the regulatory requirements have been met in the most cost effective and resource-efficient manner. ISPM 31 (2008) provides an overview of the goals and challenges of sampling and examines topics including sample size and selection, inspection efficiency, and random versus targeted sampling.

Different commodities require different sampling strategies. In Australia and New Zealand, a random 600-unit sample selected from homogeneous consignments of fresh produce (including fruit, vegetables and cut flowers) commonly is used. For most large shipments, this strategy provides a 95 % level of confidence that not more than 0.5 % of units in the consignment are infested. Inspectors at ports-of-entry in the USA select samples of imported commodities for inspection based on various protocols including flat 2 % of shipments, hypergeometric sampling or random sampling. The USDA's Agricultural Quarantine Inspection Monitoring (AQIM) programme is based on hypergeometric sampling. Hypergeometric protocols select inspection units (lots) by selecting the appropriate number of 'sample units' to provide a 95 % confidence level of selecting one or more sample units when the inspection unit is infested/infected at a rate of 10 % or higher. The USA now applies sample methods for shipments of "plants for planting" based on the hypergeometric probability distribution. Before importation, plant shipments are assigned a risk rating (high, medium, low, and risk monitoring), and sampled accordingly for inspection.

NPPOs typically use the International Seed Testing Authority procedures for drawing subsamples of seed shipments to inspect for phytosanitary risk items. This usually involves the collection of multiple random samples throughout the consignment including the top, middle and bottom of the bag/container and then blended to form a composite sample. After the sub-sample has been selected, a thorough visual inspection is completed looking for any signs of invertebrate pests, disease symptoms, weed seeds and other phytosanitary risk material. NPPOs stipulate which inspection technique to use depending on the type of product being imported and the acceptable level of protection. Inspection using statistically based sampling methods provides a level of confidence that the incidence of a pest must be below a certain level, but does not prove that a pest is absent from a consignment (ISPM 31 2008).

Optical aids commonly are used by phytosanitary officers during inspections of imported plants and plant products to help magnify pest specimens to a size suitable for detection. These aids vary in form and function and their suitability is dependent on the specific work location and function being undertaken. Relatively large invertebrate specimens and disease symptoms may require no or low magnification devices to detect them. The detection of tiny invertebrates and life stages (including eggs and nematode cysts) may require the use of a 10–30× hand lens or a microscope.

A bright light source (minimum 600 lux at the point of primary inspection) commonly is used when inspecting plants and plant products. An optical fibre or a cold light source is favoured over an incandescent light source because the former generally is brighter and does not generate heat (minimising drying and damage of the specimen). Greater magnification can be gained by using compound microscopes.

However, preparation and observation typically require a higher level of expertise and such specimens usually are forwarded to specialist entomologists and plant pathologists for assessment (Sects. 12.2, 13.5 and 13.6).

Other tools commonly used during phytosanitary inspections include: (1) White paper that provides a contrasting background and enables easier detection and collection of invertebrates during inspections; (2) Trays, sieves, paint brushes, forceps and probes to remove and collect specimens from hard to reach crevices, particularly around the calyx of fruit; (3) Vials containing 70 % ethanol or other suitable preservative to collect pests found during the inspection; (4) Plastic snap-lock bags to collect plant tissue with disease symptoms; (5) Pest interception forms with details of the pest found on the imported commodity to allow data to be collected on the risk pathway.

Border inspectors must follow correct specimen-handling protocols and preservation procedures when collecting pests because damaged invertebrates and disease specimens make identification more difficult. When collecting invertebrate pests, all available life stages (e.g. egg, larva/nymph, pupa, adult) should be collected as this assists in identification. Generally invertebrate specimens, with some exceptions, should be collected into 70 % ethanol or propylene glycol (1,2-propanediol). However, sending specimens preserved in 70 % ethanol through the mail is prohibited in most countries because 70 % ethanol is considered a 'dangerous good'. If internal feeders (beetle or moth larvae or "grubs") are detected, inspectors should leave the specimens in situ (on the plant material) and an entomologist should be contacted for advice. Likewise mealybugs and scale insects should be left attached to the plant tissue because removal can damage their mouthparts and make identification more difficult. Instead, leaf tissue around the insect should be carefully removed and placed into a dry vial or 70 % ethanol. Adult moths and mosquitoes should be collected dry in vials and placed into a freezer overnight; they should not be placed into ethanol or other liquids because the body and wing scales needed for identification will fall off. Where actionable pests are identified, consignments typically are directed for mandatory treatments including fumigation and/or insecticide dipping depending on the pest and product.

If suspect disease symptoms are observed on imported plants and plant products, then the consignment must be placed "on hold" pending advice from a plant pathologist. A representative sample showing the full range of disease symptoms should be collected and submitted to the plant pathologist by placing affected tissue in snap-sealed plastic bags. Heavily diseased samples are normally unsuitable for isolation as they usually have many saprophytic organisms (fungi or bacteria that grow on and derive nourishment from dead or decaying organic matter) making it difficult to isolate the causal pathogen. As such, samples consisting of healthy and diseased tissues are the best samples for submission. One leaf is generally not sufficient for pathologists to determine the causal agent or assess the health of the plant. Care must be taken when collecting disease specimens from live plants. Otherwise, damage can occur and lead to secondary disease infections. Secateurs or a sharp knife should be used for collecting diseased samples; instruments should be cleaned and disinfected with 70 % ethanol or other suitable disinfectants between samples.

Samples must be clearly labelled and carefully packaged, ideally wrapped in dry paper towel in snap-sealed plastic bags. When collecting samples from the field or from post-entry plant quarantine facilities, the specimens should be delivered to the plant pathologist as quickly as possible. When necessary, specimens should be stored in a refrigerator (not freezer) because the plant tissue deteriorates very quickly after collection. Deterioration makes the isolation and identification of the causal agent difficult. If plant disease samples are being sent through the post or overseas to specialists, then additional precautions must be taken including placing samples into plastic screw-top bottles to minimise the risk of breakage and escape during transit. When actionable diseases are detected, and depending on the risk status of the disease agent, the consignment may require treatment, be re-exported or destroyed. In situations where a phytosanitary disease is suspected, or has been confirmed in a post-entry plant quarantine facility, additional precautions are required including destruction of susceptible hosts and decontamination of the premise and equipment.

Detector Dogs. Detector dogs are another tool for inspection used by some NPPOs for managing phytosanitary risk pathways. Dogs used for the detection of phytosanitary risk material first began in Mexico during the 1970s and now are used in many countries:

http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/detector_dog.pdf

Detector dogs are trained to search international mail, passenger's baggage and imported cargo to discover undeclared phytosanitary risk products. Detector dogs are particularly effective in discovering phytosanitary material including live plants, seed, plant produce and soil which can be difficult to detect with visual inspection and x-ray technology. In 1984, the USDA was the first NPPO to trial beagles to work amongst people at baggage collection points at international airports. With their enhanced sense of smell and friendly nature, beagles proved popular with the traveling public and are now commonly used by border agencies in media campaigns to educate the public on the importance of quarantine.

Two types of detector dogs are commonly used by border agencies. Passive dogs are trained to simply sit next to a passenger or their luggage when they detect phytosanitary risk material, waiting for their food reward. Passive dogs are typically used in baggage halls at international airports. Active dog breeds are trained to paw or nuzzle target items and are rewarded with a game of tug-of–war. Detector dogs are used at international mail centres, cargo centres and behind the scenes at international airports.

Detector dogs are also used by NPPOs in Australia and other countries for the detection of termites in timber products and high risk shipping vessels (e.g. yachts with timber products that have visited a risk port). Termites, particularly drywood termites, are cryptic insects that are difficult to detect because they leave little or no external symptoms of infestation and a visual inspection alone will often fail to detect them.

X-ray Imaging. X-ray transmission imaging equipment is used by NPPOs to detect organic material including live plants and soil. X-ray technology is commonly used to assist in the screening of passengers' baggage at airports and seaports, mail centres and cargo examination depots and is effective in detecting items of phytosanitary concern.

Inspections using trained phytosanitary inspectors, x-ray equipment and detector dogs are widely used by border agencies. But this approach has limitations. We cannot detect all pest and disease threats that are likely to be transported on plants and plant products. Many invertebrate pests are too small to detect or are hidden beneath bark or within products (e.g. wood borers). Infestation rates of target pests in the imported goods may be too low to detect. Also, there is an inherent probability of missing pests given the use of sampling procedures for inspections and testing. Similarly, plant pathogens may infect plants and seeds internally and not express obvious disease symptoms, thus evading detection at the point of entry. Several other risk mitigation measures are used by border agencies to further minimise the phytosanitary risk associated with imported goods.

6.7.4 Treatment Options

Treatments applied to imported goods to manage the phytosanitary risks associated with plants and plant products include chemical, irradiation, physical and controlled atmosphere treatments (Sect. 10.2). Selection of the most appropriate treatment depends on the pest, commodity and intended use of the goods. Given the variability between international agencies, detail of the dosage rates, exposure times and the temperature ranges used for the treatments is not provided (specific information is available from each border agency website).

Chemical treatments. Chemical treatments used for risk mitigation purposes include fumigation, application of pesticides and disinfectants (Sect. 10.3). Fumigation is one of the most common treatments applied to imported goods particularly for managing invertebrate pests in plants and plant products. A fumigant is a chemical usually delivered in a gaseous form at a certain concentration and timeframe to be lethal to a given pest. The toxicity of a fumigant depends on the temperature and respiration rate of the target pest. Generally, the lower the ambient temperature, the lower the respiration rate of the organism which tends to make the pest less susceptible. Fumigation at lower temperatures usually requires a higher dosage rate for a longer exposure period than fumigation at higher temperatures (Anon 2010a). Methyl Bromide, Phosphine, Ethylene Oxide and Sulphuryl Fluoride are four common fumigants used by border agencies (Sect. 10.3.2).

Methyl Bromide (CH_3Br) is the most frequently used fumigant for phytosanitary treatments because it is effective against a wide variety of plant pests (including insects, mites and ticks, nematodes and snails), has good penetrating ability and is rapid acting (Sect. 10.3.2). As a phytosanitary measure, Methyl Bromide is commonly used for the treatment of durable commodities, such as bulk grains, cereals and dried foodstuffs, wood packaging materials, wood and logs, as well as perishable commodities, including live plants and fresh fruit, vegetables and cut flowers.

Plant material generally tolerates Methyl Bromide fumigation well, although the degree of tolerance varies with species, stage of growth and condition of the plant material. Methyl Bromide accelerates the decomposition of plants in poor condition and can react with excess free water to create methyl bromic acid, which can damage plants and plant products. Methyl bromide cannot generally penetrate goods covered in plastic wrapping, lacquer and paints that present an impermeable finish. Methyl Bromide may also leave residues in particular food groups with high oil content (e.g. nuts) and is not used for treating these products. Moreover, Methyl Bromide is classified as an ozone-depleting substance. Under the Montreal Protocol on substances that deplete the ozone layer (1987) Methyl Bromide is being phased out under a mandatory timetable. Methyl Bromide used by NPPOs for phytosanitary and pre-shipment purposes is currently exempt from this protocol. Several alternative treatments to Methyl Bromide fumigation have been developed (See Sect. 10.3.2) along with technology for recapture of emissions on activated carbon from fumigation chambers.

Ethylene Oxide ($CH_2.O.CH_2$) is a fumigant used by regulatory agencies for fumigating dried plant products including herbs and spices but it kills living plants and is not recommended for use on seeds. Ethylene Oxide is a broad-spectrum fumigant and unlike Methyl Bromide can penetrate plastic packaging and varnished or lacquered wood products. Ethylene Oxide is a strong alkylating agent causing the replacement of labile hydrogen with an alkyl group on hydroxyl, carboxyl, sulfhydryl, amino and phenolic groups. The alkylation of these compounds affects cellular function and structure that ultimately leads to inactivation of cellular function and pest mortality. Ethylene Oxide fumigation under vacuum (minimum 50 kPa at 1,500 g/m^3 for 4 h at 50 °C or 1,500 g/m^3 for 24 h at 21 °C) is used primarily to sterilize materials that are not designed to be exposed to heat or steam. It is very effective as a killing agent of phytosanitary pests.

Phosphine (PH_3) is a highly toxic fumigant gas that diffuses rapidly, penetrates deeply and is commonly used as a fumigant for treating stored product pests in bulk grain (See Sect. 10.3.2). Phosphine is slow acting and requires long exposure times – typically 7 or more days to control insect pests (depending on temperature). Some life stages are more tolerant to Phosphine (eggs and pupae the hardest to kill); larvae and adults succumb more easily.

Sulphuryl Fluoride (SO_2F_2) is typically used by border agencies for controlling dry wood termites and other insects inhabiting timber and wooden products. The gas has excellent dispersion and penetrating qualities that enable it to infiltrate termite tunnels and crevices and kill the insects. However, Sulphuryl Fluoride is generally not used on foods, living plants or medicines destined for human or animal consumption (See Sect. 10.3.2). Sulphuryl Flouride is an identified greenhouse gas.

Other Treatments. Some NPPOs routinely use fumigants to eradicate invertebrates associated with imported plants and plant products. Other treatments may be used to achieve the same outcome. In New Zealand, imported nursery stock is treated for insects and mites using Methyl Bromide fumigation, Hot Water Treatment (dormant material only) (See Sect. 10.5.2) or a combination of insecticides (http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf). Whole plants also undergo treatment for fungal pathogens using Hot Water Treatment and/or broad-spectrum fungicides.

Other chemical treatments are used for managing other phytosanitary risks associated with plants and plant products. In Australia and New Zealand, certain cut flowers, stems and foliage capable of being propagated (including roses, gypsophila, chrysanthemums and carnations) must be treated to render them non-viable. The herbicide glyphosate is commonly used for this purpose. Similarly, fungicides may be used to manage fungal diseases that are present on imported cut flowers.

Under World Health Organisation (WHO) guidelines, some border agencies require international aircraft to be disinfected and/or disinfested to minimise the introduction and spread of unwanted insect pests and vectors of plant disease that may be inadvertently transported. This may include any of the following treatments:

1. Residual spray treatment of interior surfaces of cabins and cargo holds at intervals not greater than 8 weeks (conducted in the absence of passengers);
2. Pre-flight cabin treatment (conducted in the absence of passengers before embarkation) that lasts for a single flight;
3. Pre-flight cabin treatment and top of descent spray (consisting of a pre-flight treatment followed by further in-flight spray of a non-residual insecticide, carried out at top of descent as the aircraft starts its descent) that lasts for a single flight; and
4. On arrival cabin and hold treatment (conducted in the presence of passengers and crew prior to disembarking).

Further information can be obtained from the WHO web site: http://www.who.int/docstore/bulletin/pdf/2000/issue8/99-0285.pdf.

Disinfectants. A range of disinfectants is used by NPPOs to mitigate risk pathways for plants and plant produce. Alcohols, chlorines and quaternary ammonium compounds are used for treating and disinfecting the external surfaces of imported seed, live plants and to disinfect inspection benches and equipment. Alcohols work through the disruption of cellular membranes, solubilisation of lipids and denaturation of proteins by acting directly on sulphur-hydrogen functional groups. The antimicrobial action of alcohols is optimal in the 60–90 % range as the highly hydrophobic nature of plant cell walls inhibits penetration of pure alcohol into the cell. Alcohols are typically used as broad-spectrum disinfectants against vegetative bacteria, fungi and some viruses although they are not sporicidal. Chlorine agents including sodium hypochlorite are widely used as broad-spectrum disinfectants particularly for treating imported seed and epiphytic infections of imported plant propagation material. Chlorine is a highly active oxidising agent and disrupts the cellular activity of proteins. Chlorine and ammonia-based disinfectants are used in combination with high-pressure water for cleaning phytosanitary risk material from containers and vehicles.

Controlled Atmosphere. In addition to the fumigation gases used for phytosanitary pest control, atmospheric gases including oxygen, nitrogen and carbon dioxide can be manipulated to preserve imported plant products, a process referred to as "controlled" or "modified" atmosphere storage. Controlled atmosphere techniques are widely used in the storage of perishable plant products to retard ripening and reduce spoilage from plant microorganisms as well as controlling some insect pests (Morgan and Gaunce 1975; Aharoni et al. 1981). The most extensive use of controlled atmospheres for regulatory purposes is on grain and similar commodities. Controlled atmosphere procedures work by depleting oxygen or increasing the levels of carbon dioxide to asphyxiate organisms. Insects are generally killed more rapidly by carbon dioxide than they are by lack of oxygen.

Physical Treatments. Physical treatments (including heating, cooling and reconditioning) are used by some NPPOs to manage phytosanitary risks associated with imported plant products (Sects. 10.4 and 10.5). Unlike fumigants that primarily target invertebrate pests, physical treatments generally have a wider application and impact against a broader range of phytosanitary pests.

Heat treatments used by border agencies include incineration, moist heat at 121 °C for 15–30 min (autoclaving) and dry heat, commonly 160 °C for 2 h or 85 °C for 8 h, to sterilize and kill plant pests. Heat acts by disrupting membranes and denaturing proteins and nucleic acids. At about 50 °C most plant parasitic nematodes are killed whereas temperatures between 60 °C and 72 °C are required to kill most fungi and bacteria (Agrios 2005). At about 82 °C, most weeds, insects, plant viruses and the rest of the plant pathogenic bacteria are killed. Heat treatment is commonly used for treating timber and wooden products and phytosanitary waste. High-temperature forced-air is used as a phytosanitary treatment in Hawaii for treating papayas for fruit flies (Armstrong 1989). The treatment involves heating papayas with forced hot air until the temperature of the centre of the fruit reaches 47.2 °C for 3–7 h. At this temperature, Mediterranean Fruit Flies, Melon Flies and Oriental Fruit Fly eggs and larvae cannot survive. Relative humidity during the treatment must be maintained at 40–60 % to prevent damage to the fruit. Following treatment, the fruit are rapidly cooled until fruit pulp temperatures are below 30 °C to help preserve quality. Heat treatment may cause damage to some imported goods potentially igniting flammable items, melting glue and plastic coverings and making some items brittle after treatment e.g. straw hats.

Hot-water Immersion is used for treating fruits that are hosts of fruit flies, propagation material including seed, and dormant plant material such as cuttings and bulbs (Sect. 10.5.2). Hot water at temperatures ranging from 35 °C to 54 °C with treatment times lasting a few minutes to several hours are used for various host-pathogen combinations (Agrios 2005). In Australia, imported dormant grapevine cuttings undergo a mandatory hot water treatment of 50 °C for 30 min to eliminate risks associated with phytoplasma diseases and Pierce's disease (*Xylella fastidiosa* Wells et al.). Hot water treatment for seed was for many years the only means to control many seed-borne diseases and is still commonly used. Hot water treatment at 43 °C for 3 h is used for treating nematodes in ornamental bulbs. Hot water treatment works on the principle that dormant plant material can withstand the treatment temperature whereas the pathogen is killed.

Heat Therapy is another temperature treatment used by NPPOs to eradicate viruses from plants for propagation. Actively growing virus infected plants are placed into growth chambers at relatively high temperatures (ca 38 °C) for 6–12 weeks. At this temperature, plants continue to grow albeit slowly while most virus multiplication is temporarily halted. Meristem tips are aseptically removed and placed into culture or tiny pieces of explant tissue are budded onto virus-tested rootstocks. Plants are grown and tested to confirm the absence of the virus.

Cold Treatment (Refrigeration) (See Sect. 10.4) is widely used for controlling post-harvest diseases and insect pests of fresh produce and has been employed for many years. Cold treatments are relatively slow and typically are used for treating commodities travelling as sea freight in refrigerated containers or "reefers" where the goods can be maintained at low temperatures for extended periods. Low temperatures do not necessarily remove all the phytosanitary risks associated with plant produce, but they do mitigate many of the pest risks. Freezing at −18 °C for 7 days is an effective pest treatment process and is used by border agencies for treating wooden or dried plant products including herbarium specimens.

Cleaning/Reconditioning of Consignment. This is another form of physical treatment used to remove phytosanitary risk of contamination. Treatment depends on the level and type of phytosanitary risk. For instance, washing or pressure steam treatment are often used for removing soil and phytosanitary debris from imported containers, motor vehicles, farm machinery and earth moving equipment. The waste is then collected and disposed of in a "phytosanitary approved" manner. Other forms of reconditioning include:

1. Upon arrival, commodity processing begins to mitigate phytosanitary risks (e.g. grain milled into flour);
2. Australia's border agency requires imported fresh taro tubers to be 'topped and tailed' to limit their ability to be propagated; and
3. Berries and fruits are generally not permitted on cut flowers, foliage or dried plant products because they may contain viable seeds or may be infected by internally feeding insect pests.

Irradiation. Ionizing radiation (irradiation) is another pest-risk management treatment used to mitigate plant risk pathways (ISPM 18 2003; See Sect. 10.6). Three types of ionizing radiation used as regulatory treatments include:

1. Electrons generated from machine sources up to 10 MeV (eBeam);
2. Radioactive isotopes (e.g. gamma irradiation from cobalt-60 or cesium-137);
3. X-rays (up to 5 MeV).

The primary objective of irradiation as a phytosanitary measure is to prevent the introduction or spread of regulated pests. Irradiation is also used as a devitalization treatment of plants e.g. seeds may germinate but seedlings do not grow; tubers, bulbs and cuttings do not sprout (ISPM 23 2005).

Irradiation has several advantages over other treatments. Temperature and fumigation treatments involve generating data for each fruit-pest combination; irradiation treatments are developed for a pest species irrespective of the commodity (Anon 2002). Irradiation is known as a 'cold process' because the temperature of the processed product does not significantly increase and the treatment is effective against most insects and mites at dose levels that do not affect the quality of most food commodities (Anon 2002). Essentially, irradiation treatment generates short wavelength energy that passes through the treated product resulting in cellular breakdown and disruption to organic processes. As a phytosanitary treatment, irradiation can result in:

1. Mortality of the target pest;
2. Sterility of the pest (inability to reproduce);
3. Inability of the pest to emerge/fly; or
4. Devitalisation whereby plant parts including seeds, tubers, bulbs and cuttings cannot be propagated.

Unlike many chemical and physical phytosanitary treatments that result in death of target pests, the use of irradiation as a phytosanitary measure presents a new paradigm for border agencies because the treated consignment may not achieve the traditional phytosanitary criteria of total mortality of the pest. Irradiation may result in the pests' inability to reproduce (sterility), inability to complete all the pest life stages or non-emergence of adults however the pests may still be alive. This presents a dilemma for phytosanitary officers who undertake pest inspections and may reject consignments due to the presence of live pests. Acceptance of irradiation treatments is dependent on sound and verifiable research to give confidence to regulatory agencies in accepting such products.

Despite these challenges, irradiation as a phytosanitary treatment is gaining increasing acceptance by NPPO's (Anon 2002). The USDA first approved irradiation in 1997 for use on papayas from Hawaii for export to the mainland, followed by Guam, Puerto Rico and the U.S. Virgin Islands (Anon 2010a). In 2002, irradiation was approved as a phytosanitary treatment for all admissible fresh fruits and vegetables from all countries. New Zealand has approved irradiation as a phytosanitary treatment for Australian mangoes, papaya and lychee fruit.

6.7.5 Post-entry Plant Quarantine

Clearly, the international movement of germplasm as live plants (including potted plants, bare rooted plants, dormant cuttings and tissue cultures) and seed for breeding and crop improvement has significantly contributed to the development of global agriculture systems. However, trade in live plants also poses substantial biosecurity risk due to: (1) Their capacity as living hosts to introduce regulated pests, which may not be obvious at the entry point and (2) Phytosanitary treatments are more difficult for this pathway. NPPOs typically strictly regulate the importation of live plants and seeds. An excellent example of the risk posed by the unregulated exchange of plant germplasm was the inadvertent introduction of phylloxera (*Daktulosphaira vitifoliae* Fitch) into Europe in the mid 1880s resulting from attempts to manage another pest. Grapevine cuttings were imported from North America into Europe for study but unfortunately the vines were infected with powdery mildew. European vines were extremely susceptible to the disease and vineyards were threatened with total destruction. Given that North American vines were immune to the disease, thousands of American vines were imported to breed with European cultivars. Unfortunately, the root-inhabiting phylloxera was introduced on these cultivars along with two additional fungal pathogens, Downy Mildew and Black Rot. As a result of these uncontrolled introductions, millions of hectares of grapevines were destroyed and the French vineyards were almost entirely destroyed (Mathys and Baker 1980).

Many border agencies require an import permit for entry of live plants and seeds for propagation. The permit stipulates specific import conditions based on the pest risk and the type of planting material. Import conditions may be as simple as visual screening on arrival for certain low risk seed and ornamental hosts imported in tissue culture to mandatory treatments and extensive pathogen testing in Post Entry Plant Quarantine (PEPQ) facilities (known as Plant Quarantine Stations in the USA). The objective of PEPQ is to allow plants to pass through a period of growth so that NPPOs can screen and/or test for phytosanitary diseases before releasing the plants from quarantine. Regardless of the specific conditions, imported live plants must meet several basic import conditions including being clearly labelled with both genus and species names and being free of obvious pests symptoms. Imported live plants usually undergo a mandatory inspection on arrival and a phytosanitary treatment to manage insect pest risks.

The phytosanitary risk posed by live plants varies depending on several factors including: (1) Host-susceptibility to significant phytosanitary pests; (2) Economic and ecological impact of the pest on crops and native plant flora; (3) Country of origin and source (e.g. collected from wild versus commercial supplier); (4) Age of plant material; and (5) Type of plant imported (e.g. tissue culture plantlet versus rooted plant).

Live Plants. Imported live plants are typically classified into two or more risk categories. Low-risk plants include many ornamental species that are not hosts of significant plant pests. These plants are imported in tissue culture under sterile conditions, and then the likelihood of regulated pests being present is largely mitigated and may simply require visual inspection on arrival. If no obvious pests or diseases are present, then they can be released without further phytosanitary intervention. If the same ornamental plants are imported as bare rooted cuttings, then we see a greater risk of phytosanitary pests being present. Additional mitigation steps, including treatment and growth in PEPQ with disease screening, may be required. This process can be completed in Government PEPQ facilities or more commonly in approved private nurseries that conform to specific criteria and provide an appropriate degree of security and containment. These arrangements benefit the plant importing industries because they can access greater quantities of imported germplasm than would be possible if imports were restricted to the limited space in Government PEPQ facilities.

High-risk plants commonly include species that are hosts of particularly damaging pests that may significantly impact an industry, the environment or location the species poses a significant weed threat. In Australia, a few examples of high-risk plants include many commercial food crops (e.g. *Citrus*, *Fragaria*, *Malus*, *Prunus*, *Solanum*, *Triticum* and *Vitis* spp.), amenity and forest tree species (e.g. *Eucalyptus, Pinus, Quercus* and *Ulmus*) and ornamental hosts of significant plant diseases (e.g. Sudden Oak Death hosts). High-risk plants usually require active testing for the causal agents of these diseases and are typically placed in Government PEPQ facilities given the higher level of containment and diagnostic expertise required to perform disease testing.

Internationally, several high health-planting sources are recognised by border agencies as centres of excellence. These centres provide a high level of compliance with regulatory requirements including scientific integrity in pest and disease screening and other biosecurity processes. The post-entry phytosanitary requirements for certain plant material sourced from these suppliers can be significantly reduced or waived. Approved sources play an important role in the biosecurity continuum. However, they must regularly be audited by desk-top studies and/or site visits to ensure ongoing compliance with the importing country's regulations.

The international transportation of live plants (particularly rooted plant cuttings moving from one climatic zone in the Northern Hemisphere to the Southern Hemisphere or vice a versa) can be detrimental to plant survival. Plant health rapidly deteriorates while in transit. Combined with phytosanitary treatments, the likelihood of successful propagation very much depends on appropriate selection, preparation and transport of the plant material before importation. Planting material should be obtained from the highest health source available, ideally a pathogen tested source.

Regardless of the source, plant material should be free of obvious pest symptoms. If tissue culture plantlets are not available, then deciduous plants should be sent as young, fully-dormant hardwood or softwood cuttings in preference to rooted plants because of the reduced risk of viable plant diseases. One-year-old cuttings are less likely to have been damaged from pruning cuts, thereby providing fewer infection sites for wound pathogens. Likewise, most wood-rotting fungi are confined to the older central wood of roots, trunks and branches. Disease symptoms are typically more obvious on young tissue. When sending evergreen plants, excessive foliage should be removed to reduce dehydration; all soil from the root ball should be removed and the roots wrapped in damp paper. Plants should be clearly labeled with their full botanical name and loosely packed in a sturdy, padded box containing shredded paper or polystyrene packaging. A copy of the import permit should be attached to the outside of the packing box and labeled as "LIVE PLANT MATERIAL".

On arrival, plants immediately must be transferred to the inspection facility to allow border agency personnel to thoroughly examine the plants for pests and treat for pests as appropriate. Post phytosanitary care of plants after treatment (particularly fumigation) is extremely important for the survival of plants. Treated plants fumigated with Methyl Bromide should be ventilated for a few hours to allow gas to escape. Roots should be kept moist and not allowed to dry; foliage should not be watered for 24 h as any traces of fumigant gas may react with the water. Plants should be kept out of bright sunlight and strong winds for 2–3 days. A good biosecurity practice for dormant plants is to surface disinfect plants using 0.5–1.0 % sodium hypochlorite for 10 min with 0.1 % wetting agent followed by triple washings in tap water to remove the disinfectant.

Cuttings are then propagated or buds are taken from the cutting and budded onto pathogen-tested rootstocks and placed into appropriate PEPQ facilities. After the propagated plant cuttings have established, they are actively tested for viruses and other transmissible pathogens using biological indicators (herbaceous and woody plants), microscopic devices (light and electron microscopes), bacterial and fungal culturing, serological (ELISA) and molecular (PCR) tests as regulated by the importing NPPO.

Tissue Culture Plantlets. Like imported plants, tissue culture (in-vitro) plantlets should be obtained from the highest health source. Tissue cultures are the preferred method for transporting plant germplasm as it typically presents a lower biosecurity risk and is more robust for transport compared with live plant material. In-vitro plantlets should be transported in clearly labelled, air tight and transparent containers to allow inspection on arrival. The growth media should be free of antibiotics and have a high quantity of agar to firmly keep plantlets in place during transport. Low-risk ornamental tissue culture plantlets may simply require visual inspection on arrival and release. Higher risk plantlets may require a period of growth and assessment in PEPQ.

Seed. The importation process for seed for sowing follows the same principles as nursery stock. Certain species of permitted seed simply require visual examination for pests and if compliant may be released with no further phytosanitary intervention. Other species are prohibited or restricted and subject to strict import permit conditions including extensive disease testing in PEPQ facilities. Seed should be free of pulp, dried and free of obvious insect pests and symptoms. Imported seed may undergo mandatory Hot Water Treatment or surface disinfected with 0.5–1.0 % sodium hypochlorite for 10 min with 0.1 % wetting agent followed by triple washings in tap water to remove the disinfectant. Many seed borne diseases do not produce obvious symptoms and may need to be grown in PEPQ with appropriate observation and testing for pathogens of phytosanitary concern. This may include moist incubation whereby seeds are placed on moisten paper and incubated at specified temperatures followed by microscopic examination for the presence of fungal fruiting structures. Another technique for detection of fungal pathogens is the agar plating method whereby seed is placed on selective media (commonly malt extract and potato dextrose agar) and pathogens identified based on their macroscopic colony characteristics. Following testing, the plants are released or seed is harvested from the plants, re-inspected and released to the importer.

Pollen. The international exchange of pollen is less common although trade is significant for some genera such as *Actinidia* and *Pyrus*. Pollen should be collected from pathogen-tested plants or may be tested for pollen borne viruses and virus-like agents by ELISA and/or PCR. Pollen should be free of other floral parts, arthropod pests and fungal pathogens of bees.

PEPQ plays a significant role in the battle against disease entry. In Australia, significant plant diseases have been detected in PEPQ include Plum Pox Virus, Cedar Apple Rust (*Gymnosporangium juniperi-virginianae*), Chestnut Blight (*Cryphonectria parasitica*), Grapevine Corky Bark Virus, Grapevine Fan Leaf Virus and Cereal Smuts.

6.7.6 Re-export and Destruction

When a regulated pest is detected or where imported products fail to comply with the import regulations and no suitable treatment or other alternative risk mitigation system is available, the consignment is either destroyed using an approved process or re-exported. Methods for destruction used by NPPOs include: (1) Deep burial of waste at an approved location at a depth of greater than 2 m; (2) heat treatment and irradiation; (3) incinerations where phytosanitary risk items are burnt to ash; and (4) autoclaving. Re-export of a commodity is typically used for high-value commodities that are prohibited or where the importer does not wish to have the commodity treated or destroyed.

6.7.7 Public Awareness and Engagement

Public awareness and engagement by all stakeholders including government, industry and the public is an essential component of an effective biosecurity programme. Without active awareness and support by the broader community to the phytosanitary regulations, ignorance and non-compliance are likely outcomes. The key to successful community engagement is to involve stakeholders in the development of phytosanitary policies taking into account their views and concerns. In some countries, individuals can register with the border agency and provide input into development of phytosanitary policies and regulations. Industry consultative committees including export and import focused committees have been established with the express aim of consultation on border services and policies. In Australia, the border agency has developed a series of workshops for the cargo import industry to help raise awareness of pests of phytosanitary concern. The free courses target industry personnel working at import cargo depots, wharves and other risk locations helping to raise awareness of the major pests and diseases of concern as well as the major phytosanitary risk pathways and most importantly what to do if pests are detected.

Public awareness campaigns promoting phytosanitary awareness consist of many different facets:

1. *Internet sites, posters and handouts* in various foreign languages highlighting the importance of phytosanitary issues and showing the impact pests can have on agriculture and the environment;
2. *Giveaways* such as hats and pens to promote the importance of phytosanitary programmes; some border agencies have used phytosanitary signage on transport-vehicle tarps and sails for yachts to increase awareness of phytosanitary programmes among target groups;
3. *Phytosanitary awards* for industry groups and individuals who display and contribute to the meaning of phytosanitation. In Australia, the international electronic trading company “eBay” was awarded a national phytosanitary award in 2006 in recognition of efforts made to highlight to buyers and sellers of their legal responsibilities in trading plants and plant products;
4. *School programmes* to target children who are the next generation of travellers and to remind their parents of the importance of phytosanitary principles;
5. *Displays at overseas tourist bureaus and international travel expos* to target travel and shipping agencies about phytosanitary regulations and to spread information about phytosanitary issues to the travelling public;
6. *Displays at agricultural and horticultural shows* to target key risk groups including the public who may be interested in importing plants and plant products and to highlight to the rural sector the potential damage phytosanitary pests may cause to their industry; and
7. *Television* can be a particularly useful medium to get the phytosanitary message across to a wide audience. Several countries have successfully documented the challenges faced by border agencies in managing phytosanitary risks at the border.

A key message that should form part of a public awareness campaign involves the complexity and difficulty in managing risk pathways and the concept of *managed risk* or *acceptable level of protection*. This message is important; otherwise the community may develop unrealistic expectations of an NPPO and become unnecessarily critical when an exotic pest inevitably enters the country.

6.7.8 Enforcement and Compliance

Importers and exporters are responsible for being aware of and complying with all NPPO regulatory requirements before and after the import/export process. NPPOs commonly have enforcement and compliance branches that contribute to the integrity of border operations through investigation and enforcement activities. NPPO’s typically have phytosanitary statutory authority and specific regulatory authority whereby regulatory officers have the power to impose fines (known in some countries as “infringement notices”) for individuals who contravene phytosanitary regulations.

Phytosanitary infringement notices are used at international airports and seaports where passengers fail to declare items of phytosanitary concern and non-compliance is minor. More critical offences including deliberate smuggling of prohibited goods, falsification of phytosanitary certificates and breaking phytosanitary seals on containers/packaging. These offences are typically pursued by Compliance Officers and may result in more serious prosecutions with court action.

Many NPPOs have compliance agreements with third parties who have approval to undertake certain low-level activities on behalf of the Agency. These agreements are regularly audited to ensure compliance; appropriate sanctions are applied if conditions are contravened. This may include prosecution by the compliance branch or withdrawal of registration as a phytosanitary approved premise.

6.7.9 Access to Specialists

Timely and accurate pest identification and risk mitigation advice is crucial to the delivery of effective border operations. In line with international agreements, regulatory action can only be justified if the pest is of phytosanitary significance. Access to plant diagnostic specialists including entomologists, plant pathologists and botanists is a key element in identifying pests, diseases and weed seeds as well as providing timely advice in the delivery of inspection and certifications services and maintaining and enhancing science-based decisions.

Access to specialist diagnosticians and taxonomists is declining and delays in responding to phytosanitary questions can increase biosecurity risks. A technology increasingly used by border operations involves remote diagnostics, which allows access to a range of offsite specialist biosecurity experts within countries and across borders (Sect. 12.4). Remote diagnostics enable rapid and high resolution images of intercepted live pests to be taken by phytosanitary inspectors by simply connecting microscopic platforms to computers via a camera attachment and communicating with offsite diagnostic specialists via the internet (Sect. 12.5). The remote identifier can discuss the identification over the telephone, giving directions regarding the diagnostic features to check in order to identify the pest. This allows preliminary findings to be reached quickly, reducing waiting time from hours/days to minutes and thus enabling appropriate action to address the phytosanitary risk. Trials are underway to further develop and utilise technology using hand-held microscope devices and mobile phones to allow field-based inspectors to send images to diagnostic specialists. In addition, remote diagnostics is being used as a platform for training of technical specialists without the need to physically bring the specialist and trainees together.

The effectiveness of inspectors in the inspection and clearance of imported and exported plant material very much depends on their capability and confidence in knowing where to look (inspection technique), what to look for (ability to recognise pests) and what phytosanitary action to take when pests are detected. Access to work instructions, standard operating procedures, guidelines, pest data sheets, manuals and posters play a key role in ensuring regulatory decisions are consistent and compliant. Likewise, access to technical training in pest recognition, inspection techniques and treatment protocols from training specialists plays a key role in ensuring the effectiveness of a plant inspector. Some border agencies are now developing e-learning packages to deliver some of this information to phytosanitary inspectors who may be widely scattered around the country.

6.8 Role of Border Personnel in Export Functions

An effective import regulatory system is of paramount importance to exporters. Border inspection gives confidence to the overseas trading country of the commitment to preventing the spread of pests through science-based phytosanitary measures. Inspection also provides overseas countries with confidence about the exporting country's pest freedom. Freedom from major pests is a clear advantage in global trade and an important attribute for a country's export sector.

In contrast to importers who must meet the requirements of their country's phytosanitary regulations, exporters must meet the requirements of another country. As part of ensuring the goods meet the importing country's requirements, NPPOs verify exported goods are safe, accurately described and meet the foreign government's requirements. NPPOs may be required to issue phytosanitary certificates for exported products certifying that the goods have been inspected and found free from pests. Importing countries may request specific additional declarations as part of the certification providing an official government assurance regarding the phytosanitary status of a plant or plant product. This may require an importing country to conduct surveys, inspections or testing for particular pests in order to provide evidence that justifies the provision of the additional declaration. The type of inspection and documentation depends on the commodity and export-destination requirements.

Many NPPOs have international agreements with trading partners and are moving away from end-point inspections of the product to "quality assurance" based systems. Exporters now have greater responsibility for their products' quality and compliance with overseas government requirements with the NPPOs auditing the system to verify compliance. Such arrangements typically require an industry sector or exporting company to develop a hazard assurance framework to identify, control and eliminate hazards for products in line with the importing country's phytosanitary requirements. The system relies on accredited persons or competent staff to undertake particular roles including end-point inspections. The NPPOs role is to negotiate arrangements with importing countries and to approve and oversee the compliance of the process with participating parties including verification of certificates and auditing of the approved arrangements.

This chapter shows that phytosanitary/biosecurity regulation at the border is complex and typically expensive to develop and maintain. However the environmental, economic and social benefits of an effective biosecurity system can be readily demonstrated particularly when the costs and losses to plant production and biodiversity are considered.

6.9 Useful Links

The following links are provided for readers interested in obtaining more specific information concerning the import and export requirements of plants and plant produce.

1. **Australia**
   Import conditions for plants, grains and horticulture: http://www.daff.gov.au/aqis/import/plants-grains-hort
   Export conditions for plants, grains and horticulture: http://www.daff.gov.au/aqis/export/plants-grains-hort
2. **Canada**
   Import conditions for plant products: http://www.inspection.gc.ca/english/plaveg/plavege.shtml
   Export conditions for plants/plant products: http://www.inspection.gc.ca/english/plaveg/expe.shtml
3. **New Zealand**
   Import conditions for plant/forestry commodities: http://www.biosecurity.govt.nz/enter/plants
   Export conditions for plant/forestry commodities: http://www.biosecurity.govt.nz/regs/exports/plants
4. **United States of America** USDA-APHIS maintains a diverse set of links for import and export conditions for plants:
   http://www.aphis.usda.gov/import_export/plants/plant_imports/plant_inspection_stations.shtml
   http://www.aphis.usda.gov/favir/ (fruit and vegetable database)
   http://www.aphis.usda.gov/import_export/plants/plant_imports/Q37.shtml (plants/seed)
   http://www.aphis.usda.gov/permits/ (permits issued by APHIS PPQ)
   http://www.aphis.usda.gov/import_export/plants/manual/index.shtml (PPQ manuals)

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Anon (2010a) United States Department of Agriculture Treatments manual. http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment_pdf/05_02_t100schedules.pdf. Accessed 17 Sept 2009

Anon (2010b) ACI world report – passenger and freight traffic, pp 11–12. http://www.aci.aero/aci/aci/file/World%20Report/2010/WorldReportJune2010-.pdf. Accessed 11 May 2009

Anon (2010c) International mail into Australia. http://www.daff.gov.au/aqis/mail. Accessed 21 Oct 2009

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ISPM 18 (2003) Guidelines for the use of irradiation as a phytosanitary measure. ISPM No. 18. FAO, Rome
ISPM 20 (2004) Guidelines for a phytosanitary import regulatory system. ISPM No. 20. FAO, Rome
ISPM 23 (2005) Guidelines for inspection. ISPM No. 23. FAO, Rome
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Chapter 7
The Biosecurity Continuum and Trade: Tools for Post-border Biosecurity

Shashi Sharma, Simon McKirdy, and Fiona Macbeth

7.1 Introduction

The increase in rapid transport systems and movement of people and goods, accompanied by climate change has enhanced the potential for pests to disperse to new regions, find new vectors, new hosts, new environments, and new opportunities to evolve into damaging species and strains (Sharma 2012). In this era of globalisation, nations are perpetually exposed to the high likelihood of invasion by exotic pests unless strict biosecurity risk management measures are implemented across the biosecurity continuum of pre-border, border and post-border.

About 70,000 pest species damage agricultural crops worldwide (Pimentel 2009). The primary function of National Plant Protection Organisations (NPPOs) in every country is to prevent introduction, establishment and spread of exotic pests, minimise spread of endemic pests and provide information to other countries about the status of different pests in the country. The NPPO provides evidence that a pest is absent from a defined area, region or country. This information is required to claim, gain and maintain access to export markets.

Options for managing exotic pest risks pre-border and at the border include quarantine, treatments, inspection to a range of other phytosanitary measures. However, despite implementation of biosecurity risk management measures pre-border and at the border, some pests manage to invade and establish in new regions. This includes introduction of pest species and strains that are recognised by

S. Sharma
Centre for Biosecurity and Food Security, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia
e-mail: s.sharma@murdoch.edu.au

S. McKirdy (✉)
Chevron Australia, QV1 Building, 250 St Georges Tce, Perth, WA 6000, Australia
e-mail: SMcKirdy@chevron.com

F. Macbeth
Blackwood and Kemp Pty Ltd, Canberra, ACT 2611, Australia

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biosecurity agencies as significant threats to the plant sector, the community, the economy and the environment. The old saying “an ounce of prevention is worth a pound of cure” holds true for biosecurity. Preventing introduction[1] (i.e. the entry and establishment of a new or exotic pest) is more efficient and effective than attempting to “cure” a pest problem post border after a pest has established.

Post-border biosecurity is an integral component of the biosecurity continuum. It keeps a vigilant eye on any new biological threat and utilises best practice pest monitoring procedures and tools for early detection of new or exotic pests to achieve cost effective eradication, containment or control outcomes. It includes the use of sub-national boundaries to monitor and restrict the movement of biosecurity risk materials, surveillance and monitoring activities for pest detection and maintenance of pest free areas, and incursion response planning. Monitoring and surveillance for pest incursions, pest spread and establishment in new regions are some of the key activities of post-border biosecurity programmes (McKirdy et al. in press).

Post-border biosecurity actions are vital to claim pest freedom status for a region or country. They are necessary to demonstrate area freedom in order to meet trading partner requirements, as well as to demonstrate successful pest eradication.

Pest surveillance programmes include targeted active surveillance, generally undertaken by pest specialists, and passive surveillance often relying on growers and the general community to report any suspect pest to the relevant biosecurity institutions. The efficiency of passive surveillance depends on the awareness and interest of growers and general public in reporting any unusual sightings of pests and their symptoms. Successful post-border containment and eradication initiatives can be difficult and careful planning and preparedness is required.

This chapter discusses various post-border biosecurity tools that enhance preparedness of the NPPO and assist in responding in a timely manner to pest incursions as well as maintaining pest area freedoms. The tools ranging from standards of the International Plant Protection Convention (IPPC) to local communication are presented in different sections that correspond to various activities in post-border biosecurity.

7.2 Standards of the International Plant Protection Convention

The IPPC is an international agreement on plant health with 178 current signatories.[1] It aims to protect cultivated and wild plants by preventing the introduction and spread of pests. The IPPC defines plant pests as “any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products”. Introduction of any biological species that meets the definition of ‘plant pest’ causes anxiety and apprehension that

[1] Terminology in this chapter is consistent with the International Plant Protection Convention’s Glossary of Phytosanitary Terms (ISPM No. 5, IPPC, 2010) available online at http://www.ippc.int.

the introduced species may cause economic damage. The economic impact analysis of such pests is predictive and in most instances based on results of research and development and impact analyses from countries where the pest is endemic.

The IPPC provides International Standards for Phytosanitary Measures (ISPMs). These are the standards, guidelines and recommendations recognised as the basis for phytosanitary measures applied by members of the World Trade Organization (WTO) under the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement). Some of these ISPMs are applicable to post-border biosecurity in building the operational framework and guiding the establishment of post-border biosecurity programmes. These ISPMs provide necessary guidance for post-border activities particularly when a pest is introduced to a new area (more information about ISPMs are in Chap. 2):

- ISPM No. 3: Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms
- SPM No. 4: Guidelines for pest free areas
- ISPM No. 6: Guidelines for surveillance
- ISPM No. 8: Determination of pest status in an area
- ISPM No. 9: Guidelines for pest eradication programmes
- ISPM No. 17: Pest reporting
- ISPM No. 29: Recognition of pest free areas and areas of low pest prevalence

Pest Risk Analysis is an important component of post-border biosecurity and the IPPC has at least two standards to guide national and regional pest risk analyses:

- ISPM No 2: Framework for pest risk analysis
- ISPM No 11: Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms

In addition to the IPPC, other organizations such as the International Atomic Energy Agency (IAEA) and the Regional Plant Protection Organisations (RPPOs) produce documents that provide standard protocol and guidance for post border pest management. An RPPO is an inter-governmental organization functioning as a coordinating body for NPPOs on a regional level. Information on RPPO can be found on the IPPC website: http://www.ippc.int.

The IAEA documents are useful for the application of sterile insect technique and management options for fruit flies. Pest management information from the IAEA is available at http://www-naweb.iaea.org/nafa/ipc/public/manuals-ipc.html.

7.3 Biosecurity Legislation

Modern and robust biosecurity legislation is a vital part of any nation's biosecurity system to meet the increasing demands of movement of people and goods and to ensure that the biosecurity system is effective in dealing with rising pest incidents and in maintaining freedom from exotic pests.

Table 7.1 Examples of biosecurity legislation relevant to the management of pest threats

Country	Name of legislation	Scope
Australia	Quarantine Act (under revision)	In this Act, quarantine includes, but is not limited to, measures for the prevention or control of the introduction, establishment or spread of diseases or pests that will or could cause significant damage to human beings, animals, plants, other aspects of the environment or economic activities
Canada	Plant Protection Act	To protect plant life and the agricultural and forestry sectors of the Canadian economy by preventing the importation, exportation and spread of pests and by controlling or eradicating pests in Canada
New Zealand	Biosecurity Act (part 5 Pest management)	To provide for the effective management or eradication of pests and unwanted organisms
United States of America	Plant Protection Act	Detection, control, eradication, suppression, prevention, or retardation of the spread of plant pests or noxious weeds for the protection of the agriculture, environment, and economy of the United States

Biosecurity legislation provides enabling powers to the NPPOs to reduce the likelihood of introduction of biosecurity threats and enhance the level of preparedness to respond to biosecurity emergencies and safeguard the industry, the environment and the community. Biosecurity legislation implemented at the national level is referred to as first tier legislation. In some countries, the states and territories implement additional second-tier legislation. Considerable understanding is required to ensure alignment of operations of first and second-tier biosecurity programmes. In Australia, the Federal (first-tier responsibility) and most of the state and territory governments (second-tier responsibility) have signed an Intergovernmental Agreement on Biosecurity to strengthen the collaborative approach between the federal and state and territory governments (IGAB 2012).

Biosecurity legislation and regulation of a nation must be consistent with international requirements described in the WTO's SPS Agreement and the IPPC. The regulatory actions can be diverse and relate to treatments that facilitate trade, movement controls that lower risk of entry of a pest in an area and surveillance of areas for regulated pests. Regulations are developed and amended to facilitate "biosecure" movement of people and goods that are potential carriers of pests. For example, regulations can specify restricted entry for commodities that pose an unacceptable level of risk of entry of regulated pests or specify standard operating procedures for testing, inspection and surveillance. These actions are described in Chap. 3. Some examples of biosecurity legislation are given in Table 7.1.

7.4 Tools That Guide Organisational Response to Detection of New or Exotic Pests

Responses to detection of new or exotic pests generally require significant commitment of resources (time, money, staff, technology, etc.). The biology of the pest and characteristics of the environment are important in determining the success of an eradication response and other contributory factors such as costs, benefits and stakeholder support are crucial for successful eradication. The NPPO often adopts standard operating procedures (SOPs) or protocols used in different national or regional emergencies such as wildfire, flood, cyclone, volcanic eruption and earthquake. The approach to managing risks and emergencies and organisational structures needed for implementation of incident responses to pest incursions are similar across sectors and involve the following (from Murray and Koob 2004):

- **Emergency planning** – emergency management related policies, strategies, plans and procedures to enable a high level of readiness.
- **Prevention and mitigation** – regulatory and physical measures to ensure that risks are minimised, emergencies are prevented, or their effects mitigated, by working with neighbouring countries, conducting import risk analyses, and border and quarantine measures.
- **Assessment and training** – personnel are able to perform their assigned tasks to accredited national competencies standards.
- **Surveillance, warning and alerting** – systems for predicting, detecting, warning and alerting of potential emergencies.
- **Co-ordination** – mechanism to ensure the integration of national whole-of-the-government and industry (affected crop growers) decision-making.
- **Emergency response** – actions are rapidly taken in anticipation of, during, and immediately after an emergency to ensure that its effects are minimised.
- **Communication** – timely information exchange before, during and after emergencies, between governments and government agencies, with industry and with the community.
- **Risk assessment** – systematic identification and analysis of hazards, exposures and vulnerabilities.
- **Knowledge management** – gathered, stored, accessible and applied information.
- **Legislation** – supporting laws and regulations.
- **Resourcing** – adequately trained people, appropriate equipment and facilities, and necessary financial arrangements.
- **Emergency recovery** – the co-ordinated process of supporting emergency affected communities in the reconstruction of the physical infrastructure and restoration of emotional, social, economical and physical well-being.
- **Continuous improvement** – enhancement of existing systems through exercising, auditing against performance standards, bench marking and debriefing following emergencies.

In Australia, this approach has been adopted for responding to incursions of exotic plant pests, and developed into national manuals approved by the industry, government and other relevant stakeholders. Plant industry bodies and the Australian and state and territory governments have established Plant Health Australia as a public company in April 2000 with the challenge of taking a partnership approach to key plant health issues and enhancing Australia's ability to respond to incidents of plant pests (Donovan 2004).

Plant Health Australia has established a 'world first' Emergency Plant Pest Response Deed (DEED) which enables equal involvement of government and plant industry members in decision making when responding to pest incidents. The DEED is underpinned by PLANTPLAN (www.planthealthaustralia.com.au/plantplan), a national emergency preparedness and response plan for plant industries (Donovan 2004). PLANTPLAN describes four phases of incident response:

1. **Investigation** – presence of a suspect new pest is reported to the Chief Plant Health Manager of the State/Territory agriculture department. The process of confirmation of identity by diagnostic experts is initiated. Additional trace back analysis defines the nature of the incident. Relevant contacts in stakeholder organisations are notified.
2. **Alert** – Pest identity is confirmed by diagnosis using local and independent experts, and the outbreak declared. A management committee comprising representative stakeholders is convened. Pending a decision that confirms the pest meets the criteria of an Emergency Plant Pest[2] the committee then evaluates feasibility of eradication. If this is also confirmed the issue is referred to a high-level management committee, consisting of representatives from Industry and Government. Its responsibility is to consider the facts and recommend an action. It has the power to authorise eradication and associated resources.

   Development of a specific response plan based on PLANTPLAN is usually referred to the "affected" jurisdiction. This includes estimates of technical and economic resource requirements. This is subject to consideration by the high level committee and, if satisfied, eradication action is approved. The lead agency and the formula for national cost sharing arrangements are confirmed.
3. **Operational** – The lead agency implements and manages the response plan and reports to a Consultative Committee on Emergency Plant Pests that provides

[2] An Emergency Plant Pest is defined as:

(a) It is a known exotic plant pest the economic consequences of an occurrence of which would be economically or otherwise harmful for Australia, and for which it is considered to be in the regional and national interest to be free of the plant pest.
(b) It is a variant form of an established plant pest that can be distinguished by appropriate investigative and diagnostic methods and which, if established in Australia, would have a regional and national impact.
(c) It is a serious plant pest of unknown or uncertain origin which may, on the evidence available at the time, be an entirely new Plant Pest and which if established in Australia is considered likely to have an adverse economic impact regionally and nationally.
(d) It is a plant pest of potential economic importance to the area endangered thereby and not yet present there or widely distributed and being officially controlled, but is occurring in such a fulminant outbreak form, that an emergency response is required to ensure that there is not either a large scale epidemic of regional and national significance or serious loss of market access.

Table 7.2 Factors to consider in deciding whether to implement a full-scale pest eradication programme

Factors favouring eradication	Factors favouring alternate action
Cost/benefit analysis shows significant economic loss to industry or the community if the organism establishes	Cost/benefit analysis shows relatively low economic or environmental impact if the organism establishes
Physical barriers and/or discontinuity of hosts between production districts	Major areas of continuous production of host plants
Cost effective control difficult to achieve (e.g. limited availability of protectant or curative treatments)	Cost effective control strategies available
The generation time, population dynamics and dispersal of the organism favour more restricted spread and distribution	Short generation times, potential for rapid population growth and long distance dispersal lead to rapid establishment and spread
Pest biocontrol agents not known or recorded in Australia	Widespread populations of known pest biocontrol agents present in Australia
Vectors discontinuous and can be effectively controlled	Vectors unknown, continuous or difficult to control
Outbreak(s) few and confined	Outbreaks numerous and widely dispersed
Trace back information indicates few opportunities for secondary spread	Trace back information indicates extensive opportunities for secondary spread
Weather records show unfavourable conditions for pest development	Weather records show optimum conditions for pest development
Ease of access to outbreak site and location of alternate hosts	Terrain difficult and/or problems accessing and locating host plants

regular reports to the high level management committee. If required, a Scientific Advisory Panel may evaluate technical effectiveness of the response and an independent auditor may assess the financial accountability of the programme.

4. **Stand Down** – This occurs when eradication is completed or when review determines that eradication is no longer feasible. Records of expenditure and technical reports are provided so that cost shares can be calculated. Activities are formally reported that summarise outputs and impact of incursion response action. This is communicated to stakeholders including appropriate international agencies and markets.

Biosecurity emergencies in urban and peri-urban areas are generally more complex than that in the rural areas due to higher population density, diversity, small land parcels, opposition to application of chemicals in urban landscapes, and the need to revisit properties during eradication.

Table 7.2 shows a description of factors considered by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) in determining whether to pursue an eradication programme for a new or exotic pest. The NPPO may elect to not manage a given pest if no effective, affordable or feasible options are available. This can include cases where the pest is not expected to have a significant impact. In between eradication at one end and “do nothing” at the other end is a spectrum of other measures and options including surveillance, control, suppression, containment and area wide pest management.

7.4.1 How to Prioritise Resources and Assess Risk of Pest Incursion

Uncertainty can be common in dealing with pest incursions when there are insufficient resources, data and time to make well-informed decisions. Prioritisation tools when operating in uncertain and resource-constrained environments are becoming increasingly important. These tools assist the NPPO in decisions on how best to use diminishing resources when faced with new pest challenges.

Multi-Criteria Decision Making tools are promising in that they offer diverse views to enter the decision making process and for the negotiation of consensus positions (Liu et al. 2011). Decision makers invariably face complex situations when responding to pest incursions with potential for their decisions to have positive consequences for some stakeholders and negative for other stakeholders. These tools assist in working out best possible decisions based on available information using qualitative and/or quantitative information. Each decision alternative is represented by its performance in multiple criteria and assists in finding the best alternative or finding a set of good alternatives. For instance, pests can be prioritised and ranked as high or low impact using multi-criteria analysis. The advantage of using a designated prioritisation process is that the evaluation is more objective and comparisons are possible because the same evaluation methods and criteria are used for different pests. Properly documented process increases transparency for communicating with stakeholders.

The Analytical Hierarchy Process (AHP) is a specific type of multi criteria analysis that weights evaluation criteria in order of importance, and then uses them to evaluate a problem (Saaty 2008). The US Department of Agriculture uses the AHP to prioritise exotic pests in the Cooperative Agricultural Pest Survey programme (CAPS), which is designed to detect new pests that have been ranked as important and high risk to US agriculture and the environment. This prioritisation identifies which pests warrant the greatest expenditure of resources for detection (USDA APHIS 2003). The AHP has been used to prioritise introduced pests and make decisions on resource allocation; the Department of Primary Industries, Victoria (Australia) has used this process to rank weeds that are candidates for control programmes (Weiss and McLaren 2002).

Risk analysis and threat prioritisation are the tools used to assess and manage the risk and likely consequences of entry, establishment and spread of pests. Exotic or new pests are usually detected as a result of surveillance. In many cases, there are pests for which the NPPO conduct regular surveillance based on pre-existing knowledge and there is clear understanding of the actions that would be taken if those pests were detected. In Australia, industry biosecurity plans summarise surveillance and monitoring for the high impact threats, and include incident response in the event of a pest incursion, containment, eradication and other management measures, research and development priorities and biosecurity communication and training needs.

There is distinction between exotic pests for which there is little experience and exotic pests for which responses have already been mapped prior to introduction.

Often exotic pests are detected by chance via passive surveillance (e.g. reported by member of community or a grower) and there is no specific preparedness for exotic pests that are not identified as high impact pests. Depending on the pest and its likely impact, the NPPO may decide to:

- Do nothing;
- Undertake surveillance;
- Suppress or contain the pest;
- Manage the pest;
- Eradicate the pest.

For example, fruit flies are a taxonomic group of pests that are considered to be economically important by many NPPOs (Chap. 15). When incursion of a fruit fly species is detected, there is usually an understanding of its importance because an analysis has already been done and a response plan is usually ready to commence eradication action. For pests that are well understood, the level of uncertainty is usually much lower as there is broad understanding of potential impacts, management strategies, costs and benefits of taking different types of actions.

For poorly understood pests there is uncertainty whether the pest will establish and spread to new areas and express significant impacts. An example is a new species of wood-boring beetle whose complete host-range and other important biological information are not known. Consequently a much higher level of uncertainty exists, and decision-making becomes more difficult. Decisions relating to ongoing progress and success of a programme invariably must be made in the absence of complete information. These situations are often described as 'damned if you do and damned if you don't scenarios', and they fall within the "choice under uncertainty" category of classical decision theory.

More importantly, by understanding the principles of decision theory, the risks of poor decisions can be mitigated. In the absence of data, desired outcomes may be achieved using several treatments or restrictions whose cumulative effects become equivalent to the preferred measure that would be applied in the presence of data. These measures may gain the desired level of confidence to ensure that eradication remains feasible. Decisions made on this basis should be well documented and recorded. Furthermore, decisions made without complete data should involve the widest possible consultation. Stakeholders not directly involved in that process should be provided with the full rationale on which the decision was made. Good practice recommends recording decisions made in a sub-optimal environment and to fully explain the logic followed in the absence of empirical evidence.

7.5 Tools for Pest Diagnostics and Surveillance

Two critical operations needed for dealing with exotic pests involve diagnosis and surveillance. They go hand in hand and are used to generate contemporary data for decision makers throughout the incursion response.

7.5.1 Types of Diagnostic Tools

In this section, types of diagnostic tools are presented rather than the specific detail of the methods for diagnosis of strains and species of pests in entomology, bacteriology, mycology, nematology and virology. (See Chap. 13 for details on Molecular Diagnostics.)

The Plant Biosecurity Toolbox (PBT) (www.padil.gov.au/pbt) is an example of a diagnostic tool site developed by the Australian Cooperative Research Centre for National Plant Biosecurity. The PBT provides detailed, web-based diagnostic information to assist with the rapid identification of exotic plant pests in the event of an incursion. It centralizes diagnostic information in recognition of the need for diagnosticians and plant health workers to have quick and easy access to accurate diagnostic resources that have been endorsed by the NPPO.

The comprehensive PBT resources include:

- Information on biology and taxonomy of the pest;
- Diagnostic morphological, biochemical and molecular tests;
- Images of the pest, host symptoms and damage.

Diagnostic science is an important tool for post-border biosecurity because it must specify methods for recognition of damage and symptoms and identification of new or exotic pests. The goal for the NPPO is to ensure laboratories adopt best practice standard operating procedures to minimise the risk of misdiagnosis. The diagnostic labs engaged in biosecurity programmes utilise accepted protocols for diagnosis of pests. The protocols include two components: (1) Recognition of symptoms and pest damage, and (2) Isolation and identification of the pest. (See Chap. 11 for a discussion of digital identification tools.)

Recognition of pest damage or disease symptoms sometimes can be surprisingly difficult. This is due to variation in symptom production on plant parts, cultivars and between plant species. High-quality imaging of diseased or damaged plants is helpful. Locating quality images that illustrate differences can be difficult and diagnostic tools such as PBT, Pest and Disease Image Library (PaDIL) (www.padil.gov.au) and Bugwood (www.bugwood.org) have been developed to provide diagnosticians with access to these valuable resources (See Sect. 12.3.6). Field-survey teams need prior training to identify the affected host plants, and recognise various types of damage on plant parts, varieties and species. Tools such as printed images of pest symptoms or mobile digital technologies with access to image libraries are essential for field based teams responding to new detections.

Lab-based diagnosis of exotic or new pests requires access to robust, reliable and accurate methodologies. Molecular approaches are increasingly being evaluated to find unique sequences of DNA or RNA that can be used to identify pests (Chap. 13). While molecular protocols are readily utilised, many traditional methods are still valuable tools for diagnosing exotic or new pests. Molecular approaches enable diagnosticians to ensure an accurate and repeatable result is obtained that can be used by the decision makers in determining and justifying actions to be undertaken.

The use of molecular (Chap. 13) and morphological (Chap. 12) protocols in tandem helps to achieve reliable diagnosis of suspect pest.

Confirmatory diagnosis is important to verify the identity of a new or exotic pest. Internationally recognised and approved methods are used to confirm pest identity and avoid false positive or false negative results. It is generally advisable to involve at least two independent national labs in pest identification and, if required, consideration should be given to involve a third independent lab based at a location where the pest is endemic. Routine diagnosis is often a shortened version of confirmatory diagnosis with emphasis on selection of robust tests with quick turnaround time.

7.5.2 Tools for Pest Surveillance

Pest surveillance is one of the most critical functions that all the NPPOs perform at the domestic level and one of the first steps in any post- border biosecurity plan. (See Chap. 11 for a detailed discussion of surveillance and ISPM No. 8.) It enables detection of new pests and pest incursions, determination of the extent of pest spread, monitoring programmes for eradication, official containment, control, maintenance of pest free areas and areas of low pest prevalence and confirmation of pest freedom after eradication.

Surveillance provides the basis for domestic phytosanitary measures including justification for quarantine regulation of plant products from foreign sources. The presentation of contemporary surveillance data enables countries, states and territories to specify pest status either 'known or known not to occur' or as 'not known to occur'. The distribution of a pest is defined by delimiting surveys, which identify the extent of spread and account for climatic, host and ecological influences. Surveillance tools range from passive to targeted surveillance. These tools enhance the ability to detect an organism when it is present. Failure to detect or the false positive detection of high impact threats may pose significant and unacceptable risks.

Post-border surveillance (Chap. 11) includes structured surveys, passive surveillance, qualitative assessment of data from various sources and passive surveillance assisted by mathematical tools ranging from formulae to assist in survey design to stochastic scenario trees and Bayesian belief networks. Self-organising maps are a type of neural network that have been used to identify species that are likely to establish, if introduced (Worner and Gevrey 2006; Paini et al. 2010). These maps compare pest assemblages from different regions around the world. When high similarity exists between two regions, pest species known to have established in one region are predicted to have a high likelihood of establishing if introduced to the other region.

Results from sentinel site surveys can be important in supporting claims of pest area freedom status (Boland 2005; McMaugh 2005). Sentinel sites are selected in locations where there is a high likelihood of a pest incursion.

Pest distribution data provide essential information to assess feasibility of intervention and there is usually a requirement for delimiting surveillance to monitor progress of containment or eradication programmes (See ISPM No. 8 "Determination of pest status in an area" for more information.) When selecting sites for pest surveys, random sampling, stratified random sampling, systematic sampling and flying insect trapping are all appropriate methods (McMaugh 2005).

Surveillance is required to confirm that the pest has been eradicated and that eradication is endorsed internationally. Incursion response activities tend to focus on areas of pest presence, but there is an equally important issue of confirming pest absence or "freedom" in unaffected areas. This information is required by industry for national and international trade.

Many tools available for biosecurity surveillance and field teams normally require tools such as:

- Images for recognition of host plants, disease symptoms, pest damage and life stages (Chap. 12).
- Survey strategies that provide information on how to survey in rural, peri-urban and urban environments, and modified strategies for targeted survey (Chap. 11).
- Vehicles, survey clothing and equipment including identification tags and geo-positioning system (GPS) for accurate location.
- Data recording methods that facilitate direct information technology input i.e. digital maps that include GPS points.
- Methods of communication with property owners/managers by survey team leader (Sect. 8.3).
- Hygiene protocols for moving on and off properties (Sects. 18.3 and 18.4).

The Department of Agriculture and Food in Western Australia (DAFWA) runs targeted and community surveillance programmes; the targeted programmes document the absence, presence or level of containment in the State of key exotic pests. The community programmes include general surveillance where specimens are actively solicited from the public and identified free-of-charge. Information gained is used to confirm the state's area-freedom of targeted pests of quarantine significance. Surveillance also monitors the status of pests that are under eradication or containment programmes. DAFWA has increased public awareness and engagement of the community in surveillance via the provision of the Pest and Disease Information Service (PaDIS) that offers a free service to identify specimens and handles any unusual sightings. Cities are 'transport end-points' for road, rail, air and sea freight, through which most exotic pests enter the State. Therefore it is important to engage the public as a resource in the detection of exotic pests.

7.6 Tools for Pest Risk and Economic Analyses

Pest Risk Analysis (PRA) is addressed in more detail in Chap. 9. PRA consider the biological and associated factors that determine options for intervention activities against a new pest in the area where it has become established. It is frequently integrated with economic analysis. Both are needed and used to make informed decisions. PRA provides the biological and technical information that guides decisions on what steps are taken after a pest is introduced. PRA normally assesses likelihood of entry and magnitude of consequences. However, in the case of new pest detection, the likelihood of entry is redundant because entry has already occurred. For example, a new organism may be detected in a light trap at a port of entry. If the PRA demonstrates that the pest is likely to be a negligible risk because the climate of the country does not favour pest survival, then the NPPO may decide not to take further action against that pest. On the other hand, if a new pest is detected in an orchard and the risk analysis shows that the pest has a high likelihood of establishment based on ability to survive and potential economic impacts then the NPPO is likely to implement a response plan. Consideration of the following factors helps to develop and implement a sound response plan:

- Potential distribution and abundance;
- Length of time present;
- Host range;
- Distribution of potential hosts;
- Biology of the pest including length of life cycle and viability;
- Potential for spread;
- Influence of climate;
- Vectoring capacity, presence/absence of vector;
- Ease of identification both in the field and in the lab;
- Legislation to enable an adequate response;
- Effectiveness of proposed treatments.

Economic analyses are important components of any decision-making framework for newly introduced pests. An economic analysis of a pest may be a “stand-alone” document, or may be integrated with PRA. The types of impacts of pest introductions include direct impacts on agricultural production, impacts on exports (e.g. loss of export markets), environmental impacts and brief reference to social, aesthetic and political impacts. Tangible monetary impacts are generally easy to assess and quantify than the non-tangible impacts which are equally, if not more, important on lifestyle, biodiversity, etc.

The NPPOs determine the economic impacts associated with either managing or eradicating a new pest and this determination includes the relative costs and benefits of different actions to be taken. The Cost Benefit Analyses help to provide economic assessment and useful information in determining whether the costs of a programme (e.g. eradication or containment or maintenance of low pest prevalence programmes) outweigh its potential benefits. In some instances eradication is technically feasible but the costs of eradication may exceed long-term benefits. In such cases, the NPPO may decide that an alternative to eradication (such as the use of existing Integrated Pest Management system or planting of resistant varieties) is preferable.

For export-oriented industries, the economic analyses include costs of potential loss of export markets and the need for additional phytosanitary treatment and certification. Pest free places of production are sometimes considered as an alternative to eradication (ISPM 10) and the recurrent costs of their establishment and maintenance should be considered.

Additional information is provided in ISPM No. 2 “Framework for Pest Risk Analysis”, ISPM No. 11 “Pest Risk Analysis for quarantine pests including analysis of environmental risks and living modified organisms” and ISPM No. 5 “Glossary of phytosanitary terms”. This in-depth analysis provides more information on the types of economic consequences that may be considered in a risk analysis.

7.7 Tools for Eradication and Pest Management

Differences exist in tools and processes for eradication and management. Management tools usually aim to reduce the population of a pest to levels that minimise its economic damage on hosts. By contrast, eradication tools aim to completely eliminate the pest population. Surveillance and diagnosis are required to check that the pest is absent and no longer detectable by best practice survey and diagnosis.

The success of eradication recommended by pest and economic analyses frequently depends on depriving the pest of susceptible hosts on which it can survive and reproduce. This involves planned programmes and tools to remove and destroy the hosts that surround the infested area. Additional survey and pest management tools are used to check and treat (i) the area where host plants have been removed, (ii) the host free buffer area that surrounds the infested area, and (iii) the nearest locations of host plants. A range of strategies is selected including hygiene management and pesticide treatments.

In some instances less drastic eradication strategies are used especially for organisms that are slow growing (e.g. wood infecting pests) and where the host is accessible to management practice such as pruning. It is possible to remove infected (infested) wood to a point where the pest cannot survive. Careful removal and destruction of affected plant material and use of selected pest management tools can result in success. The benefit of this approach is that the host plant is retained. This strategy is particularly appropriate for perennial crops that represent considerable investment for farmers.

When eradication is not recommended, alternative strategies are available either for containment or control. These options can be similar to those used for endemic pests. Containment recognises that eradication is not possible in the short term and tools are applied to effectively contain the pest to a defined area and the remaining part of the country can be considered to be “pest free”. For export industries, the markets will decide on levels of surveillance and control to justify “pest free” status. Surveillance, diagnostic and regulatory tools are used to confirm the pest infested and free areas.

In some cases, a pest management programme may be applied over wide areas and may involve multiple agencies and stakeholder groups. Such programmes are often referred to as "area-wide pest management". Such programmes are typically applied for serious pests, such as certain species of fruit flies, or pests that are important for public health (e.g. mosquitoes). Establishment of a new pest can mean adjustment to existing pest management programmes because of the likely need to introduce new chemistries whose effects on other pests and biocontrol agents are not fully understood.

Regulatory tools are frequently used to secure either infested or pest free areas. Usually these specify movement controls of people and produce that minimise risk of inadvertent transfer of the pest. Also specific quarantine that controls movement can be applied to infested areas to limit pest spread. For instance, if a fruit fly is introduced into an area, then restriction could be imposed on any host material moving out of the area where the pest was found. A buffer zone could be delimited around that area to prevent spread through movement of infested host material (e.g. Sect. 18.4). The intensity and duration of measures applied are determined by analysis of the type of pest, the likelihood of success of pest treatments and the available resources.

A simple post-border measure implemented by farmers to maintain freedom from pests is the "farm biosecurity approach", which emphasises farm hygiene necessary to prevent introduction of exotic pests into the farm from anywhere. Farmers can have a major impact on the future of their own farm output and also at a wider level by implementing biosecurity measures on their farms. Farm biosecurity measures include simple actions to minimise the entry and spread of pests. These include:

- Display a sign to inform farm visitors that all machinery, vehicles, boots, hand tools, bins and boxes must be clean before coming onto the farm.
- Establish a wash-down area near the main entrance with a sump that can be readily inspected for signs of weeds and pests.
- Check the cleanliness and quality of any seed or grain before it comes onto the farm.
- Prevent livestock coming to property from spreading infections, soil-borne diseases and weeds.
- Ensure that agricultural machinery, plants and equipment are cleaned of plant material and most soil before they are moved to a new work site.
- Consider washing footwear and hand equipment before entering and leaving high-risk work sites when working in nurseries and seed-crop areas.
- Make it easy for visitors to clean machinery, equipment and boots before they leave the property.

7.8 Tools for Communication

Communication strategies are vitally important tools for post-border biosecurity (Chap. 8). Incursions of new pests can affect a range of stakeholders both on-shore (post-border) and off-shore (pre-border). This diversity of stakeholders and associated complexity demands carefully planned communication strategies to ensure everyone involved has a shared understanding of the emerging situation. The impact of new pest detections can have economic, social and environmental consequences. The communication tools that are used include dedicated phone lines, radio, television, Internet, print and news media.

Important stakeholders include offshore markets, exporters, public, governments, consumers, industry leaders, farmers, environment agencies, regulators, technical groups and media. All stakeholders are interested in progress but each has different information requirements. Communication planning ensures that the communicator has time to interpret and summarise complex technical issues for different audiences. For example, pest incursions can threaten export trade. Communication is needed by exporters and by off shore representatives in countries that are recipient markets. Both stakeholders need relevant information on how the regulated pest affects trade, disinfestation options and (if eradication is approved), when trade can be restored.

Incursions in urban areas bring unique challenges for communicators and these include but are not limited to the use of pesticides, specific demands of different property owners in the affected area, removal of host plants and associated movement controls. The key to successful communication is to develop clear and concise messages (see Chap. 18 for citrus examples). The incursion response plan should include checklists that outline information needs for specific audiences during different phases of the response plan.

Two examples are presented here that illustrate the complexity of the problem and the difficulty of achieving shared understanding by stakeholders.

A decision by the NPPO to destroy trees on private properties for control of Asian Longhorn Beetle has been met with resistance (cf. Sect. 16.5). Frequently this raises complex issues of compensation for loss. Effective communication strategies are vital to ensure stakeholders, including affected owners, have a shared understanding of the problem that frequently extends well beyond the boundaries of their properties.

Cooperation and support are essential features of communication strategies or tools. Often stakeholders have a central and active role in pest management programmes that requires almost everything from field pest management practices to observing specific quarantines such as restricting the movement of host material in infested areas. In these cases, the NPPOs communicate and work with stakeholders to ensure that the purpose of the programme, the objective of specific actions and the respective roles of the NPPO and stakeholders are clearly identified.

Another example assumes a new species of fruit fly has been detected in a citrus grove, reported by the grower to be damaging the citrus fruit. The local department of agriculture was consulted and the detection reported to the NPPO. In this example it is assumed that the detection occurs at the peak of harvest time and that the citrus fruits from the area of the initial detection are intended for domestic markets (both as fresh fruit and for processing facilities for making juice) and export markets.

Early after detection, the first steps the NPPO takes (in cooperation with local governments) are to delimit the infestation through surveys and to quarantine any materials associated with the pest moving from infested areas. The quarantine would affect the commercial growers in that area, other property owners (e.g. homeowners with backyard trees), and other industries involved in handling citrus fruit (e.g. packinghouses, juicing facilities, local markets and transportation for moving host material such as trucks or trains). All of this would take place before a full control or eradication programme has been implemented. Growers may later comply with specific requirements such as field sanitation or pre- or post-harvest treatment programmes if they wish to move their produce out of the quarantine area (Sects. 18.3 and 18.4). Other stakeholders (juicing facilities, packinghouses, local markets, transportation) might be asked to ensure waste material (e.g. rotten fruit) is disposed of in specific ways.

This example shows a relatively simple scenario in which a single pest introduction might affect many different stakeholders. Many other individuals, industries or organisations can be impacted as well.

7.9 Conclusions

In a rapidly changing global operating environment, modern biosecurity risk management approaches, ongoing vigilance and modernisation are essential. The preservation of the biosecurity status of a nation represents a moving target. The impacts of pest invasion vary depending on factors such as virulence of the pest, host range, the nature of damage, and the rapidity of spread and climate. The NPPOs are usually expected to provide leadership in technical, policy and regulatory matters that pertain to the specific incursion but justifiable responses to the new pest would be difficult without use of tools that usually generate data and help with interpretation and management of risk.

This chapter identifies the important tools commonly used by the NPPOs to assess the biological and economic implications of pest invasions and maintenance of pest area freedoms. There is general agreement by the NPPOs to apply a standardised set of procedures that are used to guide interpretation of pest risks. It is important to recognise that tools currently in use in post-border biosecurity are under continual revision and changes can occur if and when more effective and efficient tools become available.

On-going needs to develop innovative tools will ensure that post-border biosecurity risk management planning and implementation is timely, professional, effective and will ensure business excellence and continuous improvement. Post-border biosecurity issues require adequate attention from all stakeholders otherwise the cost of living with the introduced pests would be unaffordable and the loss to economy, environment, agriculture and biodiversity would be unsustainable.

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Chapter 8
Agricultural Biosecurity Communications and Outreach

Michael Tadle and Paula Henstridge

8.1 Introduction

Effective communication is the key to any successful biosecurity programme. Communication must be structured, unambiguous and timely. For phytosanitary issues, a National Plant Protection Organisation (NPPO) has the legal authority and managerial responsibility to act on behalf of a sovereign government for matters pertaining to plant protection. An NPPO should be comprised of personnel with a level of expertise appropriate for the duties and responsibilities of the positions being occupied. NPPOs should have personnel with training and experience in performing inspections of plants, plant products and other regulated articles for purposes related to the issuance of phytosanitary certificate. They should have personnel capable of making authoritative identification of plants and plant products. NPPO personnel should also be capable of detecting and identifying pests. In addition, they should be capable of performing or supervising phytosanitary treatments required for certification; pest survey, monitoring and control activities related to phytosanitary certification; constructing appropriate certification systems and formulating instructions from an importing country's phytosanitary requirements; and auditing of accredited personnel and certification systems, where appropriate. In performing these various activities, an NPPO must pay particular attention to its ability to communicate.

M. Tadle (✉)
Policy and Program Development, APHIS, USDA, 4700 River Road, Riverdale, MD 20737, USA
e-mail: michael.a.tadle@aphis.usda.gov

P. Henstridge
PPQ, USDA, 302-E, J.L. Whitten Building, 14th & Independence Ave, S.W., Washington, DC 20250, USA
e-mail: paula.henstridge@aphis.usda.go

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Table 8.1 Regional plant protection organisations

Regional plant protection organisation	Acronym	Member countries
Asia and Pacific Plant Protection Commission	APPPC	23 countries in and around Asia/Pacific, also France
Comunidad Andina	CA	Bolivia, Colombia, Ecuador, Peru, and Venezuela
Caribbean Plant Protection Commission	CPPC	22 countries around Caribbean, Central America, also France and USA
Comité de Sanidad Vegetal del Cono Sur	COSAVE	Argentina, Brazil, Chile, Paraguay, Uruguay
European and Mediterranean Plant Protection Organisation	EPPO	48 countries in and around Europe
Inter-African Phytosanitary Council	IAPSC	51 countries in and around Africa
North American Plant Protection Organisation	NAPPO	Canada, Mexico, USA
Organismo Internacional Regional de Sanidad Agropecuaria	OIRSA	Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama
Pacific Plant Protection Organisation	PPPO	27 countries in and around Pacific, also France, UK and USA

An NPPO's ability to effectively conduct plant biosecurity activities is contingent upon maintaining an effective and systematic communications strategy. Doing so allows the NPPO to clearly express to the public any potential consequences of invasive plant pest introductions to a nation's production of food, natural resource and overall economy.

Australia's NPPO is the Department of Agriculture, Fisheries and Forestry, Biosecurity Australia (DAFF). New Zealand's NPPO is the Ministry for Primary Industries (MPI). The USA NPPO is the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS). Communication among NPPOs is encouraged through Regional Plant Protection Organisations (RPPO). The International Plant Protection Convention (IPPC), an international plant health agreement established in 1952, currently recognizes eight intergovernmental organisations that are responsible for communication and cooperation in plant protection among neighbouring countries (Table 8.1) (RPPO).

Public awareness about plant pest risks influences public policy makers within government to ensure that maintaining a robust and effective plant biosecurity system is a constant civil priority (Box 8.1).

The dynamics of communication concerning plant biosecurity are constantly shifting. Within industrialized countries, citizens are becoming more removed from agriculture and its challenges. For instance, in 2011 less than 1 % of Americans earned a living from working in agriculture and only 3.6 % of Australians worked in the agricultural sector (CIA 2012a & CIA 2012d). These estimates suggest that most people in both countries have little direct connection to agricultural production.

Box 8.1 Plant Biosecurity

Plant Biosecurity is a collection of measures designed to protect a crop, crops or a sub-group of crops from emergency plant pests at national, regional and individual farm levels. (Cooperative Research Center for National Plant Biosecurity 2009–2012).

Biosecurity is a key principle supported by the United Nation's Food and Agriculture Organisation (FAO) and embraced by the International Plant Protection Convention (IPPC) for promoting, developing and re-enforcing policy and regulatory frameworks for food, agriculture, fisheries, and forestry. Biosecurity has direct relevance to food safety, the conservation of the environment (including biodiversity), and sustainability of agriculture. (International Plant Protection Convention n.d.)

Even though agricultural production is fundamental to feeding their populations (and is a significant component of both economies), more than 95 % of the people in Australia and the USA lack the level of awareness about phytosanitary biosecurity held by people who make their living from farming. This presents a great challenge to the NPPOs in both countries as they work to garner public support to conduct plant biosecurity activities in a social environment where there are many other competing public policy priorities seeking political support and civil resources. The challenge is sometimes intensified by a growing awareness and concern among the public about the safety of pesticides used on the food they eat and in the natural environment around them.

Mitigating biosecurity risks posed by plant pests to a nation's agricultural and natural resources and biological diversity is only possible when an NPPO can *convincingly* demonstrate to its stakeholders (including the public) the validity of three conditions:

1. The plant pests of concern present a significant economic and environmental threat;
2. The NPPO has the legal authority to take protective biosecurity action;
3. Science validates the pest's threat and the efficacy, practicality, and safety of pest exclusion and management efforts to mitigate the threat. In some situations, this may require independent scientific validation.

An NPPO cannot rely exclusively upon its legal authority. For instance, APHIS obtains legal authority via the Plant Protection Act of 2000 (United States GPO 2012) and MPI obtains legal authority through the Biosecurity Act of 1993 (Parliamentary Counsel Office n.d.) to take quarantine or other biosecurity action to mitigate the risk of the introduction or establishment of a plant pest. An NPPO must have an effective communications strategy to convince its stakeholders that protective biosecurity action is in their individual and shared interest. Otherwise, even if an NPPO presents scientifically valid evidence indicating that it can safely and effectively manage an

Box 8.2 What Is a Stakeholder?

Stakeholders are individuals with a vested interest in a NPPO's actions, policies, and regulations. A stakeholder can be a person, group or organisation (Thomas 2010).

Traditionally, NPPOs considered stakeholders to include industries directly affected by its actions, policies, and regulations. For example: A wheat producer in South Australia; the New Zealand Farm Forestry Association; and commercial air carriers operating international flights (with potentially prohibited agricultural products on board) into the USA. The current concept of stakeholder has expanded to include a more extensive range of individuals and groups, including organic and specialty crop producers, sellers and users of commodities, commodity consumer groups, and conservation interests and organisations. With instant communication via electronic media and social networking, an NAPPO appropriately considers the country's general public as it stakeholders.

economically and environmentally significant pest, stakeholders may remain unconvinced and seek to impede the NPPO's ability to act.

An NPPO's ability to persuade stakeholders to accept the implementation of protective biosecurity measures that mitigate plant pest risks depends upon the NPPO being recognized by the public as a scientifically credible authority that serves the public's interest. Credibility and public trust are earned and maintained through the NPPO's *commitment to transparency* (i.e., conducting business in a manner that promotes openness, communication, and accountability) and *willingness* to give the public opportunities to provide feedback that will influence policy development and implementation.

Maintaining public trust, ensuring transparency, and engaging stakeholders in a meaningful way requires that an NPPO make its intentions understood. This is not easy. The NPPO must clearly communicate its message (typically involving technical, scientific and agricultural-specific concepts) to a diverse audience that generally has limited scientific or agricultural background.

An NPPO's reliance on a sustained communications strategy to carry out its plant biosecurity mission is especially relevant and reliant upon developments in telecommunications and mass media (e.g. the Internet and social networking). Heightened public concern about the environment and the use of chemical pesticides increases the importance of effective communication. In this climate, NPPOs find that a communications strategy focusing on traditional stakeholders (farmers, agricultural industry groups, commodity importers and exporters, state or provincial and local governments, and members of the community affected or threatened by a plant pest) is insufficient. The stakeholder base must also include interested individuals and groups that are directly or indirectly impacted.

8.2 What Is at stake?

Farmers and other people engaged in agricultural industries, naturalists and conservationists may understand the potential economic and environmental impact the introduction of an alien invasive plant pest could cause. People whose livelihood is not directly dependent upon harvesting and marketing crops, or is not grounded with protecting the environment and biological biodiversity, have limited understanding of the potential for damage a plant pest can cause. However, history clearly shows that plant pests can cause long-term devastation to a country's environment, economy, and social customs. Here we discuss two case studies to illustrate how devastating the introduction, establishment, and spread of plant pests can be on a nation.

8.2.1 Chestnut Blight and Decline of the American Chestnut Tree

Today, few North Americans remember the ecological dominance of the American chestnut tree, *Castanea dentate* (Marshall) Borkhausen, in the Eastern USA and southern Ontario in Canada. Before the early twentieth Century, stands of American chestnut extended from Maine thru the Mid-Atlantic States to Georgia and into the Ohio Valley, reaching southern Ontario. Forty years after the unintentional introduction of Chestnut Blight, these populations of American chestnut trees were virtually eliminated everywhere (Eilperin 2010).

Chestnut Blight is caused by an Asian bark fungus, *Cryphonectria parasitica* (Murrill) Barr. Chestnut Blight was introduced into North America circa 1900–1904 and first identified and reported in New York City (1904) in a stand of dying American chestnut trees. The fungus dispersed via spores carried in the air, raindrops, or by animals and entered Chestnut trees through any fresh injury in the tree's bark. The fungus spreads into the bark and underlying vascular cambium and wood, killing these tissues as it advances. The flow of nutrients is eventually choked off in sections of the tree above the infection, subsequently killing them. Only the root collar and root system of the tree are fairly resistant to the blight. Consequently, a large number of small American Chestnut trees still exist as shoots from existing root bases. However, the shoots seldom grow to reproduce before the blight attacks them (Freinkel 2007).

The socio-economic impact of Chestnut Blight's introduction was highly significant to the affected areas. Rural communities depended upon the annual chestnut nut harvest as a cash crop to feed livestock. In addition, the chestnut lumber industry was a major sector of rural economies. Chestnut wood was easily worked, lightweight, and highly rot-resistant, making it ideal for fence posts, railroad ties, barn beams, and home construction, as well as for fine furniture and musical instruments. In fact, for three centuries preceding the introduction of Chestnut Blight, most barns and homes built in the USA east of the Mississippi River were made from American Chestnut wood (Eilperin 2010).

The primary lesson learned from North America's experience with Chestnut Blight is that plant pests have the potential to be game-changers ecologically, economically, and socio-politically. Therefore, the very visible and rapid loss of the American Chestnut from the Eastern tree canopy became a symbol of the vacuum that existed in terms of the government's authority to act. Chestnut Blight was cited, along with several other pests, during U.S. Congressional hearings and deliberations about a lack of safeguards against the importation of infected and infested plants and nursery stock. The result was the passage of the Plant Quarantine Act of 1913.

While the USA apparently lost the battle with Chestnut Blight, this defeat laid the plant health regulatory groundwork to prevent future plant pest outbreaks. But, this story of the American Chestnut has not yet come to a close. Current developments in cross-breeding and biotechnology may enable the creation of resistant varieties of American Chestnut from remaining germplasm to support the eventual recovery of the tree in its native range (Shepherd 2009).

8.2.2 Tomato Potato Psyllid in New Zealand

Recent examples of devastating plant pests are evident elsewhere. When the Tomato Potato Psyllid (TPP), *Bactericera cockerelli* Sulc, was first reported in New Zealand in May 2006, very little was known about its potential effect on the country's horticultural environment. Further, nothing was known about the existence or effect of the *Liberibacter* on the psyllid vectors (Gill 2010).

TPP was detected first in glasshouse tomato crops and subsequently in glasshouse capsicums and field potatoes across four unrelated sites in the Auckland area of the North Island. Field populations at several of these sites documented the insect's ability to disperse quickly and widely. New Zealand's MPI determined that the most appropriate method of control and management of TPP was industry-based psyllid management on an individual crop/property basis. To promote this strategy, MPI prepared factsheets in consultation with industry and distributed them to growers to assist in managing the pest and to raise industry awareness of the pest (Ministry of Primary Industries 2009).

In 2007 TPP spread to the South Island where it was detected in the island's Nelson Region. In 2008 it was also found in outdoor tomato crops in the Hawkes Bay and Gisborne areas of the North Island. Ultimately, TPP's range may only be limited to those areas where winter temperatures are above 0°C, where the pest cannot survive below this temperature (New Zealand Fresh Vegetable Industry 2007).

To further complicate the situation, in 2008, a previously undetected pathogen, later named *Candidatus Liberibacter solanacearum*, was detected in glasshouse tomatoes during an investigation into the cause of unusual yellowing of tomato and capsicum plants (Liefting 2009). At the same time, reports from potato growers in the USA mentioned potato crops afflicted with a condition known as "zebra chip" (Crosslin 2010).

This condition leads to potatoes being rejected for processing and sale. A similar condition was observed in New Zealand potatoes with the same new-to-science bacterium found in New Zealand potatoes. Research showed that *Candidatus Liberibacter solanacearum* was vectored by TPP. (The New Zealand Institute for Plant and Food Research n.d.)

Initially, *Candidatus L. solanacearum* affected the fresh tomato and capsicum export markets. MPI immediately reported the detection of this organism in accordance with the IPPC agreement reporting obligations (EMPPO 2009). After researchers clarified questions relating to the mechanism of transmission of the *C. L. solanacearum*, the export trade was re-opened within a few months for most markets. The industry was relatively unaffected, although some smaller operators were impacted to a higher degree. Soon, however, a wider scale impact became apparent with potato growers seriously affected (Gill 2010). New Zealand's potato industry experienced reduced sprouting of tubers by approximately 23 % and significant loss in marketable yield by 30–40 % (Pitman 2011). The potato industry's Integrated Pest Management (IMP) programme was rendered ineffective as growers were forced to use harsher chemicals in an attempt to control TPP and subsequent impacts of the bacterium (Gill 2010).

Estimates of losses to potato production were understood to be economically significant but varied across sectors and seasons. Some potato producers were forced out of production or diversified to other crops. Glasshouse tomato and capsicum producers were largely unaffected as they could rely on physical exclusion practices to avoid the TPP (Gill 2010). Potato production in New Zealand is slowly recovering with the adoption of new approaches and the implementation of new pest management tools (Potatoes New Zealand 2011).

Both of the previous case studies make clear that the lack of complete information about a plant pest introduction often challenges an NPPO's ability to effectively eradicate or contain infestations. Little was known about the threat of Chestnut Blight to Canada and the USA early in the twentieth Century. The recent case of TPP and *C. L. solanacearum* in New Zealand illustrates the difficult decisions twenty-first Century biosecurity response managers' face when working with limited information. Out of necessity, they must operate in an environment where there will be uncertainty and they must assess risks as logically as possible and prioritize resources accordingly. Doing so successfully, as demonstrated in the case of TPP and *C. L. solanacearum* in New Zealand, requires close coordination between the NPPO, plant health researchers, and impacted growers. MPI coordinated closely with industry to:

- Monitor TPP and *C. L. solanacearum* impacts nationally;
- Inform the science community and growers of observed impacts;
- Guide the development of research programmes to investigate impacts, pathways of spread, and pest management development;
- Keep growers informed of scientific developments;
- Develop and communicate grower-level management recommendations;
- Monitor grower implementation of recommendation management and progress of control (Potatoes New Zealand 2010).

Such a communication strategy has been effective for MPI specifically and New Zealand horticultural growers, especially in situations when not a lot may be known about the potential range, host specificity, and extent of damage associated with a newly detected exotic plant pest. New Zealand is currently locked in an ongoing debate about MPI's record of success in excluding the entry of agricultural pests. Among other issues, between 2008 and 2012 the country has experiences a 14 % increase in the volume of international travelers and tourists while MPI has had to absorb a 12 % reduction in front-line biosecurity officers who screen these passengers at ports-of-entry. Nevertheless, MPI maintains a strong record of success communicating to and with growers, industry, and horticultural scientists. Further, MPI highlights the importance of biosecurity to New Zealand's significant agricultural economy and the general public (Lancashire 2012). In the face of recent economic realities and increased international trade and travel, MPI's communication strategies used in emergency pest response efforts, like TTP and *C. L. solanacearum* and, more recently, Queensland Fruit Fly, allows for the level of communication and coordination with producers and others necessary to ensure biosecurity vigilance when pest introduction occur.

8.2.3 Communicating with the Public to Offer Assurance That Pest Can Be Managed Safely

Raising public awareness about the impact of plant pests involves communicating with communities/stakeholders experiencing an outbreak. Stakeholders must understand that the tools used to manage or eliminate the pests pose no danger to public health or the environment. This becomes a critical primary message for an NPPO, and one that must be coordinated effectively with industry and scientists to ensure message consistency. Some members of the public may not realize that an exotic plant pest can do serious economic and environmental harm beyond damaging landscaping, gardens, and crops. Most members of the public know that chemical pesticides used to control pests in the past have been shown unsafe for humans and the environment. Accordingly, NPPO personnel responding to pest outbreaks must develop messages that identify public/stakeholder concerns and address these concerns. NPPO personnel should not rely on science alone, because some public/stakeholder concerns can be based on perception rather than fact. NPPO personnel should specifically learn who targeted audiences consider to be "trusted sources of information". NPPO personnel must understand how messaging can be crafted to cater to the interests of trusted sources by aligning scientific fact with the target audience's concerns and motivations. This action is often referred to as addressing the "human dimensions" of a situation.

A high level of concern for environmental protection exists in most urban and suburban communities. Misunderstanding or misinformation about the safety of pest management tools and operations used in response to an outbreak generates distrust within a community and effective management of harmful plant pests can become impossible. Popular concern for environmental issues (and potential underlying distrust of the safety of government-approved chemical/biological pest management tools) can be elevated within a community if an NPPO fails to communicate effectively and respond transparently to an outbreak.

Often an NPPO can be challenged by an underlying general distrust of government. This distrust of national-level government seems more common in the USA than elsewhere, but can still occur in each country, especially as it generally pertains to rural communities and aboriginal/first-nation communities that often maintain a legacy of mistrust and historical grievance with national governments. To help overcome distrust, NPPO officials should always seek to engage local and/or tribal officials, who are often perceived as most trustworthy within the community.

8.2.4 Origins of Public Skepticism About the Safety of Pest Management Tools

Rachel Carson's book *Silent Spring* was published in 1962. It documented and raised alarm that unrestricted use of chemical pesticides to control insect pests was negatively impacting the health and survival of animals, especially birds, and humans. This book became a New York Times best-seller and stirred widespread public concern about the environmental damage caused by chemical pesticides and other man-made pollutants since the early 1940s. The pesticide DDT was noted to be of particular concern as Carson documented it to be the cause of eggshell thinning that resulted in reproductive problems and death among exposed bird species, including the iconic American bald eagle, national symbol of the USA (Graham 1978).

Silent Spring also offered a strong indictment of the chemical manufacturing industry for concealing evidence of the harmful effects from DDT exposure, and public officials for uncritically accepting industry claims of DDT's safety. *Silent Spring* advocated the banning of DDT and other harmful chemicals pesticides. Carson recognized a need and role for helpful pesticides as long as they were used responsibly and their safety was scientifically verified before use and monitored closely for unintended environmental impacts following their application (Lewis 1985).

The international popularity of *Silent Spring* (and the important story it detailed concerning the environmental impact of agricultural pesticides) is arguably the genesis of the importance of biosecurity communications. Upon reading *Silent Spring* the public developed an acute awareness of the broader impact of agricultural pest management. Unfortunately, public awareness about the use of DDT and similar hazardous products was accompanied by a mistrust of agricultural pest management practices (especially associated with high-yield crop production) and skepticism over governments' ability to effectively and safely regulate the use of pesticides. At best, this mistrust manifests itself in public and environmental interest-groups demanding vigilant government oversight to confirm that agricultural pesticides are used safely and responsibly and pose no significant risk to human health or the environment. At worst, these groups can fan public fears to the point that low to zero tolerance of the use of government-approved pesticides can threaten the health and viability of the crops they are designed to protect.

8.2.5 Origins of National Government's Role in Environmental Protection

Since the publication of *Silent Spring*, a new global environmental awareness has developed, marking the beginning of the modern environmental movement. Carson's book elicited a public outcry in the USA for direct government action to protect America's natural environment, including the Clean Air Act of 1963. Subsequently, the U.S. Congress authorized the Federal government to "create and maintain conditions under which man and nature can exist in productive harmony," and to "assure for all Americans safe, healthful, productive, esthetically, and culturally pleasing surroundings" through the passage of the National Environmental Policy Act (NEPA) of 1969. Under NEPA, all Federal agencies planning projects with potential to impact the environment are required to submit reports, formally known as "Environmental Impact Statements" (EISs), accounting for the likely consequences of each project. During July 1970, the U.S. Federal Government created the Environmental Protection Agency (EPA) from smaller units of several Federal agencies. EPA serves as an independent agency that comprehensively regulates the pollutants that harm human health and degrade the environment. In June 1972, EPA cancelled nearly all remaining Federal registrations for the use of DDT products, essentially banning its use in the USA (Lewis 1985).

During 1972, the United Nations convened the Stockholm Conference on the Human Environment. This conference represented the first multi-lateral governmental dialogue concerning global environmental protection and conservation. The conference resulted in the Stockholm Convention, an international treaty to enact global bans or restrictions on DDT and other chemicals classified as "persistent organic pollutants." However, the Convention limited restrictions for the use of DDT to control mosquitoes to support efforts to combat the insect as a vector of malaria in the developing world (United States Environmental Protection Agency 2012a).

Other governments have also moved to establish their environmental protection authorities. For example, in 1971, Australia's Federal Government established its' first Environment Department (under Prime Minister William McMahon). After several variations through succeeding governments, the Department of Sustainability, Environment, Water, Population and Communities (DSEWPC) was established (September 14, 2010). DSEWPC develops and implements national environmental policy and programmes, including the enforcement of the

Table 8.2 Some environmental protection laws pertinent to plant biosecurity

Australia
Environment Protection and Biodiversity Conservation Act
Agricultural and Veterinary Chemicals (Administration) Act
Canada
Pest Control Products Act
Canadian Environmental Protection Act
New Zealand
Resource Management Act
Hazardous Substances and New Organisms Act
Agricultural Compounds and Veterinary Medicines Act
United States of America
National Environmental Policy Act
Federal Insecticide, Fungicide, and Rodenticide Act
Endangered Species Act

Sources: (DSEWPC 2011; Australian Government 2011); (Health Canada 2011); (New Zealand Ministry for the Environment 2012; New Zealand Ministry for the Environment 2011; New Zealand Ministry for Primary Industries 2012); (United States Environmental Protection Agency 2012b)

Environment Protection and Biodiversity Conservation Act 1999 (DSEWPC 2012). This Act is Australia's central piece of environmental legislation. It provides a legal framework to protect and manage nationally and internationally important flora, fauna, ecological communities, and heritage places defined in the Act as matters of national environmental significance (DSEWPC 2010).

DSEWPC also serves as an advising agency to the Australian Pesticides and Veterinary Medicines Authority (APVMA). APVMA is a statutory authority, established in 1993, that centralizes the registration of all agricultural and veterinary chemical products in the Australian marketplace (APVMA 2008).

These, other national environmental protection laws (Table 8.2), and their state/province equivalents, all oblige NPPOs to adhere to legal requirements scientifically designed to protect human health, the environment, biodiversity, and offer evidence that pest management tools meet or exceed the conditions or tolerances required under law.

Often, laws provide requirements for public disclosure that compel NPPOs to be transparent in adhering to environmental protection laws and monitoring the impact of pest management projects. This helps overcome public skepticism about the safety of applied pest management tools, but may not do so in some cases. Additional outreach and communication may be necessary to show how an NPPO's adherence to relevant environmental protection laws ensures that pest management is conducted with minimum impact on environmental quality and maximum safety for human and animal health. Failure to adequately communicate adherence to environmental laws can generate public skepticism about the safety and impact of an NPPO's actions. This can result in governmental or court mandates to facilitate transparency and accountability, thus requiring more resources and effort to overcome solidified public skepticism. In extreme cases this may result in governmental or court restrictions that hinder pest prevention and response efforts.

8.3 Messaging

An NPPO must communicate with stakeholders, build plant biosecurity awareness through public outreach, and influence public policymaking (Table 8.3). A simple and compelling message is the foundation of effective communications. Creating a message involves four elements: Identifying the audience; determining the purpose of the message; crafting the wording of the message; and delivering the message (Curlett 2010). Sometimes an additional step is necessary: Receiving feedback from the target audience after message delivery. Feedback is critical for guiding policymaking and communicating future messaging (Zimmers 2011).

A critical aspect of an NPPO's success in the message-building process lies in its ability to maintain close collaboration between its public relations professionals, policy and legal advisors, technical experts, and leadership during message making. The messages must be accurate, appropriate, clear, concise and consistent (Curlett 2010).

The following information provides a basic understanding of the process used to develop and deliver key messaging used by NAPPOs to communicate about plant biosecurity issues. Using these elements ensures that any future role played in developing messaging to support plant biosecurity (as a NAPPO representative, cooperator, or stakeholder) will maximize the effectiveness of the communication being delivered and achieve its purpose.

Table 8.3 Elements of effective stakeholder communications

Identify the audience:
With which stakeholders are you communicating?
Do you know what these stakeholders want or need to know?
Determine the message's purpose:
What is your objective of this communication?
What ideas do you want to get across to stakeholders?
Are you seeking to change stakeholder behaviour with this communication?
Carefully craft the message:
Is your message clear and concise?
Is your message tailored to the intended audience?
Do your stakeholders understand the terminology you are using?
Determine how best to deliver the message:
Have you selected the appropriate communication medium for the message?
Are you proactive in providing information, or is this information being delivered in reaction to an issue or event?
Obtain stakeholder feedback:
Was the message understood?
Did the message achieve your purpose?
How did stakeholders react to the message?

Source: (Curlett 2010)

8.3.1 Identify the Audience

The first step in creating an effective message involves identifying the intended audience of the message. Avoid identifying the audience in broad, vague or general terms ("general public" or "farms"). Identify the audience in well-defined groups (e.g., recreational campers, citrus packinghouse operators, Northwestern apple growers, and cut flower importers).

The message must address audience needs, preferences, and concerns. The message writer must make contact with individuals and organisations that have a clear understanding and close working relationship with the intended audience. Contacts include local level NPPO officials, State or Provincial government counterparts, or representative industry groups or associations. On-line information and social networks can also be useful reference sources for understanding a targeted audience. Additionally, depending on the time and resources available, NPPOs can work with internal or external public relations professionals to conduct focus groups or surveys to better understand an intended audience, their preferences, concerns, and core values (Curlett 2010).

8.3.2 Determine the Message's Purpose

After the targeted audience is identified, the NPPO must determine what purpose it hopes to achieve by conveying the intended message. One purpose could be to raise awareness among stakeholders about a specific plant pest threat. The message also could explain the nature and impact of the pest, offer a description, and inform stakeholders how to report such a pest when found. The message also gives stakeholders tips on appropriate biosecurity measures they can take to prevent the pest from damaging their crops, gardens, landscape, or property (Curlett 2010).

Another purpose could be to describe specific biosecurity counter-actions being planned or taken by the NPPO and cooperators to manage or eliminate a plant pest outbreak. The objective of this purpose is to build trust through transparency of actions. This includes assurance that all appropriate environmental laws are being observed related to the use of pest management tools and scientific evidence that the tools are safe and effective. The message must be specific about when and where tools are used and should include contact information for people in the area where chemicals are applied to use to seek additional information.

An additional purpose could be to influence or promote behavioural changes within an industry or community that helps enhance plant health biosecurity. The NPPO may appeal to the target audience to voluntarily change behaviours in the interest of mitigating pest risks to their own benefit; the NPPO may inform the audience that the behaviour change is stipulated by regulatory requirements for the greater good of all stakeholders. Such messaging must clearly specify the behaviour change being sought, the benefits of making the behaviour change, and the potential

Table 8.4 Responsibilities of key players in stakeholder communications

Public relations (PR) professionals: PR professionals ensure that the message is clear, concise, and appropriately targeted to the intended audience. These professionals are experts in designing a message that is attention getting, memorable, and most likely to influence the opinions and actions of the targeted audience. Through their role as the primary point of contact with external audiences (stakeholder groups, the media, and the public), PR professionals also ensure that the NPPO's message is consistently communicated
Policy and legal advisors: Policy and legal advisors ensure that the NPPO's message is consistent with all relevant legal authorities and policies to which the NPPO is bound
Technical experts: Technical experts provide the operational and scientific foundation of the information used to determine the purpose of the message, the intended audience, and the specific information underlying the messages' context. Technical experts ensure the accuracy of the message
NPPO leadership: NPPO leadership brings a political understanding that contributes to tailoring the message appropriately to the intended audience's needs and concerns, as well as fitting the message within the context of the broad goals of the NPPO's policy and mission. The NPPO leadership is the final authority in determining that the message is appropriate and will serve its intended purpose. If leadership approves the message, then it will be communicated. Otherwise, the message must be re-crafted until it meets the NPPO leadership's expectations

Source: (Curlett 2010)

consequences if individuals within the target audience fail to make the change. In cases where the change is mandated by the NPPO as a regulatory requirement, the message should clearly indicate that failure to comply will result in enforcement action and possible civil penalties (Zimmers 2011).

One, all, or a combination of these purposes may be pursued by the NPPO through messaging as a way of cultivating stakeholder and public understanding, support, and participation in plant biosecurity activities. However, in plant health emergency situations messaging must address all of these purposes through timely and consistent communications. Crisis communication is necessary to alleviate uncertainly, fear, and anger among stakeholders (including the public) in response to major pest outbreaks, other significant breaches of plant biosecurity, or reactions to plant biosecurity activities themselves.

8.3.3 Craft the Message

Effective message-building is a collaborative process that must involve an NPPO's public relations professionals, policy and legal advisors, technical experts, and leadership. Each collaborator brings an important skill or level of knowledge necessary to craft an effective message (Table 8.4).

The foundation of plant biosecurity is rooted in science and most of an NPPOs messaging will contain scientific or technical concepts. Australia, New Zealand, and the USA have a comparable basic literacy rate (99 % of a population above age 15 years meets the basic definition of literacy). Nevertheless, many scientific aspects of plant biosecurity are beyond the general public's reading and scientific comprehension (CIA 2012a, c, d). Thus, messaging must be communicated so that all stakeholders (including the public) can easily grasp the issues involved and their impacts. To achieve this level of general understanding and clarity, NPPO technical experts must work with and follow the advice of their public relations professionals.

The message map is a useful tool for crafting clear, concise and effective messages. A message map identifies three core messages about an issue, policy, or action. Then, each core message is reinforced by three supporting messages. Message mapping assumes that 95 % of all questions and concerns from a specific target audience of stakeholders can be anticipated. Three core messages can be developed to address the fundamental (overarching) aspects of each anticipated question/concern. The three core messages should be ordered by descending importance. To ensure brevity and utility as audio/visual media sound bites, each core message should be restricted to no more than 27 words total. Each core message should be easily understood by anyone with a 6th grade (primary school) reading level. Each core message should be able to stand-alone. Each core message should avoid absolutes and unnecessary negative statements (Covello 2005).

The next step to message mapping develops three supporting messages for each core message. The supporting messages must consist of factual information that, ideally, can be accredited to credible third parties and/or include sources to obtain additional information. Visual aids can also serve as strong evidence in a supporting message. Like the core message, each supporting message should be restricted to no more than 27 words and avoid absolutes and unnecessary negative statements (Covello 2005).

An example of message mapping follows:

Anticipated Question or Concern – How can our country's NPPO safeguard against the heightened risk of entry by invasive pests associated with increased numbers of international travelers/tourists arriving during the summer?

Core Message #1 – We urge summer travelers to join us in the fight against invasive pests by not bringing a pest in their luggage or clothing.

Supporting Message #1-A – Overseas agricultural products make tempting souvenirs, but invasive pests can hitchhike on fruits, vegetables, meats, processed foods, plants, and handicraft items.

Supporting Message #1-B – If invasive pests become established, then they can devastate urban and rural landscapes, potentially costing billions of dollars in lost revenue and eradication efforts.

Supporting Message #1-C – The total value of our nation's agriculture, fishing and forestry industries is about $157 billion. These resources must be protected.

Core Message #2 – We exercise the authority to restrict or prohibit the entry of certain agricultural products from foreign countries to prevent the entry of invasive pests.

Supporting Message #2-A – We will inspect your baggage when you arrive in our country to ensure that any agricultural items you carry are allowable under our biosecurity regulations.

Supporting Message #2-B – To assist us in safeguarding against invasive pests, be sure to declare all agricultural items you are carrying in your baggage at the first port of entry.

Supporting Message #2-C – Failure to declare food products can result in fines and penalties.

Core Message #3 – We encourage travelers to know restrictions pertaining to agricultural products before entering our country and to use these as guidelines when purchasing souvenirs.

Supporting Message #3-A – You may be allowed to enter with certain fruits, vegetables, animal products, plants and plant materials, depending on the item and its country of origin.

Supporting Message #3-B – All allowable agricultural products must still be declared and presented to an agricultural biosecurity specialist or customs officer for inspection.

Supporting Message #3-C – For comprehensive information on importing agricultural items for personal use, visit our Webpage for Agricultural Information for International Travelers.

8.3.4 Message Delivery

After the audience is identified, the purpose of the message is determined, and the message is crafted, the NPPO can then decide how best to deliver the message. Many optional techniques exist for message delivery. These techniques can be put in three categories of communications: Reactive, Proactive, and Sustained (Box 8.3) (Curlett 2010).

Reactive communication can follow the occurrence of a significant event, such as plant pest outbreaks or disruption of domestic exports due to phytosanitary considerations. Reactive communications involve the NPPO issuing a message or messages to clarify, explain, or interpret information related to the event. Typically reactive messages are delivered by press releases, media alerts, press conferences and official notices to State, Provincial, or Territorial agricultural regulatory officials.

Proactive communication involves planned and strategically aligned efforts to disseminate information through messages designed to enable an NPPO to achieve its objectives. Because this category of communications is not dictated by events, an NPPO has more time to target a specific audience, design an appropriate message, and deliver that message using several different integrated techniques. Proactive communication is most conducive to facilitating behaviour change within a target audience.

Box 8.3 Categories of Communications

- Reactive Communications
- Proactive Communications
- Sustained Communications

Examples of proactive communications include: (1) Alerting importers and ports about a new exotic pest pathway threat, (2) informing stakeholders about regulatory policy changes, or (3) general efforts to raise public awareness about invasive plant pests. Outreach campaigns are concerted efforts to direct messaging to a specific target audience or the general public for the purpose of raising awareness and influencing actions concerning critical issues. Outreach campaigns are an especially useful form of proactive communication.

Sustained communications consist of periodic or routine messaging to an NPPO's stakeholders, cooperators, or international counterparts on a continuous basis. Sustained communications can be carried out via regularly scheduled meetings or bilateral contacts, newsletters, dedicated and routinely updated websites and blogs.

Proactive and sustained communications are the most effective and desirable categories of communication to apply to successfully engage stakeholders, trading partners, and the public in supporting efforts designed to achieve strategic plant biosecurity goals and objectives. Reactive communications presents NPPOs with the greatest challenge due to the need to craft and deliver messages rapidly and effectively. If reactive communications is performed successfully, then the NPPO will build trust among impacted stakeholders and members of the public. This trust is necessary to respond to plant health emergencies and other critical events effectively. Otherwise, the NPPO will lose trust and the support of stakeholders and the public.

8.3.5 *Obtain Feedback*

Feedback is an important extra step intended to ensure that messaging reaches the target audience and resonates with them. Obtaining timely feedback is critical to determining whether an NPPO's communication efforts are achieving their purpose and supporting the NPPO in meeting its strategic-level plant biosecurity goals and objectives. NPPOs should routinely strive to obtain feedback after message delivery. Feedback gauges the effectiveness of recent NPPO communications and can guide the direction of policymaking and communicating future messaging (Zimmers 2011).

Gathering feedback must not be an end to the process of message development and delivery. Feedback enables programme managers and staff to understand and respond to the needs of stakeholders and the public. Obtaining feedback requires appropriate follow-up from the NPPO. Feedback must be collected, analysed, and used tactically to guide future communication efforts and strategically influence policymaking. Feedback without follow-up is a meaningless exercise.

NPPOs can solicit feedback from their stakeholders and the general public using many techniques. Collaborating with public relations professionals in organising focus groups or surveys that seek feedback from a cross-section of stakeholders is a proven method of engagement. The use of Internet-based formats such as Webpages and blogs often provide links that allow visitors to provide feedback (Curlett 2010). Laws governing regulatory processes like the Administrative Procedures Act in the USA or the Federal Regulatory Policy in Canada require that the public be given an opportunity to comment before new regulations or amendments to existing regulations are finalized. In Australia and New Zealand, this same concept is built into the public consultation obligations of their Cabinet processes; in addition to the Federal Regulatory Policy, Canada also utilizes consultation as an element of their Cabinet process (Gill 2010).

Feedback, can also be obtained less formally through routine contact and communication with stakeholders. Another significant avenue of obtaining feedback indirectly involves communication with elected officials who represent concerned stakeholders and members of the public within their constituency.

8.4 Stakeholders

Stakeholders are defined as people who have a stake (interest, responsibility) in an enterprise or who are involved in or affected by a course of action. In terms of NPPOs, the list of stakeholders is very long. The list starts with farmers and other growers who represent the production side of agriculture (growing fruits, vegetables, grain, flowers, nursery stock, or timber). Next, consider the various support industries that move or add value to production (fruit packers, grain elevator operators, processors, transporters, brokers, exporters and importers). Finally, consider the retail markets that ultimately sell agricultural commodities to consumers, and members of the general public who (as consumers, taxpayers or activists) share an interest in NPPO biosecurity efforts to safeguard the health of agricultural production. Every link in this stakeholder chain has offshoot service providers that directly or indirectly depend upon or benefit from plant production (including families dependent on salaries and communities dependent on commerce and taxation) (Zimmers 2011).

8.4.1 The Agricultural Sector's Economic Role

To realize the range of people considered agricultural stakeholders, we must understand the scope of the agricultural sector's importance to the economies of nations. For example, consider Australia, Canada, New Zealand, and the USA. Statistically, agriculture represents a significant segment of all four national

Table 8.5 Agricultural contribution to national gross domestic product (Est. 2011)

Country	Percentage of GDP (%)	Value $US (Billions)
Australia	4	$59.5
Canada	1.9	$33
New Zealand	4.8	$7.8
USA	1.2	$181
Total		$281.3

Source: (CIA 2012a, b, c, d)

Table 8.6 Agricultural composition of total labour force (Est. 2011)

Country	% Labour force	Number of workers
Australia	3.6	434,000
Canada	2	373,000
New Zealand	7	165,000
USA	0.7	1,075,000
Total		2,047,000

Source: (CIA 2012a, b, c, d)

Table 8.7 Export value of total agricultural products 2009

Country	Value $US (Billions)
Australia	$21.8
Canada	$31.1
New Zealand	$13
USA	$101
Total	$166.9

Source: (FAO UN 2012b)

economies. Together, the four countries' agricultural sectors generate an estimated $281 billion in 2011 (Table 8.5) and employ over two million workers (Table 8.6).

Globally, Canada and the USA are ranked as top producers of many key agricultural commodities. For instance, in 2010, the USA was the top producer of fresh cow milk, meat from cattle and chickens, corn, soybeans, strawberries, and eight other key commodities. Canada was the top producer of mustard seed, lentils, oats, and four other key commodities in 2010. Further, with the addition of Australia and New Zealand, the four countries are also ranked among the top five world producers of 71 other key commodities, ranging from Kiwi fruit and sheep meat in New Zealand, blueberries and cranberries in Canada, chick peas and greasy wool in Australia, and apples and oranges in the United States (FAO UN 2012a).

The value of the four countries' exports of total agricultural products in 2009 accounts for $166.9 billion (Table 8.7). Strictly in terms of crop commodities, Australia, Canada, New Zealand, and the USA are well represented as top global exporters (Table 8.8). In fact, American soybean exports are rated as the number one export commodity globally, with a total value of over $16.5 billion.

These statistics illustrate the importance of the agricultural sector in these countries for their economies, generating jobs and supporting communities.

Table 8.8 Top crop commodity exports by value 2009

Country	Commodity	Value $US (Billions)
USA	Soybeans	$16.5
Canada	Wheat	$5.3
Australia	Boneless beef and veal	$3.3
New Zealand	Dried whole milk	$1.9

Source: (FAO UN 2012b)

8.4.2 Industry Stakeholders

The producer is the foundation of the agricultural economy, whether he/she is a hobby farmer, full-time farmer, or owner of an intensive farm operation. The producer is responsible for crop production and routine on-farm biosecurity (e.g., best practices to facilitate healthy crops and protect against pests). Moreover, the producer has the most at stake, their business and their livelihood, in the face of plant health threats.

Producers act on their own behalf to promote and protect their interests. Often producers join industry associations representing their collective interests based upon the type of crops they grow and the geographic area where their production operations are located (e.g., county, state, province or territory). These collective industry associations provide member-producers more political influence in protecting common interests than acting individually, especially for producers that do not operate high-intensity or high-value farming operations. The function of industry associations are similar in Australia, Canada, New Zealand, and the USA, but their styles of operation often differ based upon size of the country, scope and diversity of agricultural production, and legal conditions for accessing and influencing policymakers.

In Australia, primary production industries have national and state/territorial organisations that advocate on behalf of their constituent members. In addition, many of the national organisations have state/territorial structures. National, state, or territorial organisations may also be organised along product-sector lines (Gill 2010).

In New Zealand, primary production industries have national organisations that advocate on behalf of their constituent members. Many of these national organisations have provincial or regional structures. National organisations may also be organised along product-sector lines. In addition, several indigenous-industry groups exist to look after the interests of Māori industry participants (Gill 2010).

Examples of prominent Australian Industry Groups:

National Farmers Federation;
Canegrowers Australia;
Australian Dried Fruits Association;
Ricegrowers' Association of Australia;
Cotton Australia;
NSW Farmers Association;
Tasmanian Farmers and Graziers Association;
Grain Producers Australia;
Growcom (Queensland Horticulture).

Examples of prominent New Zealand Industry Groups:

Avocado Industry Council Ltd;
Federated Farmers of New Zealand Inc.;
Horticulture New Zealand;
New Zealand Citrus Growers;
New Zealand Farm Forestry Association;
New Zealand Forest Owners Association;
New Zealand Kiwifruit Growers Inc.;
New Zealand Winegrowers;
Pipfruit New Zealand Inc.;
Summerfruit New Zealand.
(Gill 2010)

In the USA, primary production industries have national, regional, state, or local organisations that advocate on behalf of their constituent members. In addition, many of the national organisations have state structures. The multi-layered structure of industry groups in the USA increases the complexity of communications because these groups tend to be decentralized and different regional segments of the same industry can differ in their perspectives. National, regional, state, or local organisations may also be organised along product-sector lines. In addition, the United States government has obligations to Native American tribes that must be observed and many federally recognized tribes participate in intertribal groups that exist to look after the specific interests of tribes (Zimmers 2011).

Prominent USA Industry Groups:

Napa Valley Grape Growers Association
American Farm Bureau
Florida Citrus Mutual
Intertribal Agricultural Council
United Fresh Produce Association
American Nursery and Landscaping Association
National Potato Council
New York Apple Association
California Avocado Commission
Western Growers Association

Source: (Zimmers 2011)

8.4.3 Public Stakeholders

Agricultural producers may be an NPPO’s primary stakeholder, but the public-at-large remains the ultimate constituency in the democratic nations of Australia, Canada, New Zealand, and USA. In areas related to plant biosecurity activities, this public plays a principal, diffuse, and often divergent role in influencing policy as it relates to many aspects of public life beyond agricultural production, including commerce and trade, environmental protection, human health, transportation and travel, home gardening and landscaping, and jobs.

In rural areas, producer groups generally wield a high level of influence over public opinion, given that producers live among rural communities and contribute significantly to rural economies. However, producer influence can be significantly limited among the public in urban and suburban communities, especially in regard to environmental and public health issues related to plant biosecurity activities. Subsequently, there are instances where producer groups may overwhelmingly support the actions of an NPPO and its Regional, State, Provincial, or Municipal cooperators in managing plant pests, but broader public questions and concerns about the activity's impact on human health and the environment can take precedence over the economic consequences of infestation (Curlett 2010).

8.4.4 Social Media and Networking

The ability to organise a significant section of the public to make or break public support for or against plant biosecurity activities is now enhanced and complicated through online social networking. In the past, small groups of vocal activists could organise at the grassroots to make their views heard through letter campaigns, demonstrations, participation in public meetings, and generate media coverage. Today, however, the ability for these groups to organise rapidly and broadly and get out their message using social networking is unprecedented.

The experience of responding to the introduction of Light Brown Apple Moth (LBAM), *Epiphyas postvittana* (Walker) (Tortricidae), in the USA serves as an example of the power social networking has in influencing biosecurity policy towards the management of invasive pests. In response to the first detection of LBAM in the State of California in 2006, APHIS and the California Department of Food and Agriculture (CDFA) cooperatively initiated a management strategy that included the aerial dispersal of a pheromone in the heaviest infested areas, which happened to include wide residential areas. This straight-chain Lepidopteran pheromone was developed exclusively to disrupt LBAM's ability to mate, which would eliminate LBAM populations after several life-cycles (USDA APHIS PPQ 2008).

APHIS and CDFA maintained that comprehensive studies have shown that the LBAM pheromone, which was used safely for a decade previously in Australia and New Zealand, does not pose any risks to human health or the environment. Therefore the pheromone strategy was considered by APHIS and CDFA as a preferable pest management solution over traditional pesticides (USDA APHIS PPQ 2008). However, opposition to aerial dispersal of LBAM pheromone grew quickly among residents, environmental activists, and local elected officials who were concerned about the potential health impact of the pheromone's chemical ingredients and encapsulation material when biodegrading. This opposition coalesced quickly through effective use of the Internet and social media sites.

Opposition demanded conclusive evidence that aerial dispersal of the pheromone was harmless to humans (Wood 2008).

Websites, such as the Monterey County-based environmental advocacy group "Helping Our Peninsula's Environment" (HOPE), gathered and distributed information supporting opposition to aerial pheromone dispersal. This information included claims of adverse health effects (e.g., skin rash, headaches, nausea, chest pains, and asthma attacks) reported by members of the public after earlier aerial pheromone dispersals in Monterey and Santa Cruz Counties. HOPE also made claims countering APHIS and CDFA-accepted evidence that LBAM was not present in the USA before the 2006 California first-detection and that LBAM is a harmful pest of agriculture. Such Websites supported local elected officials who opposed the use of LBAM pheromone, informed followers about the schedule of public meetings discussing the issue, coordinated letter campaigns and petition efforts, and raised funds to support successful legal challenges against APHIS and CDFA's strategy in civil courts on environmental protection grounds (Helping Our Peninsula's Environment 2011). Court injunctions and public opposition to aerial pheromone applications resulted in LBAM spreading extensively in California. By 2010 eradication was deemed no longer feasible by APHIS and CDFA until new pest management tools (such as Sterile Insect Technology, see Sect. 14.4) were fully developed and ready for widespread use. Therefore, APHIS shifted to a control and suppression strategy to ensure that LBAM would not spread to other states or foreign trading partners (APHIS 2010).

Social networking has significant implications for the plant biosecurity regulators. Information on the Internet travels rapidly but can be inaccurate, allowing misinformation to spiral out of control before the more sluggish mechanism of government public relations efforts can respond. Social media is often used to motivate people against government action but is not used as effectively by governments to motivate people toward positive action. This situation, however, is changing as NPPOs explore the ways and opportunities of using social media as a communication tool (Curlett 2010).

Some examples of the use of social media to support networking around biosecurity and pest management communications are APHIS' Hungry Pests (http://www.hungrypests.com/), Beetle Busters (http://beetlebusters.info/) and Save our Citrus (http://www.saveourcitrus.org/) websites (Figs. 8.1, 8.2, and 8.3). These sites employ quality public service announcement (PSA) videos, pest descriptions with visual aids, quarantine maps, news updates, links to other topical Websites, and the ability to access YouTube for additional PSAs and Facebook or Twitter to become a follower (USDA APHIS 2012a, b, c; APHIS 2010).

These websites offer individuals the opportunity to obtain information about key pest threats to the USA (all invasive species, including Asian Longhorned Beetle (Chap. 16) and citrus pests (Chap. 18)) in various and sometimes interactive formats. The websites also enable individuals to become followers of the sites by subscribing to electronic newsletters (being alerted to new information postings) and provided opportunities to photograph and directly report potential pests to APHIS via a smartphone app.

Fig. 8.1 Hungry pests website (Source: USDA APHIS 2012b)

Fig. 8.2 Beetle Busters website (Source: USDA APHIS 2012a)

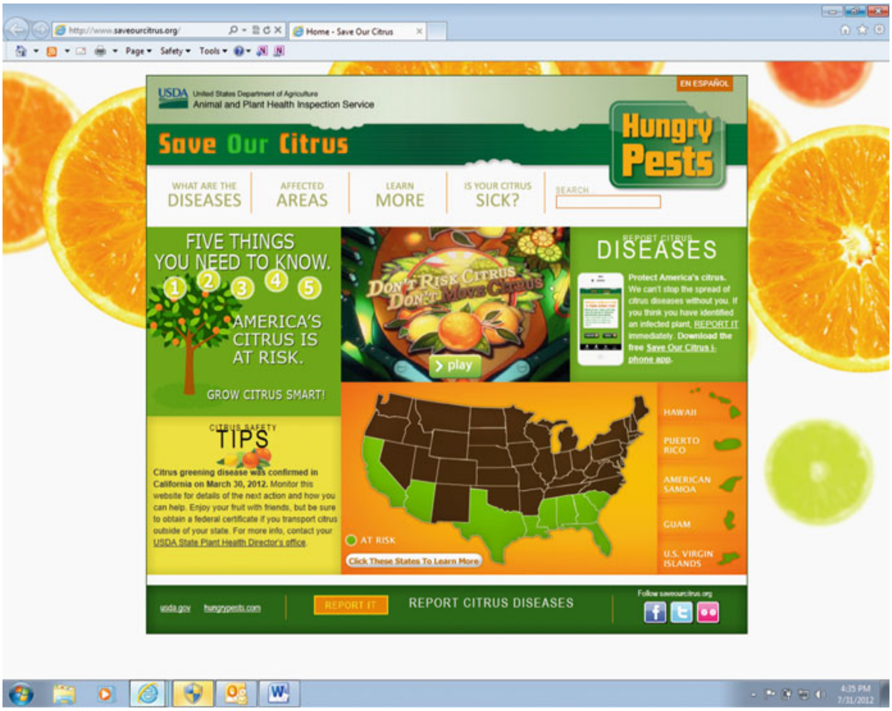

Fig. 8.3 Save our Citrus website (Source: USDA APHIS 2012c)

Social media and networking technology offers NPPOs the opportunity to connect directly with the public and clearly communicate the threat of invasive pests and the importance of biosecurity and pests management in multiple formats. This can help simplify a very complex subject for the average person, especially individuals who have no direct connection to farming and other forms of agricultural production. Nevertheless, we must caution NPPO users of social media to take great care in ensuring the accuracy and clarity of the information presented. When this information is released into the network, it certainly will be passed to many other networks throughout the Internet (Curlett 2010).

The enormous benefits and potential risk of social media and networking highlights the fundamental importance of messaging. The critical elements of messaging (identify the target audience, determine the message's purpose, carefully craft the message, determining how best to deliver the message, and obtain stakeholder feedback) should always be followed to ensure NPPOs communicate agricultural biosecurity information clearly, concisely, and effectively. To ensure communications remain consistently successful, NPPO officials must work closely with their public relations professionals in concert with NPPO policy and legal advisors, technical experts, and NPPO leadership. The NPPOs of Australia, New Zealand, and USA are committed to transparency with their constituency, both among industry and throughout the public. This commitment also extends to communicating with each other and the NPPOs of our trading partners. Communications and outreach is an essential role of a NPPO in effectively ensuring agricultural biosecurity.

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Chapter 9
The Role of Pest Risk Analysis in Plant Biosecurity

Mark Burgman, Bill Roberts, Claire Sansford, Robert Griffin, and Kerrie Mengersen

9.1 International Context for Pest Risk Analysis

The International Plant Protection Convention (IPPC) is an international treaty for plant health formed in 1951 to minimise plant pest risks associated with trade. The IPPC Commission adopts International Standards for Phytosanitary Measures (ISPMs), facilitates information exchange, provides for non-binding dispute settlement and promotes capacity building in plant health (Sect. 2.2.4). ISPMs are the standards, guidelines and recommendations used as the basis for phytosanitary measures applied by contracting parties to the IPPC. These same standards are also recognised by the World Trade Organization in the Agreement on the Application of Sanitary and Phytosanitary Measures which indicates that phytosanitary measures applied by governments in commerce must be based on international standards or risk assessment (SPS Agreement, WTO 1995). The ISPMs provide guidance to countries on the application of measures to protect plants and plant products from 'pests' (including pathogens) that can be moved in the course of trade (Hulme 2011).

M. Burgman (✉)
Centre of Excellence for Biosecurity Risk Analysis, School of Botany, University of Melbourne, Parkville 3010, Australia
e-mail: markab@unimelb.edu.au

B. Roberts
Plant Biosecurity Cooperative Research Centre, Bruce, ACT 2617, Australia

C. Sansford
Plant Protection Programme, The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK

R. Griffin
Pest Epidemiology and Risk Assessment Laboratory, Center for Plant Health Science and Technology, Plant Protection and Quarantine, APHIS, USDA, Raleigh, NC 27606, USA

K. Mengersen
Mathematical Sciences, Queensland Institute of Technology, Brisbane, Australia

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ISPMs most pertinent to Pest Risk Analysis (PRA) are ISPM 2 (*Framework for pest risk analysis*, FAO 2007), ISPM 11 (*Pest risk analysis for quarantine pests, including analysis of environmental risk and living modified organisms*, FAO 2004a), and ISPM 21 (*Pest risk analysis for regulated non-quarantine pests*, FAO 2004b). The main elements of PRA are shown in a flowchart (Appendix 1) of ISPM 2 (FAO 2007). This is reproduced in Fig. 9.1 and described further under 'Methodology for PRA' below. Terms used in the ISPMs are defined in ISPM 5 (*Glossary of Phytosanitary Terms*, FAO 2010, Table 2.1).

The SPS Agreement recognises three international standard-setting bodies: The *Codex Alimentarius* for food safety issues, the *World Organisation for Animal Health (OIE)* for animal health and the IPPC for plant health. The central objective of the SPS Agreement is to promote safe trade by ensuring that the measures applied by governments to international commerce for the purpose of protecting plant, animal, and human health have a rational, scientifically supportable basis. Surrounding this objective are several important principles to ensure the use of sanitary and phytosanitary measures in a non-discriminatory, non-protectionist, transparent and harmonized way with minimal impact on trade. In this context, trade-restrictive measures must be scientifically justified. Article 5 of the SPS Agreement (Appendix 1) refers to the assessment of risk and determination of the appropriate level of sanitary (human and animal health) and phytosanitary (plant health) protection, to address the risk. It provides general guidance on factors that should be considered in assessing the risks posed by plant pests. Jurisprudence associated with the SPS Agreement has identified an approach to setting measures based on risk assessments that includes three steps: (1) Identify the pests of concern; (2) Evaluate their likelihood of entry, establishment and spread, and the associated potential consequences; and (3) Re-evaluate the likelihoods and consequences against potential SPS measures.

In determining the measures ('protection') to be taken, the SPS Agreement refers to the *Appropriate Level of Protection* (ALP or ALOP). This is defined in Annex A of the SPS Agreement as: "*The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory*". Some countries refer to this concept as the '*acceptable level of risk*' (ALR). The term ALR was used early in the negotiation of the SPS Agreement but was replaced by ALP except for a clarifying note in Annex A. Under the SPS Agreement, countries have the sovereign right to set their own policy (ALP) in regard to how much risk they are prepared to tolerate (their ALR) within the disciplines of SPS principles. The ALP provides a guide for the application of measures based on the consistency and justification for the range of regulatory policies that may be associated with trade.

SPS jurisprudence has also confirmed that the strength of measures is justified in proportion to the assessed level of risk (Sansford 1998, unpublished; EPPO 1998). In this context, WTO members are required to demonstrate a rational relationship between the risk and required measures, and minimise any negative effects that measures might have on trade whilst protecting against risks to human, animal or plant life or health.

9.2 Concepts and Terminology

In statutory plant health related to international quarantine (termed 'plant biosecurity' in some countries), the term **pest** was taken from the IPPC (first published by the IPPC in 1951; see revised text in FAO 2007; FAO 2010), which defines a pest as "*Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products*". This broad definition covers insects, nematodes, molluscs, fungal pathogens, chromists, viruses, viroids, phytoplasmas and plants (e.g. weeds) that may have a detrimental effect on plants or plant products.

In plant biosecurity, the term **risk** is applied to the probability or likelihood of an event occurring and the consequences of that event (WTO 1995; IPPC 1997). PRA is defined as '*The process of evaluating biological or other scientific and economic evidence to determine whether an* ***organism*** *is a* ***pest****, whether it should be regulated, and the strength of any* ***phytosanitary measures*** *to be taken against it*' (FAO 2010). This means that PRAs may determine the potential status of an organism (meets the defining criteria for a quarantine pest), provide estimates of the risks, decide whether the risks are acceptable, and, if they are not, how best to manage them. The strength of risk management options varies; these options are evaluated in the risk management section of the PRA or in a separate risk management document. The conclusion of a PRA can be a recommendation to policy makers to apply or modify phytosanitary measures to manage the risks posed by the pest. Conversely, the outcome may be to not recommend any measures or modifications to existing measures, depending upon the circumstances.

The term **Pest Risk Assessment** is sometimes confused with or considered equivalent to **Pest Risk Analysis**. However, **Pest Risk Assessment** (for quarantine pests) is defined as '*Evaluation of the probability of the* ***introduction*** *and* ***spread*** *of a* ***pest*** *and the magnitude of the associated potential economic consequences*' (FAO 2010). Thus, in contrast to PRA, Pest Risk Assessment does not consider how best to manage the risks posed by the pest.

A **quarantine pest** is defined in the IPPC (1997) as '*A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled*'. In other words, it is a pest that has the potential to have an impact if it is introduced. In FAO 2010, **introduction** is defined as '*The entry of a pest resulting in its establishment*'. **Establishment** is defined as '*Perpetuation, for the foreseeable future, of a pest within an area after entry*'.

9.3 Methodology

The SPS Agreement outlines a broad framework for PRAs that apply to trade but does not prescribe the process by which it should be conducted (Baker and MacLeod 2005). Under Article 5 of the SPS Agreement, Members shall '*take into account risk assessment techniques developed by the relevant international organisations*' (Appendix 1). For plant health these are the ISPMs established under

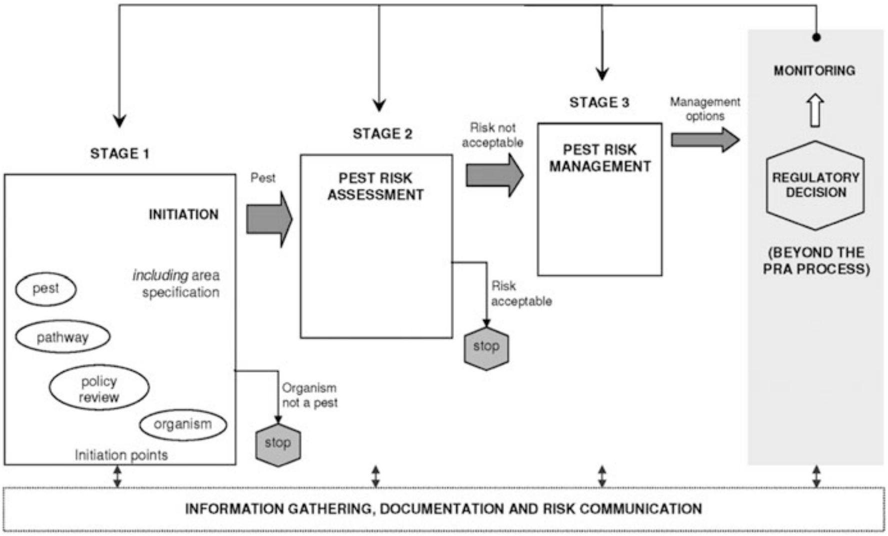

Fig. 9.1 Pest risk analysis flowchart (From ISPM 2, FAO 2007)

the IPPC. The ISPMs provide broad guidance. Detailed guidelines for conducting PRAs are prepared by each jurisdiction's National or Regional Plant Protection Organisations (NPPOs or RPPOs) based upon the ISPMs. The PRA flow chart (Fig. 9.1) illustrates the major steps, which are:

1. Initiation (stage 1);
2. Pest Risk Assessment (stage 2);
   (a) *Pest categorisation*, to determine which species qualify as quarantine pests,
   (b) *Estimation* of the probability of entry, establishment and spread,
   (c) *Assessment* of potential economic impact or consequences,[1] and
   (d) *Overall estimation of risk*, combining likelihood and consequence
3. Pest Risk Management (stage 3).

9.3.1 Initiation and Categorisation

Risk analysis for plant pests may be initiated for many reasons including: (1) Identification of a pathway that carries a potential pest hazard, (2) Identification of a pest that may require phytosanitary measures, or (3) Review or revision of phytosanitary regulations, policies, and priorities.

[1] Economic impact and consequences include environmental outcomes such as impacts on threatened species or ecosystem services, as well as social impacts. This topic is explored in detail below. Note that in some jurisdictions, human health is not evaluated in PRA.

The discussion below focuses on Pest Risk Assessment and Pest Risk Management for individual pests associated with a PRA for a commodity import. ISPM 11 (FAO 2004a) provides more detail on the initiation stage. Output of the initiation stage is to determine whether an organism is a pest and to define the PRA area (geographic region for which the risk posed by the pest is to be considered).

When the PRA process is initiated in response to a request to consider a pathway (normally, a plant or plant-based commodity), the initiation phase is preceded by the compilation of a comprehensive list of organisms of potential concern that are associated with the pathway (FAO 2007; step not shown in Fig. 9.1). That is, analysts determine which pests might be associated with the plant species that forms the basis of the commodity. For example, if there is a request to consider the risks associated with trade in wood of European beech (*Fagus sylvatica* Linnaeus), then it may be necessary to compile a global list of organisms recorded on this species. The list is based upon records of interceptions and outbreaks of pests from official sources, published literature and relevant databases on presence and absence of plant pests.

The exporting country is normally in the best position to provide information on the pests associated with the commodity in question but may be reluctant to share such details despite the information exchange obligations of both the SPS Agreement and the IPPC. Deliberately withholding relevant pest information reduces the value of risk analysis, reflects poorly on the credibility of the importing and exporting countries, and jeopardizes the viability of an operational programme. Likewise, the importing country requires adequate information on the pests present in the country in order to correctly categorize organisms on the pest list. IPPC (1997, Article VII.2.j) describes the obligation to survey for pests as a prerequisite to categorisation. Article VIII describes the obligation to share such information. If the focus is on a product from a specific country then only organisms from that country may be listed.

This list is then considered further to determine which organisms are present in the importing country. If an organism is known to be widespread and not under official control (having the objective of eradication or containment) in the importing country then this organism should not be of quarantine concern, need not be considered further and can be removed from the list of organisms considered because measures are not justified. It may also be possible to quickly reduce the list of organisms likely to be associated with the whole plant in the exporting country for further analysis if the pathway under consideration is a processed product or is intended for consumption or processing, such as fruit (i.e. rather than plants for planting). This effectively eliminates some organisms from further consideration. For example, root-infecting pathogens are unlikely to be associated with fruit, insects that do not feed or breed on fruit are unlikely to enter (except possibly as hitchhikers), and saprophytic organisms are not pests of living plants. Likewise, industry practices associated with the normal handling and preparation of a commodity for shipping should be considered when these processes are known to eliminate certain pests. The washing of mango fruit to remove sap is an example of a routine industry practice for quality purposes but also results in the removal of some external pests and contaminants that may be quarantine significant.

Of the pests that remain on the list, processing the commodity may result in their elimination. However, pests such as these may still need further assessment in the PRA process until analysts prove they cannot establish or are unlikely to cause an unacceptable impact, or where processing in the importing country is deemed an effective non-phytosanitary measure, at which point they can be disregarded. For example, processing citrus fruit for juicing under controlled conditions in countries that do not grow citrus can assure that the pests of concern will not pose a risk. This process creates a list of pests that should be categorised**.** PRAs are also triggered by the identification of a pest that may require phytosanitary measures. If so, the PRA process commences with pest categorisation.

Pest categorisation aims to establish that the pest of concern is a valid, unique taxonomic entity, determines its presence or absence in the PRA area where this has not been clarified in the initiation phase, describes its regulatory status (i.e. listed in statutory regulations or recommended for regulation), identifies potential pathways (in the case of a PRA initiated by a pathway, additional pathways may be identified), and determines whether the pest could establish and spread in the PRA area and, if so, whether there is potential for unacceptable economic consequences (including environmental and social consequences) should it do so. Typically, this is not a detailed process but in effect it is a short version of a pest risk assessment. The outcome is to determine whether the pest fulfils the defining criteria for a quarantine pest; if so, it should then be subjected to the more detailed process of a full PRA (outlined below) to determine the level of risk and the strength of measures (if any) required to achieve a country's ALP or ALR.

9.3.2 Probability of Entry, Establishment and Spread

Probability of entry. The probability of entry describes the likelihood that a pest could arrive in the PRA area in a viable state on the identified pathway. Pests may enter on host commodities, 'hitchhike' on machinery, passengers or containers, or enter naturally (2.2.1.1 in ISPM 11; FAO 2004a, b). In some import PRAs, all relevant pathways for a specific pest are identified in the initiation phase, and if appropriate, are considered separately. Factors influencing the likelihood of pest entry into a PRA area include:

- The likelihood of association of the pest with the pathway at origin accounting for the life stages of the pest, the season and environmental conditions in the country of origin;
- Prevalence of the pest on the pathway accounting for cultivation and commercial practices in the country of origin and pre-existing phytosanitary measures (that is, activities not specifically designed to reduce the probability of entry of the pest in question, but which may affect it and that are part of routine, ongoing activities; as well as measures that are in place for other pests);
- Volume and frequency of trade along the pathway;

Box 9.1 Cumulative Risk

In some PRAs, pests and pathways are considered in isolation. Some countries do not outline or employ an explicit method for combining the risk of entry (or establishment) of a pest over numerous pathways, or evaluate how to assess the risks associated with a commodity, when it may harbour many pests, when each is a low risk. That is, most PRAs overlook cumulative risks. In Europe, the EPPO scheme examines all the pathways on which an individual pest may enter the PRA area. Analysts rate them separately but give an overall risk of entry in qualitative terms before determining the risk of establishment. However, the methodology for doing this is not specified.

When undertaking a PRA that has been triggered by a new trade pathway, intuitively, a commodity that hosts a single low-risk pest is less risky than a commodity that hosts many low-risk pests. More formally, if the probability of entry of a pest on a specified pathway is low (say, $p_{entry} = 0.001$ per annum, given a volume of trade), then the probability of entry on a commodity that hosts a single pest organism is 0.001 per annum. If another commodity supports 50 different pest organisms, each with a probability of entry of 0.001, then assuming independence, the probability that at least one of these pests will enter is $p = 1 - (1-p_{entry})^n = 0.049$. Similar logic may be applied in the assessment of probabilities of entry (or establishment) of a single species over many pathways.

Explicit application of ideas suggested above would be impractical because, if cumulative risks were evaluated, decisions in one PRA may affect decisions in other PRAs. The practical difficulty of tracking all pest risks over all commodities, over time, over changing volumes has resulted in a more tractable focus on individual pests and pathways.

- Species' biology in relation to survival and reproduction during pre-export/post-import storage or during transport (accounting for conditions during storage and transport);
- Detectability of the pest in infected or infested commodities (including visible damage or symptoms on the commodity) at the origin or destination; and,
- Likelihood of the pest transferring to a suitable host which for commodity pathways includes consideration of how widely the commodity is distributed in the PRA area, arrival at a suitable time of year and the intended use of the commodity.

Entry (as well as establishment and spread; considered below) is usually considered to be a series of events with associated probabilities. If the events are independent, then probabilities may be multiplied to give an overall assessment. For example, if the probability of any event is negligible, then probabilities for events on the pathway beyond this point become negligible at most. Not all countries apply rules that are consistent with this simple, probabilistic interpretation. The assumption that events are independent is explored further below.

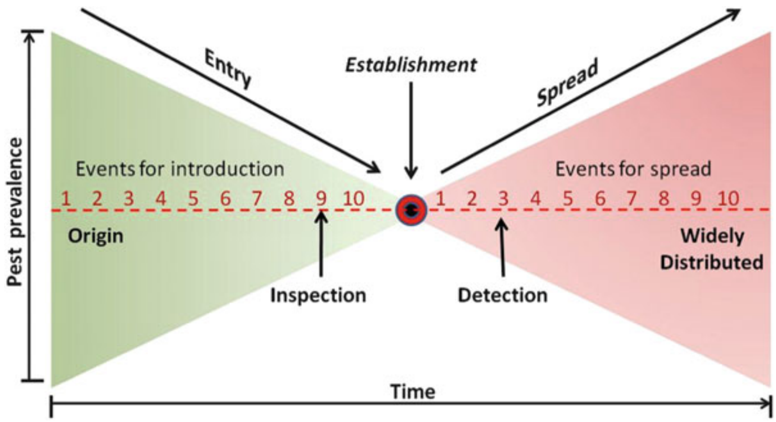

Fig. 9.2 **Pathway continuum**. The figure represents (in general terms) expected pest prevalence on pathways (see text for an explanation)

One approach to estimating the probability of entry is to develop a scenario tree showing the key steps in commodity import (for plants and plant products) and associated pest movement up to the point of import, including production, processing, transport and clearance at the point of entry in the PRA area. This technique has the added benefit of also facilitating risk management because points in the scenario where mitigations may be applied are more easily identified and analysed. Data may be available on inspection of the commodity at export or from the experience of other countries importing the same commodity from the same country. However, the prevalence of a pest in the commodity and volume of trade are often assumed, based on expert judgement.

In some situations, phytosanitary measures (see below) and industry practices are already in place in the exporting or importing country for a particular pathway to deal with other pests that coincidentally negate the risk posed by the pest on that pathway. If so, such pathways may be eliminated.

Figure 9.2 illustrates in general terms the expectations for the prevalence of pests on the biosecurity continuum. Pest prevalence and variability may decline from the source area to the point of entry because of the natural processes of birth and death in populations, and the generally adverse conditions associated with quarantine measures (if these are already in place), commercial production, sanitation and transport that can affect the organism. Post-border, if the receiving environment is conducive, then pests may establish in new habitats or on host species and spread by natural and human-mediated dispersal to occupy available habitats thus increasing their prevalence. This is considered under the assessment of the likelihood of establishment and spread. The points 1–10 on each side of the fulcrum in Fig. 9.2 can be used to define levels of risk and/or consequence, as well as critical control points such as inspection and detection.

At the end of the assessment of entry, the overall probability of entry of the pest of concern should be described and the probability of entry on each different

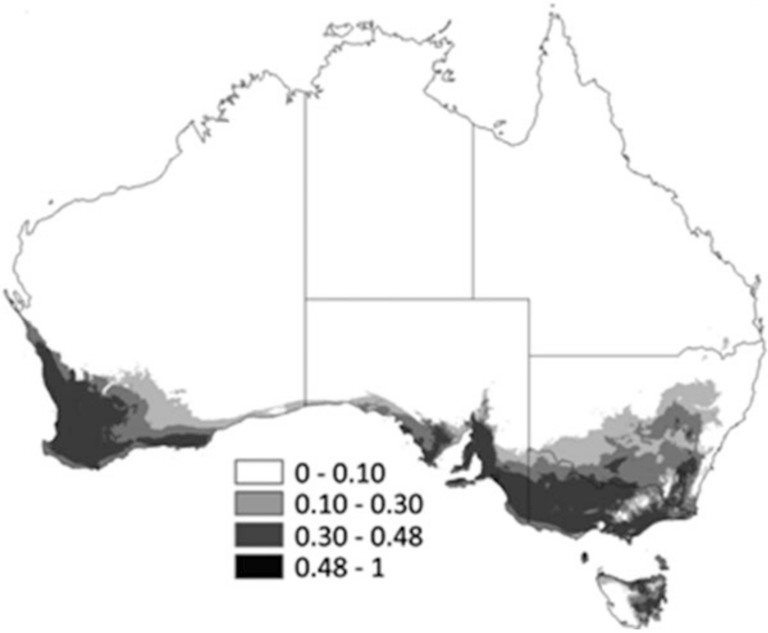

Fig. 9.3 Species distribution map for the red-legged earth mite, *Halotydeus destructor* (Tucker), an important invasive plant pest

pathway should be specified along with the associated uncertainties. If the probability of a viable pest or pests entering the PRA area in association with all of the pathways being assessed is negligible (i.e. the risk is acceptable), then the PRA can be terminated.

Probability of establishment. Factors influencing the likelihood of pest establishment in a PRA area include:

1. Availability of suitable hosts, habitats, alternate hosts and vectors (where needed);
2. Suitability of the environment in relation to the pest's biology and lifecycle including climatic conditions (outdoors and under protection for pests of protected crops), abiotic factors (e.g. soil conditions, topography);
3. Presence and likely efficacy of natural enemies;
4. Pre-existing pest management practices;
5. Frequency with which the pest has been introduced into new areas outside its original area of distribution, and
6. Potential to survive eradication programmes.

Assessment of the probability of establishment can include the use of Geographic Information System (GIS) data layers of environmental variables and databases of host distribution, together with statistical or automated learning methods, to determine how similar the conditions for establishment are in the area of origin of the pest to the PRA area. These tools can be used to generate maps that identify where the pest is likely to establish and spread (Fig. 9.3). Another example is given by the maps of the potential for establishment of *Phytophthora ramorum* Werres et al. in Europe produced in the PRA under the EU Sixth Framework Project RAPRA (Sansford et al. 2009).

The shaded areas depict the probability that the habitat is suitable for this species. The model was developed by linking relevant climate layers to presence records of the species, through the modeling tool Maxent (Elith and Graham 2009). Recent changes in distribution may be due to climate change, change in agricultural practices or adaptive evolution.

We may conclude the analysis during the assessment of the probability of establishment if, for example, there are no suitable hosts in the importing area or the environment is clearly unsuitable for survival or reproduction of the pest (although in such circumstances, the pest may not have passed the categorisation stage). At the end of the assessment of the likelihood of establishment, the overall probability of introduction (entry and establishment) should be estimated. Ideally, assessments should include estimates of uncertainties associated with each probability, and with the overall estimate.

Probability of Spread. **Spread** is defined as '*the expansion of the geographical distribution of a pest within an area*' (FAO 2010). Estimates of the potential for spread help determine how quickly any impact might occur as well as the ease with which a pest could be contained if it was introduced. These questions are especially relevant to pest management and discussed below. Spread potential depends on a range of factors, many of which also affect establishment, such as the distribution of suitable hosts. Natural spread depends in part upon the capacity for pest dispersal (e.g. wind-blown fungal spores, insect flight capacity, vectors if needed). Human spread depends on movement of commodities (plants for planting, plant-based products, fruit etc.) and unintentional spread (e.g. soil movement with machinery, vehicles, or on footwear).

9.3.2.1 Summarising the Overall Probability of Entry, Establishment and Spread

At the end of the likelihood assessment stage of the PRA, analysts estimate the probability of entry and establishment (= '*introduction*'; FAO 2010) and may also analyse spread. The analysis will define the part of the PRA area that is endangered by the pest based on ecological factors. The '*endangered area*' is defined as '*the area where ecological factors favour the establishment of a pest whose presence in the area will result in economically* (includes environment) *important loss*'.

Methods for estimating and combining the probabilities of entry, establishment and spread include narrative methods, point-scoring systems, rule-based methods, and quantitative (probabilistic) methods. One or all of these tools may be used for specific elements of the analysis. In most cases, the choice of methodology will be driven by information, tools and skills available and conventions of the relevant NPPO or RPPO, PRA method used, as well as resources available to produce a PRA that is fit-for-purpose. Whatever method or methods used, the type and degrees of uncertainties should be specified.

9.3.3 Tools and Schemes for PRA

Some of the tools that have been used in PRAs include Scenario Trees, Likelihood-Consequence Tables, Point-Scoring Systems and Monte Carlo Analysis (some of these and other examples are explained in Box 9.2). For routine analyses, Biosecurity Australia (2008) and the USDA (2000) specify numerical intervals that correspond to terms for likelihoods and provide rules for combining the implied probabilities.

PRA schemes can be qualitative but may deploy some quantitative tools for some parts of the analysis. In the qualitative EPPO scheme, the analyst decides how to score and what the scores mean, as well as how to present them. The analyst also estimates the overall risk. EPPO does not currently prescribe a means of doing this; however, this scheme and methodologies for PRA are being developed in the EU Seventh Framework Project Pratique (Baker et al. 2009; https://secure.fera.defra.gov.uk/pratique/).

Several European countries have developed rapid risk assessment schemes. In 2010, England and Wales moved to the use of a Rapid Assessment scheme in some situations to accelerate the PRA process. It is based upon the UK PRA scheme and assists risk managers to decide relatively quickly on a response to a new or revised pest threat. It does not constitute a full PRA but includes advice on whether it would be helpful to develop such a PRA and, if so, whether the PRA area to be considered should be the UK or the EU, and, whether to use the UK or EPPO PRA scheme. Theoretically this should help reduce the need to conduct a full PRA for every pest that comes to the attention of the UK NPPO. Biosecurity New Zealand's scheme (Biosecurity New Zealand 2006) is less prescriptive than EPPOs. It does not define terms for probabilities precisely and does not indicate the rules for combining likelihoods.

Words and phrases often mean something different in various countries. For example, 'low' risk for USDA (2000, Table 1) means a probability of less than 0.01. For Biosecurity Australia (2008), 'low' risk means a probability between 0.05 and 0.3. The intervals don't even overlap! Such potential contradictions may not be a problem providing, within a country, the words are defined and used in a consistent manner and the measures are justified by the analysis. Provided risk analysts adhere to the definitions in the Glossary of Phytosanitary Terms (FAO 2010) and are explicit about the method they use, then an NPPO or RPPO can provide consistent PRAs, and enable reviewers to evaluate consistency between decisions.

Box 9.2 Tools for PRA

Scenario Trees. Recommended for use in PRAs by Biosecurity New Zealand (2006), Scenario Trees are closely related to Logic Trees, Decision Trees and Cognitive Maps. Scenario Trees can be used to map complex 'exposure'[2] pathways and identify critical control points for effective intervention strategies (Hayes 2002). Decision Trees may also be incorporated into structured question protocols (see below). Scenario Trees and related structures can be translated into Probability Trees and Bayes Nets by adding marginal and conditional probabilities. These trees show the analysts' understanding of the cause-effect relationships among the most important variables in the system of interest (Fig. 9.4).

Qualitative categories. Many PRA schemes use qualitative (narrative) categories to represent probabilities. Usually, the words are ordered and probabilities are combined into an overall estimate subjectively or by using rules that can be defined in a risk matrix. Sometimes, categories are linked directly to a range of probabilities (e.g., Table 9.1).

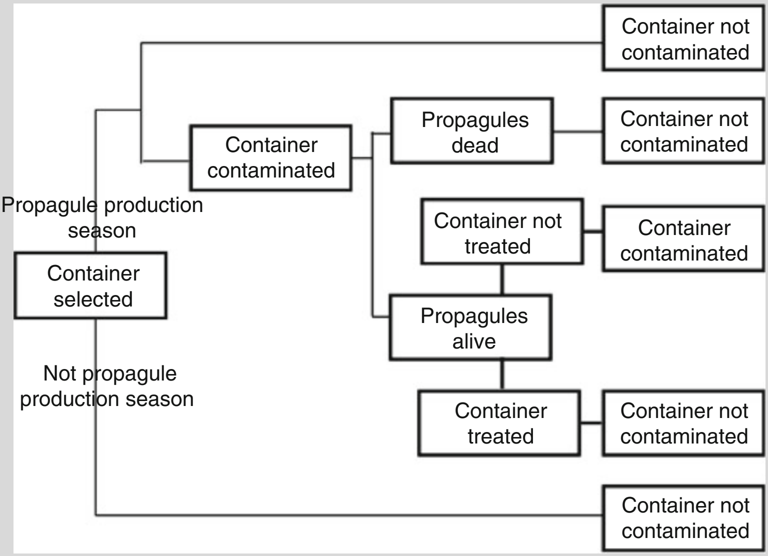

Figure 9.4 Scenario Tree for contamination of cargo containers by a plant pest

(continued)

[2] The term 'Exposure' is used in New Zealand PRAs for consistency between animal and plant health PRAs. The term is used routinely in animal health risk assessments and refers to the mechanisms by which the environment or other receptors become exposed to a potential hazard.

Box 9.2 (continued)

Table 9.1 Likelihood definitions employed by USDA (2000). Biosecurity Australia (2007) uses an ordinal scale with 6 categories whereas USDA (2000) uses 3

Category	Probability interval	Score
Low	<0.01	1
Medium	0.01–0.1	2
High	>0.1	3

In other applications, analysts interpret the words without specifying a quantitative meaning. For example, the most recent published version of the EPPO PRA scheme (EPPO 2009) is divided into two sections. The assessment in section A (initiation and pest categorisation) is a Binary Decision Tree that takes the form of a sequence of questions. If the risk analyst concludes that an organism may be a quarantine pest, then the pest is evaluated in greater detail in section B. Each relevant pathway of entry is assessed separately. Analysts provide information and data in response to each question in Section B and assign likelihoods to the responses, assigning one of five words that range from (for example) '*very unlikely*' to '*very likely*' or '*minimal*' to '*massive*'. In past applications of the EPPO scheme, these words have not been tied to specific probabilities or categories.

Bayesian Networks. Like Scenario Trees, Bayesian Networks begin with a graphical depiction of cause and effect relationships. In a Bayesian Network the system variables are called "nodes" and the dependencies between variables are represented by arrows. Unlike qualitative models, however, the relationships between variables are strictly one-way because they represent a conditional probability distribution that describes the relative likelihood of each value of the "child" node (end of the arrow) conditional on every possible combination of values of the "parent" nodes (start of each arrow) (Fig. 9.5).

A path cannot pass through a variable more than once. Thus, Bayes Nets cannot easily represent feedback systems. Furthermore, estimating parameters in the conditional probability tables that lie behind Bayes Nets can be difficult, especially when many parent nodes exist. Their advantages are that they can accommodate expert judgement and data, can be used to build models for systems such as exposure pathways relatively quickly and intuitively, and may be updated as new information is acquired.

(continued)

Box 9.2 (continued)

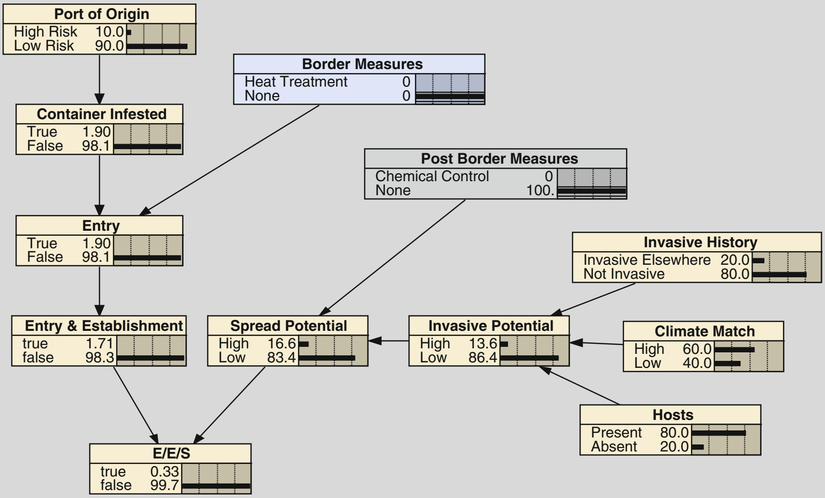

Fig. 9.5 Bayesian Network for estimating probability of entry, establishment and spread (E/E/S), and the effectiveness of border and post-border treatments of a plant pest that may be associated with commodities in sea containers

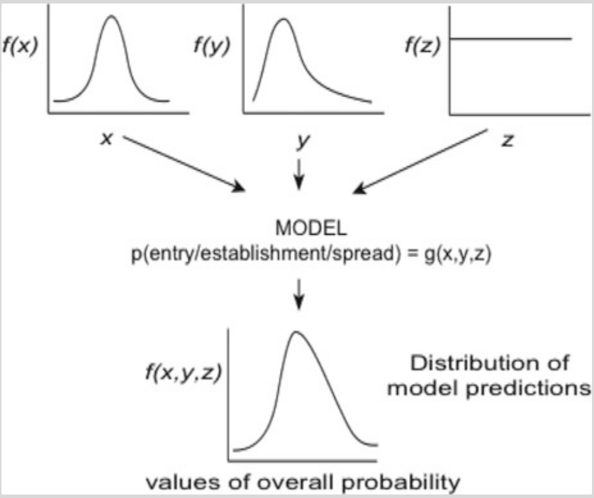

Fig. 9.6 Representation of a first-order Monte Carlo simulation for a PRA

(continued)

Box 9.2 (continued)

Monte Carlo. Estimates may be available of the probabilities of the pest being present on each of the steps of the exposure pathway. Monte Carlo simulation provides a method for combining the probability density functions, even in arbitrarily complex situations, into an overall estimate (Fig. 9.6). In this hypothetical example, the overall probability of entry, establishment and spread of a pest is the combination of three independent variables. The model is run thousands of times. On each occasion a variate is chosen randomly from the distributions specified by the analyst. The independent distributions are multiplied. The results are collated into a histogram that shows the overall variability of the probability estimate (Burgman 2005).

Comparisons between PRAs produced by different NPPOs/RPPOs may not be so easy. Nevertheless, the transparency of phytosanitary decisions would be enhanced if terms were used in a standard way internationally.

Analysts may interpret the words describing categories of risk differently, especially if the words are not anchored explicitly to a probability scale. Unfortunately, in many countries, the words are not defined against a national quantitative standard but are left to the analyst to interpret. In such situations, even experienced analysts operating with standardised guidelines often interpret probability terms differently and inconsistently (Budescu et al. 2009). MacLeod (2010) noted that probability scales would help analysts to provide consistent results when used by different analysts, or even by the same analyst at different times but using the same information. The empirical results suggest that probability scales result in more consistent and more accurate estimates (irrespective of data conditions) because they eliminate arbitrary misunderstandings that arise through different interpretations of words.

Estimates of the probabilities of entry, establishment and spread must be combined by the analyst into an overall judgement. Methods for combining probabilities differ between jurisdictions. For example, likelihoods of '*entry*', '*exposure*' and '*establishment*' in New Zealand of squash bugs (*Leptoglossus gonagra* (Fabricius)) on citrus fruit imported from Samoa were estimated to be '*very low*', '*high*' and '*low*', respectively (Biosecurity New Zealand 2008, pp. 124–125). Potential consequences were estimated to be *low*. From these assessments, analysts concluded that the risks were sufficiently high that New Zealand's ALP was not satisfied, and risk management measures were justified. If the same terms were applied in the Australian system, analysis would result in a '*very low*' risk, satisfying Australia's ALP with no measures required. Such contrasting outcomes may reflect different methods for combining judgements, or different definitions of terms.

As for the estimates of probabilities associated with entry, establishment and spread, such potential contradictions may be alleviated if, within a country, the processes for combining probabilities are defined and used in a consistent manner. Provided risk analysts are explicit about methodology, it may be possible to provide consistent PRAs and to allow reviewers to evaluate consistency between decisions. Inconsistency can lead to indefensible results, which are more susceptible to challenge. Transparency of documentation of these processes differs between countries. In some countries, the process for combining probabilities is unspecified.

9.3.4 Consequences

The consequences of pest introduction or spread are distinct from the impacts of measures that may (or may not) be applied. Article 5 of the SPS Agreement identifies relevant economic factors that must be considered by WTO members to include: (1) Potential damage in terms of loss of production or sales in the event of entry, establishment or spread of a pest or disease; (2) costs of control or eradication in the territory of the importing Member; and (3) relative cost-effectiveness of alternative approaches to limiting risks. Additional guidance for estimating consequences is provided in ISPMs, which refer to economic, environmental and social impacts associated with establishment and spread within the endangered area.

Care must be taken to ensure transparency regarding worst-case assumptions, which can exaggerate impacts and therefore bias risk. Likewise, analysis of consequences must balance the magnitude of impacts with severity. For example, severe impacts in small areas may be less important than moderate but very extensive impacts. If certain specialist sectors are affected, even narrowly, then distributed impacts may not be acceptable. One example of this is Camellia Flower Blight caused by the fungus *Ciborinia camelliae* L. M. Kohn; only growers of camellias would be affected by this pathogen, but this is a high-value specialist crop in some countries and individual businesses could be severely affected when outbreaks occur.

In some cases there may be no need to undertake a detailed analysis of economic consequences. Sometimes basic information is sufficient. Even when data are unavailable (especially where a pest has the potential to cause environmental impacts which are difficult to evaluate), we may reasonably conclude that consequences may be unacceptable. For example, the recent spread of *P. ramorum* into timber plantations in the UK (first detected in 2009) (Webber et al. 2010) and the subsequent death of Japanese larch (*Larix kaempferii* (Lamb.) Carrière) make it self-evident that this pest has potential to cause unacceptable impacts in some areas of Europe outside the UK, where it is present but has not established in the wider environment. Information from similar pests already in the country (or in another country with a similar environment) can be used to estimate consequences arising from the pest of concern. Analysts may assess whether such a pest would add significantly to the damage caused by another, similar pest that is already present. For example, if a fruit fly is present in the importing country with a similar host range and biology that is being effectively controlled, then will the impact of a new fruit fly species be significant if it is subjected to the same control regime?

Consequences can be separated into various elements including direct impacts on agricultural/horticultural/silvicultural production, environmental and social impacts and impacts related to loss of markets. These elements can be estimated separately and then combined to produce an overall estimate of consequences. NPPOs seek to minimise impacts on private sector profitability (through assessing the potential for direct impacts on plant yield or quality, costs of non-phytosanitary control, loss of markets), public sector costs (through eradication and containment costs and associated research, administration and publicity), environmental values (through impacts on ecologically important species and ecosystem processes such as erosion, fire frequency, nutrient balances, pollination and water quality and quantity), human health consequences (where assessed) and social impacts (such as impacts on tourism and employment). The validity and importance of each factor depends on specific circumstances. Not all criteria are assessed in all jurisdictions. The methods employed by different jurisdictions (Box 9.3) make a range of assumptions about the criteria, and how they should be assessed and combined into an overall judgement. The validity of any approach will first be judged by consistency in its application and conclusions.

Economic methods for combining impacts of different kinds include cost-benefit analysis, cost effectiveness analysis, multi-criteria decision analysis and simple scoring systems (ISPM 11; FAO 2004a). The SPS Agreement limits assessments to the negative impacts from a pest and cannot be a national interest test. Relatively little use has been made of structured decision making in formal economic or environmental analysis, even in high-profile and contentious cases. Rather, within the frameworks outlined above, most jurisdictions employ expert-based subjective estimation of the severity of impact. This arises from a lack of data on the likely consequences of a pest, an inability to measure true consequences due to the pre-existence of phytosanitary measures, or a lack of trade-specific data. Beale et al. 2008 (pp. 108–109) argued for greater use of formal economic analysis to quantify consequences of pest and pathogen incursions (e.g., Abdalla et al. 2005). They acknowledged the need to deal explicitly with non-market values and economic values in multi-attribute analyses. They advised that estimates of consequences should take into account adjustment options available to producers in the event that they are affected by such an incursion, and should focus on net consequences to avoid overestimation of consequences. They recommended PRAs focus on the absolute net value of production at risk. This view is employed by many NPPOs when prioritising threats and biosecurity measures nationally and is one that the current authors support.

In some jurisdictions, a few individual high-profile pests have been subject to more detailed economic analysis including cost-benefit analyses of the effects of taking phytosanitary measures to determine the best route to either prevent entry or to manage pests that are currently absent. One example of this is the EU Fifth Framework project '*Karnal bunt risks*' within which analysts evaluated the potential socio-economic impact of *T. indica* in the EU arising from a small and a large outbreak scenario in a wheat-growing area of the UK (Brennan et al. 2004a, b). They concluded that it was better to prevent an outbreak occurring (by preventing entry) than to attempt to contain such an outbreak (this supports the current EU phytosanitary measures for *T. indica*; Anon 2000).

Box 9.3 Example Applications of Consequence Estimation

Qualitative methods (NZ). Biosecurity New Zealand (2006) suggests that consequence assessment should estimate spread, the potential biological, environmental, economic and human health consequences, and the likelihood of these consequences. Detailed analysis of consequences is not necessary if '*there is sufficient evidence*', or '*it is widely agreed*', that the introduction of a hazard will have unacceptable consequences. Otherwise, Biosecurity New Zealand (2006) recommends that analytical techniques be used in consultation with experts in economics to complete detailed analysis of the potential economic effects. For non-commercial and environmental consequences, they suggest the use of qualitative information about consequences, and that analyses document areas and degrees of uncertainty in the assessment, and indicate where expert judgement has been used. Economic and environmental impacts are assessed separately, and an overall, subjective judgement of the severity of consequences is provided at the conclusion of a PRA.

Subjective assignment of ratings (EPPO). The EPPO Decision-support scheme (EPPO 2009) estimates economic consequences through a series of questions with replies in the form of evidence as well as the selection of one of five words: '*Minimal, minor, moderate, major and massive*'. In the case of evaluating the likelihood of loss of export markets, analysts select one of five words/phrases: '*Impossible/very unlikely, unlikely, moderately likely, likely, very likely/certain*'. The analyst specifies uncertainty at one of three levels for each question. In this section of the PRA, the efficacy of controls is assessed based upon five words or phrases ranging from '*very easily*' to '*impossible*'. The overall level of pest consequences is expressed in words and in relation to the endangered area (where the pest can establish). In the EPPO scheme, the analyst determines how to select the word scores and how to present them to the reader. Currently, EPPO does not prescribe a procedure for doing this, although outcomes of recent research may lead to more detailed specification of uncertainties (Baker et al. 2009).

Point-scoring (USA). The USA plant PRA protocol (USDA 2000) follows initiation and categorisation with a step termed '*Assess Consequences*'. It results in a score termed a Cumulative Risk Rating that is considered to be an indicator of the potential of the pest to establish, spread, and cause economic and environmental impacts (termed '*Risk Assessment*' in some PRAs, e.g., USDA 2007). Consequences are assessed subjectively against five '*Risk Elements*', each assigned a score of between 1 and 3. They include:

1. Suitable hosts and climates exist in the USA,
2. Host range (1 for monospecific pests, 3 for pests of multiple families),
3. Dispersal (spread) potential (including reproductive potential and movement capabilities),

(continued)

Box 9.3 (continued)

4. Economic impact (damage to host crops, commodity value or loss of markets), and
5. Environmental impact (including ecological disruption, effects on threatened species or habitat, or the indirect impacts of control actions).

If no suitable hosts or climates exist in the USA, then the PRA ceases. Otherwise, the scores for each risk element are added and compared with a Cumulative Risk Rating scale;

- Low: 5–8 points
- Medium: 9–12 points
- High: 13–15 points

Rule-based assessment (Australia). Biosecurity Australia (2007) suggests that PRAs consider direct and indirect economic and environmental consequences. Direct pest effects include consequences for plant or animal life or health, and '*other*' environmental aspects. Indirect effects include the consequences of eradication and control measures, effects on domestic and international trade and the environment. For each criterion, the extent of consequences is estimated over four geographic levels: Local, district, regional or national, and the severity of the potential consequence is described using four categories ranging from '*indiscernible*' (pest impact unlikely to be noticeable) to '*major significance*' (expected to threaten the economic viability through a large increase in mortality/morbidity of hosts, or a large decrease in production; expected to severely or irreversibly damage the intrinsic '*value*' of non-commercial criteria). Values are translated into a qualitative impact score. Consequence estimation assumes that spread has occurred to its maximum potential. The overall consequence for each pest is achieved by combining the qualitative impact scores for each direct and indirect consequence using a series of decision rules. These rules are mutually exclusive, and are assessed in numerical order until one applies.

9.3.5 Risk Estimation, ALP and ALR

Risk involves a consideration of the likelihood of an event and the severity of its consequences. Typically, in probabilistic PRAs a risk analyst generates a measure of risk by multiplying a probability (defined within some time frame) by a measure of consequence. That is, decisions about the acceptability of risks depend on a synthesis of consequences, weighted by their likelihoods. The EPPO scheme does not currently specify a time frame but this issue is being examined in the ongoing European Union PRATIQUE project (Baker et al. 2009).

International standards for risk analysis (ISO 31000 2009) use likelihood-consequence tables as a framework for summarising and communicating the

Likelihood of pest entry, establishment and spread	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Consequences of pest entry, establishment and spread

Fig. 9.7 Risk estimation matrix (Biosecurity Australia 2007, 2009)

relative magnitude of risks. This convention has been adopted explicitly by Australia (Fig. 9.7) and is implicit in the protocols employed in other jurisdictions. At present the EPPO scheme has no structured mechanism to combine risk elements. After scoring many detailed aspects that inform risk, most on a five-point scale (Boxes 9.2 and 9.3), it asks assessors to review the assessment and draw conclusions, thus providing a narrative conclusion for overall risk. This issue is also being addressed within PRATIQUE (Schrader et al. 2010).

In the USA, the analyst commences by first estimating the potential consequences. If the estimate is above a threshold then the analysis focuses on the probability of entry, establishment and spread. If the estimated consequences are below the threshold then no further analysis is needed – the risks associated with importation do not exceed the ALP. In Europe, New Zealand and Australia, the normal process following the initiation and categorisation stage of a PRA is to determine the risk of entry, establishment and impact; if there is a risk of introduction (entry and establishment) and if the consequences (impact) are not acceptable (above an ALR) then the analyst determines which measures would reduce the risk below the ALR and which, where possible are not trade-restrictive.

In Europe, the ALR is not consistently low nor is it strictly defined. It varies according to the sector that is affected and whether the pest is already present in some areas and can be managed without phytosanitary measures.

Most decisions do not require complex or detailed analyses of probabilities or consequences, either because the probabilities and consequences are self-evidently above or below acceptable levels, or because similar expected consequences have been found to be acceptable in the past. A small number of cases are more equivocal and require more careful analysis. Most countries employ a tiered system of analysis in which straight-forward decisions are fast-tracked based on risk management policies already developed for the same or similar products. Borderline or high-profile cases are analysed more carefully.

ALP/ALR is determined by circumstances relevant to each jurisdiction. Consistency is achieved by the technical justifications and adherence to the criteria and procedures applied by each jurisdiction. ALP/ALR cannot be arbitrary or capricious but requires rational justification, particularly because it must survive cross-examination in the WTO, if challenged.

9.4 Risk Management

Risk management identifies options to reduce risk. This often includes attempting to prevent entry to the PRA area by requiring an exporting country to take measures to ensure a commodity originates in a pest-free area or place of production (e.g. for the EU, Anon 2000). Management may involve specifying treatments such as fumigation, radiation, chemicals, heat/cold, or inspection and culling. The exporting country may propose alternative, scientifically verified measures that provide an equivalent level of protection. The measures suggested by the importing country should be feasible (i.e. operationally practical and cost-effective).

Measures taken by the exporting country to comply with the importing country's requirements may include reducing infestation in the crop through a system of measures (e.g. chemical treatment of the crop, cultural practices, resistant cultivars, treatment of the consignment), inspection and testing to check for contamination or infection/infestation at different points on the pathway. Other measures include tools to enable traceability of a commodity back to its source production or processing area, registration of the quarantine status of source areas, pest and pathogen inspections in the importing country and restrictions on end use.

The effectiveness of any proposed measure (or combination of measures) is evaluated to ensure it reduces the risk for the pest to a level that meets a country or region's ALP (Australia/New Zealand) or the ALR (Europe). ISPM 11 (FAO 2004a) provides details of appropriate risk management options and notes that the choice of measures should be based on their effectiveness in reducing the probability of entry of the pest, and should be the least trade-restrictive. Analyses of the efficacy of measures should reflect how much the assessed level of risk is reduced by a measure. Analysts (or risk managers) should revise the stringency of measures over time by auditing the efficacy of each measure. On occasion, it may be necessary to impose a ban on commodities on some pathways where it is clear that an exporting country is unable to meet the importing country's requirements, for instance, when a country repeatedly intercepts a non-indigenous damaging pest from an exporting country. The specifications for risk management measures are necessarily case-specific, depending on the risk posed by the pest, the capabilities, regulations and practices of the exporting country, and the nature of the commodity.

9.5 Dealing with Uncertainty

The SPS Agreement is clear about basing measures on scientific principles and evidence but provides little guidance on handling uncertainty. Clearly, uncertainty is a fundamental element of risk, but a central aspect of risk analysis in the SPS framework is estimating the risk based on the evidence and treating the uncertainty separately in order to be able to understand the extent to which measures are based on evidence and how uncertainty has influenced the decision making process. As a result, the handling of uncertainty in PRA becomes a process itself with multi-faceted approaches that are subject to the same disciplines and criteria applied to the analyses done on the evidence.

Three kinds of uncertainty affect PRAs. Incertitude (or epistemic uncertainty) in PRAs arises from lack of knowledge or lack of data. Information gaps may be about the biology of a pest, trade volumes, crop production, the efficacy of protection measures or any other element of the biosecurity system. Incertitude may be reduced by further study and sampling (Burgman 2005). This contrasts with natural variation, which is unpredictable variation in biological and physical processes. It is better understood but does not diminish as it is studied further.

Lastly, PRAs are affected by linguistic uncertainty, which arises through misunderstanding in language because words can be ambiguous, vague, context dependent or underspecified (Regan et al. 2002). Many PRAs rely heavily on language-based analyses, and as noted above, words relating the probabilities and consequences may be interpreted differently. The consequences are that different analysts will generate different judgements of risk, even when using the same data and the same risk analysis system. The problems can be mitigated to a large extent by defining terms, creating precise definitions, specifying context, providing guidelines for interpreting information consistently, and verifying assessments by independent assessors (Regan et al. 2002).

ISPM 2 (FAO 2007) suggests that uncertainty should be taken into consideration when conducting PRAs. Specifically, it recommends that *the nature and degree of uncertainty in the analysis should be documented and communicated, and the use of expert judgement indicated. . . . Documentation of uncertainty contributes to transparency and may also be used for identifying research needs or priorities*. The main sources of uncertainty in PRAs listed by ISPM2 include missing, incomplete, inconsistent or conflicting data, natural variability of biological systems, subjectiveness of the analysis and sampling randomness.

Despite this recommendation, PRAs rarely deal adequately with uncertainty. Many PRAs deal with uncertainty superficially, or not at all. ISPM 2 does not suggest a separate characterisation of variability (arising from nature), incertitude (arising from a lack of knowledge) and linguistic uncertainty. It may be useful to distinguish these sources of error because different kinds of uncertainties warrant different kinds of analysis (Regan et al. 2002).

In general, Biosecurity New Zealand (2006) adopts a '*semi-quantitative*' approach similar to that described by the OIE (2010). A common approach to combining various qualitative estimates is to assign numbers to them (in the form of probability ranges or scores), to produce a summary measure. Systems like this are employed by Australia, Canada and the USA, in a variety of forms, detailed above. The OIE (2010) states that numbers, ranges, weights and methods of combination are usually arbitrary and need careful justification. They argue that semi-quantitative assessments often give a misleading impression of objectivity and precision and that these methods *do not offer any advantages over a well-researched, transparent, peer-reviewed qualitative approach* (OIE 2010, p. 36).

Good information may exist about some steps in an exposure pathway, and poor or no information about other steps. When '*significant uncertainty*' exists in an estimate of risk, Biosecurity New Zealand (2006, p. 28) suggests *a precautionary approach to managing risk may be adopted. However, the measures selected must nevertheless be based on a risk assessment that takes account of the available scientific information*. Article 5.7 of the SPS Agreement specifies that measures should be based on scientific evidence, stipulating that '*where relevant scientific evidence is insufficient, a Member may provisionally adopt … measures on the basis of available pertinent information…*'. It is likely that, in the absence of specific advice or guidelines on how to handle uncertainty, all analysts occasionally adopt a conservative position. Unfortunately, this position usually will be hidden beneath the standard operations of a risk analysis, leading to uncontrolled and unspecified levels of conservatism in PRAs, and making it difficult for others to understand and interpret the results of a PRA.

In the EPPO Decision-support Scheme (EPPO 2009) analysts list uncertainties associated with every question and summarise these at the end of each section. Similarly, in the UK PRA scheme, a table of uncertainties is presented at the end of the document with a list of recommendations of what work would be required to address these. For example, where there is uncertainty on the distribution of a pest, there may be a proposal for surveillance. Thus, uncertainties are identified and risk management measures, where necessary, are tailored to reflect the risk posed by individual pathways. While such guidelines go part way to alleviating the problem, they do not prescribe a thorough treatment of uncertainty (see below). MacLeod (2010) noted part of the difficulty in using the current EPPO scheme is that potential users recognise the subjectivity inherent within the scheme, both in assigning scores to questions and combining elements of risk to draw conclusions. Suggestions to reduce this element of subjectivity are being considered in the PRATIQUE framework (Baker et al. 2009).

Subjective judgements are susceptible to a range of individual, psychological and contextual biases (Kahneman et al. 1982; Slovic 1999; Burgman 2005). Many studies have shown that substantial differences in ranks (categories) assigned by analysts are attributable to differences in risk perception and linguistic uncertainty even when people have identical information at their disposal (Burgman 2005). Subjective rules for combining judgements can serve to reinforce the conservatism of judgements associated with individual steps in a PRA. This can be compounded in PRAs to generate outcomes that are overly-conservative (risk-averse to an unknown extent).

Wherever uncertainty is identified in a PRA, it should be carried through the chains of reasoning in a coherent and repeatable way and be presented together with the final, overall assessment. This can be achieved in different ways for the different methodologies adopted in various jurisdictions (see examples in Box 9.4). When uncertainties are identified, their importance should be evaluated.

Box 9.4 Sensitivity and Uncertainty Analyses for PRAs

An Australian PRA. In its Import Risk Analyses, Biosecurity Australia assesses the probability of entry, establishment and spread (between Negligible and High) at four stages: (1) importation, (2) distribution, (3) establishment and (4) spread. The value assigned at one of these steps can be said to be 'sensitive' if, when that one step is varied (but the others are kept the same), it results in a different decision being taken regarding that pest. For example, if changing the probability of importation of a pest from 'Very low' to 'Low' would have resulted in the unrestricted risk value of that PRA being 'Low' rather than 'Very low', it would no longer meet Australia's ALP. We can say that the pest species is insensitive to misclassification at that stage. In the example below, the probability of importation of the pest is not sensitive to misclassification (by a single category) but the estimate of the probability of distribution is sensitive to misclassification.

For a species;

Step 1. Provide best estimate of risk

$$\begin{aligned}\text{Risk} &= \text{probability of (importation, distribution, establishment, spread)} \times \text{consequence}\\ &= (\text{Moderate} \times \text{Low} \times \text{Low} \times \text{Moderate}) \times \text{Moderate}\\ &= \textit{Very Low}\ (\text{satisfying Australias ALP})\end{aligned}$$

Step 2. Vary each estimate in turn upwards or downwards by a single category, in the direction that could lead to a different decision.

Step 1a. Increase importation estimate

$$\begin{aligned}\text{Risk} &= \text{probability of entry, establishment and spread} \times \text{consequence}\\ &= (\mathbf{High} \times \text{Low} \times \text{Low} \times \text{Moderate}) \times \text{Moderate}\\ &= \textit{Very Low}\ (\text{satisfying Australias ALP})\end{aligned}$$

Step 1b. Increase distribution estimate

$$\begin{aligned}\text{Risk} &= \text{probability of entry, establishment and spread} \times \text{consequence}\\ &= (\text{Moderate} \times \mathbf{Moderate} \times \text{Low} \times \text{Moderate}) \times \text{Moderate}\\ &= \textit{Low}\ (\textit{not}\ \text{satisfying Australias ALP})\end{aligned}$$

. . . and so on.

A USA PRA. The USDA (2000) arrives at a summary of entry, establishment, spread and consequence assessment by summing the two Cumulative Risk Rating scores, each of which is determined by scoring several factors on a scale of 1–3. This generates a score termed the "Pest Risk Potential" that is defined by the following scale:

Low: 11–18 points
Medium: 19–26 points
High: 27–33 points.

Any assessment that scores in the 'low' category may not require quarantine measures. To conduct a sensitivity analysis, the analyst may manipulate

(continued)

Box 9.4 (continued)
estimates in each probability and consequence category by a single value. Any assessment that scores 18 or 19 points is sensitive to misclassification in a single element. An assessment that scores 17 or 20 points is sensitive to misclassification in two elements, and so on. The analyst may then consider the reliability with which each assessment was made, to determine if any misclassifications are plausible, and what information would be needed to substantiate them more reliably.

Uncertainty analyses. Sensitivity analyses may be supplemented by more comprehensive uncertainty analyses. In these, if any classification of a probability or consequence estimate is uncertain, then the analyst records the full range of plausible categories, and carries these uncertainties through the logic or procedures of the system in a transparent and repeatable way.

For instance, the judgement of a probability of entry under the Australian system may fall near the boundary between 'very low' and 'low', say a value of 0.04. Uncertainty in this estimate may make it plausible (say, within 90 % credible bounds) that the true value is as high as 0.06, or as low as 0.01. The judgement of 'very low' remains the best guess but 'low' is possible, given the current state of the analyst's understanding and data. However, we must recognise that although a range of values is plausible, typically there is greater probability associated with the values toward the middle of the range than those near the boundaries.

The analyst may record the range of possible values for this step on the pathway as '[very low, low]'. In the Australian and USA system, any uncertainties that cross category boundaries may be recorded similarly. The analyst may then repeat the analysis for all combinations of these categories, generating a range of potential outcomes. If any of these outcomes result in a different classification relative to Australia's ALP, the uncertainties matter. The analyst may decide to try to collect additional information to reduce these (epistemic) uncertainties, or they may make a transparent, risk averse decision in the interim, and indicate the data required to resolve the decision. If assessment outcomes are the same, despite the uncertainties, then uncertainties can be disregarded.

An analogous approach may be implemented for any procedures used in any jurisdiction. Generally, subjective judgements of probabilities and consequences (together with their credible bounds) may be combined using the rules of interval arithmetic, to provide robust, transparent and general treatments of uncertainty (Burgman 2005). In the few cases when sufficient data are available to characterise uncertainty distributions and dependencies more fully, Monte Carlo techniques may be more appropriate.

Sensitivity analysis is a standard method of analysis in which uncertainty in models or procedures is examined systematically. Parameters or other inputs, together with the structures and procedures in which they are used, may be evaluated by examining how a model's output responds to changes in inputs. In PRAs, this would mean assessing how sensitively a decision about the acceptability of a pest may change, if estimates or judgements are changed around the analyst's estimates, or if the rules for combining the estimates were changed. More generally, this is equivalent to asking, "if an input is changed by a small amount in the region of the best estimate, what is the magnitude of change in the output?" This perspective on sensitivity analysis can help to understand which inputs determine outcomes most strongly. The analyst may explore sensitivities with a view to providing advice on further field studies, so the analysis may concentrate on those parameters that are amenable to further study. In general, the details of the analysis depend on the kind of analysis, the context of the problem, and the kinds of risks being considered.

We must distinguish between 'precautionary' estimates that contribute to a risk assessment, and precaution in management decisions. The SPS Agreement specifies that measures should be based on scientific evidence, but Article 5.7 allows for the adoption of provisional management measures where substantial uncertainty exists in a risk assessment, stipulating that *where relevant scientific evidence is insufficient, a Member may provisionally adopt … measures on the basis of available pertinent information…In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk*…. This wording indicates that the risk assessment must identify the uncertainty so that it can be resolved. It also identifies the country applying provisional measures with the full responsibility for collecting the information necessary to re-evaluate the measures.

Biosecurity New Zealand (2006) attempts to outline this distinction with an instruction to identify critical uncertainties for future research as a part of the risk assessment process, while noting that the risk management decision must meet the requirements of the SPS Agreement. However the distinction between making conservative estimates in a risk assessment and making provisional risk management decisions is not particularly clear.

In the absence of clear advice or guidelines on how to handle uncertainty, all analysts occasionally adopt a conservative stance in the assessments. Where the risk assessments themselves adopt a conservative approach, this position usually will be submerged beneath the standard operations of a risk analysis, leading to uncontrolled and unspecified levels of conservatism in PRAs.

Conventionally, PRAs estimate the probabilities of entry, establishment and spread, and combine these estimates into an overall judgement, as outlined above. In those jurisdictions where the steps for combining probabilities are explicit, the procedures assume the probabilities are independent.

Another way of looking at the assessment is that essentially, analysts predict the prevalence or abundance of unwanted organisms on a pathway. This view is implicit in Fig. 9.2. Such a view makes it easier to see that it will be rare for the probabilities associated with the individual steps on a pathway to be independent of one another, for two main reasons. Firstly, abundances of organisms make the probabilities between steps dependent on one another. Consider a pulse of pests, the result of a mast reproductive event in the source country. When there are many organisms in source areas, if not controlled, there may be relatively many in packing facilities, and, if the consignment is not treated, relatively many may be transported to the pest risk area. That is, if the probability of entry in a particular year is relatively high, then the probabilities of establishment and spread may also be relatively high given favourable conditions in the PRA area. The second reason for a lack of independence is that many of the biological factors that affect the probability of entry and establishment are the same as those that affect the probability of spread. For example, we noted above that the probability of spread is affected by many factors, several of which also affect the probability of establishment, including host availability, climate suitability and the presence of natural enemies. PRAs may be improved by assessing the distribution of the expected numbers of pest organisms, rather than the probabilities of entry, establishment and spread.

9.6 Qualitative Versus Quantitative PRAs

An uncomfortable and artificial dichotomy has arisen in some biosecurity literature that implies distinct '*qualitative*' methods exist for PRA that are largely subjective and '*quantitative*' methods that depend on data. In fact, all quantitative methods rely on subjective judgement to formulate models and estimate parameters. Likewise, all sound qualitative methods are answerable to data and the fundamental rules of probability and formal logic. Varied quantitative and qualitative schemes and tools are employed for PRAs, from subjective reasoning based on knowledge of biological systems, trade pathways and impacts, to methods underpinned by a quantitative system but expressed qualitatively, to point-scoring systems, logical rules and Monte Carlo simulation (Fig. 9.8). Thus, we see a continuum ranging from implicit to explicit formal reasoning, each element of which may be associated with tools or schemes that express inputs, results and uncertainty with varying degrees of numerical representation. Sansford (2002) reviewed the topic and concluded that the WTO SPS Agreement (WTO 1995) does not prescribe the methodology to be used. Rather, the management, production, development and communication of PRAs should be fit for purpose and no more complex than is technically justified. PRAs that depend on non-numerical analysis are the most common type of assessment for routine PRA decision-making in all authorities reviewed here.

Biosecurity New Zealand (2006) guidelines maintain that qualitative risk analysis (defined as *a reasoned and logical discussion of the relevant commodity factors and epidemiology of a hazard* where likelihood and consequences are expressed using non-numerical terms such as high, negligible or non-negligible) is suitable for most PRAs. The examples of PRAs from Biosecurity New Zealand reviewed here do not use numerical representations.

Biosecurity New Zealand (2006) and USDA (2000) note that in some circumstances it may be desirable to undertake a quantitative risk assessment. For example, assessors may wish to gain further insights into a particular problem, to

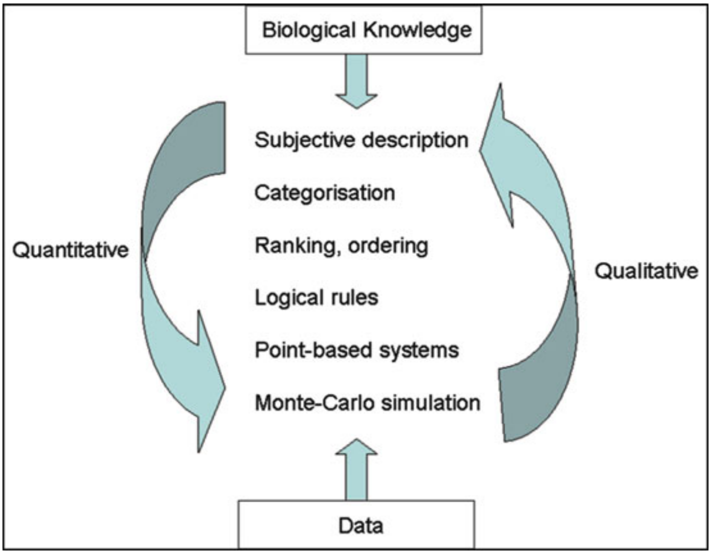

Fig. 9.8 Continuum of quantitative and quantitative schemes and tools for PRA, with different emphases on data and biological knowledge

identify critical steps, to compare measures or to be more explicit about uncertainties. Quantification may involve developing a mathematical model to link various aspects of the epidemiology of a pest, which is expressed numerically. The results, which are also expressed numerically, invariably present significant challenges in interpretation and communication.

Methodologies termed ‘semi-quantitative’ by OIE (2010) assign numbers to qualitative assessments (in the form of probability ranges or scores) to produce a summary measure. Systems like this are employed by Australia, Canada and the USA, in a variety of forms. The OIE (2010) states that numbers, ranges, weights and methods of combination are usually arbitrary and need careful justification to ensure transparency. Further, semi-quantitative assessments often give a misleading impression of objectivity and precision and that these methods *do not offer any advantages over a well researched, transparent, peer-reviewed qualitative approach* (OIE 2010 p. 36; Biosecurity New Zealand 2006 p. 27).

Systems that use categories without linking them explicitly to scales invite analysts to use vague and inconsistent interpretations (for example, Budescu et al. 2009). Aven and Renn (2009) suggest that when data are lacking, larger deviations arise among experts’ subjective probability assignments and participants become more convinced that many unlikely causes could lead to the undesired result. In their opinion, this creates an imperative for including in the risk assessment process tools other than subjective, qualitative assessment. They recommend semi-quantitative methods that include construction of case scenarios, analogies from other related fields, brainstorming and/or Delphi-type exercises.

Our view is that strident opinions regarding types of risk analysis create false methodological dichotomies. Most PRAs involve a mixture of qualitative and quantitative methods because generally we cannot quantify all sources of variability and uncertainty. An appropriate choice of tools should be determined by the context, data, skills, time frames and resources available. As noted above, even protocols that rely entirely on linguistic representations of probability and consequence are answerable to the rules of arithmetic, probability and logic. Perhaps the most important general point is that all PRAs should include a systematic consideration of both quantified and unquantified sources of variability and uncertainty to evaluate how they might affect the outcome. Our experience is that the choice of any tool or scheme used for PRA does not preclude a thorough and transparent treatment of uncertainty and variability. PRAs that omit them are incomplete and not transparent.

9.7 Desirable Properties of a PRA

Analysts and stakeholders want to know whether the predictions in a PRA are accurate and whether any proposed risk management options (when implemented) work. Measuring the effectiveness of PRA is difficult because long time lags may occur between establishment of a new pest and its discovery; often we cannot trace the incursion back to a regulated pathway. However, when PRAs are viewed as predictions of the number of organisms expected to be present at different points along the exposure pathway, validation becomes possible. While it may not be plausible to assess the effectiveness of biosecurity measures in general, each PRA makes verifiable predictions regarding the number of organisms expected at each step along the biosecurity continuum (MacLeod et al. 2005). Audit and inspection data collected strategically could be used to validate PRAs.

We conclude our evaluation of PRA by listing properties that are generally desirable, based on recommendations of ISPM 11 (FAO 2004a) and our own experience:

- PRAs should clearly identify pathways that are being assessed and should provide clear specification of how frequency and volume of trade are accommodated.
- Guidelines for PRA should take care to define as precisely as possible the terms used to express likelihood and consequence.
- Rules for combining likelihoods should be consistent with the rules of probability.
- Analyses should include transparent economic, social and environmental impact assessments.
- Guidelines for PRA should recommend and provide examples of how to present uncertainties.
- Sensitivity analyses are not normally routine elements of pest risk assessments, neither for the qualitative or and quantitative elements, but could be considered for future use.

- Analysts should avoid making ‘conservative’, precautionary or risk-averse judgements in the absence of evidence (within the body of an analysis) and reserve interpretations to a transparent step at the end of the analysis.
- Attitude to uncertainty of the importing country could be expressed by its interpretation of the bounds on likelihood and consequence, relative to its ALP or ALR.
- Explicit expression of ALP would assist stakeholders to understand and interpret a PRA.

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Appendix 1: SPS Agreement, Article 5. Assessment of Risk and Determination of the Appropriate Level of Sanitary or Phytosanitary Protection

1. Members shall ensure that their sanitary or phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to human, animal or plant life or health, taking into account risk assessment techniques developed by the relevant international organizations.
2. In the assessment of risks, Members shall take into account available scientific evidence; relevant processes and production methods; relevant inspection, sampling and testing methods; prevalence of specific diseases or pests; existence of pest — or disease — free areas; relevant ecological and environmental conditions; and quarantine or other treatment.
3. In assessing the risk to animal or plant life or health and determining the measure to be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk, Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks.
4. Members should, when determining the appropriate level of sanitary or phytosanitary protection, take into account the objective of minimizing negative trade effects.
5. With the objective of achieving consistency in the application of the concept of appropriate level of sanitary or phytosanitary protection against risks to human life or health, or to animal and plant life or health, each Member shall avoid arbitrary or unjustifiable distinctions in the levels it considers to be appropriate in different situations, if such distinctions result in discrimination or a disguised restriction on international trade. Members shall cooperate in the Committee, in accordance with paragraphs 1, 2 and 3 of Article 12, to develop guidelines to further the practical implementation of this provision. In developing the guidelines, the Committee shall take into account all relevant factors, including

the exceptional character of human health risks to which people voluntarily expose themselves.

6. Without prejudice to paragraph 2 of Article 3, when establishing or maintaining sanitary or phytosanitary measures to achieve the appropriate level of sanitary or phytosanitary protection, Members shall ensure that such measures are not more trade-restrictive than required to achieve their appropriate level of sanitary or phytosanitary protection, taking into account technical and economic feasibility.
7. In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organizations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time.
8. When a Member has reason to believe that a specific sanitary or phytosanitary measure introduced or maintained by another Member is constraining, or has the potential to constrain, its exports and the measure is not based on the relevant international standards, guidelines or recommendations, or such standards, guidelines or recommendations do not exist, an explanation of the reasons for such sanitary or phytosanitary measure may be requested and shall be provided by the Member maintaining the measure.

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Chapter 10
Phytosanitary Treatments

M.K. Hennessey, L. Jeffers, D. Nendick, Ken Glassy, L. Floyd, J.D. Hansen, W.D. Bailey, I. Winborne, D. Bartels, C. Ramsey, and C. Devorshak

10.1 Introduction

A phytosanitary treatment is an official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalisation (FAO 2009). These treatments are for plants or plant material. Many treatment options are available including chemical treatments (dips, sprays or fumigations), and physical methods (cold, heat and irradiation treatments). Ideally, phytosanitary treatments used for quarantine purposes should produce efficacy equal to or greater than Probit 9 (Sects. 5.6 and 11.2). Probit 9 is a concept developed by Baker (1939) that allows for a minimum of 99.9968 % mortality of pests associated with commodities. In other words, at a 95 % confidence level, after the treatment of 93,600 pests there are no survivors (Heather and Hallman 2008b).

Generally, Probit 9 is the required efficacy for a stand-alone phytosanitary treatment and is a standard for treatment effectiveness having its origin in fruit fly mortality research (Sect. 15.3). This standard has been adopted by the United States Department of Agriculture (USDA) and other countries for economically important fruit flies and other pests. Following this acceptance, Probit-9 has been used as a yardstick for many international quarantine treatments (Johnson and Hansen 2008; Schortemeyer et al. 2011).

However, Probit 9 is not the international standard or desired endpoint for treatment efficacy. According to ISPM 11 (FAO 2004), *The conclusions from pest risk assessment are used to decide whether risk management is required and*

M.K. Hennessey (✉) • L. Jeffers • L. Floyd • J.D. Hansen • W.D. Bailey • I. Winborne
D. Bartels • C. Ramsey • C. Devorshak
Center for Plant Health Science and Technology, Plant Protection and Quarantine, Animal Plant Health Inspection Services, United States Department of Agriculture, Raleigh, NC, USA
e-mail: michael.k.hennessey@aphis.usda.gov

D. Nendick • K. Glassy
Plant, Food & Environment Directorate, Standards Branch, Ministry for Primary Industries (MPI), Pastoral House 25 The Terrace, PO Box 2526, Wellington, New Zealand

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the strength of measures to be used. Since zero-risk is not a reasonable option, the guiding principle for risk management should be to manage risk to achieve the required degree of safety that can be justified and is feasible within the limits of available options and resources. Pest risk management (in the analytical sense) is the process of identifying ways to react to a perceived risk, evaluating the efficacy of these actions, and identifying the most appropriate options. The uncertainty noted in the assessments of economic consequences and probability of introduction should also be considered and included in the selection of a pest management option.

The strength of a measure is relative to the level of efficacy required. The higher the level of efficacy required, the stronger the measure required. The required level of safety for a treatment may be prevention of establishment or spread by the target pest. The level of safety is demonstrated by replicated lab and field-testing of the method against the pest. The level of survivorship allowed after treatment is determined by how many or what stages of the pest would be required to initiate a population that could become established. A Probit 9 level treatment with 95 % confidence is a very strong treatment. Even a Probit 9 level treatment can be less than effective if the pre-treatment infestation rate is exceptionally high. Powell (2003) discussed this in light of a treatment failure for Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) in imported "clementines" (the mandarin orange, *Citrus reticulata* Blanco), subjected to a required, proven Probit 9 cold treatment where live maggots or "wigglers" were intercepted post-treatment. In this example, where infestation rates approach 100 % of the host material, a level of control provided by a treatment even to Probit 9 levels would be considered inadequate to prevent target organisms escaping from the host material and possibly establishing in a new area.

The following works are recommended reading as aids to understanding strength of measures required: Mangan et al. 1997 assert that a Probit 9 treatment is effective in preventing a breeding population of fruit flies from becoming established; Follett and McQuate 2001 state that low infestation rate and volume of a commodity may allow for an applied treatment with lower than Probit 9 efficacy to prevent establishment of the pest; and Follett and Neven 2006 advocate risk-based systems approaches rather than Probit 9 efficacy stand-alone treatments. Systems approaches are also discussed later in this chapter. A systems approach requires two or more measures that are independent of each other, and may include any number of measures that are dependent on each other (FAO 2009). Processes, which may function as treatments, may be parts of systems approaches. These may include fruit or commodity washing to remove pests, which is also discussed later in this chapter.

Inspections used by USDA are designed to be part of a systems approach. An advantage of the systems approach is the ability to address variability and uncertainty by modifying the number and strength of measures to meet the appropriate level of phytosanitary protection and confidence. Measures used in a systems approach may be applied pre- and/or post-harvest wherever NPPOs have the ability to oversee and ensure compliance with official phytosanitary procedures. Thus, a systems approach may include measures applied in the place of production, during the post harvest period, at the packinghouse, or during shipment and distribution of the commodity (FAO 2002). In this way the point of entry/exit inspections, and subsequently any required treatments, can play an integral part of a systems approach to pest management alongside good agricultural practices, preclearance treatments, or the applications of pesticides during the growing season, etc. One benefit of a systems approach is that redundant safeguards are built into the process. That is, if one mitigating measure fails, other safeguards are in place to ensure that the risk continues to be effectively reduced and managed (Miller et al. 1995).

Pest mortality is not always the endpoint of a quarantine treatment. For irradiation treatments, pest sterility is equally effective from a plant quarantine standpoint. In fact, the FAO lists a range of options that may be specified in which the required response is the inability of the pest to reproduce. These may include: Complete sterility; limited fertility of only one sex; egg laying and/or hatching without further development; altered behaviour; and sterility of F1 generation (FAO 2003).

This chapter does not describe all types of treatments in detail, but introduces the reader to widely used treatments. For the USA, consult current official USDA publications to determine which treatments have been approved by USDA.

10.2 International Treatment Standards

10.2.1 International Standards for Phytosanitary Measures

The International Plant Protection Convention (IPPC) is an international agreement on plant health with the goal of protecting plants by preventing the introduction and spread of pests (See Sects. 2.1 and 2.2). International Standards for Phytosanitary Measures (ISPMs) are adopted through the Commission on Phytosanitary Measures (CPM), which is the governing body of the IPPC. Adopted standards include guidelines and recommendations concerning many aspects of plant protection, but three standards in particular relate to phytosanitary treatments. Two examples of these standards (ISPM 15: Regulation of wood packaging material in international trade, and ISPM 28: Phytosanitary treatments for regulated pests) are described here.

10.2.2 ISPM 28: Phytosanitary Treatments for Regulated Pests

The IPPC recognizes certain phytosanitary treatments as international standards in an effort to achieve harmonization, enhance mutual recognition of treatment efficacy by NPPOs, and facilitate trade. NPPOs and Regional Plant Protection Organisations (RPPOs) may submit data and other information for evaluation of treatments to the IPPC Secretariat. Requirements for submitting treatment research data and other information are described in detail in ISPM 28.

Treatments submitted for recognition in the standard should already be approved for national use. Submissions are reviewed by the Technical Panel on Phytosanitary Treatments (TPPT) that makes recommendations to the Commission on Phytosanitary Measures (CPM). The CPM adopts or rejects the treatment as an international standard. If a treatment is adopted as a standard, then it is added as an annex to ISPM 28. However, NPPOs are not obliged to use treatments that are adopted as international standards and may require the use of other treatments instead, even for the same pests or regulated articles.

All submissions should include a detailed description of the treatment, efficacy data, and the relevance or reason for the submission. A contact person should be indicated and credentials of those conducting the research should be provided. Information concerning feasibility and applicability should be included as well. Treatment data may be based on research conducted in a lab or controlled settings, or may be based on trials conducted under operational conditions. Ideally, when research is conducted under controlled conditions, it should be validated by tests conducted under operational conditions as well. Submissions of treatment data and other information based on research conducted under controlled conditions should include the following types of information:

Summary information should be submitted by NNPOs or Regional Plant Protection Organisation (RPPOs) to the Secretariat and should include:

- Treatment name;
- NPPO/RPPO contact information;
- Contact individual;
- Treatment description – commodity, target pest(s), parameters;
- Reason for submission;
- Credentials of those conducting research.

The submission should include all efficacy data in support of a phytosanitary treatment, including:

- Source of data;
- Lifecycle/stage of target pest(s);
- Statistical level of confidence;
- Statistical Methods used;
- Dose/efficacy curves (if applicable);
- Additional information to support extrapolation (if applicable).

The following information about the target pest should be included:

- Species, strain, biotype, race etc. (if applicable);
- Conditions under which pests are cultured/reared;
- Weight;
- Stage of development;
- Health;
- Method of infestation/infection;
- Most resistant life stage.

The submission should also include information pertaining to the regulated article/commodity:

- Type of regulated article;
- Intended use;
- Size/shape/weight;
- Stage of maturity (if applicable);
- Storage conditions after harvest/quality (if applicable).

All parameters of the experimental design should be described in detail; this information includes but is not limited to the following:

- Facilities used;
- Equipment used;
- Calibration of equipment and accuracy of measurements;
- Experimental design;
- Level of confidence;
- Conditions;
- Critical parameters;
- How effectiveness was measured;
- How phytotoxicity was measured;
- Dosimetry (if irradiation).

Submissions of treatment data based on research conducted only under operational conditions should include the information listed above. In addition, factors that affect the efficacy of the treatment (such as the way a commodity is packaged or stacked) should be included. Information should be provided concerning the monitoring of critical parameters, such as exposure time, dose, and temperature. For example, the placement of temperature or humidity sensors, or of gas sampling lines, if applicable, should be addressed. If special procedures are necessary to maintain the quality of the commodity, then they should be explained as well.

All submissions should include information on the feasibility and applicability of the treatment. Examples of this sort of information are:

- Ease of use;
- Risk to operators;
- Technical complexity;
- Training/expertise required;
- Equipment/facilities required;
- Cost of treatment facility;
- Commercial relevance;
- Versatility of treatment;
- Phytotoxicity data;
- Effects on humans, non-target organisms, and the environment;
- Information concerning how resistance will be managed;
- Whether other NPPOs have approved the treatment;
- Whether the treatment stands alone or is part of a systems approach.

Data and other information included in submissions to the IPPC Secretariat should be previously published in a peer-reviewed scientific journal. Statistical review methods should be reproducible and recognized, or accepted as international practice. Treatments must not be phytotoxic or have other adverse effects and must be feasible and applicable for practical use in international trade. Treatments are adopted only for the commodities and target pest(s) and conditions under which they were tested, unless there is sufficient data for extrapolation.

10.2.3 ISPM 15: Regulation of Wood Packaging Material in International Trade

Wood packaging materials (WPM) – items such as crates, boxes, pallets, dunnage, etc. – are often used to support, protect, or carry a commodity in international trade. The potential for the spread of quarantine pests via WPM presents unique challenges. Often, determining the origins of wood used for WPM is difficult. Moreover, this material is predominantly of low relative value, which makes the process of risk analysis difficult. To address these challenges, the Commission on Phytosanitary Measures adopted ISPM 15 *Regulation of Wood Packaging Material in International Trade*. The measures required by ISPM 15 apply to WPM made from raw wood. These measures do not apply to WPM made from materials such as plywood that are already processed in such a way that they are free from, and extremely unlikely to be re-infested with quarantine pests. Additional exceptions include particleboard, sawdust, WPM made entirely from thin wood (≤6 mm) and wooden components permanently attached to freight vehicles or containers. Measures described in ISPM 15 are not intended to target contaminating pests such as “hitchhikers”.

Two basic measures are required for WPM used in international trade: Debarking and treatment. WPM should be composed of debarked wood. In addition to debarking, WPM should undergo heat treatment or methyl bromide fumigation. Heat treated WPM must be heated to at least 56°C for 30 continuous minutes. The treatment temperature should be reached throughout the entire profile of the wood, to the core. Multiple processes including kiln drying, chemical pressure impregnation, and microwaving are acceptable as long as the necessary temperature is maintained for the appropriate period of time.

Fumigated WPM must be treated with a certain concentration of methyl bromide for at least 24 continuous hours. The exact gas concentration will vary depending on the temperature at which the WPM is treated. Typical considerations for conducting a chemical fumigation, such as air circulation, and proper sealing of the treatment area should be addressed.

Measures described in ISPM 15 should be accepted, without additional requirements, by NPPOs of importing countries. Further requirements must be technically justified. If WPM is deemed non-compliant with the measures outlined

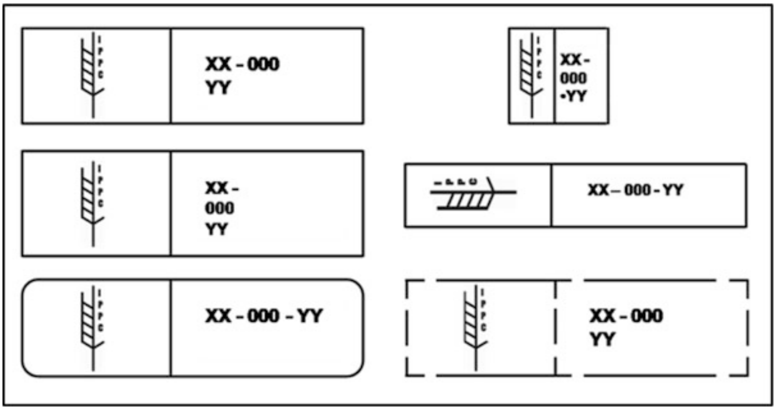

Fig. 10.1 Examples of acceptable variants of the mark used to indicate that WPM has been treated according to ISPM 15

in ISPM 15, then the importing country's NPPO is responsible for notifying the appropriate parties and securely disposing of non-compliant WPM. Acceptable disposal methods include incineration, deep burial, processing, and re-exporting.

All WPM that has been debarked and treated according to ISPM 15 should be marked with a symbol (Fig. 10.1). The symbol includes a country code, producer code, and treatment code. Country codes are two-letters and are accredited by the International Organisation for Standardisation (ISO). Producer codes indicate where the WPM was produced and/or treated. These codes are assigned by the NPPO. Treatment codes are either "HT" for heat-treated WPM or "MB" for WPM fumigated with methyl bromide.

10.3 Fumigation Treatments

10.3.1 Treatment Overview

A fumigant is a substance that exists in the gaseous state in sufficient concentrations that is lethal to pests when applied in a gas-tight enclosure for a designated period of time. Because fumigants are gaseous, the molecules can penetrate the interior of a commodity and then exit the commodity after fumigation. Toxicity of the fumigant depends on the respiratory rate of the target pest. As temperatures increase, pest respiration increases and subsequently the pest is more susceptible. A fumigant treatment schedule is defined by fumigant dosage and duration of treatment (Fig. 10.2). Fumigations are used as condition of entry treatment and as an emergency measure when non-target quarantine pests are detected.

T101-a-1	Apple and Pear[1]			
	Pest: External feeders			
	Treatment: **T101-a-1** MB at NAP—tarpaulin or chamber			

Temperature	Dosage Rate (lb/1000 ft^3)	Minimum Concentration Readings (ounces) At:	
		0.5 hr	2 hrs
80 °F or above	1.5 lbs	19	14
70-79 °F	2 lbs	26	19
60-69 °F	2.5 lbs	32	24
50-59 °F	3 lbs	38	29
40-49 °F	4 lbs	48	38

Fig. 10.2 Treatment schedule T101-a-1. From some countries, the USA requires a condition of entry methyl bromide fumigation when importing apples or pears

10.3.2 Fumigants

In the USA, only three fumigants are authorized for quarantine treatment: Methyl bromide, phosphine, and sulfuryl fluoride (See Sect. 6.7.4).

Methyl bromide (CH_3Br) (bromomethane) is a colorless, odourless, nonflammable gas with a boiling point of 3.6°C (40.1°F) and specific gravity of 3.27. Methyl bromide is a broad spectrum, highly effective fumigant used to treat a wide variety of plant pests associated with diverse commodities. Currently, methyl bromide is the most frequently used quarantine fumigant. It is compressed and stored in metal cylinders as a liquid. Liquid methyl bromide is heated in a volatiliser to speed up its conversion to a gas. The compound is about three times heavier than air and requires fans to ensure upward movement and equal gas distribution. After the international adoption of *The Montreal Protocol on Substances that Deplete the Ozone Layer*, the phase out of methyl bromide production and worldwide use was initiated. Despite the progress made, the international community still relies heavily on methyl bromide for mitigating the pest risks associated with imported goods. In 2010, more than half a million pounds of methyl bromide were used in PPQ-monitored, port-of-entry fumigations on nearly 150 commodities from about 100 countries (Fig. 10.3).

Phosphine (PH_3) (phosphorous hydride) is a colorless, odourless gas with a boiling point of −87.4°C (−126°F) and a specific gravity of 1.214. Pure phosphine is odourless, but often there is a garlic-like odour due to associated impurities like diphosphine. At concentrations above 18,000 ppm, phosphine forms explosive and self-flammable mixtures with air. Phosphine circulates easily throughout fumigation enclosures and can be aerated rapidly. However, phosphine is highly corrosive on copper, brass, gold, and silver; at high humidity it is slightly corrosive towards other metals.

Sulfuryl fluoride (SO_2F_2) is a colorless, odourless, nonflammable gas with a boiling point of −55.2°C (−67°F) and a specific gravity of 2.88. Sulfuryl fluoride

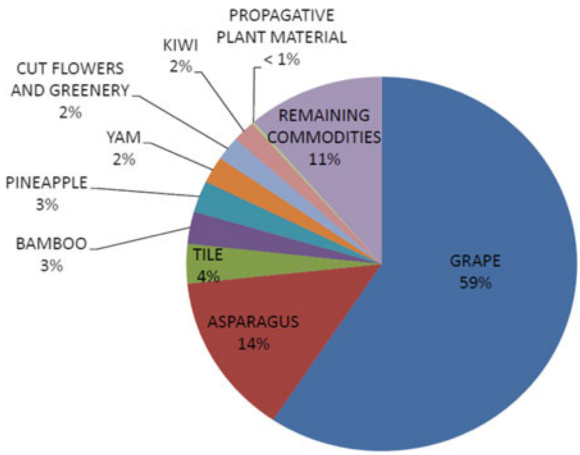

Fig. 10.3 More than 680,000 lb were used for PPQ-monitored methyl bromide fumigations on imported goods in 2010

is effective against many wood pests, but is not registered for use on living plant material. It penetrates wood well and is non-corrosive towards metals.

10.3.3 Labeling

By law, pesticide use must comply with all directions found on the product label. In the case of fumigants, each label lists the commodities on which the product can be used. The label also lists the dosages and durations allowed for each product. These rates are approved by the regulatory body for each nation that oversees pesticide use. Administered by the Environmental Protection Agency (EPA), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provides the overall framework for the United Stated Federal Pesticide Programme.

If a non-labeled use for a fumigant arises, then several types of exemptions can allow use. In the USA, the most common example of this is the FIFRA Section 18 Quarantine Exemption. "Section 18" exemption allows the treatment of commodities that are at risk for carrying Federal quarantine pests. The exemption applies to commodities imported into the USA as well as domestic commodities grown in quarantine areas.

10.3.4 Enclosure Types

A gas-tight enclosure is required to conduct a fumigation treatment. The most common fumigation methods to provide the required environment are:

- Chamber (normal atmospheric pressure (NAP) and vacuum) (Fig. 10.4);
- Tarpaulin (break-bulk) (Fig. 10.5);
- Tarpaulin (container) (Fig. 10.6).

Fig. 10.4 Fumigation chamber

Fig. 10.5 Tarpaulin fumigation of break-bulk commodity

Fig. 10.6 Placement of monitoring leads and temperature sensors before a containerized tarpaulin fumigation of logs

10.3.5 Certification of Fumigation Sites and Chambers

In New Zealand (NZ), approval of fumigators providing quarantine and pre-shipment (QPS) treatments is closely regulated. Fumigators must meet the standards of the Ministry of Agriculture and Forestry (MAF) and the NZ Treatment Supplier Programme. Fumigators are closely audited and assessed for competence under this programme. The programme covers exported and imported goods. The roles and responsibilities for all parties are outlined in the programme and detailed work instructions are included when required. QPS facility approval occurs after fumigator approval is granted, and the location is registered as a MAF transitional facility (TF).

Ports of arrival in NZ include designated areas for QPS fumigation where imported goods must be treated before being permitted to enter the country. To avoid cross contamination, treated and untreated goods are segregated. If fumigation does not occur at the port of first arrival, then imported goods must be securely transported to the TF of an approved operator for subsequent treatment under specific direction from MAF. TF operators must monitor and verify success of QPS treatments.

Treatment suppliers also are required to meet the NZ Environmental Protection Agency (NZ EPA) conditions for the use of hazardous substances such as approved handler requirements, the establishment of and maximum fumigant concentration levels in and around specific buffer zones associated with QPS treatment areas. NZ EPA's guidelines specifically cover methyl bromide fumigations (in containers, ship holds and under tarpaulin) in relation to:

- Air quality monitoring and comparison to Tolerable Exposure Limits (TELs);
- Buffer zones;
- Notification of fumigations;
- Reporting where TEL requirements are exceeded; and
- Annual reporting of use.

10.3.6 Operational Procedures

Fumigation operational procedures are outlined by each country's NPPO. For example, the operational procedures for the USA are listed in the PPQ Treatment Manual. This document is available at the following public site:

http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf

Additionally, the fumigation operational procedures for New Zealand are listed in the Approved Biosecurity Treatments. This document is available at the following public site:

http://www.biosecurity.govt.nz/files/regs/stds/bnz-std-abtrt.pdf

Monitoring. To ensure efficacious treatments, fumigant concentrations in the fumigation enclosure are monitored during the course of a treatment. Depending on the fumigant used, three types of monitoring technologies are used to determine fumigant concentrations: (1) Thermal conductivity gas analysers, (2) infrared spectroscopy gas monitoring devices, and (3) gas detector tubes. If required, minimum concentrations are listed in the fumigation treatment schedules. Normally, gas concentration readings are not required for chamber fumigations.

Aeration. After fumigation, the fumigant concentration in the enclosure must be reduced to safe levels before the commodity can be released; this ensures commodities are safe for handling during storage and transportation. For example, the concentration of methyl bromide in a fumigation enclosure must be 5 ppm or less before the commodity can be released to responsible parties. Depending on the fumigation enclosure and commodity, different aeration procedures are required. In the USA, private fumigation companies monitor most fumigations. The USDA Plant Protection and Quarantine (USDA-PPQ) usually does not supervise the aeration of the commodity unless the commodity is fumigated under a Section 18 crisis exemption or if the fumigation occurs at a USDA-PPQ Plant Inspection Station.

10.3.7 Packaging Requirements

Commodity packaging must not interfere with the circulation, penetration, and aeration of a fumigant during a treatment. To ensure gas movement, most countries maintain lists of approved packaging types. For example, the following packaging types are allowed for methyl bromide fumigations in the USA:

- Dry cloth;
- Dry, non-waxed or non-painted cardboard;
- Dry, non-waxed, non-painted, or non-glossy paper;
- Dry woven fabrics and plastics;
- Perforated plastics with evenly distributed holes on all sides and 0.93 % open area of surface;
- Plastic clamshells with evenly distributed holes on all sides and 0.93 % open area of surface.

10.3.8 Treatment Data Systems

In an effort to replace paper-based systems, some countries are trying to move to electronic treatment data systems. In the USA, the Commodity Treatment Information System (CTIS; https://treatments.cphst.org/tqau/) is a secure, online web-enabled data system that collects, stores, and creates reports from phytosanitary treatments. The Fumigation Form 429 Database collects treatment data from PPQ-monitored fumigations in the USA.

10.4 Cold Treatments

10.4.1 Treatment Overview

Since the early 1900s, sustained cold temperatures have been used to control the Mediterranean fruit fly, *C. capitata* (Heather and Hallman 2008b). From that time, cold treatments were employed to mitigate the pest risks associated with diverse commodities. A cold treatment schedule is defined by the temperature and duration of the treatment (Cf. Sect. 6.7.4; Fig. 10.7). Cold treatments generally are used as a condition-of-entry treatment. On rare occasions, cold treatments are used as an emergency measure when a non-target quarantine pest is detected. Most often, the target pests for a cold treatment are fruit flies from the genera *Ceratitis, Bactrocera*, or *Anastrepha*. The USA also has cold treatment schedules for the moths *Thaumatotibia (Cryptophlebia) leucotreta* (Meyrick) (false codling moth) and *Conopomorpha sinesis* (Bradley) (litchi fruit borer), the beetles *Tomicus piniperda*

T107-d

Apple, Grapefruit, Kiwi, Orange, Pear, Tangerine (includes Clementine)

Pest: *Bactrocera tryoni* (Queensland fruit fly)

Treatment: **T107-d** Cold treatment

Temperature	Exposure Period
32 °F (0 °C) or below	13 days
33 °F (0.56 °C) or below	14 days
34 °F (1.11 °C) or below	18 days
35 °F (1.67 °C) or below	20 days
36 °F (2.22 °C) or below	22 days

Fig. 10.7 Treatment schedule T107-d. The USA requires one of several treatment parameters to be met when importing apple, grapefruit, kiwi, orange, pear, or tangerine from countries where *Bactrocera tryoni* is known to occur

(Linnaeus) (common pine shoot beetle) and *Curculio caryae* (Horn) (pecan weevil), as well as several families of snails.

Most arthropods have evolved physiological responses to prevent damage caused by ice formation. Mechanical damage caused by ice crystals, osmotic removal of unfrozen cellular water, and the resulting cellular dehydration and membrane damage are the primary causes of mortality. However, most quarantine cold treatments to do not involve freezing, and the mode of action of sub-freezing cold treatments is not well understood. Denlinger and Lee (1998) propose that chilling of the cell fluid causes changes in membrane permeability, reduction of membrane-bound enzyme activity, and separation of membrane proteins and lipids leading to damage and death.

10.4.2 In-Transit Vessel and Container Cold Treatments

A significant drawback of non-freezing cold treatments is the long treatment duration. To circumvent this problem, cold treatments are often performed in self-refrigerated containers or refrigerated compartments of vessels during transport of the commodity to its country of destination. This practice is known as in-transit cold treatment. In-transit cold treatments can last several days up to several weeks.

10.4.3 Vessel Approval and Certification for Cold Treatment

According to USDA import requirements, all vessels must be approved and certified by the USDA before they can be used to perform a cold treatment. Before the start of vessel construction, the following specifications are collected during the vessel approval process:

- Diagrams of refrigerated compartments;
- Printouts from the temperature recording system (must be hourly and include date, time, temperature unit, and vessel name);
- Diagrams of air and pulp sensors for each compartment;
- Make and model of refrigeration unit;
- Make and model of the temperature recorder/control unit;
- Make and model of the sensor (including diagram of air and pulp sensors for each compartment, number of sensors is dependent on size of area).

Following vessel approval and construction of the vessel, an on-site certification will confirm the specifications collected during the approval process. Special attention is paid to the location and number of sensors in the refrigeration compartments. Each temperature sensor is calibrated using an ice-water slurry. Vessel certifications must be performed every 3 years. All USDA-certified vessels are listed in the Vessels and Containers Database, which is part of the Commodity Treatment Information System (CTIS). This is a public site located at:

http://treatments.cphst.org/vessels/.

10.4.4 Container Certification for Cold Treatment

As with vessels, all refrigerated containers must be certified by the USDA before they can be used to perform a cold treatment. The following specifications are collected during the container certification process:

- Contact information for container owner/manufacturer;
- Container BIC number, an internationally recognized label assigned to each shipping container for tracking purposes (identifies owner and type of container);
- Air flow rate;
- Container size;
- Make and model of refrigeration unit;
- Make and model of the temperature recorder/control unit;
- Make and model of the sensor (three sensors are required for each container);
- Container certifications last for 15 years. Containers are not re-certified. All USDA-certified containers are listed in the Vessels and Containers Database.

10.4.5 Warehouse Cold Treatments

In-transit cold treatments are not conducted in cold- treatment warehouses. Warehouse cold-treatments are often required when transit time cannot accommodate the necessary treatment duration. Warehouse cold-treatments are also used after in-transit cold treatment failures, for combination treatments (e.g. commodities that require a condition of entry fumigation and cold treatment), or as part of a preclearance programme in the country of origin. In the USA, all warehouses must be approved and certified by the USDA before they can be used to perform a cold treatment. Before the start of warehouse construction, the following specifications are collected during the warehouse approval process:

- Warehouse location;
- Contact information for warehouse owner;
- Diagram of treatment area (including dimensions, cubic capacity, and door locations);
- Diagrams of air and pulp sensors for each compartment;
- Make and model of refrigeration unit;
- Make and model of the temperature recorder/control unit;
- Make and model of the air circulation system (including number of air exchanges/unit of time and direction of air flow);
- Make and model of the sensor (including diagram of sensor location);
- Method for segregating fruit under treatments from other foreign and domestic articles.

Following warehouse approval and construction of the warehouse, an on-site certification will confirm the specifications collected during the approval process. Special attention is paid to the location and number of sensors in the treatment area. Each temperature sensor is calibrated using an ice-water slurry. Warehouse certifications must be performed every year. Additionally, many countries use the USDA vessel, container and warehouse approval and certification standards for their own cold treatment programmes.

10.4.6 Operational Procedures

Cold treatment operation procedures are outlined by each country's NPPO. For example, the operational procedures for the USA are listed in the PPQ Treatment Manual. This document is available at the following public site:

http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf

Precooling. Before an in-transit or warehouse cold treatment begins, the internal temperature of the commodity must reach the prescribed treatment temperature. The internal temperature of the commodity is monitored with pulp temperature-probes. Precooling is critical because depending on size, density, and temperature of the commodity, several days to weeks may be required to reach the prescribed treatment temperature. Commodities should not be precooled in the conveyance in which the commodity is to be shipped. Rapid cooling of the commodity is necessary to minimize physiological acclimation of the insect.

Fig. 10.8 Placement of pulp sensors before in-transit cold treatment of citrus (Image courtesy CPHST, APHIS, USDA)

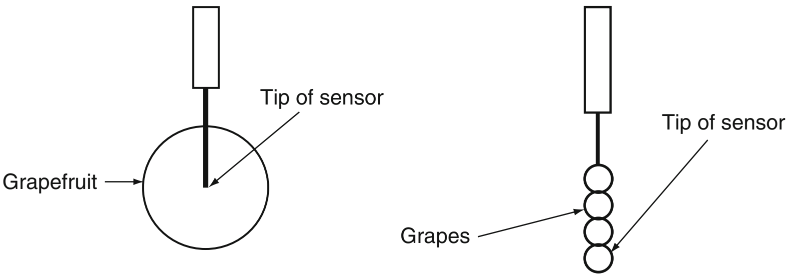

Fig. 10.9 Placement of pulp sensors into large and small fruit

Inspection of temperature recording equipment. Temperature recording equipment must be inspected before loading the commodity. Temperature sensors are calibrated using an ice-water slurry before each cold treatment.

Commodity Loading. The commodity is loaded directly from the precooling area to the container or vessel so internal temperatures do not rise significantly during the transfer. Each container or cold treatment compartment must contain only one type of fruit in one type of carton. As the container or compartment is loaded, temperature probes are placed into the commodity (Fig. 10.8). Temperature probes are inserted into the centre of larger fruit or through several smaller fruit (Fig. 10.9). The treatment does not start until all temperature probes reach the prescribed treatment schedule temperature (Fig. 10.7).

Treatment requirements. In addition to maintaining temperatures at or below the parameters outlined in a cold treatment schedule, some countries require that temperatures be recorded at intervals of one hour or less. In addition, some countries also minimize variation between temperature probes in a container or vessel compartment. For example, the USDA does not allow variation between pulp temperature sensors to exceed 0.39°C (0.7°F).

Document preparation. During a cold treatment, the USDA creates several types of documents, including but not limited to:

- Calibration of temperature probes;
- Instruction to Captain;
- Location of temperature sensors;
- Foreign site certificates;
- Shipping manifest;
- PPQ Form 556 (in-transit).

10.4.7 Treatment Data Systems

The USA uses the 556 In-Transit Cold Treatment Database (one of several databases in CTIS). The 556 was designed to manage cold treatment data for commodity imports. With the advent of electronic systems, auto-analysis of the cold treatment data greatly increases productivity of the certifying official.

10.5 Heat Treatments

10.5.1 Treatment Overview

Heat treatment is the process by which a commodity is heated until it reaches a minimum temperature for a minimum period of time according to an official technical specification (FAO 2009). Heat is a physical method for controlling agricultural pests of commodities after harvest. Heat treatment takes advantage of the difference in biological susceptibility between the pest and its host. Heat treatments are ideal for tropical fruits and vegetables or for surface pests. In some cases, the margin of efficacy and commodity tolerance is narrow, thus requiring a high level of precision.

The mechanisms of thermal death are poorly known and vary with the target pest. Specific mechanisms include protein denaturation, cell wall damage, accumulation of toxic products, and asphyxiation. Pest death may be immediate (*acute*) or delayed (*chronic*). In some cases, heat can lead to sterility or a weakened condition, making the pest susceptible to predators and pathogens.

T102-e **Limes**

Pest: Mealybugs (Pseudococcidae) and other surface pests

Treatment: **T102-e** Hot water immersion

1. Fruit must be treated in a certified hot water immersion treatment tank, and the treatment must be monitored by an inspector.
   A. Fruit must be submerged at least 4 inches below the water's surface.
   B. Water must circulate continually and be kept at 120.2 °F (or above) for 20 minutes. Treatment time begins when the water temperature reaches at least 120.2 °F in all locations of the tank.
2. Cooling and waxing the fruit are both optional, and are the sole responsibility of the processor.

NOTICE Phytotoxic damage (increased yellowing) may occur if the temperature reaches 125.6 °F or if the treatment duration significantly exceeds 20 minutes.

Fig. 10.10 Treatment schedule T102-e. Hot water treatment for surface pests

Efficacy of the treatment depends on the physiological state of the pest and its location (e.g., internal or external feeders). Factors that may weaken a pest are environmental preconditioning (e.g., cold storage), humidity, starvation, lack of temperature acclimation, and age. If these factors can be incorporated into a treatment, then potential heat damage to the commodity can be reduced.

Constraints on heat treatments depend on complexity, cost, time, handling, and thermal sensitivity of the commodity. Although simplicity is a goal, some treatments may require precise control by complex equipment controlled by sophisticated computer programmes. Obviously, the cost of the treatment cannot be so high as to make the commodity unmarketable. In some cases, holding limitations and marketing strategies require rapid treatment, which preclude long-term pretreatments like cold storage. Reduced handling is generally preferred, but some treatments require commodity movement to assure uniform thorough heating.

10.5.2 Heat Treatment Approaches

Hot Water (>40°C) baths and dips are simple heat treatments. The commodity is submerged for a specified time to assure that the core obtains the required temperature (Fig. 10.10). A sequence of dips at different temperatures can be completed to prevent surface scalding, such as against fruit flies in Hawaiian papayas (Couey and Hayes 1986). Hot water dips are used for bulbs, acorns, and tropical/subtropical fruits. The USA has an extensive hot water programme for imported mangos from South and Central America and the Caribbean (Fig. 10.11).

Vapour Heat (Fig. 10.12) and Forced Hot Air (Fig. 10.13) treatments use heated air to warm fruit to temperatures that are lethal to target pests, primarily fruit flies. Vapour Heat treatments differ from Forced Hot Air only in the relative humidity of

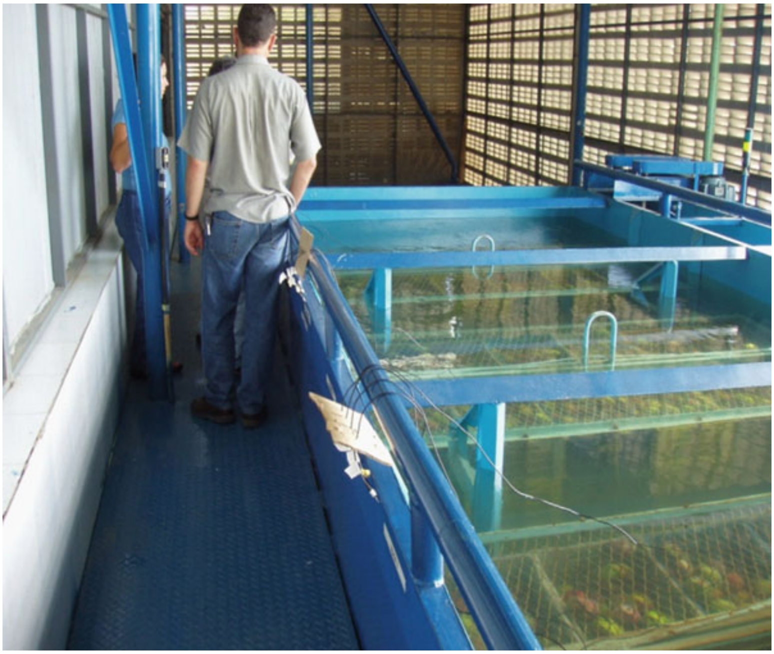

Fig. 10.11 Mango hot water treatment facility (Image courtesy CPHST, APHIS, USDA)

T106-f

Litchi from Hawaii

Pest: *Ceratitis capitata* (Mediterranean fruit fly), and *Bactrocera dorsalis* (Oriental fruit fly)

Treatment: T106-f Vapor heat

Heat Up Time:	1 hour
Heat Up Recording Interval:	5 minutes
Minimum Air Temperature:	N/A
Minimum Pulp Temperature at End of Heat Up:	47.2 °C/117.0 °F
Dwell Time:	20 minutes
Dwell Recording Interval:	5 minutes
Cooling Method:	Cool water spray

Fig. 10.12 Treatment schedule T106-f. Vapor heat-treatment for Litchi from Hawaii

the air in the treatment chamber. Vapour Heat treatments, not to be confused with steam, have a long history, including treatment of fruits and vegetables against the Mediterranean fruit fly, starting in the early 1930s. Forced Hot Air is more applicable to tropical/subtropical commodities than temperate ones.

Dry Heat. Dry Heat uses low humidity hot air (<100°C), primarily to control stored product pests of grain, nuts, and dried fruits. Dry Heat was employed in France for stored grains as early as 1792 (Fields and White 2002). It is also used to

T103-c-1

Mango from Mexico

Pest: *Anastrepha ludens* (Mexican fruit fly), *Anastrepha obliqua* (West Indian fruit fly), and *Anastrepha serpentina* (black fruit fly)

Treatment: T103-c-1 High temperature forced air

Heat Up Time:	N/A
Heat Up Recording Interval:	2 minutes
Minimum Air Temperature:	50.0 °C/122.0 °F
Minimum Pulp Temperature at End of Heat Up:	48.0 °C/118.0 °F
Dwell Time:	2 minutes
Dwell Recording Interval:	2 minutes
Cooling Method:	Forced air or Hydrocooling
Size Restrictions:	Fruit weight must **not** exceed 1 1/2 lbs. (700 grams)

Fig. 10.13 Treatment schedule T103-c-1. High-temperature forced air on Mangoes from Mexico

Fig. 10.14 Niger seed treatment facility (Image Courtesy, CPHST, APHIS, USDA.)

decontaminate storage structures and to devitalize weed seed contaminants in Niger seed, *Guizotia abyssinica* (L. f.) Cass., a birdseed food product imported into the USA from Ethiopia and India (Fig. 10.14).

Solarization. Solar heating is the simplest heat treatment. Besides using direct sunlight, this method often employs plastic to absorb and contain heat. Grains and dry products, like beans and lentils, can be treated while in storage. Solar heating has potential in rural areas and in developing countries, but problems exist with temperature control and maintaining consistency.

Fig. 10.15 Radio frequency heating unit showing top electrode plate and fruit rotation (Image courtesy James Hansen, ARS USDA.)

Electromagnetic Energy. Electromagnetic energy can also be used as a heat source. Infrared (0.3–430 THz) has been examined for efficacy against stored product pests, but it has not been popular for the last couple of decades. Microwaves (1–100 GHz) have been applied to a wide range of products, from soil and museum artifacts to fresh fruits. Recent efforts have been to control pests of grain and stored-products (Wang and Tang 2001). Radio frequencies (13.56 MHz, 27.12 MHz, 40.68 MHz) generate internal heat by resistance from the very rapid change in molecular polarity. The treatment is very fast, can penetrate deep into the target material because of its longer wavelength, may produce possible differential heating between the product and the pest, and does not produce toxic residues. Suitable products for radio frequency are tobacco, seeds and grains, flours, cereal breakfast foods, and nuts because these items have low moisture content (Wang and Tang 2001) (Fig. 10.15).

For additional information on heat treatments reference: Paull and Armstrong (1994), Sharp and Hallman (1994), Mangan and Hallman (1998), Tang et al. (2007), Vincent et al. (2003), and Yahia (2006).

10.6 Irradiation

Irradiation treatments involve any type of ionizing radiation (FAO 2009). Irradiation is a safe and effective method for phytosanitary treatments (See Sect. 6.7.4). Some types of radiation have been used for several decades to reduce microbial loads on food products. The use of irradiation for phytosanitary treatments is more recent and increasing in application. An extensive range of pests are neutralized by

T105-a-2 **Various Commodities**

Treatment: T105-a-2 (IR @ 400 Gy)

Pests: Fruit flies from the family Tephritidae and all insect pests **except** adults and pupae of the order Lepidoptera

Treat using a minimum absorbed dose of 400 Gy, **not** to exceed 1000 Gy.

Table 5-2-14 Origin and Approved Commodity List for 400 Gy

Origin	Commodity
Ghana	Eggplant, Okra, Pepper
Hawaii	Banana, Breadfruit, Cowpea (pod), Curry Leaf, Dragon fruit, Guava, Jackfruit, Mangosteen, Melon, Moringa pods (Drumstick), and Sweet Potato
India	Mango
Malaysia	Papaya, Rambutan
Mexico	Guava
Pakistan	Mango
South Africa	Grape, Persimmon
Thailand	Dragon Fruit, Litchi, Longan, Mango, Mangosteen, Pineapple, Rambutan
Viet Nam	Dragon Fruit, Rambutan

Fig. 10.16 Treatment schedule T105-a-2. Irradiation for various commodities

irradiation, making it a suitable alternative to methyl bromide and a tool for eliminating potential phytosanitary trade barriers. Consequently, NPPOs around the world are developing collaborative standards, regulations and policies to support the use of irradiation as a phytosanitary treatment (ASTM 2004, 2006; IPPC 2003).

The use of irradiation as a phytosanitary treatment has increased significantly during the past 10 years. In 2000 the first purpose-built phytosanitary irradiator was constructed in Hilo, Hawaii. This facility continues to treat tropical fruit and sweet potatoes grown on the island of Hawaii. In 2007, the USA initiated its first preclearance programme utilizing irradiation in India. Following the Indian mango programme, Thailand, Viet Nam, and Mexico have established programmes with over 16 million Kg of fruit treated by the end of 2010. One facility in the USA has used irradiation to ship limited amounts of tropical fruits within domestic quarantine areas. Australia has also shipped irradiated mangoes to New Zealand.

10.6.1 Treatment Overview

Types of radiation include heat, visible light, and radio waves. Ionizing radiation is used for phytosanitary treatments, which breaks chemical bonds within exposed organic matter. Phytosanitary irradiation treatments require relatively low doses in comparison to food safety or sterilization applications (Fig. 10.16). Most phytosanitary treatments target acute insect mortality as the endpoint. Irradiation treatments target neutralization of the organism, which may result from sterility, cessation of development, impaired emergence (from egg or pupa), or death. The mode of action for phytosanitary irradiation treatments is a combination of direct mutagenic effects on DNA and general tissue damage resulting from free oxygen radicals generated during the treatment. The contribution of generating free radicals to the success of irradiation treatments is significant, although not yet fully understood (Hallman 2004).

Ionizing radiation is charged particles and electromagnetic waves that, as a result of physical interaction, create ions by primary or secondary processes (FAO 2009). Radioactive isotopes or machine sources generate ionizing radiation. Radioactive isotopes emit gamma radiation as they decay to a more stable form. Cobalt-60 is the primary source of radioactive isotopes for commercial irradiation facilities. Cesium-137 is less frequently used due to safety concerns but is found in some sterile insect release irradiation facilities. Machine sources generate electron beams (e-beam), which can be applied to the target directly or at a material composed of a high atomic number element where it is converted to X-ray radiation. Cobalt-60, e-beam, and X-rays are all currently utilized for phytosanitary irradiation. A major difference between machine sources and radioactive isotopes is that isotopes emit radiation continually while machine sources can be turned on and off. This is one of many factors that facilities must take into consideration when selecting a source.

Regardless of the source type, radiation is measured in units of gray (Gy). The Gy is the unit of absorbed dose where 1 Gy is equivalent to the absorption of 1 J per kilogram ($1\ \text{Gy} = 1\ \text{J.kg}^{-1}$) (FAO 2009). Radiation is expressed as absorbed dose. The absorbed dose is the quantity of radiating energy (in Gy) absorbed per unit of mass of a specified target (FAO 2009). Therefore, an absorbed dose of 400 Gy is equal to 400 J delivered to 1 kg of matter. Dosimeters are used to indirectly measure absorbed doses. Many types of dosimeters exist, each type reacts in a known and measurable way when exposed to radiation, which may then be related to the absorbed dose using a simple linear equation. Indirect measurement systems require careful calibration, and national labs such as the U.S. National Institute of Standards and Technology (NIST) and the National Physics Laboratory (NPL) provide calibration services for this purpose.

Radiation is attenuated as it passes through matter. When a commodity is irradiated, the outer areas of the load receive a higher dose than inner areas. Additionally, some radiation sources deliver doses in an uneven distribution. Point(s) within a load that receive the lowest dose are termed D_{min}, while those that receive the highest dose are termed D_{max}. The dose absorbed at the D_{min} must be equal to or greater than the minimum dose established by the regulating NPPO to satisfy phytosanitary requirements. D_{min} is the localized minimum absorbed dose within the process load (FAO 2009).

Before commercial treatments, each load configuration must be dose mapped to determine the dose distribution, D_{min}, and D_{max}. Dose mapping is the measurement of the absorbed dose distribution within a process load through the use of dosimeters placed at specific locations within the process load (FAO 2009). During the dose mapping process, dosimeters are placed throughout the load and the load is irradiated.

A dosimeter is a device that, when irradiated, exhibits a quantifiable change in some property of the device which can be related to absorbed dose in a given material using appropriate analytical instrumentation and techniques (FAO 2009). The dosimeters are then analysed and the dose distribution is determined. The D_{min} location can then be directly monitored during routine treatments, or the D_{min} value can be estimated through the use of reference ratios. Dosimetry is a system used for determining absorbed dose, consisting of dosimeters, measurement instruments and their associated reference standards, and procedures for the system's use (FAO 2009).

10.6.2 Treatment Facilities

Irradiation treatment facilities generally resemble warehouses with commodities staged for treatment, an area with a protected source, and commodities awaiting shipment after treatment. Operational treatment processes vary by facility and source type but most irradiation facilities share some common procedures. Because of the high levels of radiation, sources are protected, usually in bunkers or "cells" constructed of concrete several meters thick. Untreated products are loaded onto conveyors or carriers (also referred to as "totes") that allow the product to be exposed without endangering workers (10.17). Dosimeters are placed in or on the load to verify dose and the product is carried through the bunker and exposed to the source. The product exits the bunker and is unloaded after treatment.

Verification of irradiation treatments differs from verification of other phytosanitary treatments. This difference exists for two reasons. Irradiation treatments do not impart radioactivity to other materials, therefore no residue is made to the commodity during the treatment, and acute mortality of the target pest is usually not the treatment endpoint. This means that methods used to verify other treatment types, such as cutting fruit to verify pest mortality or residue testing, are not appropriate for irradiated commodities.

A systems approach should be used to provide confidence that treatments are applied effectively. This system includes: Use of pest proof packages to exclude untreated pests; pretreatment pest inspection to ensure low infestation rates; physical barriers between treated and untreated products at irradiation facilities to ensure untreated products are not mixed with treated; rigorous inspection and certification of irradiation facilities to ensure procedures are in place to effectively apply treatments; labeling of each treated package with traceback information; and a data management system that allows quick access to treatment records. In addition to providing verification of treatment by inspection of records, this system reduces the number of instances when a regulatory response necessitating verification is needed.

Fig. 10.17 Irradiation facility where "totes" filled with mangoes move along a conveyor to the protected cell (*left*), where the cobalt-60 source is located (Image courtesy CPHST, APHIS USDA.)

Fig. 10.18 A pallet of mangosteen awaiting irradiation. Note the red-capped dosimeter Radura on each box. The Radura is an international symbol indicating a food product that has been irradiated (Image courtesy CPHST, APHIS, USDA.)

10.6.3 Irradiation as a Methyl Bromide Alternative

Significant interest exists in expanding the use of irradiation treatments. The international phase-out of methyl bromide under the Montreal Protocol necessitates the growth of alternatives, including irradiation. While the future of irradiation phytosanitary treatments is bright, challenges do exist. These include costs associated with facility construction and irradiation, concerns over consumer acceptance of irradiated products, and licensing issues. For more in-depth overviews of phytosanitary irradiation treatments, please consult Heather and Hallman (2008c) and Sommers and Fan (2006). (Image courtesy CPHST, APHIS, USDA).

10.7 Removal of External Pests from Fruit

10.7.1 Treatment Overview

Most fruit and vegetable commodities pass through a packinghouse before being shipped to their final destination. The main purpose of a packinghouse is to clean, sort, and package the produce for shipment to the market place. In recent years, packinghouse equipment has become more sophisticated in the removal of damaged or discolored products. Vision sorting systems are being incorporated into processes to remove damaged produce. Some infrared systems can detect bruising that is not visible to the human eye. Although highly dependent on the type of pest and fruit, the process of cleaning and packaging provides the ideal opportunity to remove many unwanted pests from the outside of the commodity.

Typical packinghouse procedures start with the unloading of the field boxes. Usually a large screen or sorter will allow field debris, such as twigs and leaves, to be removed. After the initial screening, the product can be moved into any combination of wash tanks, brushing with or without a washing solution or pressure washing. After the cleaning step, a culling step is conducted to remove damaged or misshapen produce. This step is usually done by hand, but computerized vision sorting systems are also used. Following the culling, the produce may then be waxed, labeled, sorted by size or grade before the final packaging.

A range of techniques (reviewed by Jamieson et al. 2009) is used to remove insects from fruits physically. A significant advantage of treatments that remove insects (compared with those that simply kill insects) is that absence of insects on arrival in overseas markets means that the product line is much more likely to pass official phytosanitary inspection.

10.7.2 Brushing

Use of rotating brushes during packing (generally before quality checking and grading) is a common practice in most fruit crops. This is carried out either on dry brushes or with lightly wetted brushes. This treatment removes dirt and other material from fruit, and in doing so the process also removes exposed pests such as thrips, mites, beetles and Collembola (Stevens and McKenna 1997). However, they will not remove insects and mites that are well protected by either their structure (e.g. scale insects or insects within cocoons fastened to the surface) or because they are protected by structures of the fruit (e.g. under calyx).

10.7.3 Pressure Washing

Several developments involve water-blasting (or pressure washing) systems for fruit products. These can be carried out for longer durations (10–20 s) over rotating brushes, or for very short times without brushes. Published information indicates that the first water blasting of fruits was developed in South Africa during the 1970s by L.J.K. Theron (Honiball et al. 1979). This process was then taken up by other countries and further research was published in the USA (Walker et al. 1996, 1999).

Moderate pressure/high volume washing. Moderate pressure/high volume water systems were developed successfully for citrus to dislodge scale insects and are used in several countries including the USA, New Zealand, Israel and South Africa. Walker et al. (1999) found that a 20-s treatment at 325 psi was optimal for removing California red scale, *Aonidiella aurantii* (Maskell). Higher pressures resulted in unacceptable external damage to the fruit. Similar systems operating at 50–120 psi for 10–15 s over rotating brushes were successful for disease reduction in apples from Washington State (Bai et al. 2006).

In New Zealand, moderately-pressurized washing treatments are used on nearly all apple exports. This treatment achieves up to 90 % removal of mealybug as well as reductions in *Eriosoma lanigerum* (Hausmann), scale, leafcurling midge, mites, leafrollers and a range of hitchhikers such as weevils and spiders, without causing apple damage (Walker personal communication 2005). Recent work has aimed to standardize this treatment and the target is 80–120 psi for 13–17 s over rolling brushes (Patterson et al. 2006).

High pressure/low volume washing. Research during the 1990s examined the use of high pressure (400–2000 psi) water washers on New Zealand apple, kiwifruit and avocado exports (Whiting et al. 1998a, c; Jamieson et al. 2000). The fruit were rotated precisely, thus ensuring a great accuracy of coverage and pest removal efficacy. Use of high pressures (500 and 800 psi for 1–2 s) was shown to remove a substantial proportion of *Epiphyas postvittana* (Walker) and mealybug removal from apples, despite artificially high infestations (Whiting et al. 1998a). Scale insects were difficult to remove from kiwifruit, even at 2000 psi with a hot water drench (55–65°C) for 30 s before water blasting (Whiting et al. 1998b). While useful for disinfestation, this high-pressure system that rotates fruit is technically more challenging and tends to have lower throughput and higher running costs, than other more simple fruit washers.

Avocado is a crop for which higher pressure/low volume treatments is successful (Woolf et al. 1998, 1999a, b; Jamieson et al. 2005). This work was targeted at removal of leafroller egg rafts, an important quarantine pest for export to Australia. However, the treatment was also very effective at removing all crawling pests such as leafrollers, thrips and mites (Jamieson et al. 2000). Optimum treatment was 1 s with two fruit-rotations, and a pressure of about 800 psi.

10.7.4 Air-Blasting

Air blasting is used for mite removal (John White, personal communication) on green kiwifruit. A crucial limitation is that large compressors are expensive. An additional challenge is that efficacy of the air-flow decreases rapidly with distance, more so than for water blasting. Thus, differences in fruit size and orientation will have more impact on insect removal.

10.8 Disposal and Decontamination

10.8.1 Treatment Overview

Disposal and decontamination of phytosanitary waste is rapidly becoming a critical issue due to increased international travel and trade. Regulated waste may be agricultural products, contaminated goods, such as water or soil, or airport or seaport waste. Disposal systems should be integrated and designed under a national programme that can effectively sterilize or decontaminate a wide range of contaminated materials before disposing of the waste in a municipal facility or landfill.

Currently many decontamination technologies available for treating agricultural products, including steam sterilizing (autoclaving), quick freeze, chemical treatment with high level biocides, and chemical digestion. The primary methods for phytosanitary waste disposal are incineration and burial.

10.8.2 Disposal

Incineration. Air curtain incineration (ACI) works by forcefully projecting a curtain of air across an open chamber in which combustion occurs. The products of combustion are mixed with air from above the air curtain to give a smokeless exit gas discharge into the air above the ACI unit. The powerful curtain of air created in this process traps unburned particles under the curtain in the high temperature zone where temperatures can reach 1000°C.

The very high temperature attained ensures complete destruction of all organisms, pathogenic and otherwise, yielding a sterile ash. ACI systems can also be used to help combat the spread of devastating beetles and other pests at the locations of infestation. The system is mobile and can be transported from place to place, which gives the option of taking the ACI to the diseased area as an alternative to transporting the material, reducing the risk of quarantine pests spreading.

ACI systems have been used for land clearing, burning trees and other foliage, forest maintenance and clearing, forest-fire prevention, and post-fire clean-up. They have been used in emergency cleanup for the removal and disposal of wastes resulting from events such as high winds, floods etc. ACI's are widely used in the USA forestry industry for disposal of waste wood. They comprise petrol or diesel engines driving a caged fan. The air-flow is forced across the top and angled downward into a trench, pit or fire-box (hence "air curtain"). The air curtain swirls into the pit increasing combustion efficiency and the burning rate due to increased oxygen being fed to the fire and greater air turbulence. The process provides 4–6 times faster burning rates than open fires and reaches temperatures of 1000–2000°C. The air curtain ensures that emissions (smoke and odour) are minimal. The incinerators are able to burn whole, unopened carcasses. They require an experienced operator, a pit of accurate dimensions, and an external fuel source (e.g. wood or coal).

The site must have the following requirements:

- Good weather access to allow equipment, fuel, deposal material;
- Sufficient space to house equipment, fuel stockpile and carcasses;
- Adequate separation distances from site to sensitive neighbours (at least 200 m);
- Required consents obtained for the site;
- Groundwater below 10 m and no possibility of future subsidence.

Burial. Deep Burial of quarantine waste is an option for disposal of infested or prohibited plant products and garbage removed from ships and aircraft when other options (such as steam sterilization or incineration) are not available, not suitable or cost prohibitive. Most burial sites are contained within municipal landfills for garbage or at least have the consents from the relevant authorities. Care must be taken during transport to the burial site; the material must also be loaded, transported and unloaded in a secure manner to prevent spillage and exposure to the environment.

Contaminated material should be buried in a pit of a sufficient size to contain the volume of material. Note that loose material is often double the volume of the compacted weight. The burial site should be at least 20m from any deep-rooted plants such as trees and waterways; the bottom of the pit must be above the groundwater level. After unloading, the material must be covered as soon as possible by at least 2 ms of fill to prevent pathogenic spores, insects or weed seeds from spreading and scavenging by animals or people.

Fig. 10.19 Shredder/Steam Sterilizer (Image courtesy CPHST, APHIS, USDA.)

Equipment should be washed and decontaminated at the burial location immediately after dumping. This should consist of an initial wash down with water, followed by rinsing with a suitable disinfectant.

The burial site should remain undisturbed with the exception of normal practices for addition of further deposits on top of the previously buried material. The location of the site should be recorded precisely for future reference.

10.8.3 Decontamination/Disinfestation

Shredding and Steam Sterilization. This method of disposal involves shredding the biologically contaminated plant products followed by a steam treatment. Since 1995, this method has been used by hospitals, labs, and commercial treatment facilities to decontaminate medical waste (Fig. 10.19). The basic concept of the system involves shredding all materials into homogeneous particles so that the containment materials and biologic waste are fully exposed to direct steam treatment (Fig. 10.20). The particles are very quickly elevated to the boiling point of water (100°C) and held at that temperature for 60 min The shredder/streamer system is a continuous feed system (not a batch treatment system) allowing for optimum throughput of waste materials.

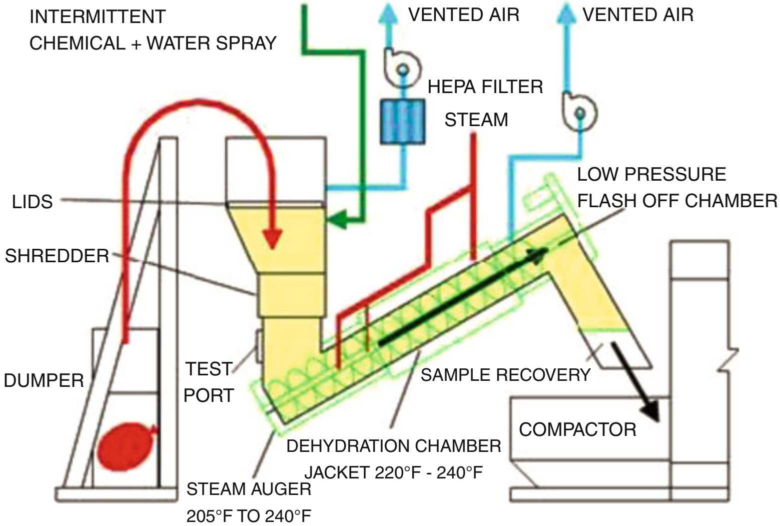

Fig. 10.20 Schematic diagram of Shredder/Steam Sterilizer (Image courtesy CPHST, APHIS, USDA.)

The shredder/steamer system has several advantages. First, steam generation and treatment produces a relatively small carbon footprint. Second, there is high microbial efficacy because the treatment method does not rely on conduction or convection of moist heat through multiple barriers (sealed bags, boxes, etc.) typically associated with conventional autoclaves. Finally, steam can easily penetrate and quickly elevate the targeted materials to proper treatment temperatures and correct time requirements because all waste and waste containers has been shredded into small particles. In 2010, USDA-APHIS demonstrated that shredding and steam treating of hay resulted in a 6-log reduction in colony forming units when compared to untreated hay samples. A seed germination test was also conducted during this study, which resulted in average germination rates of 0 % and 36 % for steam treated and control seeds, respectively.

Chemical Digestion. The use of chemical digesters has rapidly emerged as the preferred sterilization method when complete biological devitalisation is required for extreme high risk or difficult to kill pathogens. Alkaline digesters use potassium or sodium hydroxide, high temperature (150°C), high pressure, and powerful pumps to completely liquefy products.

APHIS-PPQ has tested the efficacy of alkaline digesters for destruction of seeds and microbial sterilization. Digestion tests for several types of hard coated seeds resulted in germination rates of 0 % and 74 % for the digester treated and untreated seeds, respectively.

Potential disadvantages for digesters include transportation costs (although this may also be true for other treatment types), batch capacity limitations, high initial digester establishment costs, and treated waste disposal issues. The advantages of using digester systems include complete sterilization for the most highly infectious

Fig. 10.21 Chemical decontamination of equipment (Image courtesy Craig Ramsey CPHST, APHIS, USDA)

and difficult to kill organisms and a diversity of contaminated materials can be treated including soil and water. In addition, there are usually few regulatory restrictions on such facilities and waste can be converted into biofuels or used to power electrical production.

Chemical Decontamination. Field equipment, farm machinery, first responder equipment, military equipment, recreational vehicles (boats, ATV's, etc.), cargo containers, cars and trucks represent a significant dispersal mechanism for pests (Fleming 2008). Chemical disinfectants are the preferred method of cleaning bulky equipment, especially under variable and dirty field conditions. Several classes of disinfectants may be used, including alcohols, aldehydes, ethylene oxide, halogens (chlorine, iodine, etc.), metals, ozone, oxidants, phenolics, and quaternary ammonium compounds.

Oxidants such as hydrogen peroxide and chlorine dioxide are broad-spectrum biocides, but are generally labeled as disinfectants. For example, chlorine dioxide will control zebra mussels (*Dreissena polymorpha* Pallas) with water concentrations at 5 ppm (Matisoff et al. 1996); and 7 % hydrogen peroxide will kill most herbaceous plants if sprayed on the foliage (Ramsey, unpublished). The primary advantage of oxidant disinfectants includes a short half-life, broad-spectrum activity, low health risk, and material safety. The disadvantages include limited surface residual activity, limited life after activation, and side-reactions with organic tissue or organic debris mixed on the contaminated surface.

Effective decontamination of field equipment should include pre-washing with a high-power pressure washer, followed by complete coverage with a high-level disinfectant/biocide (Fig. 10.21). Currently, commercial mobile pressure-washers

T110-b Treatment: T110-b — Quick Freeze for Destruction

Important

T110-b may ONLY be used with permission from CPHST Treatment Quality Assurance Unit.

Contact 919-855-7450 for official approval.

1. Initially, lower the commodity's temperature to 0 °F (-17.77 °C) or below.
2. Hold the commodity's temperature at 20 °F (-6.66 °C)or below for at least 48 hours.

   The commodity may be transported during the 48-hour treatment period, but at no time may the commodity's temperature rise above 20 °F (-6.66 °C) prior to release.
3. After treatment, transport the commodity to a landfill for deep burial.

This treatment is considered an acceptable method of destroying most commodities in lieu of returning them to the country of origin, with the exceptions listed in the Figure 5-2-8 on page-5-2-87 at the beginning of this treatment schedule.

Fig. 10.22 Treatment schedule T110-b. Quick freeze for destruction of tropical/subtropical pests and diseases

are designed to remove unwanted organisms from forest fire-fighting equipment. One washer-system includes a large, inflatable mat with sides that collect all rinse water, which is then passed through three filters to remove particles to 10μ. The filtered water is then recycled and stored in 200-gallon tanks (Divittorio et al. 2010; Fleming 2008).

Quick Freeze. In the USA, a freeze treatment is available for commodities originating in tropical and sub-tropical regions. This is a highly regulated treatment because the low temperatures can damage many commodities or may not be efficacious against pests from temperate regions (Fig. 10.22).

10.9 Systems Approaches

The use of integrated measures for pest risk management (also referred to as "systems approaches") is gaining more attention from NPPOs as an alternative to more traditional single approach phytosanitary treatments. Three factors drive the interest in developing systems approaches as a way to manage pest risk. First, many countries are trying to reduce/eliminate the use of certain chemical treatments (e.g. methyl bromide). Second, the types of products (and associated pests) traded globally are increasing in diversity. Traditional treatments may not always be available, effective or data may be lacking to demonstrate efficacy. Finally, countries are recognizing the benefit of applying measures throughout the production chain as a useful strategy for managing pest risk, in particular by managing pest risk at the source rather than post-entry (Jang and Moffitt 1994; Liquido et al. 1997; Heather and Hallman 2008a).

"Systems Approaches" are defined as "the integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests" (IPPC 2009). Systems Approaches are typically applied in cases where a single measure (such as a particular phytosanitary treatment) is either not available or is not likely to achieve the appropriate level of phytosanitary protection or in cases where the only other alternative is prohibition of importation. The cumulative and often synergistic effect of combining independent measures can regularly provide the necessary level of phytosanitary protection where no other alternatives are available.

Systems Approaches typically include independent measures, redundancy and safeguarding. Combining measures that act independently of each other has the advantage that if any one measure fails, the overall system maintains a high level of efficacy. Also, including redundant (overlapping) measures means that if one measure applied at a particular point in the production chain fails, another measure directed at that same point will assure that mitigation of risk still occurs. Finally, measures that do not necessarily reduce pest prevalence, but are aimed at preventing new risk from being introduced into the system are called "safeguards" (IPPC 1999; Follett and Neven 2006).

The types of measures that are applied in a Systems Approach can occur anywhere in a production chain, from pre-planting and pre-harvest through distribution and final end-use of the commodity (Fig. 10.23). Many different measures can be applied to manage risk before the shipment of a commodity to its final destination. Therefore, the main advantage of Systems Approaches is that the risk is managed primarily at origin thereby reducing the level of risk for the importing country and the requirement for treatment on arrival. Systems Approaches can also be used to mitigate the risk of pests that are not normally associated with the commodity (e.g. hitchhikers or contaminating pests), or pests that are not accounted for in the PRA process (unknown pests) (MAF 2008).

The measures employed in a Systems Approaches can range from very simple combinations (e.g. two independent measures such as low pest prevalence combined with fumigation) to highly complex "control point" systems (IPPC 1999, 2002). This is dependent on the:

- Level of risk involved;
- Cost, feasibility and efficacy of possible measures;
- Suitability of any given management option for managing that risk;
- Availability of information for the pest(s) and associated commodity; and
- Appropriate level of protection (or acceptable level of risk).

In general, the use of a Systems Approach requires a very good knowledge of the production practices, the biology of the target pest(s) and its relationship to the host(s), and post-harvest practices. In many cases, a Systems Approach can be highly flexible in the number and type of measures applied (as long as at least two measures act independently), even if specific data on efficacy is lacking. Less often, most or all of the specific points in the production chain can be well defined, the

Pre-harvest	Harvest	Post-harvest handling	Shipping	Distribution	End use
Pest free areas or areas of low pest prevalence	Harvesting at specific times	Post-harvest treatments (*e.g.* chemical, heat waxing, washing brushing, *etc.*)	Treatment in transit (*e.g.* cold treatment)	Restrictions on ports of entry	Restrictions on end-use
Resistant cultivars	Culling infested products	Testing	Speed and type of transport	Restrictions on time of year	Post entry processing
Healthy planting material	Field sanitation	Culling	Sanitation	Post-entry quarantine	Packaging*
Certification schemes	Harvest technique	Packing house Inspection	Pre-shipment inspection	Post-entry Inspection	
Testing	In field chemical treatments	Method of packing*	Type of packaging*	Post-entry treatment	
Protected conditions*	Field surveillance	Screening*		Packaging*	
	Tarping*	Sanitation*			

Fig. 10.23 Examples of measures that can be applied in a systems approach

hazards and mitigations can be measured, each point can be controlled and finally the efficacy of each mitigation step can be verified and documented. In these cases, a "control point" system can be applied – this being the most rigid type of Systems Approach used for phytosanitary risk management (IPPC 1999).

Since 2010, Systems Approaches have been employed by many countries for a range of pests and commodities. Tropical tephritid fruit flies (*Ceratitis capitata*, *Anastrepha* spp., *Bactrocera* spp.) are a frequent target of systems approaches on commodities such as papaya, mango, avocado, citrus and other tropical/sub-tropical fruits (Podleckis 2007). A few examples of other pests or commodities managed through systems approaches include species of Lepidoptera (Follett and Neven 2006; Grove et al. 2010), various plant pathogens (NPB 2002), pests of grain and other stored products (Robbins 2006) and a range of "hitchhiking" or contaminating pests (MAF 2008) on different commodities.

NPPOs continue to be challenged by the risks of moving pests through trade and gain more experience with systems approaches. The role for systems approaches will grow in importance, and the types of pests and commodities managed through systems approaches will continue to expand. As with individual phytosanitary measures such as treatments, the need for scientific data to support regulatory decisions remains a crucial challenge for all countries.

10.10 Summary

Phytosanitary treatments are essential measures in the continuum of biosecurity. They are crucial for the effective management and facilitation of international trade in plants and plant products. Phytosanitary treatments mitigate the risks associated with unwanted organisms or contaminants associated with goods such as cut flowers and greenery, fresh fruit and vegetables, propagative plant material, and stored products.

Phytosanitary treatments may be simple, single-step processes or they can be complex and time consuming in application or operation. Treatments can occur in the country of origin, during transit to the importing country or on arrival at destination. Multiple component treatments are often formed into an integrated sequence of activities and processes in a complex “Systems Approach”. Systems Approach activities can occur during pre-plant, field/plantation growth, harvesting, processing, transportation, and on-arrival in destination country. Single treatments or multiple processes involved with a systems approach must meet efficacy requirements and not destroy or significantly degrade the commodity with regard to quality or shelf life.

For specific products, regulatory authorities may require certain treatments to be conducted as a mandatory pre-shipment processes (which could begin at any stage before this point), but in many other cases they are performed as emergency eradicant treatments upon interception of pests (following inspection on arrival in the importing country).

Frequently new or revised phytosanitary treatments are used on a national basis and then championed through regional plant protection organisations before submission to the IPPC. The trend has been to make treatments more widely acceptable internationally through the IPPC and guidelines are available to enable regulatory authorities to submit new treatment processes through a validation process. This provides a transparent, consistent and aligned method of acceptance for worldwide use of new treatments for replacement or supplement of existing treatment methodology. Finally, this approach ensures that new and existing phytosanitary treatments are available, can be used consistently and extended for application to new host plants and plant products after testing, validation and scientific scrutiny.

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Chapter 11
The Role of Surveillance Methods and Technologies in Plant Biosecurity

Tom Kalaris, Daniel Fieselmann, Roger Magarey, Manuel Colunga-Garcia, Amy Roda, Darryl Hardie, Naomi Cogger, Nichole Hammond, P.A.Tony Martin, and Peter Whittle

11.1 Introduction

Countries design biosecurity systems to protect their animal, plant, and environmental resources from invasive alien species. Countries maintain biosecurity systems to safely manage trade and prevent the introduction of invasive pests (insects, diseases and weeds) through numerous pathways of entry.

Plant biosecurity programmes seek to exclude exotic organisms from becoming established on agricultural crops, ornamental plants and "natural" areas. Without barriers for entry, invasive organisms can expand their range, colonize new territory and cause considerable economic and environmental damage (Magarey et al. 2009). Spatially, one country's biosecurity efforts may be categorised as "pre-border", "border" and "post-border" when describing that country's attempts at minimising

T. Kalaris (✉) • D. Fieselmann • A. Roda
U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine, Center for Plant Health Science and Technology, Raleigh, NC 27606, USA
e-mail: Tom.M.Kalaris@aphis.usda.gov

R. Magarey
Center for IPM, North Carolina State University, Raleigh, NC, USA

M. Colunga-Garcia
Center for Global Change and Earth Observations, Michigan State University, 1405 S. Harrison Rd., East Lansing, MI 48823, USA

D. Hardie • N. Hammond • P.A.T. Martin
Department of Agriculture and Food Western Australia, Baron-Hay Court, South Perth, Western Australia 6151, Australia

Plant Biosecurity Cooperative Research Centre, LPO Box 5012, Bruce, ACT 2617, Australia

N. Cogger
Massey University, Tenndent Drive, Palmerston North 4474, New Zealand

P. Whittle
Queensland University of Technology, 2 George St, Brisbane, Qlds 4000, Australia

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the movement of unwanted organisms. Countries collaborate internationally on a range of interrelated biosecurity activities to confront these exotic invasive species. Surveillance is a key component of that continuum.

The International Plant Protection Convention (IPPC) defines surveillance as an official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures. The diverse purposes of surveillance include:

- Promote early detection of pests to facilitate eradication or management;
- Support trade by demonstrating areas of pest freedom or low pest prevalence;
- Describe the distribution and prevalence of risk organisms already present;
- Delimit the full extent of pest population following a detected incursion;
- Measure the success of biosecurity systems;
- Enable management and cost benefit decisions;
- Develop a list of pests or hosts present in an area;
- Monitor progress in a pest eradication campaign;
- Enable reporting to other organisations.

National Plant Protection Organisations (NPPO) and other regulatory agencies conduct different types of survey programmes to fulfil these needs. In addition, these Plant Protection agencies often rely on outreach to passively surveil partners who report pest detections. For example, in New Zealand most new pest detections are reported by industry, researchers, and the public via a toll-free telephone number (Froud et al. 2008).

The success of plant protection programmes depends on the ability to detect pests. To conduct a survey, a large number of associated tools and technologies are required (Fig. 11.1). Some of the tools/technology involve statistics, GIS, data management and risk mapping, and will be discussed in this chapter. However, effective surveillance tools and technology are often lacking. When no effective insect trap or lure exists, officials must rely on visual surveys. Detecting plant diseases often presents an even greater challenge. The combination of high costs and inadequate technology leads to survey programmes that are less than optimal. As a result, pests frequently are introduced and become established before timely detection. With delay in discovery of invasive pests, the likelihood of eradication decreases while the cost of control/management/eradication increases dramatically.

Figure 11.2 shows the hierarchy of surveillance activities and the flow of information. The flow of information starts at the point of collection in the field. From that point, the information is integrated and tailored to meet the needs of various end-users. For a fruit fly trapping example, regulatory officials collect, clean and compile survey data for managers to use to control fruit fly outbreaks (Chap. 15). For another application, industry collects survey data as part of the day-to-day commercial operations. This data is then used as a basis to run predictive models that can help industry understand the movement of emerging pests or pests of phytosanitary concern (Chap. 9). The same data might also be used by growers or regulatory officials to take action in support of surveillance or eradication.

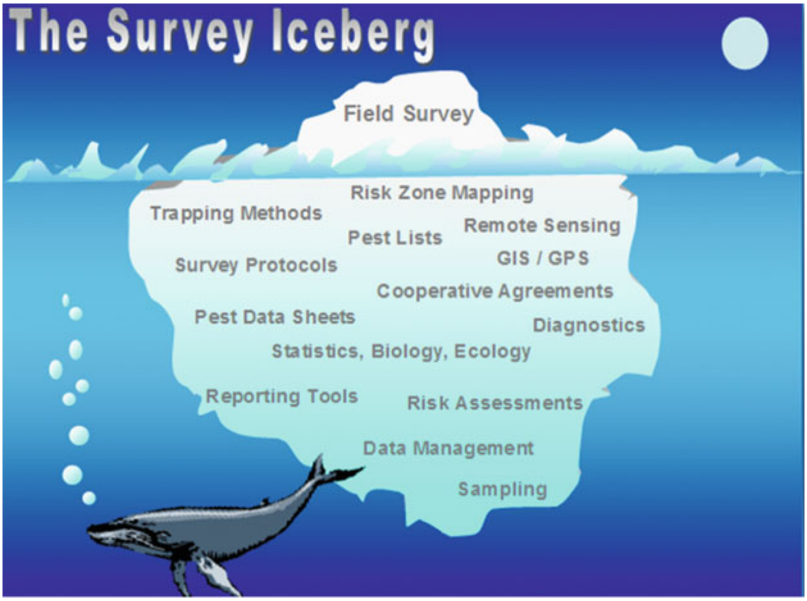

Fig. 11.1 The "survey iceberg" illustrating the need for tools and technologies to support surveillance programmes (Image courtesy of Dan Fieselmann)

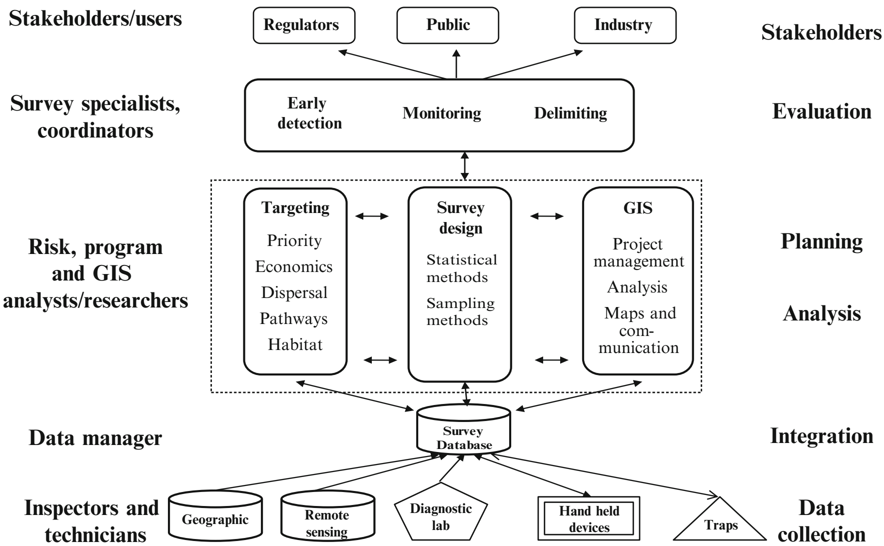

Fig. 11.2 Information architecture for surveillance (Magarey et al. 2009). *Arrows* represent the directed flow of information

This chapter outlines types of survey operations and provides a review of survey design, information management, data integration, modelling, and GIS. Surveys may be structured around high-consequence target pests. Other surveys may focus on commodities and the survey of exotic pests that may be found associated with that commodity. Still other surveys may target high-risk areas. The USDA, APHIS PPQ Cooperative Agricultural Pest Survey (CAPS) serves as an example of a large surveillance programme that demonstrates various surveillance concepts in practise.

11.2 Survey Operations

11.2.1 Detection Surveys

The goal of detection surveys to is discover an invasive species while its populations are numerically small and geographically confined. Early Detection Surveys locate species that are in the process of establishing in an area An Early Detection Survey could, for example, determine whether non-native snails are present in an intermodal container yard.

A Targeted Detection Survey focuses on areas more likely to experience new pest introductions. Targeted surveys are based upon phytosanitary data such as emergency action notifications or pest-interception data. In the USA, the High Hazard (or "Hotzone") Programme is designed to enhance the ability of the CAPS Programme to identify and target high-risk areas and sentinel sites within the USA. The methodology for a High Hazard Survey decides upon which species or high-risk pathways to search, and which areas should be the focus of a survey. In the programme, three types of high-risk sites are identified: Primary; secondary and tertiary (Watkins and Messineo, personal communication).

Primary risk sites include business, organisation, operation, or areas that have functioned as a destination or waypoint for commerce that has a confirmed record or recent association with invasive organisms or pests. These sites have a high potential for pest introduction and/or establishment and therefore should be of highest priority for survey efforts. Primary risk sites require intensive monitoring. For example, in large metropolitan areas (especially where high volumes of international trade and/or travel exist) a primary hot zone may include such areas as an international airport, seaport and their environs, a foreign trade zone, a container yard, or trucking terminal.

Secondary risk sites have physical descriptions or characteristics similar to a primary risk site but with no known history of invasive organisms or pest interception and/or introduction. The key characteristic of this zone is that it is documented as having recently received potentially infested commodities directly or indirectly from a primary hot zone. Secondary hot zones contain moderate risk and require routine monitoring to evaluate whether there is a change in status.

Tertiary risk sites share similarity in commerce or commerce-related activities with known hot zones and that have no known record of invasive organism, pest interception and/or introduction. The key characteristic of this hot zone is that it is not documented as having recently received potentially infested commodities from either a primary or secondary hot zone. Tertiary hot zones are of undefined yet suspected risk and therefore require periodic monitoring to evaluate for a change in status. This is similar to a scheme developed in Australia that involves a four-level classification system for risk sites (Self and Kay 2005).

11.2.2 Monitoring Surveys

The International Standards for Phytosanitary Measure (ISPM) 5 defines monitoring survey as an ongoing survey to verify the characteristics of a pest population. By this definition, monitoring surveys apply where a pest is known to be present and the survey is planned to examine aspects of the pest population such as prevalence of the pest and changes in pest prevalence over time (Evans and McMaugh 2005). Two reasons for using a Monitoring Survey are: Survey to assist with pest management and to develop and maintain an area of Area of Low Pest Prevalence (ALPP) status. One example of the former is the APHIS PPQ area-wide pest management programme for Glassy Winged Sharpshooter (GWSS, (*Homalodisca vitripennis* (Germar)) in California. The GWSS is a vector for Pierces Disease (caused by *Xylella fastidiosa* Wells et al.), which is very damaging to grapevines. Citrus is a reservoir for GWSS, so monitoring is used to determine when citrus groves should be sprayed to prevent the build-up of damaging GWSS populations that could impact grapevines (Hix et al. 2003). Another example involves monitoring for Asian Soybean Rust (caused by *Phakopsora pachyrhizi* Syd. & P. Syd.) in the USA (Isard et al. 2007). Sentinel plots were deployed initially across the entire soybean belt and later mostly in southern states. Sentinel plots were funded by government and industry and maintained by university extension specialists. The sentinel plots in southern states provided a warning system that allowed northern-state producers (where most soybeans are grown) to avoid application of fungicide sprays.

11.2.3 Delimiting Surveys

Delimiting Surveys are another type of survey (see ISPM 6 IPPC 1997) used to establish the boundaries of an area considered to be infested by or free from a pest. Delimiting Surveys are conducted to determine the extent and distribution of a pest incursion, and to determine whether the pest is eradicable. A good example of a Delimiting Survey is the survey used to determine the extent of Citrus Greening (HLB) (caused by '*Candidatus Liberibacter*' spp.) outbreaks in Florida. The discovery of HLB in Florida City, Florida immediately prompted a survey to delimit the infection (Gottwald et al. 2007). East–west transects were surveyed every five miles northward from Florida City in an attempt to determine the northern extent of HLB distribution. A positive detection within a transect immediately prompted a survey in the adjacent transect 5 miles to the north. HLB distribution was quickly confirmed to extend northward to the Fort Pierce residential area, 120 miles (193 km) from the initial detection.

11.2.4 Pest-Free Areas

The definition of a Pest-free Area (PFA) for the purposes of this chapter is: An area prescribed by the IPPC Secretariat and relevant ISPMs that covers this broad area. All PFA and Systems Approaches must be science based. PFAs and Systems Approaches are generally highly regulated processes that are "officially maintained" and can be cost and management intensive. PFAs can deliver low-risk pathways that allow high-value plant-based commodities to be traded with confidence from areas outside or within an endemic area for a particular pest (see ISPM 10, IPPC 1999). Ideally these approaches should allow the delivery of a quality commodity without the need for drastic endpoint measures like fumigation that may cause residue issues, physiological changes in the produce and reduce the shelf life of a commodity.

A PFA approach may be used to indicate that a pest does not occur within a designated area/property (or above a stipulated level of prevalence) and these need to be confirmed for trade purposes. Conversely, the PFA does not mean that a pest is not present within a prescribed area. Rather, it means that a pest cannot be or never has been detected at the agreed level of surveillance/sampling to allow trade.

SPS Agreements define the Appropriate Level of (sanitary or phytosanitary) Protection, (ALOP) as the level of protection deemed appropriate by the member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory. This concept is also referred to as the "acceptable level of risk".

PFAs are created through a science-based agreement between two trading (country/regional) partners generally for a specific pest. PFAs are not multi-lateral agreements that can be applied automatically to other pests and situations. PFAs rely on goodwill from both partners with respect to transparent compliance and auditing procedures. Nevertheless, an existing agreement could be used as the basis of a new agreement.

11.2.5 Scenario Tree Modelling for Evaluation of Surveillance Supporting Pest-Free Status

We cannot prove that an area is free of any pest. Proof would require testing of all susceptible individuals with a perfect test (a test free of error), and then would only provide freedom for that point in time. The best that can be done is to estimate the probability that the area is "free" of the pest, based on the available evidence. In this context, "freedom" from the pest must be interpreted as "there is less of it present than some agreed low but detectable threshold level" (Cameron and Baldock 1998; Martin et al. 2007).

Evidence for estimation of probability of area freedom comes in two forms: (1) Historical records of pest occurrence and eradication, and (2) negative results of more current surveillance activities aimed at detecting the pest. Often, such surveillance activities are non-random and designed to look for the pest in places where it would be most likely to occur if present. Such biased surveillance information is not amenable to standard techniques for analysing representative surveys. Biased surveillance often is ignored in quantitative evaluations of claims of pest-free status. Similarly, that the pest "has not been seen" or is "not known to occur" (i.e., has not been detected in the general, passive surveillance process) is also ignored.

Scenario Tree Modelling is designed for evaluation of animal health surveillance, and may be used for evaluation of evidence supporting pest-free status. In this approach a surveillance system for pest detection comprises multiple components, each of which has a probability of detecting the pest, when present at or above the threshold level. The process by which each component detects the organism (when present in the area) is described using a stochastic Scenario Tree Model (Martin et al. 2007). This model defines the activities of the component and includes all factors that affect probabilities of pest presence and pest detection in a surveillance unit (often an area of land, a tree, or a sample of grain) selected at random from among those "processed" in the surveillance activity. The probability is calculated that this representative unit gives a positive outcome. Then, the probability can be calculated that the surveillance component will give one or more positive outcomes when the pest is present at the threshold level. In calculating this *sensitivity* of the surveillance component, any clustering of the pest can be identified along with other causes of lack of independence among surveillance units and detection processes, and differential risk of infestation among surveillance units. Sensitivity calculations are based on the assumption that in surveillance for a pest that is not present, false positive results are not acceptable, and will always be resolved into true positives or negatives (Martin et al. 2007).

The sensitivities of surveillance components are then combined to give a surveillance-system sensitivity. This process may be repeated for sequential surveillance time periods (often months or years). The probability that the area is "free" from the pest may then be estimated from the surveillance sensitivity and a prior estimate of the probability of freedom. For sequential time periods, these prior estimates are best based on previous surveillance evidence for pest freedom.

The posterior estimate of probability of freedom for one time period is used as the basis of prior estimate for the following time period, adjusted for possibility of pest introduction during the period between rounds of surveillance (Martin et al. 2007). Thus, past surveillance evidence is accumulated over time to give a current probability of pest freedom.

11.2.6 Statistical Power Approach to Pest-Free Areas

Certification of a country (or a region within a country) as pest-free enables it to avoid trade restrictions that another country may impose on importation of commodities associated with the pest in question. This certification is a matter between the two trading countries, which occurs under rules that should be compliant with international phytosanitary standards (IPPC 2009b).

These standards and reports enable the establishment and evaluation of pest freedom claims, but the claims have a complex mix of quantitative and qualitative components, which must somehow be integrated for a single decision. Inescapably, this decision is partly subjective and it would be desirable to have a more empirical and less subjective measure of a claim's merits. With diverse sources of quantitative and qualitative information, we cannot place an overall measure on the quality of knowledge about pest freedom.

A quantitative component is provided by the surveillance programme, which should be compliant with ISPM 6 (IPPC 1997). This identifies two major types of surveillance systems: General Surveillance and Specific Surveys. General Surveillance refers to sources of information of many types that may lend weight by inference to the pest freedom claim. Specific surveys collect direct data on pest status. The information obtained from General Surveillance rarely provides empirical evidence of pest freedom, but is more of the nature of "*the pest in question has never been observed*", without quantification of the surveillance effort made. Empirical evidence in the form of "*the pest is known not to occur*" normally is derived only from specific surveys. Specific surveys tend to be simple designs (e.g. specified number of trees and fruit that must be sampled in a district of given size) to give a certain level of confidence (e.g. 99 %) of pest freedom at a certain design prevalence (e.g. 1 %). Other sources of evidence (observations of farm consultants, negative diagnostic samples in a district lab, etc.) tend to be ignored or at most, provided as general surveillance information.

11.2.7 Systems Approaches as an Adjunct for Pest Free Area Status

In Australia, regulatory officials are withdrawing *dimethoate* and *fenthion* for post-harvest treatments of horticultural products, especially for commodities with edible skins. Consequently, alternative methods must be developed to control plant pests. “Systems Approach” is among the options considered. The ISPM defines System Approach as…‘The integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests’ (FAO 2009).

For the Systems Approach to pest management to gain widespread acceptance in international trade, new analytical methods are required to demonstrate that the efficacy of the multiple risk-reduction measures in a system are equivalent to a single post-harvest treatment with *dimethoate* and *fenthion.*

A major issue facing advancement of a Systems Approach is the need to determine how efficacy of measures in a Systems Approach will be determined. Historically, ‘Probit 9 statistical standard’ (Baker 1939), has been used as the basis for evaluating efficacy of measures. When a single post-harvest measure (e.g. post-harvest dip, forced hot-air) has been used to manage phytosanitary risk, their acceptability has been evaluated against a standard. For example, the ‘Probit 9 statistical standard’ has long been required by the USDA for demonstrating the efficacy of phytosanitary treatments applied to certain pests, especially tephritid fruit flies (See Sect. 15.3; Baker 1939).

In contrast, ‘Probit 8.7’ is widely accepted throughout the Asia-Pacific region as the basis for approving quarantine treatments for tephritid fruit flies. None of these statistical standards makes operational sense when evaluating systems approaches because they focus on survivorship of insects rather than determining the level of infestation and its acceptability. Quantitative risk assessment could be used to demonstrate equivalence for the purpose of international and domestic trade, evaluate the potential of risk reduction strategies and prioritize research/information needs (See Sect. 5.5).

A quantitative risk assessment can be defined as a mathematical model in which inputs and outputs are expressed numerically (Evans and Olson 1998). In some quantitative models, referred to as Stochastic or Probabilistic Models, some or all of the inputs are probability distributions (See Sect. 5.5; Evans and Olson 1998). Complexity and resource requirements are greatest for a Stochastic Model. However, the benefit of using a Stochastic Model is that we can explore variability and uncertainty.

In conclusion, Probit 9 measures do not make operational sense when evaluating systems approaches because they focus on survivorship of insects rather than determining the level of infestation and its acceptability. The most appropriate model depends on the purpose of the model. Consequently, regulators must agree on other approaches for the evaluation of the efficacy of measures, when more than one measure is combined in a systems approach.

11.3 Survey Planning

11.3.1 Targeted Surveillance Overview

National Plant Protection Organisations do not have resources to conduct surveillance programmes for all crops, forests and natural environments that are vulnerable to exotic plant pests. Thus, surveillance programmes require spatial and temporal targeting to identify regions, areas and locations, and seasons or periods where and when exotic pests are most likely to be detected. Spatial Targeting enables resources to be allocated in the most efficient manner. Temporal Targeting enables traps or personnel to be deployed during periods of time when the pest is most active.

Suitable survey sites are selected by:

- Geographical distribution of production areas and/or their size;
- Pest management programmes (commercial and non-commercial sites), cultivars present;
- Points of consolidation of harvested commodity (e.g., a nursery, pallet or container storage-area, or a dense area of host material);
- Sampling technique appropriate to type of harvested commodity;
- Previously reported presence and distribution of pest;
- Biology of pest;
- Climatic suitability of sites for pest.

Timing of survey procedures may be determined by:

- Life cycle of pest;
- Phenology of pest and its hosts;
- Timing of pest management programmes;
- Whether pest is best detected on crops in active growth or in harvested crop.

Additional Points:

Surveyors need identification aids for suspects or target species for which they will be searching (Chap. 12). Images on websites such as CABI, EXFOR, or Invasives.org may be useful. Chose a survey interval in which your target species is active and visible.

Decontaminate surveyors and survey equipment! (For plant diseases, utilise a suitable decontaminant.) This action helps prevent invasive pests, from spreading to new sites (See Sects. 18.3 and 18.4). Report findings to your local Plant Protection Organisation or NPPO.

More sophisticated targeting information can be provided by a combination of techniques and data derived from pest data sheets, pest risk maps and pest models. Targeting information can be created by habitat, climate pathway, economic and prioritization models. The Habitat (host) Model represents where an invasive organism is likely to find suitable hosts or which hosts are available at a given location. The Climate Model provides information about where or when an invasive organism is likely to survive and reproduce. The Pathway Model provides the most likely entry points for an invasive organism. The Economic Model integrates habitat, climate and pathway models to determine costs and benefits of a particular surveillance programme. Individual maps or models can be summarized using techniques such as the Multi Criteria Decision System (BRS 2007), which allows users to weigh individual risk layers. Pareto Ranking is being evaluated for this purpose in the USA and Canada (Magarey et al. 2010).

Prioritization is the process of determining invasive organisms that are most important to target in a particular area. Here, we describe only habitat, climate and pathway models because they are the most essential components for a surveillance programme.

11.3.2 Habitat Models

These models are used to determine areas most likely to be points of introduction and establishment for an invasive organism. Habitat Models can be challenging to construct because many sources of information are needed including crop and forest inventory data, land-use data and species distribution data. Complexities may arise in constructing maps for a pest species that attacks crop, forest and environmental hosts of importance because the data may be in different formats. For example, crop distribution data may be in acres, forest inventory in species per acre, and species distribution in presence/absence for a geographical location or jurisdiction. We discuss data sources in turn and indicate some of their limitations or advantages.

A land-use database is the fundamental data source for constructing a host risk-map. This data source can be used in the absence of other information or used to downscale other data sources to a finer resolution. A typical land-use database will describe classes including cropland, deciduous, evergreen and mixed forests, rangeland and urban areas. Land-use databases are usually derived from satellite imagery. Consequently they are widely available. For example, a global land-use database, derived from satellite imagery, is now available at a 300 m resolution (European Space Agency http://due.esrin.esa.int/prjs/prjs68.php).

In the USA, crop inventory data is supplied by the USDA's National Agricultural Statistics Service (NASS) and collected under the Agricultural Census Programme. Data is available at the resolution of a County and is available for 127 crop types. Several different units of host availability are recorded including acreage, production and economic value. Usefulness of this approach is limited because the data is available in 4-year installments (2007 is latest), with a lag-time of 1 year or more before release. For some commodities, we must consult industry or commercial sales databases to determine the best surveillance locations. Commercial sales databases may require georeferencing before the information can be mapped. In countries where spatially explicit information about crop production is not available, one option is to take broad-scale information such as production figures for a state or country, and distribute the production to cropland areas from a land-use database.

In the USA, forestry data is available on the USFS Forest Inventory data site (FIDO) site (http://fia.fs.fed.us/). These data sources include the acreage of individual forest species by county. The FIDO database does not record the distribution of understory species that may be important hosts for some invasive organisms, and this is a limitation.

In forest and natural environments, the distribution of many host-plant species can be estimated from species distribution databases such as the Global Biodiversity Information Facility (GBIF, http:www.gbif.org). GBIF is a data portal that contains worldwide georeferenced species distribution records from museums and herbaria. GBIF is a powerful resource with two major limitations. First, the data confirm presence only and the abundance of the host species is typically not known. Second, only a proportion of the known species distribution data are present in GBIF. Country data sources may contain more records.

In the CAPS programme, a summary host risk-map is made for each exotic pest that is on its priority list. The host risk-map is created by estimating host-density within a county, which is a function of total acreage of susceptible hosts and total acres in that county. Primary hosts (as determined by a pest database such as the CABI Crop Compendium) and secondary hosts are included in the model. A one-to-ten scale describes the proportion of total host acreage per county.

11.3.3 Climate Models

Climate models are used to determine a pest's potential range and to help estimate the best time for survey activities. Climate is a major influence on a pest's phenology, reproduction, dispersion, and overwintering survival, and a critical component of pest targeting. Climate-based modeling may be useful for pathogens that are weather-driven or moisture-dependent, but may not be helpful for pathogens that have broad requirements or are pests of indoor environments (e.g. greenhouses). In addition, crops growing in unique microclimates or grown under irrigation present additional challenges to modelers. The three most important components of climate models are climate databases, model selection and interpretation.

Climate databases (including those with global extents) are now widely available. In recent years, we have seen a shift to grid databases and away from those based on station datasets. Databases can be discussed in terms of variables, resolution and period. Date in a modern agricultural weather database includes native variables and data derived from models of native variables. The most important variables for pest risk modeling are degree days and extremes in temperature. Leaf wetness (which can be derived from commonly collected native variables), relative humidity and precipitation are of primary importance for predicting infection periods of certain fungal pathogens and bacteria or periods when mollusks are active. Other variables such as wind speed and direction are important in predicting the dispersal of diseases such as rusts, but this is beyond the scope of the section.

Time period is the second important component for a database. Many risk maps are created using 10 or 30-year climate data. This allows a user to predict the most likely pest behaviour in an average year. Maps with low spatial resolution may miss climate variability driven by topographical features such as mountains. Decision-makers wish to see the highest possible spatial resolution, but it comes at increasing costs.

Several climate-based risk mapping systems (CLIMATE, CLIMEX, NAPPFAST, and MAXENT) have been used for plant pest risk analysis (Venette 2010). Climate risk-mapping tools will use either deductive or inductive mapping approaches (Baker 2002). Deductive approaches (e.g., CLIMEX compare locations, NAPPFAST) use experimental data to create biological models that predict a pathogen's distribution from weather or climate data. Deductive techniques work best when there is adequate information available about the biological requirements (e.g., day-degree thresholds, cardinal temperatures for growth, cold or heat-killing thresholds, leaf wetness or relative humidity requirements for infection etc.) of the pathogen. Many of these requirements are available from databases such as the CABI Crop Compendium or may be estimated from a closely related species. Inductive techniques (e.g. MaxEnt, CLIMEX match climates) are based on a statistical match of a pathogen's observed distribution and climate variables.

11.3.4 Pathway Models

Pathway risk mapping and modeling is perhaps the most important component of targeting. Pathway risk maps define location at most risk to the introduction of exotic pests. The main sources of data for the creation of these maps include phytosanitary data (e.g., interceptions, emergency actions, fumigations, commodity or nursery import data), trade and freight data and commercial sales data sets. The simplest method to create pathway risk maps is to map locations or ports by frequency of pest interceptions or emergency actions. An excellent example is the USDA APHIS PPQ CAPS Florida Tile Warehouse Survey that resulted in the discovery of several new (previously undetected) species/genera (Beckwith 2004). Sometimes, species new to science are discovered in regulatory surveys. Surveillance may also be conducted at other high-risk or hazard zones such as airports, rail yards or national parks (Self and Kay 2005).

More recently in the USA, techniques have been developed to create pathway maps using the Freight Analysis Framework (FAF) database (Colunga-Garcia et al. 2010a, b). Pathways in FAF were classified according to seven international regions of origin and 43 broad import commodity classes. Imports entering the USA were distributed to 131 regions including major metropolitan areas, remainder of state and border areas. For each pest, the introduction pathways were assessed from its international distribution and from its trade (HS) import commodity association. The final risk classes represent the tonnage of potentially infested commodities (hosts) coming from countries where the pest has been reported (Colunga-Garcia et al. 2010a, b).

11.3.5 Statistical Methods and Sampling

Introduction. The success of plant protection programmes depends on our ability to detect pests. However, often we cannot survey all potential locations or every host plant in a given area where a pest may occur. Survey designs and sampling methods are used to provide a level of confidence that the pest is not present or make inferences about the pest population. Here we describe the basic principles of surveying for detection (presence/absence of a pest) versus survey for information (level of infestation) and give examples of developing a sampling plan that will support the surveillance programme.

Several key points must be considered:

- *Confidence Level*: Our level of accuracy (confidence) in our results (conclusions). When we say we are 95 % sure a shipment is "pest free" we mean that (on average) given our methods and assumptions, we are correct at least 95 % of the time. For agricultural regulatory work, confidence levels are seldom less than 95 % but often may be higher, e.g., 99.9 %.
- *Detection Level or Threshold*: The smallest infestation rate at which we can detect a pest, given our sampling effort and confidence level.
- *Sample Unit*: The basic unit we are inspecting to find the pest. Depending on circumstances this could be a tree, a single leaf, a box of fruit, or a bouquet of flowers.
- *Sample Size*: The number of sample units inspected, e.g., the number of traps placed in a field.
- *Sampling Efficiency*: A rate or percentage that reflects the accuracy of our inspections. If a pest is in a sample unit (e.g., a fruit or bouquet), then how likely is it to be found? Historically many people have assumed this to be near 100 %, but for many inspections this is not realistic. A low sampling efficiency must result in a larger sample size for any stated conclusions to be correct.
- *Important Observation*: We can never say that we are 100 % certain that an area (or population) is pest free unless we inspect 100 % with 100 % efficiency. This standard is rarely realistic. The best we can do is to say that we are sure (at some Confidence Level) that a pest is present in a population at less than a level of detection.

How do all of the above points fit together? Confidence Level, detection threshold, and sample size are all interrelated. Any two features determine the third. Most often we start with a Confidence Level and Detection Threshold and from that determine the appropriate sample size. For example: Say we are visually inspecting an orchard with 1,000 trees for an insect pest. If our inspections are all negative, we can make the following statements:

- If we inspected 18 trees, then we could say with 95 % confidence that the infestation rate is less than 0.16 (16 %).
- If we inspected 72 trees, then we could say with 95 % confidence that the infestation rate is less than 0.04 (4 %).

Table 11.1 Summary of two basic types of sampling conducted by biosecurity agencies

	Sampling for information	Sampling for detection
Goals	Determine level of infestation	Find pest, if present
Confidence Level	Clearly stated, a priori	Often vague, hard to determine
Sample Unit	Clearly stated, e.g., a fruit	Usually clearly stated; sometimes vague
Detection Level	Clearly stated	Often vague, typically presence/absence
Sample Size	Clearly stated	Reflects level of effort. In many programmes it is # traps/area

- If we inspected 205 trees, then we could say with 95 % confidence that the infestation rate is less than 0.01 (1 %).
- If we inspected 86 trees, then we could say with 99 % confidence that the infestation rate is less than 0.05 (5 %) (Table 11.1).

Sampling for information vs. *sampling for detection*. To conduct a statistically sound survey, the sampling method must be clearly detailed, so trading partners can decide whether the method is valid. An invalid method can cause sampling error, which would call the results of the study into question.

Typical Methods:

- *Simple random*: Each sample has an equal probability of selection: The population is not subdivided or partitioned (example: Survey 10 trees in a grove of 100 trees, using a random number table to select the 4, 12, 13, 27th etc. tree);
- *Systematic*: The target population is arranged according to some ordering scheme and then samples are selected at regular intervals through that ordered list (example: Survey every 10th tree in the grove);
- *Stratified*: When the population can be divided into distinct categories and then the samples are randomly selected from that 'strata' (example: The grove is divided into lime, orange and grapefruit trees and 10 trees are randomly selected from each type);
- *Cluster*: The sample is selected from certain areas only, or at certain time-periods only (example: Citrus groves in Orange Co.);
- *Convenience sampling* (Accidental or Opportunity Sampling): When the sample is drawn from part of the population that is nearby (example: Survey a citrus tree along a road). With this type of sample we cannot scientifically make generalizations about the total population from the sample because it would not be sufficiently representative. This method of sampling is most useful for pilot testing.

Survey Design: Sampling for Detection. Surveys can be designed for the detection of pest (presence/absence) or created to give information about the pest (e.g. its abundance). Surveying for detection only requires sampling up to the discovery of the pest. The surveyor using this design can only make inferences about the sample taken. This type of survey can be used for early detection of pests to facilitate eradication or management, to support trade by demonstrating areas of pest freedom or low pest prevalence or to delimit the full extent of a pest following an incursion. After a pest is found, other types of monitoring programmes may be established to quantify the prevalence of a pest in an area in order to make conclusions about the pest population. This type of monitoring programme can be used to describe the distribution and prevalence of pest already present.

Although surveys based on convenience sampling are commonly used in detection and eradication programmes, they can never be used to confirm that a species is absent from a location. Was the species there but not detected, or was the species genuinely absent? At times, a species can be present in an area but go undetected due to random chance. Therefore, detection threshold or confidence limits are used to provide a tolerance limit or acceptable risk that a pest may be present in an area.

Detection thresholds. Detection thresholds refer to the minimum level of pest that can be detected given the sample size, detection methods, and conditions of the survey. It is used when the prevalence is thought to be near-zero. Even if a pest is never found, there is a chance that some or a few of the pests are still present. Because they are in very small numbers and do not appear to be causing damage, their presence could be tolerated. Threshold is usually based on scientific analysis, policy decisions, and risk assessment by all parties involved. If the survey involves trading partners, then the detection threshold will be set equal to the negotiated tolerance level.

Declaring an invasive species eradicated is usually based on a prescribed time for a series of negative results ("no finds"). For instance, three consecutive years of negative survey results may be used. However, such an approach has limitations, especially when the species becomes more rare as eradication efforts become successful. It also does not consider the cost of the surveys relative to the cost of premature declaration of eradication.

Confidence of Detection. If a pest is not found, then a degree of uncertainty still exists concerning the plants or areas that have not been examined or tested. A statistical confidence statement expresses the probability that the actual pest prevalence will be no more than the detection threshold. For example, after sampling a 95 % confidence interval for a detection level of .01 states that there is a 95 % probability that the actual prevalence is .01 or less. The relationship between confidence and sample size is simple – the more sites surveyed the more certain one can be about the detection results.

As a general rule a confidence level of at least 95% is considered acceptable, but in some cases, a confidence of up to 99.9 % may be necessary. For example, trading partners may require a particular level of confidence that the pest would be detected in a survey, independent of any logistical or financial constraints.

Sampling for Information. A selection of the population is used to make generalised conclusions about the entire population. Many planning decisions must be made before the first samples are acquired. When you go out looking for plant pests you have already decided where to survey, how many samples to take, and what type of data is necessary.

The sampling process comprises several stages:

- Defining the specific survey objective (detect, delimit or determine prevalence);
- Defining the population of concern (country, pest-free area, commodity);
- Specifying a sampling unit or events to measure (individual insect, box of fruit, individual plant);
- Specifying a method for selecting sample unit (random, systematic, stratified/targeted);
- Determining the sample size (number of plants, number of farms);
- Implementing the sampling plan;

Sampling and data collecting:

Sampling Units. The population must be divided into sampling units before a sample can be collected. The units must cover the entire population and must not overlap; that is, the sampling units must be mutually exclusive. In this sense every element in the population must belong to one and only one sampling unit. Sometimes the selection of the sampling unit is obvious: A population of insects in which the unit is a single insect. Sometimes there is a choice of unit: Sampling commodities entering the country in which the sampling unit may be the individual commodity unit. For instance, a potato, an apple etc. or a box or crate of the commodity with a complete sample of all the individual items it contains. Alternatively, it could be a selected shipping container with a subsample of the units contained therein. In sampling an agricultural crop, the unit might be a field, a farm, or an area of land whose shape and dimensions are at our disposal.

- *Sample size*. Sample size is the number of sites or sampling units required to survey in order to detect a specified proportion of pest infestation with a specific level of confidence. Sample size is based on detection threshold, the accuracy of the methods and the confidence required. The specified proportion of infestation (detection threshold) required may be set by you or your trading partners. Generally, the larger the sample size, the better the results. However, time and resources usually limit the number of units sampled.
- *Implementing the survey plan*. A survey plan should be well documented and the results should represent the actual pest status. Most important, the plans should be physically and financially acceptable. There is no one-way to design and implement a survey and determine the correct number of samples. Consequently, the reasons for choosing design steps must be transparent. The most successful surveys are designed and implemented through a close collaboration of biologists, statisticians and other relevant parties. The biologist has expert knowledge of species and system of interest with an appreciation of field techniques that could be employed; the statistician has knowledge of appropriate analytic techniques and awareness of data requirements.

11.4 Spatial Analysis (GIS)

11.4.1 Introduction

The term and technology of GIS has existed for more than 25 years, and GIS has taken on several meanings. The acronym originally meant "Geographic Information Systems" and referred to the integrated software that enabled the collection, manipulation, analysis, and presentation of spatial data. Subsequently, as the numbers of users and applications grew, GIS was defined by some practitioners as "Geographic Information Science" stressing the analysis and rigor behind many of the applications used today. They point to areas of GIS such as remote sensing, modeling, and spatial statistics to show where GIS, under either definition, has matured from creating simple, static maps to a system that can produce dynamic, interactive applications. In this section we use GIS in the classic definition (Geographic Information Systems) but hope to present examples of GIS applications that include more involved analysis.

11.4.2 GIS as a Project Management Tool

All biosecurity data has a spatial component, hence it can be represented on a map. As such, a GIS is critical to any control/management/survey programme conducted by a biosecurity agency. A good illustration of a GIS as a management tool is the Citrus Canker Eradication Programme that occurred in Miami, Florida, during 2003–2006 (see Sect. 18.3). Critical information that the programme needed to track for each individual property included:

- Is citrus on the premises?
- When was a particular property last surveyed?
- Were suspect trees found?
- Have the canker finds been confirmed by a pathologist?
- Have homeowners given permission for tree removal?
- Were trees removed and destroyed?
- Are there other properties within 200 m with citrus trees?

This programme encompassed more than 100,000 properties and a million people. The ability of a manager to visualize the data on a map is invaluable. A GIS linked to the programme's database can assist with answering these kinds of questions:

- Where have we surveyed and where will we need to survey next?
- How has a new positive detection changed our surveys?
- In which areas will we need more personnel?
- Which areas are ahead of or behind schedule?

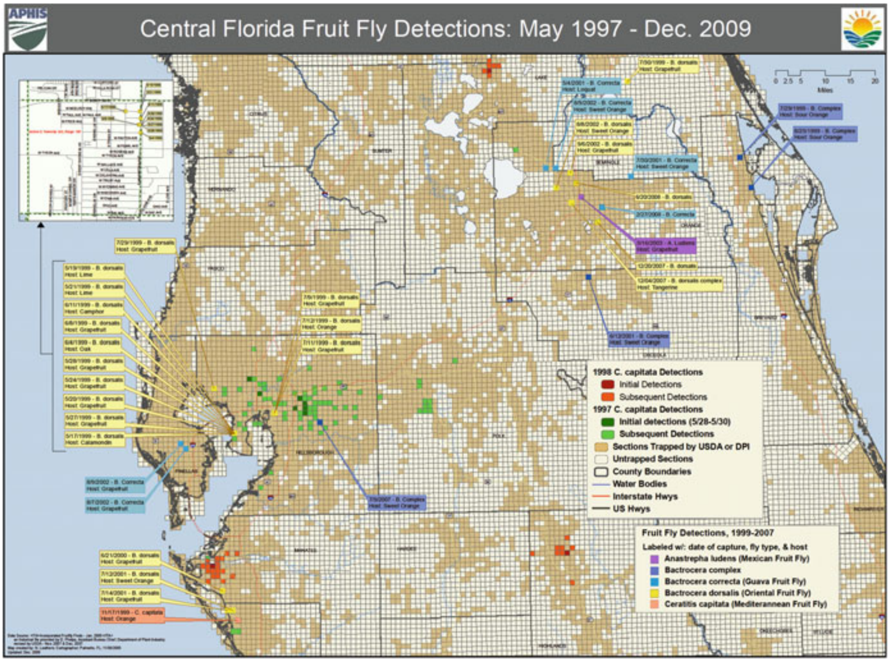

Fig. 11.3 Fruit Fly Detections in Central Florida, 1997–2009 (Map produced by Nancy Leathers, PPQ Florida)

- How is the pest spreading?
- Where are our management procedures not working?
- What areas are of greater risk?

GIS can be an effective tool for data management and project oversight. GIS can help collect field data with tools like ArcPad, it can be used for QA/QC, and it can assist in managing personnel and equipment.

Figure 11.3 shows an example from a 2010 fruit fly monitoring programme in Central Florida. It uses a GIS to coordinate, manage, and report its survey activities. This map shows the history of fruit fly finds over a 10-year period.

The Florida Fruit Fly Detection (FFD) programme is a cooperative effort between the Florida Department of Agriculture & Consumer Services' (FDACS), Division of Plant Industry (DPI) and the USDA APHIS PPQ (see Sect. 15.6.3). The programme was designed to detect new introductions of exotic target flies before they become established, reproduce, and spread. State and federal survey technicians monitor nearly 56,000 traps dispersed across 9,300 square miles of the state.

Traps are serviced at 2–3 week intervals depending on the risk assessment of the county in which they are located. In an average month, federal survey technicians visit over 56,000 trapsite locations. Global Positioning System (GPS) coordinates have been captured for all trapsites. This information gives programme officers and supervisors the ability to see and measure the spatial distribution of traps and ensure that targeted high-risk areas have a sufficient number of traps. Fruit Fly Detection data, including geographic coordinates, are collected in the field via PDAs and stored in a central database (ETRAP) that is accessible to officers, supervisors, programme coordinators and supporting staff.

Each FFD office has at least one installation of ArcGIS so local staff can create maps using a customized toolbar developed for those who have little or no GIS software experience. The maps contain a dynamic link to the ETRAP database so current trapsite locations can be viewed at any time.

11.4.3 GIS as an Analysis Tool

GIS provides tools that allow a more inclusive and complex analysis to support managers and programmes. Data may come from many sources and may include: Point data (survey data); line data (rivers and roads); polygon data (quarantine areas); or remotely sensed data (aerial photographs and satellite images). GIS allows an analyst to incorporate various kinds of data into a model to help predict introduction of a pest, the spread of a pest, the best quarantine boundaries, etc.

One example of this kind of work is an analysis of Asian Gypsy Moth (AGM) trap locations in Washington and Oregon states. The goal of this analysis was to support trap placement by the two states' Departments of Agriculture. The analyst created a map showing areas of likely introduction. This "model" was based on many layers and combines data on population, trade, roads, ports, waterways, hosts, and intermodal facilities to estimate the risk on introduction of Asian Gypsy Moth into Washington and Oregon. The red areas are of higher risk (Fig. 11.4).

The map from the model was compared to the current (previous year's) trap locations. For the project managers in Washington and Oregon, this was a valuable comparison to see where their map agreed with their trapping and where the two differed. It helped them evaluate their trap locations and trap densities. Note that the GIS model does not dictate to the manager where to place their traps. It is a tool to assist managers in the allocation of their resources.

11.4.4 GIS as a Communication Tool

The third area of focus for this brief overview of GIS involves using maps as a communication tool. Most people can read and interpret maps, which make maps ideal reporting, and summarizing tools. The legal description of a quarantine area is ponderous and difficult to visualize. In contrast, a map can clearly show the area. Maps can also show a manager or supervisor areas that have been surveyed, the locations of positives, and the locations that the programme will survey next.

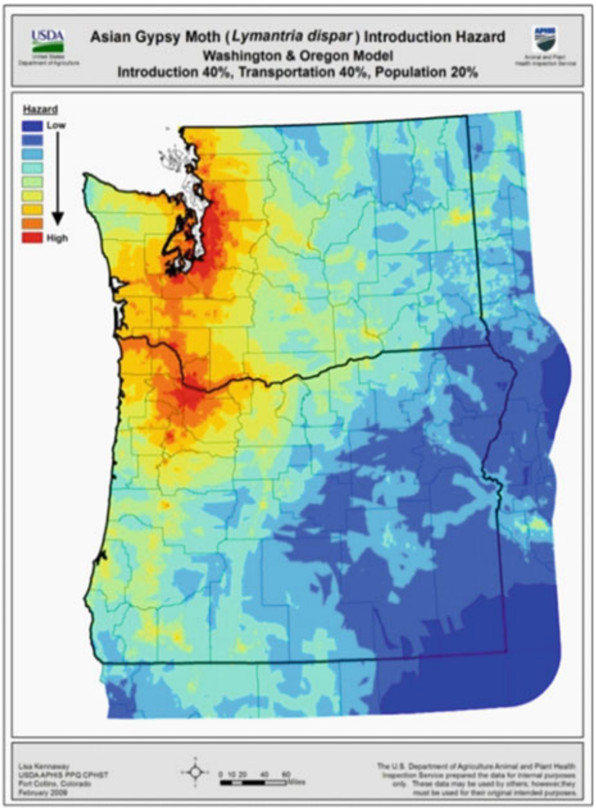

Fig. 11.4 AGM introduction risk map (Map & analysis produced by Lisa Kennaway, CPHST APHIS USDA)

A map can summarize a risk or models into an easily understood package. Most people would rather look at a map than a table of figures. A map can't replace a detailed tabulation, but it can make the numbers more easily understood. The key is to produce maps that present their message in a clear, concise, and understandable manner.

Below, we give two examples of maps trying to present the same data. The "bad" map is clearly poor, but it is not that different from many maps presented routinely. Many map-making software products are commercially available, but using these tools doesn't guarantee a good result anymore than using MS Word guarantees a clearly written report. Creating a map should be like constructing a good paragraph.

Consider the two maps in Figs. 11.5 and 11.6. Either map may work for someone who is actively involved in a specific programme, but would anyone not involved in the project understand the map in Fig. 11.5? Does everyone know the area, know the insect of concern, when the map was made and by whom? Most people would say the second map communicates more effectively.

Guidelines for a good map:

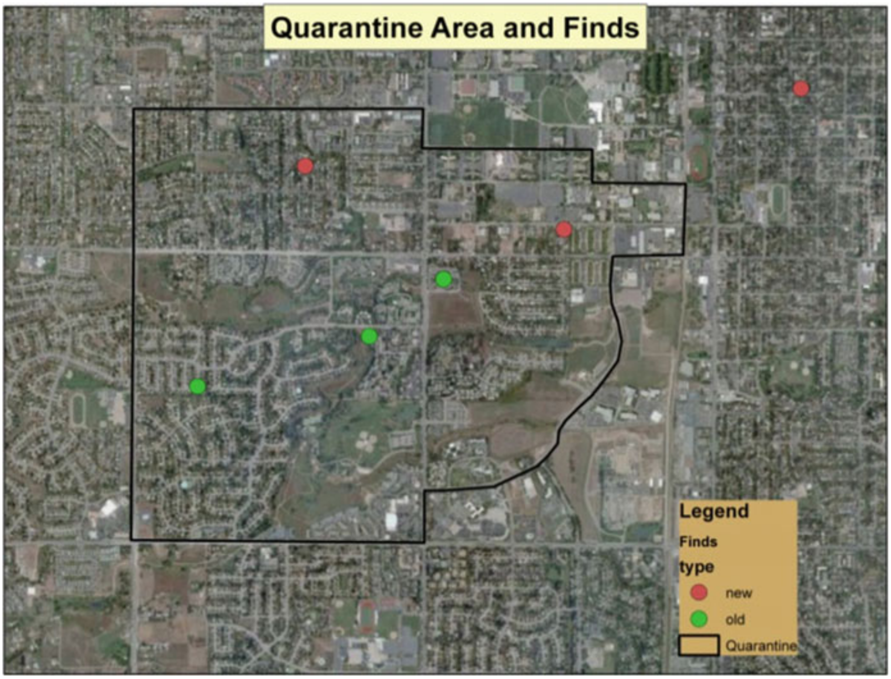

Fig. 11.5 Map 1 – Example of a poor map (Map courtesy of Lisa Kenaway, CPHST, APHIS, USDA)

- What is the purpose of the map and what information must be included?
- What map projection is most suitable?
- What characteristics are not relevant to the map's purpose?
- Can we reduce the map's complexity?
- What is the best way to convey the map's message?
- Data is the focus of the map. Is the eye is drawn to data before other places?
- Title is an appropriate size/length and descriptive of map's data?
- Scale bar, North Arrow, Legend and Supplemental Information are adequate in size and do not detract from data.
- Reference location data are included in the map (counties, cities, roads, etc.).
- Symbology of data is logical: Low values coloured green, high values coloured red.
- Supplemental Information includes: Author, date of creation, data source and Projection.

Features of bad maps:

- Data is overwhelmed with other items on map;
- Background colour detracts from data;

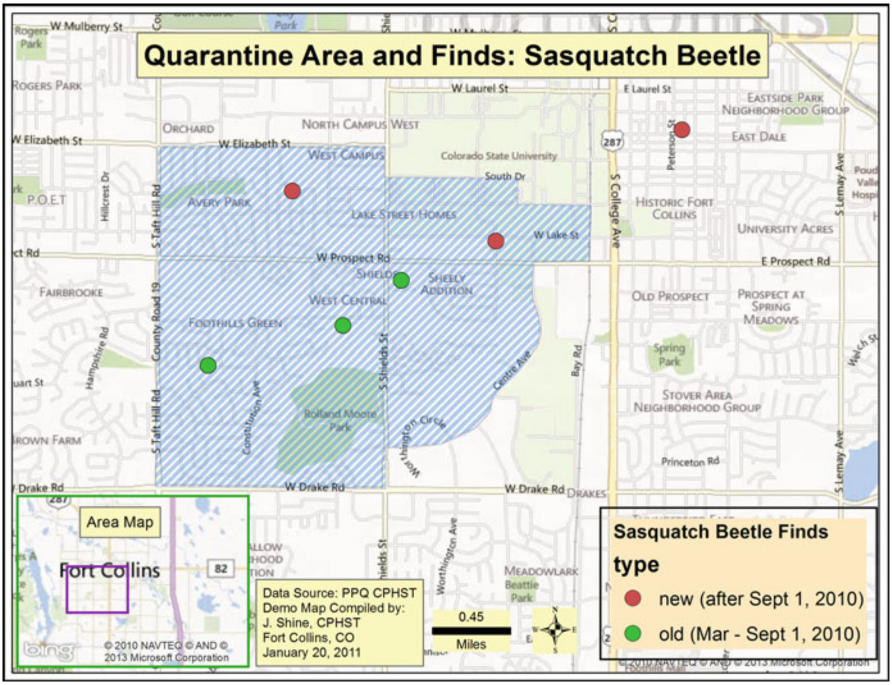

Fig. 11.6 Map 2 – Example of a better map (Map courtesy of Lisa Kenaway, CPHST, APHIS, USDA)

- Other items detract from data (legend, scale bar, etc.);
- Vague title or legend values (not logical);
- No reference data.

Good practise: Have all your maps proofread by another person for clarity and design – just as you would for a written document.

11.5 Data Integration: Cyber Infrastructure

11.5.1 Computer Infrastructure (Cyber Infrastructure)

Computer infrastructure (Cyber infrastructure) is needed to organise the large quantity and diversity of data sources required for targeted surveillance. Cyber infrastructure helps (a) collect, manage and share data, (b) create pest risk models and analyses, (c) interpret data and risk products for national and local needs, and (d) manage the distribution of information products to end users including managers, field staff and stakeholders (Fig. 11.2) (Magarey et al. 2009; Barrett et al. 2010). A cyber infrastructure should be designed to integrate heterogeneous data sources for (a) tabular data such as phytosanitary records and trade data, pest observations, pest biology, meteorological station records, and crop statistics, and (b) cartographic data including all baseline information (bio-physical and administrative boundary maps) remote sensing based maps, and spatial modeling outputs (e.g., weather maps). A cyber infrastructure also supports people (e.g. inspectors, data managers, risk analysts, programme managers and industry or extension specialists) and their responsibilities through a role-based access system. Role-based access is defined by a user's organisation, geographical responsibility (e.g. state, regional) and a user's job title. Role-based access determines what data or pest programmes a user will see, and which on-line tools they will have access.

Examples of emerging cyber infrastructure include the USDA-APHIS iPHIS system, the NAPPFAST system (Magarey et al. 2009) and the Australian Biosecurity Information Network (ABIN) (http://www.abin.org.au/). Both iPHIS and NAPPFAST system support the integration of pest observations and risk maps. The main objective is to provide decision support data for government, industry and researchers.

Data sharing is one of the greatest challenges in the development of a cyber infrastructure. This challenge results from a lack or complexity of data-sharing protocols and standards especially between federal and state governments, between countries and with industry. The incorporation of industry into cyber infrastructure is especially important because industry may provide long-term funding.

11.5.2 Data

The most common data fields usually collected or recommended for collection (ISPM 6), in surveillance programmes are: Data source ID, dates of collection, identification and verification, location, pest (scientific name, Family/Order names and pest code if available), host (scientific name and host code if available), means of collection (e.g. attractant trap, soil sample, sweep net), sample and diagnostic information. Location information may include latitude and longitude, country, state, city, zip code/postcode, address and the owner's contact information. Address and contact details may often not be included because it may violate privacy legislation. Pest information may include pest ID (or scientific name), phenological stage, quantity, and diagnostic determination. Survey and diagnostic information may also include diagnostic method, sample ID, laboratory ID, inspector ID and survey or trap method. Additional information may also be included as comments, e.g., nature of host relationship, infestation status, growth stage of plant affected, or found only in greenhouses; references (if any) and reports of pest occurrence on commodities.

11.6 Cooperative Agriculture Pest Survey (CAPS)

11.6.1 Introduction

In the USA, the post-border detection of exotic plant pests is the responsibility of the USDA, Animal Plant Health Inspection Service, Plan Protection and Quarantine (USDA-APHIS-PPQ) and its cooperators (Magarey et al. 2010). Here, CAPS, a joint Federal and State pest detection programme for exotic plant pests in the USA plays a major role (Wheeler and Hoebeke 2001).

CAPS has a multi-tiered structure with national and state level committees with representation from a diversity of organisations, including federal and state agencies, universities and industry. The first set of detection activities conducted by CAPS is targeted surveillance, also known as "Hotzone," "Risk Point," or "High Hazard" surveys (Wheeler and Hoebeke 2001). These surveys examine high-risk pathways based on the analysis of phytosanitary data including pest interceptions and emergency actions.

A second set of detection activities conducted by CAPS are pest and commodity surveys. CAPS committees select national and state survey targets from multiple sources including an annually prioritized list of about 50-60 pests. The prioritized list is selected from a larger PPQ pest list developed from sub-lists compiled from professional societies (e.g., American Phytopathological Society, Entomological Society of America) and from PPQ records. From this larger list, the prioritized list was developed using the Analytical Hierarchy Process (AHP, Saaty 1994). Expert opinion is used to answer questions regarding pest biology, pathways and impact for each pest. Pests are then prioritized by AHP using criteria weights selected by PPQ programme managers or other cooperators.

The CAPS 2011 pest list included 33 arthropods, 12 nematodes, 10 pathogens, 3 mollusks and 1 weed (Magarey et al. 2011). Some of the CAPS targets are selected at the generic rather than species level. Examples of CAPS national targets are the Giant African Snail (*Achatina fulica* Bowditch), the Summer Fruit Tortrix Moth (*Adoxophyes orana* (Fischer von Röeslerstamm)), and the False Columbia Root-knot Nematode (*Meloidogyne fallax* Karssen). Recently, CAPS began to implement commodity-based surveys that allow inspectors to sample for multiple pests during a single visit, potentially enhancing the survey process. The CAPS survey data are collected using the Integrated Plant Health Information System (iPHIS) and also archived in a central database known as the National Agricultural Pest Information System (NAPIS).

The USDA APHIS PPQ Center for Plant Health Science and Technology (CPHST) provides information on pest biology, survey methods, and risk analyses for many of these targets to help the CAPS programme cooperators plan surveys (Magarey et al. 2010). These surveys examine pathways identified as high risk on the basis of the analysis of phytosanitary data such as emergency action notifications or pest-interception data (USDA PESTID, formerly known as the Port Information Network, or PIN). Emergency Action Notifications are documents produced as part of an electronic reporting system used to enforce APHIS treatments and regulations.

When a high-risk pathway is identified, individual sites are ranked on the basis of data such as the type of establishment, phytosanitary history, logistical distance from the importation pathway, and sales data (Self and Kay 2005). For instance, targeted surveillance was carried out on ceramic importers and distributors in Miami, Florida (Beckwith 2004). After an analysis of past records of pest detections in Miami, 76 warehouses receiving tile shipments were selected for an in-depth survey. The survey resulted in the detection of new exotic arthropod species, including two new continental records and several new state and county records. The survey also found more than 20 genera from the Orders Coleoptera and Hemiptera (including Homoptera) that were not in the USDA PESTID database.

USDA APHIS PPQ's Smuggling Interdiction and Trade Compliance (SITC) is another programme actively involved in targeted surveillance. APHIS created SITC in the mid-1990s in response to the growing volume of smuggled or improperly imported agricultural products entering the USA. The mission of the SITC programme is to identify the unlawful entry and distribution of prohibited products that may harbour exotic plant and animal pests. SITC was responsible for seizing and destroying more than 2.7 thousand tons of prohibited material in 2002 (USDA-APHIS-PPQ 2004).

11.6.2 CAPS: Looking Toward the Future

Two areas with potential for future enhancement of the CAPS programme are aimed at industry and the citizen scientist.

Industry has extensive data-gathering capabilities that have not been widely incorporated into general surveillance systems (Magarey et al. 2010; Whittle et al. 2009). For example, seed companies routinely collect data from activities including: (1) Pest scouting of research and seed production fields, (2) phytosanitary field inspections; and (3) disease diagnostic testing for their customers. Another potential source of industry surveillance data would be voluntary contributions from crop consultants. In the USA, thousands of crop consultants routinely scout agricultural fields. Although many industry observations would require validation, some industry diagnostic labs are now certified by national or international agencies.

Citizen Scientist is a term used for projects or ongoing programme of scientific work in which individual volunteers or networks of volunteers (many of whom may have no specific scientific training) perform or manage research-related tasks such as observation, measurement or computation. The use of citizen scientist networks often allows scientists to accomplish research objectives more feasibly than otherwise would be possible. A citizen scientist programme is potentially one of the most important cooperative survey programmes. Citizen scientists have been responsible for the first detection of many important exotic pests including Light Brown Apple Moth (*Epiphyas postvittana* Walker) and the Asian Longhorned Beetle

(*Anoplophora glabripennis* Motschulsky) (Chap. 16). In the case of LBAM, retired UC Berkeley Professor of Entomology (a moth taxonomist) first detected LBAM in his Berkeley backyard on July 19, 2006. In the case of Asian Longhorned Beetle, beetles were first found by an unsuspecting landlord of an apartment building in Greenpoint area of Brooklyn, New York in the USA during 1996 (Sect. 16.2).

A citizen scientist programme could be created using existing observer networks, professional societies and school students to collect data on exotic pests. An example of an observer network that might be co-opted for exotic pest detection is the National Phenological Network (http://www.usanpn.org). Examples of professional societies whose members might cooperate include the Entomological Society of America and the American Phytopathological Society. APHIS currently is investigating the use of citizen science programmes for Asian Longhorned Beetle. Citizen scientist programmes might target high-risk pests or high-risk commodities such as specialty crops. Recently, we noted that specialty crops in urban areas (with high concentrations of potential participants) are at high risk of introduction to exotic pests (Colunga-Garcia et al. 2010a, b). A cyber infrastructure, described earlier in the chapter, can provide the information technology to build a system for collecting, integrating and analyzing citizen scientist data.

11.7 Conclusions

This chapter has introduced many methods and ideas concerning Surveillance and biosecurity, but it has only scratched the surface in a rapidly changing field. Even as this chapter was being written, there were advances in "sniffer" technologies, databases that allow real-time tracking of commodities, and cloud-based platforms that can deliver modeling applications into a port or field for better monitoring of resources. These can rapidly change how an organisation works. Many of the changes are technology based, and that will continue to be the case into the future. With a general worldwide trend toward tighter governmental budgets, increasing global trade, and climate change disrupting traditional patterns, technology offers a hope to allow countries to keep up with the increasing pressures on their biosecurity organisations.

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Chapter 12
Digital Identification Tools in Regulatory Science and Practice

G.A. Norton, T.W. Walters, J. LaForest, K. Walker, M. Taylor, S. Winterton, and G. Kong

12.1 Introduction

A quarantine officer's ability to identify an intercepted organism and to determine whether it constitutes a quarantine risk remains a critical attribute of any plant biosecurity strategy. Figure 12.1 shows the points at which identification is critically important, including pre-border (as part of quarantine procedures) and post-border (as part of surveillance operations) (Norton 2005).

G.A. Norton (✉) • M. Taylor
Queensland Alliance for Agriculture and Food Innovation (QAAFI),
University of Queensland, Brisbane, Australia
e-mail: G.Norton@uq.edu.au

T.W. Walters
Identification Technology Program, USDA/APHIS/PPQ-CPHST,
Fort Collins, CO, USA

J. LaForest
Center for Invasive Species and Ecosystem Health, University of Georgia,
Tifton, GA, USA

K. Walker
Museum Victoria, Melbourne, VIC, Australia

S. Winterton
California State Collection of Arthropods, California Department of Food & Agriculture,
Sacramento, CA, USA

G. Kong
Plant Biosecurity Cooperative Research Centre, Canberra, Australia

G. Gordh and S. McKirdy (eds.), *The Handbook of Plant Biosecurity*, DOI 10.1007/978-94-007-7365-3_12,

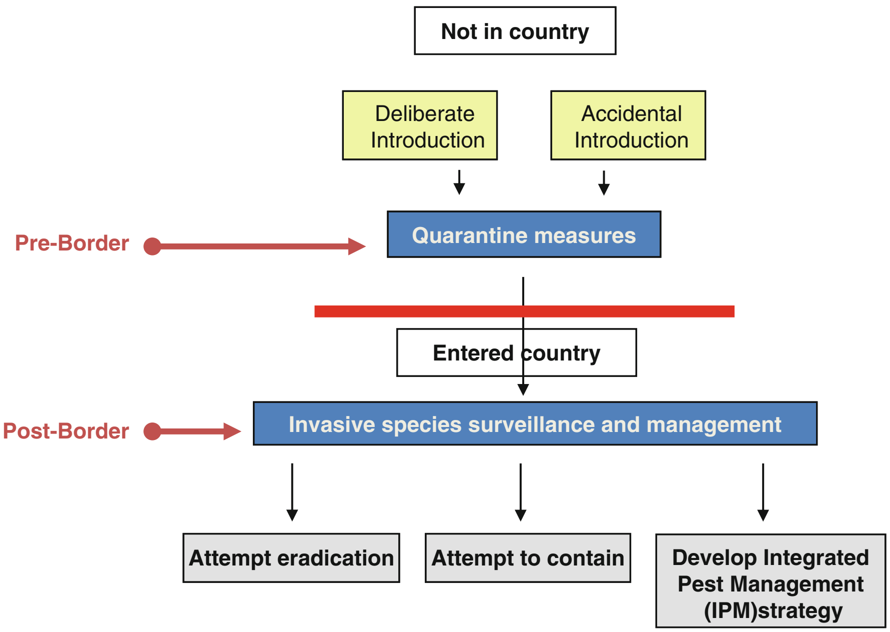


Fig. 12.1 Role of identification in plant biosecurity

12.1.1 Identification Based on Anatomical Features of the Organism

For the past several hundred years, the identification of plants and animals has relied on the systematic description of visual, anatomical features/characters. More recently, modern molecular techniques provide novel and in some cases definitive ways of identifying organisms based on selected sections of DNA code (Sect. 13.1). In the future, visual identification techniques, will be used independently or in combination with molecular diagnostics, and remain important tools for plant biosecurity, pest management and biological research.

Anyone wishing to identify an organism without access to an expert (such as a plant or insect taxonomist) must rely on some form of identification aid. Traditional identification aids match visual features of the specimen to be identified with a database of features associated with named organisms. One traditional identification aid (field guide) involves a gallery of pictures and descriptions that can be taken into the field to help the user identify a selection of plants or animals. These field guides and associated databases are often organised in easy-to-follow formats (such as shape, colour, or geographical location). Field guides can be very useful aids for identification, particularly when involving specimens where the number of likely species is limited, such as bird species in a specific location or the identification of certain invasive species.

Box 12.1 Section of a Dichotomous Key to Insect Orders

1(a) Well-developed wings present, though often folded along body and inconspicuous – 2.
1(b) Wings absent, or present only as small, functionless pads or scales – 14.
2(a) One pair of wings present – 3.
2(b) Two pairs of wings present; hind wings may be concealed beneath protective forewings – 4.
3(a) Forewings membranous; hind wings reduced to tiny club-like structures (halteres) – **Diptera**
3(b) Hind wings large, membranous; forewings reduced to tiny, strap-like structures – **Strepsiptera**
4(a) Forewings hard, opaque, in repose forming covers for hind wings; forewings entirely without branching veins or only at apex – 5.
4(b) Forewings either transparent or with branching veins over most of surface, or not forming covers over hind wings – 8.
. . . Etc.

The user answers the first question by choosing the component of the couplet that correctly describes the specimen under identification. This leads to further couplets. In the example given, if couplets 1(a), 2(a) and 3 (a) apply, then the specimen is identified as belonging to the Order Diptera.

This type of key is called "dichotomous" in reference to word's meaning... "two branching". However, in practice dichotomous keys often have questions with more than two choices.

Conversely, consider the problem of identifying an insect using a pictorial field guide for an area where hundreds of related or similar looking specimens reside. Clearly, an alternative method of identification is needed, namely, a systematic way of describing the unidentified specimen and comparing it with a descriptive database of species. In the case of an unidentified insect – does the unidentified specimen have wings? If so, how many pairs? . . . If a plant is to be identified, is it a tree, shrub, vine, or herbaceous plant? What shape are the leaves? . . .

12.1.2 Dichotomous (Text) Keys

The traditional form of identification aid, which has been used for several hundred years, is the dichotomous or pathway key system. This is still the most common form of key encountered today and is usually found in printed and/or Adobe PDF formats. Dichotomous keys may be laid out in various ways, but usually form a series of numbered questions arranged in "couplets". Box 12.1 shows a section of a dichotomous key to identify the Order to which an insect specimen belongs.

Dichotomous keys suffer from several problems, including the use of specialized terms to describe taxonomic features and the “unanswerable couplet” problem. The latter occurs when a user is working through a dichotomous key to identify a plant specimen and is asked to choose between specific flower features, such as flower colour. If the plant specimen is not in flower, then an unanswerable couplet is encountered. At that point, the identification process is often abandoned. Specialized terms in couplets create similar difficulties for the non-specialist user in distinguishing between character states and often result in the user rejecting the identification tool.

Recent Information Technology and Communication (ITC) developments have revolutionised approaches to specimen identification. The analytical powers of computer hardware and software, the opportunities provided by developing and accessing various types of multimedia, and the Internet, have made possible the development of new ways to design, format, search, deliver, and utilise digital identification aids. In the following sections we review two types of digital identification aids: Digital Key Systems, and Digital Image Database Systems.

12.2 Digital Key Systems

Two types of Digital Key Systems exist – those based on the traditional dichotomous or pathway key and those that use a matrix database at the core of the system.

12.2.1 Online Dichotomous Keys

Many dichotomous keys are now available on the Internet and take various forms. Some of these keys use the same text format as a paper-based key. Others use HTML format to present the user with an initial couplet, usually with the respective characters illustrated. The user selects the character that matches the specimen to be identified by clicking on it, and is then taken to the next screen showing the next relevant couplet, and ultimately to a screen that provides information on the identified species or taxon. Online keys are more accessible, easier to use and often contain images to illustrate the features in the couplet. But the digital dichotomous keys still suffer from the problem of the “unanswerable” couplet.

To deal with this issue, and provide other features that make digital dichotomous keys easier to use, the Phoenix Dichotomous Key System (University of Queensland 2011a) has been developed. Phoenix DKS consists of a dichotomous key builder and player. Any published dichotomous keys can be scanned and transformed into digital form using optical character recognition software. This digital file can then be imported into the Phoenix builder and automatically checked for any logical inconsistencies. When inconsistencies have been resolved, the key is ready for online use by converting it into the Phoenix player, which presents the user with each relevant couplet in turn.

When faced with an unanswerable couplet, the Phoenix DKS allows the user to skip that couplet and to continue choosing from remaining couplets to the end of one branch of the key. It then returns to the skipped couplet to enable the user to continue working down the other branch of the key. In this way, with one skipped couplet, the user ends up with two possible identifications that can be explored further. Examples of Phoenix keys can be accessed at University of Queensland (2011b).

12.2.2 Matrix Keys

The Phoenix DKS addresses some of the shortcomings of printed dichotomous keys, especially by allowing users to skip unanswerable couplets. However, the user still must follow an arranged pathway structure, and the sequence set by the key's author. This is a problem. Matrix Keys (multi-access keys) were developed to avoid this problem and allow users to navigate through a key in their own way (choosing characters easiest to distinguish first).

This new and novel approach to identification aids has been made possible using modern software and multimedia technology. Several identification platforms have been developed in recent years, including DELTA (Dallwitz 1980), Linnaeus (ETI BioInformatics 2011), Lucid (University of Queensland 2011c), and others (Dallwitz 1996). See Appendix 1 for screenshots of matrix keys developed in various software packages and applications. Most of these systems consist of two parts: (1) A key builder – which enables the author to construct the key and add images and text descriptions of characters – and (2) A key player for users wishing to make an identification.

The Matrix Key Builder. The data required for a matrix key consists of a list of entities/taxa (genera, species), a list of features/characters (wings, leaves . . .) and character states (wings absent/present; leaf shape oval/palmate, etc. . . .) that can be used to describe those entities. Data is scored in the cells for each entity in the matrix, and indicates the feature/states that apply to a specific entity. Images and other supporting material can then be linked to the matrix to provide assistance to the user in determining which feature/state best applies to the specimen under identification. The matrix format allows new taxa or new features (including location, habitat and anatomical features) to be added simply by creating a new column or a row of scores. This added information is automatically integrated into the matrix key.

The screenshot in Fig. 12.2 shows the matrix and scores associated with a section of a Lucid Matrix Key being constructed in the Lucid Builder. The taxa are shown on the horizontal axis and characters with their states on the vertical axis. The cells of the matrix can be scored in various ways including presence/absence, common/rare, and misinterpretations. Misinterpretations arise when a character state is scored for a specific taxon, not because the taxon has that character state but because the user can mistakenly think it does have that character state.

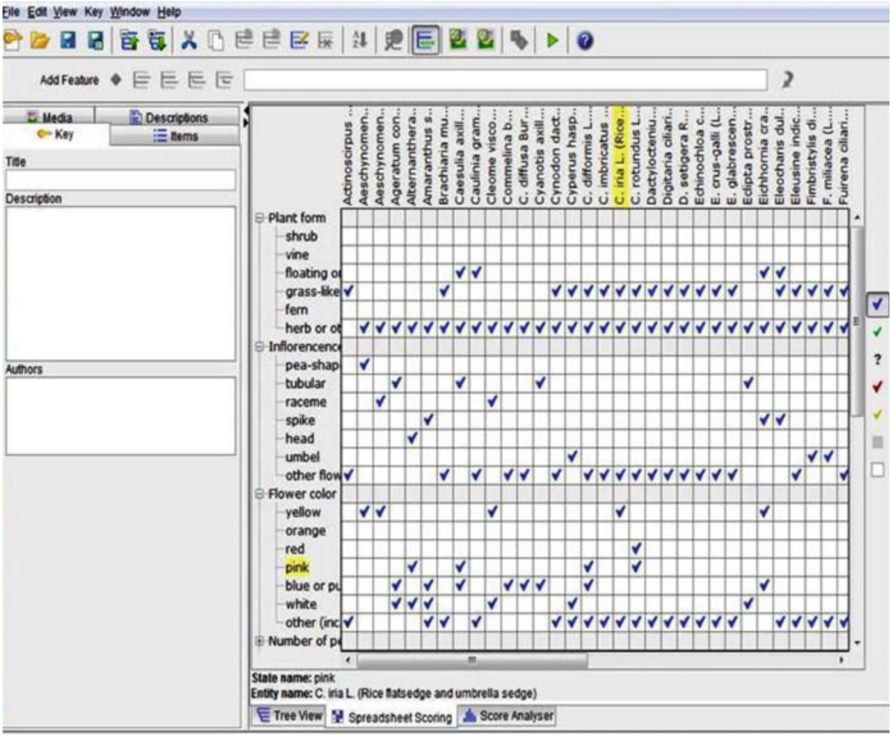


Fig. 12.2 Example of a data store in the Lucid Builder

The Matrix Key Player After a key has been constructed in a Matrix Key Builder, users through a Matrix Key Player via CD, DVD, can then access it or online, using an online applet or an online key deployed in the user's Internet browser. New multimedia technology also provides an opportunity to display line drawings and photographs to help users distinguish specific character states. Compared with Dichotomous Keys, Matrix Keys provide users with new ways of navigating through the identification process by enabling them to determine the order in which to address features in the matrix and choose those feature/states first that are easiest for them to distinguish. Moreover, Matrix Key Players also offer other features to assist the identification process, such as routines that users can "click on" that calculate the best feature to examine next in order to have the best chance of reducing the "short list" of taxa.

An example of the type of interface that a user (farmer, advisor or local authority/inspector) can use to identify weeds is shown in Fig. 12.3. By using images and text to help determine character states associated with the weed to be identified, as well as selecting other features (location and habitat where the weed was found), the user reduces the list of likely weed species. Linked weed images and fact sheets for each species allow the user to narrow down the likely species, to

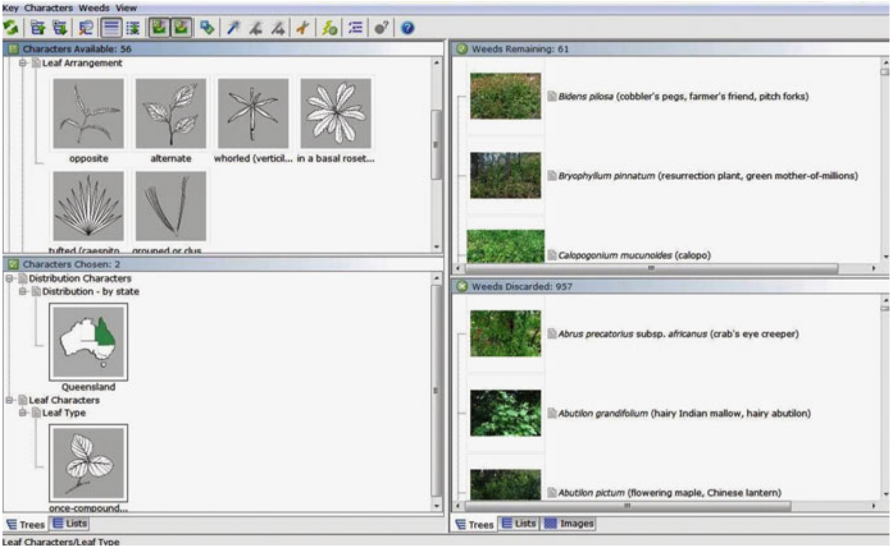


Fig. 12.3 User interface for the Lucid key to Environmental Weeds of Australia (University of Queensland 2008)

verify the identification and to obtain useful information about the weed (ecology, dispersal mechanism, management, etc.)

Matrix Keys offer a much easier way of making an identification compared with Dichotomous or Pathway Keys, but some guidance is needed to capitalize on the wide range of options and functionality that Matrix Keys provide. Best Practice Guidelines for making an identification with a Matrix Key – specifically the Lucid Matrix Key – is presented in Appendix 2.

To learn how a Matrix Key to weeds works, visit the following web site to identify the weeds shown using a simple key to garden weeds:

http://www.cbit.uq.edu.au/UQCentenary/TryoutanIdentificationKey.aspx

12.3 Digital Image Database Systems

Recent developments in software and multimedia have enabled new features to be incorporated into image collection systems to enhance their role in identification and supporting pest diagnostics. An image-collection system consists of three components: (1) A collection of images, (2) a database of information pertaining to those images, and (3) an interface to allow users to utilise the information in the database to return appropriate images. Each component of the system directly contributes to the value of the image database and its overall utility.

12.3.1 Image Collections

Images are non-moving, visual representations of a subject. Images include illustrations, photographs, drawings, or annotated graphics. If images are high quality and accurately represent the subjects, then they can provide a wealth of instructional value. If images are not representative or not well composed to best highlight the features of the subject, then users will be frustrated as they sort through the images. The innovations of the digital age and the ability to produce digital images have dramatically affected the way images are created, stored, organized, and retrieved.

12.3.2 Database of Image-Related Data

An impressive collection of images with no data, limited data, or poorly structured data (i.e., only a set of keywords pertaining to each image) may look attractive when viewed on a user's interface but will result in underutilization of the collection's potential due to the difficulty of finding pertinent images or the incomplete return of results to the user. In the worst case, the user is presented with an image gallery rather than an image database and is forced to examine screen after screen of images to find something that fits what the user was seeking.

12.3.3 Interface with the Image Database

Like images with no data, an impressive collection of images with detailed information about them will be wasted if the interface to access and sort through the images is too cumbersome or the interface does not make effective use of the information in the database to allow the user to efficiently narrow the returned results. Such resources initially frustrate users until they learn the quirks of the system or find another resource.

Pest image databases vary greatly in terms of the taxa they seek to illustrate. Some databases focus on specific groups of taxa, commodity groups, or a geographic region. Understanding the scope of the resource will provide the context necessary to interpret the results produced by browsing or searching: The broader the scope, the greater the requirement for navigational organisation.

Image and subject layers A major difference between keys and image databases is the increased focus on information pertaining to specific images rather than the large amount of data being dedicated to describing a taxonomic group. The need to develop subject-based and image-based information as part of an image database (especially for systems that include taxa from multiple taxonomic groups) results in

Table 12.1 Data elements typically found in the image and subject layers of an image database

Image layer	Subject layer
Life stage	Higher taxonomy
Image orientation	Taxonomic synonyms
Image setting (field, lab, museum)	Scientific name
Image resolution (height and width)	Common names
View	Nativity
Location	Quarantine status
Photographer and citation	Identifying characters
Commodity subject is featured	Commodities the subject can affect
Host that subject is affecting in image	Hosts that subject can affect

the segregation of data into two layers: The image layer and the subject layer (see Table 12.1 for examples of the content in each layer).

The **image layer** enables a user to quickly find images of an organism in a particular life stage or habitat (e.g., larvae in a cotton field; adult feeding on a cotton plant) rather than specifically identifying a specimen. Information such as the life stage in the image, the commodity or host plant of the pest, or place the image was taken can be helpful in limiting returned results. This leads to better match the habitat of the specimen or the type of specimen being identified. Other information in the image layer (photographer or citation) is not necessary for identification but is important if the user wants to use certain images for publications. Without proper data to describe the individual images, users may be able to obtain the subject of interest but must sort through many irrelevant images.

The **subject layer** is designed to provide information pertaining directly to the organism regardless of the setting, life stage or feature represented in the images. A wide range of data could be included in the subject layer, including the full taxonomy for the subject or the status of the organism as a biosecurity threat for a region. The subject layer may also include the same type of identification-level information that is found in keys. However, few image databases have included this level of detail since the information is not available in a format that is readily integrated. Providing the database structure needed to allow for disparate taxonomic groups that use different characters for identification can be extremely challenging.

Video, audio, and other forms of media Many concepts that apply to image databases can pertain to the organization of other types of media including video, audio, presentation materials, animations, and 3-D models. Developers may be tempted to make one database that includes all of these forms of media since there are many pertinent data elements in the Image Layer. However, each media type has additional data elements that are unique to that medium. For example, videos have a specific resolution, subjects and a creator, but they can also have duration, audio language, subtitles, and encoding codecs. Including these elements in a "universal media layer" can significantly increase the number of data elements included in the database. This approach leads to confusion for users trying to make sense of the database design and reduces the efficiency of content delivery. For these reasons, a system that catalogues many different types of media should incorporate a data layer for each type of media (e.g. Video layer, Presentation layer, etc.). Each media layer can still utilise information in the Subject layer since that information does not vary regardless of the media to which it is linked.

12.3.4 User Interface

The interface to an image database provides the user with the ability to manipulate and filter information to return the most pertinent images. Use of the image database interface to diagnose the organism requires the user to apply their own level of knowledge while the results are dependent on the images that have been entered into the system. Three approaches to creating user interfaces for accessing the image database are: (1) Direct searching, (2) Navigational searching, and (3) Faceted searching.

Direct Search The user crafts a query to directly search all information in the database via a "key word" search. This can provide quick results if you know what you are looking for, but should not necessarily be the first choice on a site when trying to identify something. Most systems are designed with a rationale and structure that can be used to produce more accurate results than those found through a text search of all fields in the database. Also, a user can easily create a query that returns no results. For many users this can be extremely frustrating, especially when users are certain that the database contains images that may be relevant but they are unable to create an effective search term. Direct search also has the potential to turn an image database into an image gallery, where finding the image you want is more related to perseverance of looking through many screens of images rather than systematically limiting the returned results to find images that are the best matches.

Navigational Search The user is presented with different data elements available in the database. The user can navigate along one element or a hierarchy of elements in an attempt to narrow the results. A prime example is a navigational interface using taxonomic data: The user can select any level from Kingdom to Subspecies and easily move to neighbouring taxa. This can be extremely powerful, but the user needs a significant amount of background knowledge to effectively navigate this type of interface.

Faceted Search Faceted searching uses all of the data entered in the image database to allow users the most flexibility in crafting a search that will efficiently return the desired results. A Faceted search is often used in e-commerce websites (such as eBay, Amazon, car auction sites, etc.) where the facets reflect the contents of a stock catalogue. Well-designed e-commerce websites never allow users to request an item that is not in the stock catalogue (i.e., a dead end).

Each data element from the Image and Subject layer can be used as a facet to filter which images are returned. Facet variables are created by determining all of the distinct values that are available for a given data element (e.g., some insect life stages would include the facet variables adult, larvae, and pupae), or allowing the user to supply a keyword that is matched against that data element (e.g., "beetles" being used to filter the "Common names" data element).

Effectiveness of a faceted search is achieved by the automatic removal of redundant characters and unrelated information. Each time a user selects a facet variable, the system rebuilds the menu of relevant facets and facet variables to only display those pertaining to the returned results. For example, if a user begins a search by selecting the "common name group" variable "beetles", then all variables in the Scientific Taxonomic Hierarchical facet that are not "beetles" will be removed. Similarly, variables in all other facets that are not associated with beetles (e.g. "host" or "distribution" etc.) are removed before the user can make another query. The user always is presented with relevant choices based on previous steps in their search and cannot reach a dead end where no images are returned.

Faceted searches have many benefits. The user can select facet variables because they are easier to use. If the specimen has no head or legs, then the user can select any other facet character available. Complex user-defined queries can be progressively built knowing that the user is never allowed to select a new query term that does not relate to the species returned to all of the previous query variables. Faceted search also allows the user to check the progress of the query. Automatic removal of redundant characters and character states means that values remaining in the facets accurately describe species returned to multiple queries. For example, after selecting "mango" from the facet "host", the system rebuilds the list of "host" facet variables to show all other hosts associated with the species that feed on "mango". The system also will rebuild all other facet variable lists based on what still is relevant to "host = mango". The user can scan these variables for obvious errors before continuing to query. If the user finally reaches a single return species, then every variable in every facet should match the single returned species. This feature is particularly helpful when using morphological facet variables.

Faceted search allows all available data to be brought to bear to provide the most flexibility in crafting a search. By adding individual specimen data to the faceted searches, the user can create very detailed search strings that include: Begin with a user-defined spatial and temporal search, combined with taxonomic, host, distribution, exotic status facets and then refine the query parameters further using morphological facet. For example, return any species within a user-defined spatial area and temporal period, recorded on several hosts, known to be exotic established species from nominated countries and the insect has blue wings. Only faceted search will allow a user to explore and navigate a dataset with such a complex query without reaching a dead end. The only "dead end" the user would reach is if their character variable (i.e., blue wings) was not an option in the wing-colour facet.

12.3.5 Limitations of an Image Database

When using an image database we must assess the limitations of that system based on the scope and completeness of the image database. These systems continue to grow as images are added. For instance, consider a user searching an image database for images of Lepidoptera larvae feeding on tomatoes but cannot find an image that matches the specimen. This situation does not necessarily mean that the specimen is a newly discovered species; it only means that the consulted image database did not have an image of that species. For this reason, organizations and individuals must cooperate to make the collection of images as inclusive as possible to enable identification of the organism and to include valuable information such as whether the organism is a problem. Cooperation also can help standardize terminology used across a series of image databases to reduce the learning curve of new users who have had experience with related systems.

12.3.6 Examples of Image Databases

Two examples of broad-based image databases that specifically focus on taxa of importance to biosecurity are: (1) The Bugwood Image Database at the Center for Invasive Species and Ecosystem Health (CISEH 2011) in the USA, and (2) the Pests and Diseases Image Library in Australia (PaDIL 2011). Their approaches are different concerning information stored in the underlying database and the methodology used to deliver this information to users.

PaDIL (2011) allows direct and faceted search. PaDIL exemplifies the transition of morphological taxonomy from a museum-based science to an information-based science by delivering an image database to a non-specific user group. PaDIL allows users to navigate through standardized images of pests with special attention to capturing diagnostic characters for the group. By focusing on the taxonomically relevant features of a group, PaDIL also serves as an educational tool to direct a novice user to find the corresponding view on the specimen they are attempting to identify. PaDIL is very strong in the subject layer of information including the host range of a particular organism, presence of diagnostic-level characters, reported geographic distribution, and taxonomic description. While PaDIL has a centralised image/information database, it allows the creation of separate and unique web interfaces that are designed and populated by the authors of the discrete datasets. A general pests web interface is designed to provide broad coverage for multiple groups, across multiple bioregions, across multiple host types, and multiple exotic and endemic status types. However, other library interfaces provide more targeted facet searches for individual countries or individual taxa. For example, PaDIL contains separate libraries for Thailand and New Zealand biosecurity but also contains the Australian Smut Fungi library that consists of all 300 known endemic and exotic species that occur in Australia. Each of these libraries provides the user with the best fit of facets to enable effective user exploration and navigation of the dataset (i.e., product catalogue). To merge all facets and datasets into a single web interface would substantially diminish the user's interactions with the multiple datasets.

In contrast, the Bugwood image database (CISEH 2011) is a centralized image database designed to serve a system of websites aimed at different user groups (Fig. 12.4). Each website is an extension of the Faceted Search methodology (Fig. 12.5) to allow the same images and content to be delivered to distinct user

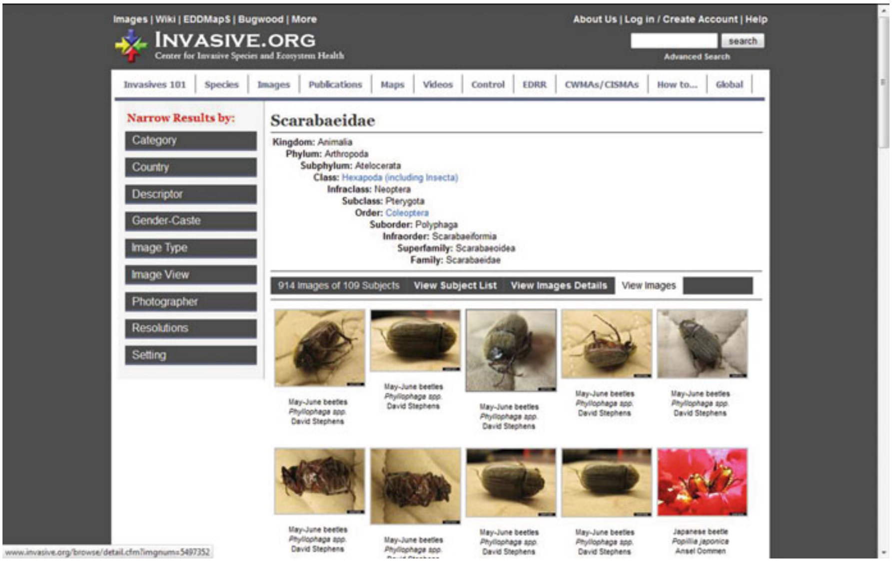


Fig. 12.4 Browsing for invasive species in the CISEH image collection by taxonomy, with other filter categories available in the left panel (CISEH 2011)

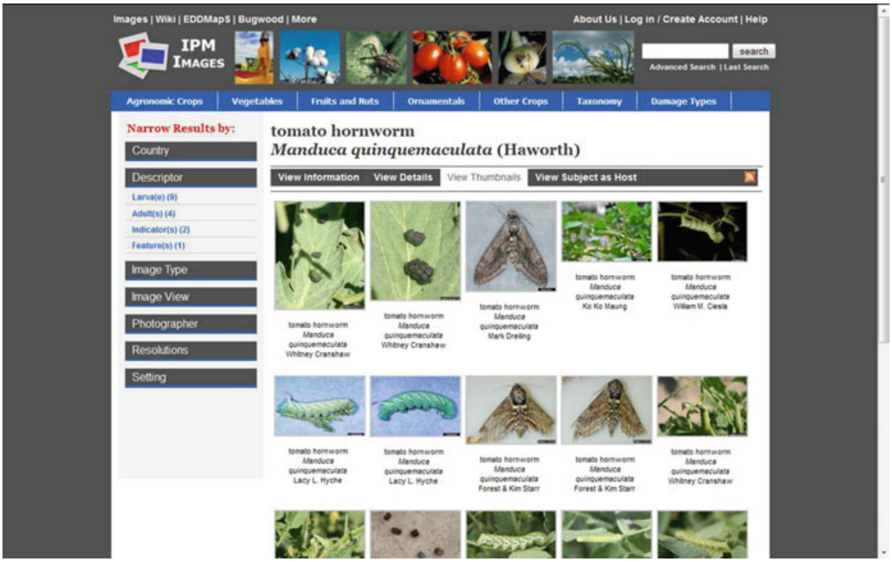


Fig. 12.5 Results of a search for tomato hornworm images in the CISEH image collection, showing additional filter categories such as Country and Life stage (CISEH 2011)

groups according to an interface that fits their perspective of how an image database should be organized and function. For example, www.invasive.org was created to focus on the organisms listed by various organizations as being currently or potentially invasive in North America. The target audience for the site includes individuals working with regulatory or other concerned organizations to identify and report the occurrence of these species. Most of their work will be focused on specific survey targets for coordinated detection programmes or public relations efforts to raise awareness of new threats. Farmers and crop consultants concerned with pests found in a particular commodity including emerging threats, would be at a significant disadvantage if they were not aware of the various lists, survey programmes, and host ranges for a particular organism. These users go to www.ipmimages.org since that site provides the same images and content but is navigable through commodity-specific selections. If users find that what they are seeing in their field is potentially a new find of an emerging pest, then they can find the information necessary to report it and obtain assistance in confirming their suspicions.

The CISEH image database also focuses on all images and aspects for an organism rather than only the identification quality images of the organism. The resource is focused on providing users with all images a user would need to educate others about the pest. . . including the damage caused in the field, control measures, life stages, and identifying characters. In that spirit, all of the images in the system are freely available for non-commercial, educational use as long as the images are properly cited. Design of the interfaces focuses on helping users quickly find images they need for the work they are doing and downloading high-resolution copies for publications. The strong focus of helping users find the appropriate images required for a specific use required a robust image information level, with a taxonomic backbone on the subject level to provide organization. The utility of the interfaces to reduce the number of images returned, and potentially identify an organism, is dependent on the system containing an image of the pest in the context the user found the specimen. For example, if a user has a specimen found in a cornfield and uses the IPM Images site to browse through all images of adult beetles feeding on corn leaves, then they may not find an image that matched. The user would have no idea that the pest also feeds in cucumber and if they had looked in that crop, or had not included the commodity as part of their faceted search, they would have found a matching image. This problem can be further amplified if the assumptions that the user made based on their knowledge were wrong (e.g., the specimen was a true bug, NOT a beetle).

Despite their different approaches to delivering information to end users, the CISEH and PaDIL image databases do cooperate to share images and information to broaden the availability of the content and the inclusiveness of the results returned to end users. Expansion of such models can serve as a blueprint for other projects to collaborate and share available information.

12.4 Recent Advances in Digital Identification Aids for Biosecurity

Digital identification aids for biosecurity are evolving at a rapid rate to meet the growing demands of the global plant protection community. This is made possible through:

- Availability of new hardware (tablet and mobile phone technologies);
- Increasing resolution and decreasing cost of digital cameras and scanners;
- New software technologies; and
- Applications that offer the potential to deliver a wide range of identification aids on diverse electronic devices anywhere and at any time.

The availability of open-source web applications and increasing options within web browsers, along with decreasing processing time for uploading, downloading, and sending information, are driving identification aid design and development for delivery via the World Wide Web. As technologies and access speeds improve and Internet accessibility is becoming widely available, identification aids are now offering users diverse digital media (keys, images, documents, links, etc.) to support their identification processes. For instance, the Lucid development team at the University of Queensland has taken advantage of faster processors and Internet connections available to their users to develop the Lucid Key Server. This is a web-based suite that includes the Lucid On-line Player that uses the latest in web 2.0 technologies to provide an intuitive, interactive user experience. Users only need their web browser to play keys instantly without waiting to download the key (see http://idtools.org/id/mollusc/index.php). Keys accessed through the Lucid Key Server can be used on many operating systems and on mobile devices.

12.4.1 Broadening the Scope of Identification Tools

With increasing use of matrix keys and the increasing demand for broader support for individuals with varying biosecurity-related identification responsibilities, key developers are now learning that matrix key systems offer considerable flexibility in increasing the key's scope and circumscription. Matrix keys are now used in different ways to support biosecurity and pest management decisions. Two basic types of matrix-based keys are being developed to support biosecurity: identification keys (based on taxonomic features) and diagnostic keys (based on symptoms).

The scope of a taxonomy-based identification key may be determined purely by taxonomy, such as a key to all species within a genus/family. Identification keys may also be a taxonomic sub-set based on other criteria, such as a key to the most important thrips or a key to insect pests found on particular crops/commodities. Examples of taxon-based identification resources include *Scale Insects* (Miller et al. 2007) and *Invasive Mite Identification* (Walter 2006).

Fig. 12.6 *Left*, screenshot of digital identification tool home page of *LBAM ID* (Gilligan and Epstein 2009). *Note the availability of resources available to the end-user in the left menu. Centre*, screenshot of one fact sheet (LBAM) within *LBAM ID*. *Right*, screenshot of one of the four Lucid keys (adult Lepidoptera) in *LBAM ID*

Diagnostic keys are typically crop or commodity focused. These keys are generally more difficult to construct, because they are based on the symptoms shown by the crop or commodity in response to pest or disease attack, or due to physiological or abiotic disorders.

Lucid key developers also are taking advantage of web-based technology to develop entire digital sites surrounding their matrix keys. These sites, referred to as identification tools, incorporate an interactive Lucid key or keys, and typically include a home page with other informational Html pages not directly attached to the matrix features and entities. These tools can include pages that contain information on ecology, systematics, taxonomy, image galleries, fact sheets, or molecular searching.

The following three examples illustrate the broader approach being adopted for the design, development and use of digital identification aids.

Light Brown Apple Moth (LBAM; *Epiphyas postvittana* (Walker) Tortricidae, Lepidoptera). When LBAM, was first detected in California, regulatory staff involved with survey, screening, identification, and verification required identification aids to support detection of the moth. Taxonomic expertise, resources, and funding were limited for the rather large-scale detection and identification process. Many staff involved with the detection of LBAM were not experts on Lepidoptera, especially larval identification. Staff also experienced difficulty in separating LBAM from other morphologically-similar moths within California. Verification of specimens was often difficult due to the condition of specimens (partial specimens) after they were removed from traps. The survey generated an extremely large number of specimens, and it became immediately clear that the field triage process had to ensure that experts only focus their time and energy on potential LBAM specimens.

To provide identification support for all staff involved with the survey for LBAM (including non-entomologists, expert entomologists but non-lepidopterists, and expert lepidopterists) USDA CPHST's Identification Technology Program (ITP) designed, developed, and delivered the identification tool *LBAM ID* (Gilligan and Epstein 2009).

LBAM ID includes four Lucid keys (Lepidoptera adults, Lepidoptera larvae, Tortricidae adults, Tortricidae larvae), an image identification comparison page, fact sheets for all relevant taxa, and a DNA sequence search page (Fig. 12.6).

Fig. 12.7 *Left*, screenshot of home page for a commodity-based identification tool *A Resource for Pests and Diseases of Cultivated Palms: Screening Aid to Pests* (Redford et al. 2010). *Centre*, screenshots of the Lucid key and *Right*, F fact sheet within the identification tool

LBAM ID was extremely successful in supporting field personnel with survey and detection, and, reducing the number of specimens requiring expert examination. In 2010 when European Grapevine Moth (EGVM, *Lobesia botrana* Denis & Schiffermuller) was detected in California, *LBAM ID*'s DNA sequence page was used for the initial identification. With the success of *LBAM ID* and the likelihood of future tortricids entering the USA (with requests for support tools like *LBAM ID* by other states), CPHST ITP initiated the development of TortAI, an identification tool to tortricids of agricultural importance to the USA. TortAI was released in 2012 and will include all of the components contained within *LBAM ID*.

Cultivated palms. An initial commodity-based identification tool – *A Resource for Pests and Diseases of Cultivated Palms: Screening Aid to Pests* (Redford et al. 2010) was specifically designed for use by non-entomologists (See Fig. 12.7). This tool has now become part of a broader identification resource for cultivated palms (Walters et al. 2010), involving a series or collection of identification and diagnostic tools related to palms (Anderson 2011; Broschat et al. 2010; Redford et al. 2010). This example illustrates the way in which identification tools might be combined and developed in the future for identifying the pest, disease, and host material associated with a specific commodity and so enhance and make survey/detection processes more efficient at ports and in the field. Other crop/commodity-based tools that have been developed include rice, cantaloupe, citrus, and sweet potato.

Wood-boring beetles (WBB) of the world. In 2011, an on-line portal to the genera of wood-boring beetles (WBB) of the world was released (Nearns et al. 2011). This portal contains numerous types of identification aids (keys, image galleries, fact sheets, species description pages, etc.) to support the screening and identification of taxa within the nine families containing wood-boring beetles (Fig. 12.8). WBB offers users various search options, such as geography and family, so they can quickly locate the specific aid or tool required for an identification process. If users do not know the family of the taxon they are trying to identify, then the portal provides an identification tool to identify wood-boring beetle families. The portal continues to be developed in cooperation with beetle experts and WBB end-users from around the world.

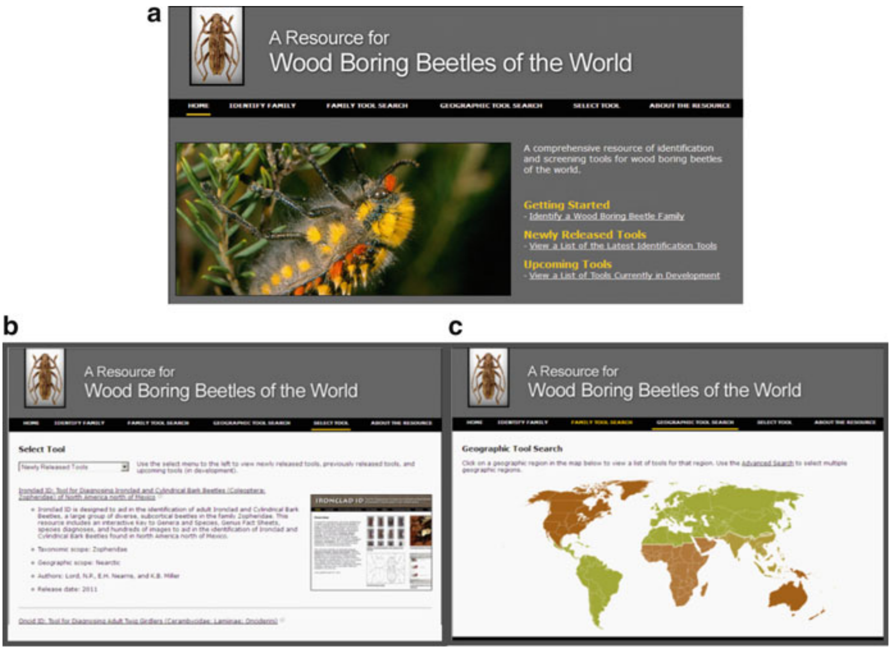


Fig. 12.8 *Top* (**a**), screenshot of home page for *A Resource for Wood Boring Beetles of the World* (Nearns et al. 2011). *Below*, screenshots of "select tool" (**b**) page and "geographic tool search" (**c**) page

Since major features of recently developed digital interactive keys include images of characters and taxa (and image collections now include search functions often using anatomical features to narrow the search for relevant taxa/images), WBB provides users with links to both digital interactive keys and image collections (CISEH 2011; PaDIL 2011).

12.4.2 A Portal for Accessing Digital Identification Aids

Digital identification aids can provide valuable assistance for a range of biosecurity or plant protection purposes, but learning whether identification aids exist for particular taxonomic groups or commodity groups can be a major constraint. Generic search engines may not be effective in finding relevant aids, and some aids may not be suitable for making a correct identification. To provide the USA biosecurity community with easier access to online digital identification tools, USDA/CPHST's Identification Technology Program (ITP) has developed *ID Source* – a gateway to information tools for plant pest, weed, and disease groups of concern for plant protection and quarantine (Fig. 12.9). *ID Source* contains keys,

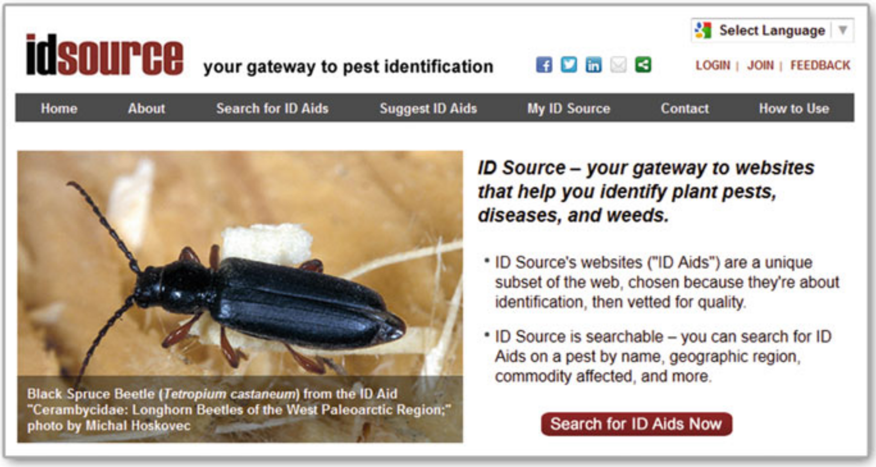


Fig. 12.9 Screenshot of the home page of *ID Source* (ITP 2011))

Fig. 12.10 ID Source offers two ways to search. Users most often enter into the Text Search scientific and common names of pests. Users can also choose values from seven Filter options to find the best matching ID Aids. The "Key Program" Filter is here expanded to show the many types of keys found among ID Source's ID Aids. *Terrestrial Mollusc Tool* is an ID Aid result for a Text Search on "Giant African Snail" with the "Lucid3" Filter value chosen

fact sheets, screening aids, image galleries, and other aids specifically designed for facilitating identification. Because it has a specialised focus, searching with ID Source leads to more helpful results than standard Internet search engines can provide (Fig. 12.10). The ID aids reached via ID Source are only those that ITP and ID Source users determine meet a certain standard of quality and usability.

12.5 The Future of Digital Identification Technologies

Taxonomic expertise and capacity has declined and continues to decline globally. Academic and research positions in taxonomy are not filled after an expert has retired, partly due to budget limitations but, more importantly, due to a lack of trained individuals with the required expertise. At the same time, the need for taxonomic services continues to increase. In these circumstances, taxonomic experts involved with biosecurity are required to identify specimens beyond their area of expertise. Consequently, the availability and access to new identification technologies and software will become critical to accurate and timely identification of intercepted organisms in the future.

Administrators of regulatory agencies are aware of these needs and of the shortages in taxonomic services, but budget constraints limit options. In the future, access to user-friendly, digital identification aids that provide a wealth of media types (images, keys, illustrations, video, etc.) that support different levels of knowledge and expertise will be essential to maintain and enhance the detection and identification of invasive species.

12.5.1 Collaborative Development of Identification Aids

Pooling resources and expertise is one way in which biosecurity agencies and organizations throughout the world are responding to the increasing demands for biosecurity identification aids. For instance, the USDA APHIS Identification Technology Program (ITP) leads a team within the biosecurity agencies of Australia, Canada, USA and New Zealand to collaboratively develop and share diagnostic aids. Such collaboration shares taxonomic expertise, eliminates redundancy in key development, reduces time for delivering aids and reduces costs associated with aid development. Examples of this collaboration include the recently released Lucid key to invasive ants of the Pacific (Sarnat 2008); the development by Australia and the USA of an identification tool to flat mites of the world, and Canadian and USA collaboration in developing an identification tool to weed seeds. Australia's Lucid development team and USA's ITP continue to develop the underlying technology to simplify and improve the efficiency of key development and enhance the user interface experience (design, content, functionality, and navigation) when using digital identification aids.

A website – IdentifyLife (University of Queensland 2011d) – has been designed to facilitate and support collaborative development and deployment of identification tools (Fig. 12.11). Working in collaboration with the Atlas of Living Australia (http://www.ala.org.au/) and the Encyclopedia of Life (http://eol.org/), the objectives of this Open Source project are to:

1. Combine a wide range of identification tools, to help people throughout the world identify living organisms.

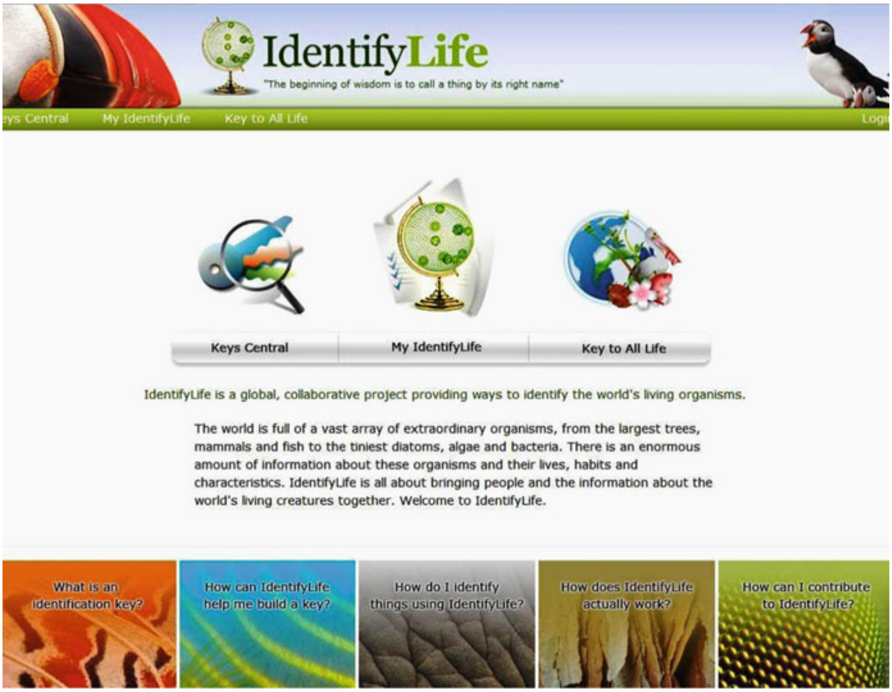


Fig. 12.11 Home page of the IdentifyLife website

2. Create a collaborative space (MyIdentifyLife) where the world's community of experts and enthusiasts can use IdentifyLife to store, manage and disseminate identification keys and descriptive information.
3. Develop an ambitious key – the Key to All Life – to all the world's living organisms.

IdentifyLife offers potential for supporting worldwide collaborative development, sharing and delivery of Biosecurity identification tools.

12.5.2 Cybertaxonomy

We noted that the decline of taxonomic expertise is a major motivation for developing digital identification aids. The development of matrix keys such as Lucid can also improve the efficiency of the taxonomic process. A character matrix, such as that constructed in a Lucid key (cf. Fig. 12.12) or in IdentifyLife, represents an atomised set of characteristics and states in their simplest form, describing a given taxon set. This information provides a useful descriptive statement that is computer-readable as metadata, allowing it to be exported as natural language taxonomic descriptions for use in taxonomic monographs and information fact sheets.

With recent proposed changes to the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 2008), taxonomic descriptions of new species are moving from traditional models of publication (solely in scientific journals) to electronic publications with links to informatics resources being embedded within new species descriptions. These informatics resources include links to online databases, such as image databases (e.g. Morphbank), names databases (i.e. Zoobank), DNA sequence repositories (i.e. Genbank), literature databases (e.g. Biodiversity Heritage Library) and various specimen databases with collection-label data. Recent publications utilizing these online resources include Johnson et al. (2008) and Deans and Kawada (2008). Winterton (2009) used the Lucid Builder to generate highly standardised taxonomic descriptions of new species by exporting natural language descriptions directly from the character matrix in Lucid. Provision of such links to online resources within taxonomic descriptions is bringing unprecedented levels of value-adding to documenting biodiversity discoveries, beyond static, paper-based species descriptions.

12.5.3 Developing Linkages Between Digital Information Sources

As noted above, identification resources are being developed in which both images and keys can be accessed from one site. Indeed, digital keys and searchable image collections have a lot in common. Key and image developers are now sharing information and images to support the use of their specific aids.

In the future, improved programmatic access to image databases through web services and Application Programme Interfaces (APIs) will enable other systems to automatically post requests for relevant content and have that material quickly delivered in a form that can be easily displayed to end users. The greatest limitation to developing these linkages is accepting a common terminology. For example, using the scientific name to request images of a certain species can work, but often disagreements ensue concerning the valid name for an organism. Also, names continue to change as taxonomists revise various groups. Slight differences in abbreviations used in scientific names (“ex. subsp.” and “ssp.” both mean “subspecies”) or differences in the conjugation of the specific epithets (ex. *Archips argyrospilus* vs. *Archips argyrospila*) lead to systems not being able to access all relevant resources. The use of unique identifiers (Life Science Identifiers (LSID) and proprietary database identifiers) and systems that allow for the lookup of synonymous taxonomy has helped to resolve some of these issues but a universally accepted solution remains elusive. Development of effective linkages requires personnel associated with each project to discuss the structure of their systems and develop solutions to ensure that the systems are utilizing a common terminology. This leveraging of image and key database resources makes the most of limited resources and improves the ability of other groups to utilise the existing resources.

Fig. 12.12 Shows how an expert can access a pest image from remote microscopy hardware connected to the Internet

12.5.4 Remote Microscope Diagnostics

For several years, physicians have been using communications technology to create the concept of Telemedicine. Patient consultations are conducted from a distance, particularly for people living in isolated communities or remote regions where access to specialist or expert opinion is limited. The constraints of distance and expertise are very similar for plant pest diagnostics where we are faced with a global decline in taxonomic expertise and in the numbers of pest diagnosticians. Since experts tend to be concentrated in major cities whereas many introduced pest species are intercepted in rural and remote areas, the diagnostic problem is at some distance from the nearest expert. Remote microscope diagnostics for pests, like telemedicine, uses the Internet and other communication technologies to present microscope images of a pest, symptoms, or damage caused by pests, to an expert in a different location.

A typical lab setup for remote microscope diagnostics (RMD) includes a microscope (dissection or compound), a video camera attached to the microscope and a computer or internet server with a graphical interface. Images captured from the video camera can be shared by connecting the computer or server to the Internet.

Systems that capture their images from the microscope to a computer need special software to capture and share the image over the Internet. This software usually includes image editing and archiving and requires the allocation of a static IP address to the computer so that images captured on the computer can be seen by internet users who simply have to type the IP address into their web-browser. Examples of this system include Olympus NetCam software and the Leica Network LAS module. Alternatively, Nikon offers the DS-L3 web-server with graphical interface which captures the microscope image and offers it directly to the internet without any computer device or additional software. The DS-L3 has its own unique

Fig. 12.13 Smart phones and handheld computers connected to USB microscopes can capture and share images from almost anywhere

operating system through which users can select their own IP settings. It also has its own image editing suite and tools for annotating and highlighting the live image as well as a touch screen for easy operation.

In both types of system, image sharing can be restricted to a local network or a particular work group. If a static Internet IP address is allocated to a computer there is the potential that users or hackers external to the site could infect or tamper with the computer operating system or other files. This presents a significant security risk to organizations that have large networks of interconnected computers and so IT security measures need to be put in place before such a system could be safely activated. In contrast, the Nikon web-server system poses minimal security risks because it has a unique operating system and does not store files in the system or connect to any networked devices.

Mobile RMD Hardware and Software. The development of handheld computers, USB microscopes, phone cameras, wireless broadband and extensive mobile network services now makes it possible to capture highly magnified, high resolution images from almost anywhere, and to share them over the internet or via phone networks (Fig. 12.13).

Real-time communication applications such as Skype can be used to share live images from a remote location with an expert, creating the potential for a rapid identification, decision and response. Alternatively, static images can be emailed to an expert to obtain a similar outcome, albeit a little slower, as has been done for the Pacific and South East Asia for several years (PestNet 2012).

PestNet (2012) http://www.pestnet.org/

The ability to share live microscope images with Internet users across many locations presents an ideal opportunity for remote training. By sharing microscope images over the Internet, diagnostic experts and taxonomists can demonstrate how to identify pest species or distinguish symptoms or features characteristic of a particular pest. Additional communication devices can be used to provide a video-conferencing environment where there can be discussion, “white boarding”, chat, documentation and image, video and audio capture. These provide a lasting record of the training that can be accessed for future reference by the participant. The result is interactive learning that is cheap and effective, where participants improve their skills by accessing an expert from their own office.

12.5.5 Mobile Technology

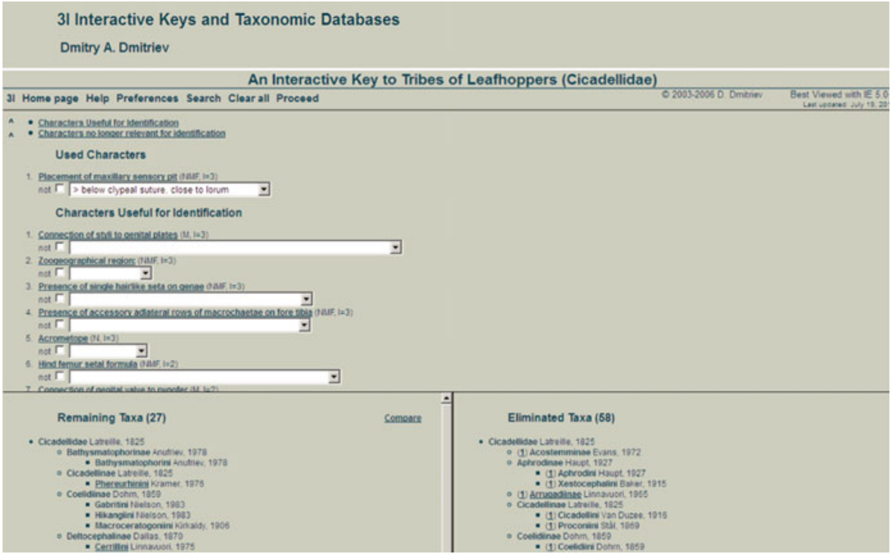
The mobile phone or “smartphone” is another technological development likely to have a significant impact on the detection and identification of invasive species. The Smartphone is an important tool by which people receive and send information. With a loss of taxonomic expertise and decrease in other resources available to biosecurity agencies, we see increasing potential for the public to provide valuable support for pest detection. For this collaboration to succeed, the biosecurity community must first provide educational opportunities and provoke interest about the value of detecting pests on farms, forests, natural areas, and other environments. At the same time, the public will need visually appealing, non-expert aids to assist them in detecting and identifying invasive species. Online computer-based identification tools have an important role to play, but smartphone applications have the potential to be a more effective and efficient way to reach the public.

Identification aid developers are increasingly looking at “app” development for their digital aids as the new way to send and receive information from the public. Mobile applications can provide interactive teaching tools, educate the public about pests, offer links to other relevant applications or websites, help them distinguish suspect organisms from natives, and report potential pests to a local office or agency. Mobile applications to support screening and detection will also be valuable for field personnel within the biosecurity community by providing access to detection information while screening ships, warehouses, planes, and commercial stores. We predict that the number of mobile applications designed, developed, and delivered to support pest detection and identification will show a dramatic increase during the next decade.

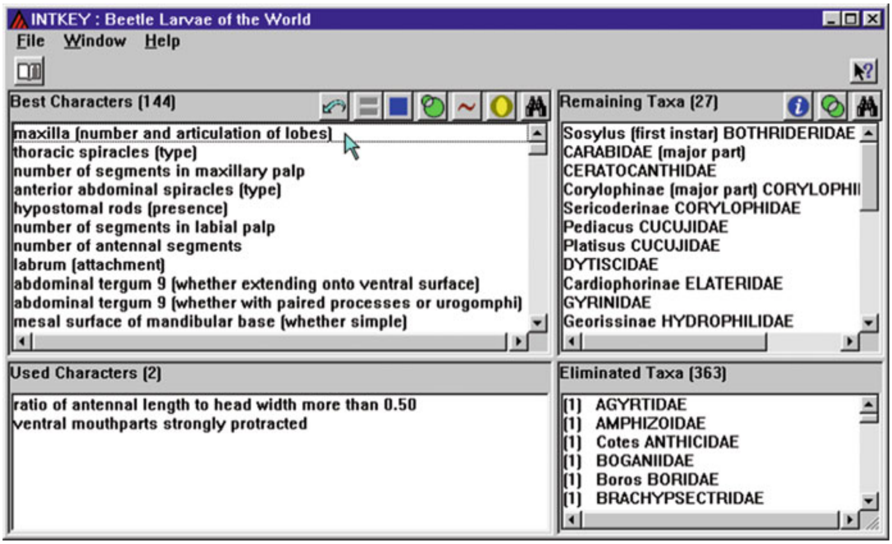
The rapid improvement of still and video capture by mobile phones, and improvements in software availability for image processing and sharing has future implications for remote diagnostics. We also see a move towards auto-identification where images captured on a mobile device can be uploaded to a cloud database where computer vision software analyses the image and offers one or several identification possibilities. An interim step towards this future may be intelligent query databases where the computer analyses the image then asks the user a series of questions that will help it make an identification. We should note however, that none of these developments could occur without taxonomists; there will be a continuing need for experts to feed knowledge into these digital systems.

Appendix 1: Screenshots of Matrix-Based Digital Keys Developed in Various Software Packages and Applications

An interactive key to tribes of leafhoppers (Cicadellidae) (Dmitriev 2003)

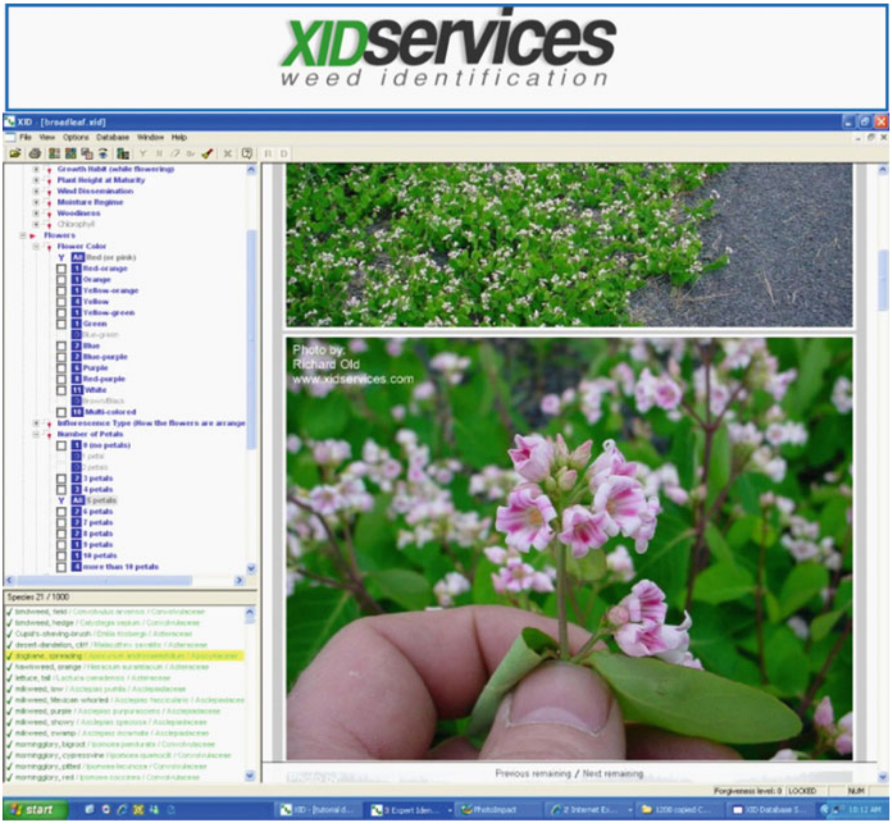


Beetle larvae of the world (Lawrence et al. 1999)



North American bee-associated mites (OConnor 2003)

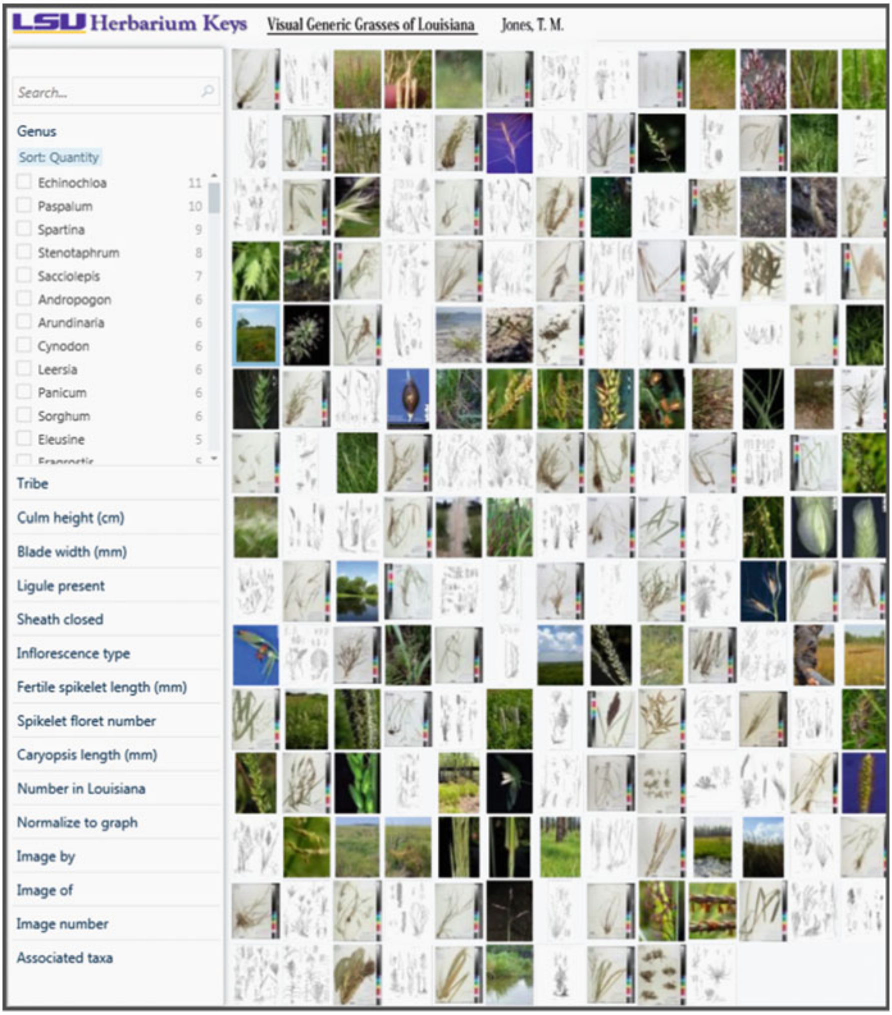
1,200 Weeds of the 48 States & adjacent Canada (Old 2011)



Key to adults of beetles common in the European part of Russia and North Palaeartic (Lobanov 2005)

An interactive key to North American *Amelanchier* (Campbell 2008)

Visual generic grasses of Louisiana (Jones 2011)



Appendix 2: Best Practice Guidelines for Making an Identification Using a Matrix (Lucid) Key

During an identification session, Lucid allows you to choose any question (i.e. a feature and its states) in its list at any time, but "stepping" through the key in a structured and sensible way will make your task of identification easier.

Familiarity with the specimen:
First, become familiar with the characteristics of the specimen you wish to identify. If you are also familiar with the Lucid key that you will use, then you may already know many of the specimen's characteristics. Briefly reviewing these before you start will make it easier for you to proceed through the identification.

Note and use distinctive features:
In any key, some taxa may possess particularly distinctive features. Use of these may allow the taxon to be keyed out in a very few steps. At the very least, starting with particularly distinctive or striking features for the first character states selected may quickly reduce the list of Entities Remaining.

Answer easy features first:
Browse the list of Features Available and address easy features first. The principles of dichotomous keys, in which the couplets must be answered in a preset order, are very familiar to most key users who often automatically apply these principles to a matrix key. Although Lucid3 lists the features of a key in an initial sequence in the opening window, this does not mean that the features must be selected in that order. You can select any feature from any position in the list. *[However, note that in some keys, where positive dependencies are used, you may be forced to answer specific questions before others become available.]*

Most Lucid3 keys will have a wide variety of features, ranging from those dealing with obvious and simple features to those dealing with features that are minute, obscure or difficult to interpret. Always start by browsing the list of Features Available for obvious features that you can quite quickly answer, as opposed to getting stuck on the first one. Lucid is designed to overcome problems associated with difficult and obscure features.

Choosing multiple states:
Always choose multiple states (more than one state of a feature) if you are uncertain which state is the correct one to choose for a particular specimen. Lucid is designed to allow you to choose as many states as you require from any one feature. Within the programme's logic, these states will be connected by an "or" link. This will cause Lucid to search for all taxa with the any of the states you select. As a general rule, if you are unsure which of two or more states your specimen has, then choose them all: that way, you can be sure that your target taxon will remain in Entities Remaining.

Finding the best feature to address next:
When you have dealt with all the obvious features, use Lucid's "Best" function to suggest the best remaining feature. The Lucid Player has two "Best" modes, Find

Find Best and Sort Best:

Find Best. In the Lucid3 Player, clicking the Best button will cause the Player to move to and open the best available feature. Next Best and Previous Best buttons on the toolbar allow navigation through the Features list, if you have difficulty in addressing the first feature nominated. If the list of entities in Entities Remaining changes after choosing a feature as suggested by Best, you should click the Best button again to recalculate the next best feature to address.

Sort Best. Sort Best will reorder the Features Available list so that features are sorted from best to worst. After a Sort Best, scan the top of the list for features that you can answer most easily.

Note that Sort Best only works using List View, as a tree representation of features cannot be sorted.

What if no taxa remain?
This will happen sooner or later in one of your Lucid sessions. If no taxa are listed in the Entities Remaining window, then it means that no taxa in the database match the selection of states you have made. Several explanations are possible; some of the most common are:

- You made an error in one or more states that you have selected. This is the most likely error for any situation in which no taxa remain.
- The taxon may be undescribed. In this case Lucid cannot identify the specimen because its features are not represented in the key's data tables.
- The key author may have made an error when constructing the key. This is unlikely, but it can happen. If, after carefully checking all the features and states and checking that the specimen you are attempting to identify would be expected to be included in the key, then a key construction error may be present.

Whichever of the above situations is suspected, you must very carefully review your chosen features and determine which ones you are uncertain about. Try deselecting uncertain states one by one to see what effect each has. One or more taxa may move back into the Entities Remaining window. In difficult cases, you may need to "play" with the key, adding or deleting states progressively to try to find the best matching taxon.

What if several taxa remain?
Never assume that you will always end up with one taxon remaining. Some taxa in the key may be very hard to differentiate, except when using difficult or obscure features. Sometimes, after you have addressed all the features you may have a short list of taxa remaining instead of just one taxon. You are still much closer to an identification than you otherwise would have been. You may then have to carefully check the specimen against associated information (descriptions, images etc. for the remaining taxa) or refer to more advanced or specialist reference sources.

Checking the result:

When you have made a preliminary identification, check the other information (such as notes, descriptions or images) provided for the taxon. Getting a possible name for a taxon from a key is not the end of an identification. You may have made errors, or you may have a taxon that is not in the key. In these cases, the key may have provided you with the wrong name. The associated information will often give you a good indication as to whether the answer is correct.

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Chapter 13
Molecular Diagnostic Techniques and Biotechnology in Plant Biosecurity

Laurene Levy, Patrick Shiel, Geoffrey Dennis, C. André Lévesque, Gerard Clover, Harvinder Bennypaul, Norman Barr, Amy Roda, Rodney Young, Jacek Plazinski, and Jane Moran

13.1 Introduction and Overview

Identification of plant pests, including the causal agents (i.e. "Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products" – International Plant Protection Convention (IPPC)) of diseases is at the core of plant biosecurity and is the basic concept driving the disciplines of entomology, plant pathology, and weed science (Chap. 4). The framework of a plant biosecurity programme as expressed in the risk analysis (Chap. 9), surveillance (Chap. 11) and mitigation of outbreaks (Chap. 10) is all dependent on the precision and

L. Levy (✉)
USDA-APHIS-PPQ, Center for Plant Health Science and Technology (CPHST), Unit 152, 5C03.50, 4700 River Rd., Riverdale, MD 20737, USA
e-mail: laurene.levy@aphis.usda.gov

P. Shiel • G. Dennis
USDA-APHIS-PPQ, Center for Plant Health Science and Technology (CPHST), 1730 Varsity Dr., Suite 400, Raleigh, NC 27606, USA

C.A. Lévesque
Agriculture and Agri-Food Canada, Government of Canada, KW Neatby Building, 960 Carling, Ottawa, ON K1A 0C6, Canada

G. Clover
Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand

H. Bennypaul
Canadian Food Inspection Agency, Government of Canada, 93 Mount Edward Road, Charlottetown, PE C1A 5T1, Canada

N. Barr
USDA-APHIS-PPQ, Center for Plant Health Science & Technology (CPHST), Moore Air Base Bldg. S-6414, 22675N. Moorefield Rd., Edinburg, TX 78541, USA

A. Roda
USDA-APHIS-PPQ, Center for Plant Health Science & Technology (CPHST), Subtropical Horticulture Research Station, 13601 Old Cutler Rd, Miami, FL 33158, USA

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accuracy of identification of the relevant pest. The diagnostic methods and technologies used for identification are driven by taxonomy and pest biology. However, the ultimate selection and implementation of specific diagnostics for any programme is influenced by the circumstances confronting each plant protection organization. The diagnostic method used and the uncertainties in taxonomy or identification can be a subject of disagreement between importing and exporting countries when implementing their safeguarding efforts. Our goal is to protect agriculture and the environment. We also see a responsibility to provide safeguards while maintaining open trade. Governments worldwide have put regulations in place that balance these two concepts, but the artifacts of some regulations can result in inadvertent and improper use of diagnostics or can be misused to uphold trade barriers.

Traditionally, plant pests have required visual detection of signs or symptoms followed by morphological identification based on internationally recognized taxonomic systems supported by codes of nomenclature (Fig. 13.1). Typically, accurate morphological characterization can be used to confirm the identity of a pest. However, morphological identification can be difficult if the appropriate life stage or specimen of the plant pest is not available. Several examples where useful morphological features are limited include immature arthropods, immature mollusks, immature nematodes, weed seeds or mycelia of fungi. Even more challenging is the detection and identification of submicroscopic organisms such as fastidious bacteria and viruses where morphological identification cannot be used, or when the pests are latent in consignments of dormant plant materials.

Implementation of diagnostic assays that supplement or supersede traditional identification techniques has been demonstrated to improve the accuracy of plant-pest identification. Several regulated species require a taxonomic resolution that can only be achieved using advanced diagnostic assays that rely on the presence of differentiating target DNAs, proteins or other biochemical components such as volatile signatures. Subspecies levels of identification are often needed for some organisms that may have native populations. For instance, the plant bacterium *Ralstonia solanacearum* (Smith) Yabuuchi et al. in the USA only has Race 3 biovar 2 specifically regulated. Canada and the USA regulate Gypsy Moth (*Lymantria dispar* L.) of European origin (which are naturalized) but attempt eradication of another Gypsy Moth of Asian origin. Still, the greatest challenge to regulatory programmes and the scientists that deliver advanced diagnostic assays involves organisms new to science,

R. Young
USDA-APHIS-PPQ, Building 308, Room 319, BARC-East, Beltsville, MD 20705, USA

J. Plazinski
Department of Agriculture, Fisheries and Forestry, GPO Box 858, Canberra, ACT 2601, Australia

J. Moran
Biosciences Research Division, Victorian Department of Primary Industries, Private Bag 15, Ferntree Gully, VIC 3156, Australia

Fig. 13.1 A regulatory scientist in New Zealand examines a sample submitted for morphological identification (Image courtesy Gerard Clover, MPI, Auckland, NZ)

especially those that pose threats to agriculture when introduced to a new area. A good example is the identification of the plant pathogen *Phytophthora ramorum* Werres et al. (Chap. 20). Before *P. ramorum* was officially described in 2002, the causative agent of Sudden Oak Death was unknown in California (Werres et al. 2001).

This chapter describes the components that currently drive method development and deployment of systems for regulatory identification and diagnosis, including: (1) Criteria for method selection, development, and transfer to the field; (2) quality management of diagnostic test results; (3) reliance upon and use of reference collections; (4) serological methods used for detection; (5) molecular diagnostics based on DNA; and (6) new technologies that may be fit for regulatory diagnostics such as use of volatile signatures, DNA barcodes, recombinant monoclonal antibodies and DNA arrays.

Many of the advanced technologies described in this chapter are derived from in vitro diagnostic developments in clinical medicine, point-of-care diagnostics and detection of bio-warfare agents that have been adapted for use in agriculture. Agriculture benefits from these developments, but we must always be cognizant that the expense associated with development and deployment of these technologies is justified by the science and risk of the plant pest to agriculture.

This chapter demonstrates that molecular and biochemical diagnostic assays can supplement morphological and visual identification as well as independently provide accurate identification of regulatory pests. How and where these diagnostics will be implemented and the impact on regulatory policy may eventually need to be considered prior to development. As much information as possible is needed to assist scientists in fitting the diagnostic to its intended use to avoid improper use or assay failure once implemented.

13.2 Regulatory Impacts and Response

Several factors drive the development of diagnostic assays for the detection of regulatory plant pests. Often the target assay is first described in peer-reviewed journals from basic research that provides a foundation for regulatory use (EPPO 2007). However, often newly published assays are developed for research purposes and have not been adapted for use with a large number of samples, have not been evaluated on a comprehensive range of related and unrelated species, and their performance using matrices likely to be encountered in routine use may not have been evaluated. The information from these parameters is needed to provide confidence for effective screening or to confirm regulatory samples. Some assays have been adapted for detection of the pest within a narrow range of samples and related species present locally or regionally but do not include related species present in large areas or entire countries that may confound the assay.

In some cases the appropriate diagnostic assay has not been developed. To ensure that assays are ready for implementation before the incursion of a regulatory pest, regulatory scientists responsible for method development must constantly screen literature associated with potential pests and use prioritized lists or predictive models to determine which assays should be developed. Regulatory scientists also develop professional relationships with subject matter experts (typically from areas where pests are endemic or effective regulatory programmes have occurred). Regulatory scientists then gain knowledge of unpublished assays for exotic pests, which is essential for maintaining an effective regulatory diagnostic programme. The "worst case" scenario is that an assay has not been developed for a new pest incursion and is needed immediately.

A diagnostic assay may be developed de novo or adapted from existing research. The most important aspect of the assay is whether it is fit-for-purpose and performs within its design scope. For example, an ELISA (Enzyme-linked immunosorbent assay) developed/adapted to detect *P. ramorum* from symptomatic leaves cannot be relied upon to detect *P. ramorum* from twigs of dormant plant materials without further validation. Testing new kinds of samples without a thorough assessment of performance characteristics is out of the design scope and not the intended use of the assay.

Assays that have been previously developed are adapted for regulatory purposes by generating experimental data to assess and quantify relevant performance characteristics. Consideration must be made during the adaptation process to: (1) Physical and biological nature of survey samples and target; (2) issues related to scale-up of the assay for use on large sample numbers; (3) limitation or expense of reagents; (4) potential of the assay to cross react with related or unrelated species; and (5) use with affordable or user-friendly equipment platforms. Molecular diagnostic tests must be developed with accurate taxonomic, systematic and genetic information and must be publicly available. The existence and availability of reagents (such as suitable antibodies for serological tests like ELISA, primers and probes for DNA tests like real-time polymerase chain reaction (PCR)) are critical for biochemical and molecular assays.

For development and/or adaptation of a diagnostic assay for regulatory use, a work plan with logical, achievable goals and timelines must be constructed. The plan should address how to obtain financial resources to complete the work as well as a thorough understanding of the diversity of hosts and environmental conditions that may be encountered for all intended uses of the assay. Diagnosticians must assess the availability of plant pest reference-materials because exotic plant pests are typically difficult to obtain and may require bio-containment in specialized regulated facilities. The plan should consider targeted users of the assay, the setting where the assay will be used (i.e. technical lab, plant diagnostic clinic, or in the field), and the intended use of the assay. The plan should also define whether the assay would be used for survey, detection, identification, as a screening tool, or as a confirmatory assay to initiate regulatory actions.

Peer-reviewed scientific publications are often used by regulatory agencies as a source of methods for regulatory programmes because the wider scientific community considers these reports reliable. However, published reports often lack sufficiently detailed information needed for further development/adaptation by regulatory agencies. Published reports often require extremely specialized equipment or are otherwise not practical for regulatory use. Published reports are sometimes not implemented because they lack universally accepted standards/criteria for regulatory methods. The International Codes of Zoological and Botanical Nomenclature provide rules for the naming of animals and plants but do not provide guidance on the accuracy of identification. The IPPC has published an International Standard for Phytosanitary Measures (ISPM 27, Diagnostic Protocols for Regulated Pests 2006), but to date only three annexes (*Trogoderma granarium* Everts) (*Thrips palmi* Karny and Plum Pox Virus) have been published with specific molecular diagnostic protocols. We expect future opportunities to establish rigorous standards for molecular protocols that will lead to accurate identification of organisms important in regulatory programmes.

In some ways, de novo development of a test can be quicker than the adaptation of an assay already in existence because regulatory agencies have made immediate fit-for-purpose an objective for assay development. The challenge for a newly developed assay is that acceptance by the scientific community may not have been achieved by publication in a peer-reviewed journal. Sometimes this can be expedited if a subject matter expert has a method in development. However, scientists can often be reluctant to provide the details of diagnostic assays before publication because of loss-of-ownership, liability if the assays are inappropriately used, organizational policies or proprietary technologies limiting disclosure that prevent the release of data before publication.

A regulatory agency may develop a de novo method and publish it, but acceptance from a trade partner may require a more rigorous demonstration of assay performance. In addition, sample results may be scrutinized in a court of law. The complexity of a diagnostic protocol has increased over the last several years. Use of a single diagnostic assay for confirmation testing in regulatory diagnostics has shifted to reliance on two or more assays to fulfill regulatory requirements, e.g. multiple nucleic acid targets and proteins from different gene regions (Roessler et al. 2012).

13.2.1 Assay Validation

Regardless of whether a diagnostic assay is developed in-house or adapted from an existing method, successful implementation of the assay is more likely if an appropriate validation is conducted. The term "validation" has several definitions, some of which are not applicable for the types of bioassays discussed here. For regulatory purposes, assay validation can be defined as 'a process that confirms by examination and development of objective evidence that a set of particular requirements for specific use are fulfilled'. Through this process, data is compiled to show that the method can detect, identify and (in certain assays) quantify a target (such as a phylogenetic trait, specific gene sequence, or protein) in all analyzed matrices (such as infected plant parts, pathogen culture, or insect parts) with demonstrated sensitivity, specificity, accuracy, trueness, ruggedness and precision (reproducibility, repeatability, and intermediate precision) (ICH Q7 2000; ICH Q2 2005). All of these points must ensure that results (Fig. 13.2) are appropriate for decision-making in a regulatory environment.

A validation begins with a documented protocol that includes the scope of the assay, a scientific description of its mechanics, references to the sample type and literature, a history of pre-validation, the method that will be used for testing, and the expectations of how the assay will perform including how it will be monitored. A separate Work Instruction (WI) describes sample collection, preparation, preservation, processing and results analysis. Both documents (the validation and WI) should include: (1) A description of reagents; (2) outline critical reagents; (3) provide instructions on how the assay should be performed; (4) identify necessary equipment; (5) explain how results should be interpreted and reported; and (6) list information that exists regarding limitations and known performance characteristics. Adapted assays should be validated especially if the authors did not validate the original assay, test to the level of confidence needed to perform the assay, or represent an improvement over previous industry standards (Nowatzki et al. 2008). In addition, re-validation may be needed if new matrices are encountered, changes occur to the basic science of a method (such as discovery of cross-reacting species (Werres et al. 2001) or assay inhibitors), or critical reagents or equipment platforms have changed (EPPO 2010; OIE (n.d.) 2008).

Performance specifications used to validate a diagnostic assay will vary depending on the intended use of the assay, type of assay (qualitative or quantitative) and extent to which the assay will be used by other labs. Validation of quantitative assays establishes confidence in the measurement of target in a sample and should include the minimum quantifiable concentration (Limit of Quantitation, LOQ), detection limit (Limit of Detection, LOD), sensitivity, specificity, accuracy, trueness (including measurement of uncertainty), precision, linearity and ruggedness.

Replicate analysis will document changes in precision of the assay through repeated operation and provide information on repeatability of an assay. Generating data on ruggedness/robustness will document changes in assay performance over a range of matrices, reagents, labs or analysts performing the assay. For example, a

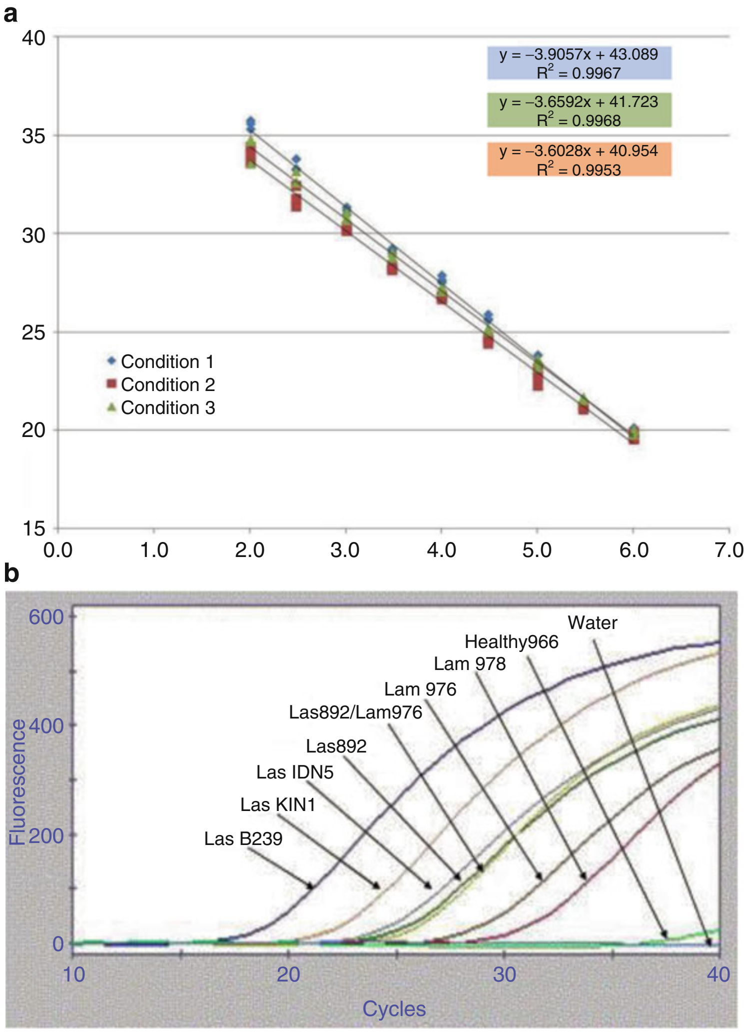

Fig. 13.2 Standard curves (**a**) comparing quantitative cycle time (y-axis; C_q often discussed as C_t, or "cycle time") compared with log copies (x-axis) for three different conditions generated using quantitative polymerase chain reaction (qPCR). During a validation, one test may be able to address multiple validation aspects of a method. In this test, Limit of Quantification (LOQ), intermediate precision (3 replicates per curve point), and ruggedness or specificity may be tested with range and uncertainty described. Ruggedness may be tested using three different concentrations of an assay component, or specificity may be tested using three different targets. Resulting signal (**b**) generated using qPCR for multiple species of Ca. Liberibacter simultaneously detected using amplisets HLBasampr and COXfpr showing method specificity for the desired target

ruggedness test may involve changes of salt concentration in a sample extraction buffer, pH in a reagent, amounts of a required reagent, or changing the time specified for use with a required instrument. Depending on these measurements, changes to the assay may require re-validation. This can be determined by running the assay with the changed parameter according to the validated protocol and comparing this data with results obtained using the parameter measured in the validated assay. If performance characteristics are consistent with the validated assay, then the new parameter is verified and re-validation is not needed. However, if changes in the established performance characteristics occur and are out-of-range of the validated measurements, then the assay may require re-validation or further adaptation.

A complete description of validation components and their interactions is too complex to describe here. However, any validation will at least measure results obtained from the anticipated sample matrices that are spiked with known concentrations of the target, with at least three levels of the target (high, medium and low), known reference standards (positive and negative), and a number of sample replicates, matrices, operators and labs appropriate for the level of validation required for the intended use of the assay.

Recent advances in development of validation standards for use in plant pest diagnostics are occurring internationally. A government initiative in the USA, known as the Department of Homeland Security (DHS), Integrated Consortium of Laboratory Networks (ICLN) attempts to coordinate lab diagnostic networks. Within this broad framework, guidelines created by the ICLN Methods Subgroup for chemical, biological and radiological targets have posited using a 4-level validation procedure (ICLN 2008). Level 1 is limited to a single lab, with a single target, and using one or more matrices. At this level, the lab conducting the validation would determine which of the quantitative or qualitative parameters of validation are important for the intended use of the assay. Level 1 validation can be particularly important during a new plant pest outbreak as the basis of emergency validation for a timely response. Level 2 is similar to Level 1, but requires that all relevant validation parameters are measured. In contrast, a Level 3 validation would require more time and involves a validation study using 2–7 labs and one or more matrices. A Level 4 validation requires a larger study using 8–10 labs and at least five matrices.

Other international efforts to standardize validation for use in regulatory diagnostics are in recent guidelines published by the European Plant Protection Organization (EPPO). The EPPO guideline (EPPO 2010) for plant pest diagnostics states that an assay is considered fully validated when data for the following performance criteria are provided by the method developer: Analytical specificity, analytical sensitivity, reproducibility and repeatability. The guideline later explains that data on reproducibility and robustness can be gleaned from lab-performed validation and inter-lab comparison. This publication includes several useful appendices with detailed guidance for validation processes for bacteriology, botany, entomology, nematology and virology related to plant pest diagnostics.

The International Standards for Phytosanitary Measures Number 27 (ISPM No. 27) describes guidelines for the structure and content of diagnostic protocols for regulated pests. Therein are outlined specific requirements for pest and taxonomic information, the components of identification and detection procedures as well as information on record management. This document states that diagnostic protocols should "contain the minimum requirements for reliable diagnosis of the specified regulated pests and provide flexibility to ensure that methods are appropriate for use in the full range of circumstances". Sensitivity, specificity and reproducibility are identified in ISPM No. 27 as the selection criteria for diagnostic protocols (https://www.ippc.int/index.php?id=13399).

13.2.2 Work Instruction Usage and Transfer

If an assay is validated for use in regulatory programmes, then the next steps are technology transfer and implementation of the assay by the intended users. This process is multi-faceted and begins even before the validation process is completed so that preparation for release of the assay to the end-user can occur. The availability of a detailed and clear WI is very important for successful multi-lab use. A WI is not synonymous with a Standard Operating Procedure (SOP). The WI is prescriptive and describes to the end-user: (1) Equipment and reagents required; (2) how to make and prepare the reagents; (3) how to combine the reagents and targets; and (4) under what physical conditions (including equipment settings), the assay will be conducted. In addition, the WI describes the collection of data, interpretation of data and the appropriate terminology (taxonomic or statistical) for reporting results or requirements for retesting to reach a diagnostic determination. Unlike the WI, an SOP describes a subset of instructions involved with portions of the WI. For example, an SOP will describe how to store an individual reagent, calibrate an instrument, or track physical conditions in the applicable lab related to and referenced in the WI.

As experience is gained in transfer of diagnostic assays to the end-user, often the WI (no matter how prescriptive) may require additional adaptation to the end-user so that the interpretation of diagnostic assay results is clearly delineated. Diagrams (process map, decision map, decision tree) are used to aide end-users in the interpretation, troubleshooting or root cause analysis (an analysis that involves searching backwards from a problem or error to its cause(s) and addressing those causes), and monitoring of results. This feature is often added at the request of the end-user.

The most difficult aspect of WIs for diagnostic assays is to ascertain that the most current version is in the hands of the diagnosticians for use in regulatory programmes. This is necessary for the integrity of the test results and is also a requirement of accreditation for most quality management systems. Current WI versions reflecting the revision number and authorization must be made available to diagnosticians. This requirement can often be fulfilled through the use of websites where the most recent version is available or through an internally accessible database. In addition to deployment of WIs, other aspects of technology transfer must occur for user labs to process regulatory samples. Reference materials in the form of positive and negative controls must be produced and verified; hands-on lab training of diagnosticians must be prepared and conducted; in some circumstances labs must be accredited to determine whether they have appropriate management, document control, equipment and space, and trained diagnostic staff; a determination of whether the diagnosticians will require proficiency testing; the development and distribution of an assay-specific proficiency test panel; and finally a release date for the assay.

13.3 Diagnostic Labs and Networks

The environment and settings of diagnostic labs has changed since September 11, 2001 in the USA and other parts of the world. Biosecurity has become the predominant priority resulting in a strengthening of diagnostic labs in the USA for human, animal and plant diseases. Several networks have been created to distribute diagnostic protocols among these diagnostic labs. The development of the USDA-sponsored National Animal Health Laboratory Network (NAHLN) and the National Plant Diagnostic Network (NPDN) increased the USA capability to provide greater biosecurity of its agricultural assets [See: National Plant Diagnostic Network (n.d.)] As mentioned above, in the USA a consortium of all lab diagnostic networks (ICLN) was formed to catalogue and exchange methods, develop common languages for validation (sampling, proficiency testing and lab accreditation), encourage computer simulations (response scenarios and minimum data elements for tracking outbreaks), and cross training tools to prepare networks for cross-network surge capacity. The ICLN was implemented in 2005 by a memorandum of agreement between 10 government departments and agencies as signatories (ICLN 2008).

> The implementation of the NPDN reversed a trend of dwindling resources dedicated to diagnostics of plant-related problems. The NPDN has brought a renewed emphasis to, and enhancement of, diagnostics at Land Grant Universities, and has started to provide an infrastructure for the rebuilding of the linkages between the diagnostic laboratories and extension, regulatory agencies, and the broader community of agricultural practitioners. (NPDN 5-year review Executive Summary 2007).

Deployment of the NPDN redirected diagnosticians from a primary focus on local and year-to-year existence towards a regional and national focus for long-term evaluation of plant pests in states, regions and nationally. This refocus opened the labs to coordination as a network by assisting with diagnostics from other states in their regions under a surge situation, and for coordinated assistance with federal regulatory surveys, eradication programmes and annual surveillance for new plant pests.

The challenge for the federal government in utilizing this rejuvenated resource was the assessment of the capability of NPDN diagnosticians to perform regulatory diagnostics for coordinated programmes. This involved the biosecurity situation of each lab and the physical and personnel resources of member labs. Once created and resourced, the NPDN initially concentrated on developing and focusing its infrastructure and communication assets. Animal and Plant Health Inspection

Service (APHIS), Plant Protection and Quarantine (PPQ) has become a primary stakeholder for NPDN services, and fostered the NPDN on lab needs from equipment to hands-on training, as well as quality assurance procedures common to other lab networks.

The APHIS PPQ National Plant Protection Lab Accreditation Program (NPPLAP) was created to assist external diagnostic labs in performing and improving diagnostic testing needs, including equipment, hands-on training, and quality assurance. Diagnostic proficiency assays are distributed by NPPLAP throughout the USA in an attempt to decrease the number of samples requiring federal confirmation, strengthen the capabilities of USA labs and build a strong diagnostic network for plant pests that is unique in agriculture.

The current process begins with exchange of documents between NPPLAP and the participating lab. NPPLAP provides documents that describe the components of the programme, including the inspection checklist and the WIs for making valid diagnostic determinations for regulatory purposes. The participating lab provides documents on the expertise of the analysts and the infrastructure of the lab. This is followed by a site visit by an NPPLAP-coordinated team of scientists to inspect and evaluate the lab facilities. The inspection checklists are used to examine the lab's equipment, personnel, chain-of-custody, facility infrastructure, and equipment maintenance.

After successful completion of the inspection phase, a blind Proficiency Test Panel (PTP) is sent to participating analysts within NPPLAP-approved labs. Proficiency testing (PT) is an assessment of a lab's testing performance by means of inter-lab comparisons (Eurachem 2007; ISO/IEC 2005), creating a standard measure of diagnosticians within a lab typically used by regulatory agencies. This test evaluates and verifies the proficiency of the analyst's technical skills in the lab, and also measures the overall capabilities of the lab and its personnel to interpret and accurately report proficiency-test data. The PTP is designed to mimic actual samples received by the national lab for final determinations. A diagnostician requesting certification for a specific diagnostic method is assessed using a method-specific PTP on a yearly basis. PT assessment of diagnosticians also occurs when new diagnostic methods are being implemented. Use of a validated method in a PT programme that uses a controlled set of samples containing related and unrelated targets at different concentrations provides data about the lab personnel assessed and the performance characteristics, such as repeatability and robustness of the validated method. This data may satisfy the requirements to achieve higher levels of method of validation (levels 3 and 4) as defined by the ICLN because they are performed by 7–10 labs.

Australia and the EU also have active committees on the development, validation and use of plant pest diagnostic methods (McGrath et al. 2008). The Subcommittee on Plant Health Diagnostic Standards (SPHDS) aims to sustain and improve the quality and reliability of plant diagnostics for plant pests throughout Australia. The EU has the European Plant Protection Organization (EPPO) that creates guidelines for labs in Europe (Le Ministre Plenipotentiaire 1951).

13.4 Reference Collections and Materials

Collections of biological specimens and associated material are valuable to the research community worldwide. Scientific collections strive to improve their management and care. Collections are distributed among government agencies, universities, institutes, researchers, private organizations, and public-private entities. In 2005, the White House Office of Science and Technology Policy (OSTP) and the Office of Management and Budget (OMB) in the USA called on federal agencies to focus on the support of scientific collections. From this focus an Interagency Working Group on Scientific Collections (IWGSC) was formed and co-chaired by the Smithsonian Institution and the USDA to examine federal collections (NSTCCS 2009). The IWGSC noted that collections serve several functions:

- Confirms earlier observations that critical analyses and observations have been performed (Voucher Specimens),
- Provides standards for reference materials (biological reference standards or Type-specimens) that are retained for future identification and validation of species,
- Includes sources of specimens for biological research such as type-culture collections, seed banks and germplasm,
- Act as repositories for rare objects, and
- Hold materials and study specimens for educational purposes.

The report states, "scientific collections by nature are backward-looking, record history and confirm past findings, but that they are created and maintained to benefit the future." In addition, federal agencies must maintain diverse collections that are essential to supporting the agency mission.

Reference collections are of great value to regulatory agencies. They ensure accurate identifications can be made and accurate plant pest-specific diagnostic protocols can be developed. Despite the condition of these collections and the expense to maintain, care, and populate them with voucher samples, their value continues to increase over time. Some collections may be local to labs, however most are centralized to preserve resources. Large scientific collections occur in Australia, New Zealand, the Netherlands, the U.K., France, Belgium, Germany, Canada and Brazil. In the USA, collections are maintained at locations including USDA ARS (Beltsville, MD), the Smithsonian Institution (Washington, DC), National Center for Agricultural Utilization Research, ARS (Peoria, IL), National Center for Genetic Resources Preservation (Fort Collins, CO), the American Type Culture Collection (ATCC), and at universities or other educational institutions. Scientists may "borrow" a collection or set of specimens, even outside their country from an international herbarium or repository.

Scientific collections are used as a working reference for visual/microscopic identification and the development of DNA barcodes for genetic identification of regulatory plant pests and seeds. In 2010, the Australian government presented a case for re-inventory of their plant pathogens to confirm species that are endemic and species that are exotic, possibly requiring quarantine measures (Hyde et al. 2010). Based on molecular data, scientists discovered that some “species” were actually complexes of cryptic species (two or more morphologically indistinguishable biological groups that are incapable of interbreeding). However, herbarium collections could not provide specimens of the quality needed for molecular analysis and further characterization of these species. Continuing identification of cryptic species and the need for higher quality DNA means that scientists must collect additional specimens throughout Australia for molecular evaluation and referencing vouchers.

13.4.1 Scientific Collections for Seed Identification

The APHIS National Botany Identifiers at the Seed Examination Facility (SEF) in Beltsville MD are responsible for the determination of plant interceptions from foreign and domestic sources. The SEF maintains plant reference collections for comparison with interceptions. Seed collections are especially valuable because most botany interceptions involve seeds or fruits. The main collection was originally compiled by the USDA, Agricultural Marketing Service (AMS), Seed Regulatory Branch and was transferred to the SEF in 1982. That same year a collection of seeds from exotic weed species was started by USDA, Agricultural Research Service (ARS) for the evaluation of noxious weed seed. That collection is now part of the SEF holdings. The seed specimens were taken from pressed plant sheets housed at the US National Herbarium and Smithsonian Institution in Washington DC. The SEF also maintains a pressed plant herbarium. This collection consists mainly of exotic introductions, which have been forwarded to the SEF for identification by PPQ and state cooperators. The herbarium contains several state and country records for some species.

Specimens are added to the collections whenever new identifications are made of interceptions. These identifications are often verified by comparison with specimens at the USDA Herbarium at the US National Arboretum in Washington DC or the US National Herbarium. Taxonomic specialists at such institutions as the US National Herbarium, the New York Botanic Garden, or the Missouri Botanic Garden sometimes make verification of the identification of new specimens. These determinations can usually be made from digital images of the interceptions sent by email to the specialists. Whenever a new species is added to the U.S. Federal Noxious Weed List, seed and plant specimens must be obtained for the SEF and collections at Ports of Entry. International cooperators have made contributions of specimens to these collections from countries such as New Zealand, Australia, Brazil, Germany, and Switzerland.

Another valuable resource for identification is the US National Seed Herbarium. This collection was developed by taxonomists in cooperation with the USDA, Agriculture Research Service (ARS) in Beltsville, Maryland and has seed and fruit specimens representing most of the genera of flowering plants. From this collection, guides for the identification of fruits and seeds of the Fabaceae

(Leguminosae), as well as an online guide to the fruits and seeds of the families of flowering plants, were produced. The ARS collection suffers from a similar fate of other specimen collections worldwide in that as financial support for research in classical plant taxonomy declined so did the financial support for maintenance of the US National Seed Herbarium. As a result, ARS relocated the collection to the USDA Herbarium at the US National Arboretum.

13.4.2 Scientific Collections for Invertebrate Identification

Reference collections of plant pests and related species are important for developing morphological and molecular identification tools and training staff that perform official pest identifications. USDA uses scientific collections of invertebrate specimens and academic researchers to develop diagnostic keys for plant pests, assist in training of identifiers, and recognize new species. This is possible because of a long history of specimen vouchering to meet standards of publication and an established biological repository infrastructure to support international research (http://www.biorepositories.org/). Although institutional standards and practices to curate and catalogue holdings can vary, we must ensure that collections are accessible to morphological experts in National Plant Protection Organizations (NPPOs). In addition to voucher specimens that are stored at museums and associated with scientific literature, smaller reference collections at APHIS Plant Inspection Stations (PIS) and labs are important for training staff and comparison of diagnostic characters of pest and non-pest species (Fig. 13.3).

The co-location of USDA taxonomists at institutions with relatively large holdings of species (such as fruit fly collections at the Smithsonian Institution and gastropod collections at the Academy of Natural Sciences of Drexel University) enables experts to verify PIS identifications and quickly assess whether detections represent emerging pest problems. As with other organisms, the method of preservation of collections (i.e., alcohol, formaldehyde, propylene glycol, slide mounting) will depend on the type of tissue and specimen size. For example, soft-bodied insect larvae and slugs are stored in a lower percentage of alcohol than many adult insects with harder tissues. High percentages (95–100 %) of alcohol are best for preservation of DNA in samples, but can shrink soft-tissue structures or preclude dissection of internal anatomy. For snails and slugs lower percentages (70–80 %) are adequate for DNA studies and dissection, if correctly collected, killed and preserved. Reference collections must include specimens with the correct information pertaining to collector, identifier, collection locality (especially GPS), collection date, environment, and associated plant hosts. Specimens that have this information as part of their documentation are considered “vouchered specimens”. Identifiers and collectors should refer to collecting manuals and textbooks to verify the appropriate preservation methods and collection information before submission to a reference collection or repository.

Fig. 13.3 Collections help taxonomists and regulatory scientists make accurate identifications. Collections also assist molecular biologists in molecular identification of a sample by providing vouchered specimens for the development of diagnostic methods such as DNA barcoding and PCR (Image courtesy Gerard Clover, MAF, New Zealand)

13.4.3 DNA Collections for Plant Pest Identification

In addition to the physical scientific collections of organisms, efforts to preserve collections of DNA and DNA sequences have begun. The long-term stability of DNA (Paabo et al. 2004) lends itself to physical storage. The storage of DNA from an organism requires less space and less variability of conditions of storage. The effort to store DNA from specimens, even vouchered specimens, is occurring in local and international DNA banks or repositories. For example, the Royal Botanic Gardens Kew DNA Bank contains almost 40,000 samples of plant genomic DNA. The Australian Plant DNA Bank is a comprehensive collection of DNA from Australian native and crop plant species. These DNA banks are now linked in a DNA Bank Network (http://www.dnabank-network.org/) for the preservation of non-human DNA to be made available to users. Methods are also being developed so that DNA can be collected from voucher specimens without disrupting important morphological characteristics. For example, insect bodies can be delicately pierced allowing for DNA to diffuse into buffer solutions or alcohol (Gilbert et al. 2007).

DNA data is also held in public sequence-databases. The most well known is GenBank, which is maintained by the National Center for Biotechnology Information (NCBI) as part of the National Institutes of Health (NIH). The database was originally created at the Los Alamos National Lab as their sequence database. In 1982 the database became public as GenBank and was acquired in 1992 by NCBI. Recent lowering of sequencing costs and increases in scientists working with molecular biology has led to an exponential increase of DNA sequences deposited into GenBank. In many cases, the GenBank accession is not derived from reference isolates or voucher specimens for verification of the data, and scientists often must be discerning in their analyses to remove potential "junk sequences" and use only sequences that are truly informative (Bridge et al. 2003). Although DNA sequence databases like GenBank provide an excellent resource for sequences associated with taxonomy, there is no review mechanism that prevents accessions from containing technical errors in the sequencing and editing process either due to poor sequence reliability or because of misidentification of the organism providing the DNA. Errors must be considered when using any database. For example, errors have been reported in human mitochondrial sequence accessions in GenBank (Forster 2003; Harris 2003). For fungal species, estimated error rates of up to 20 % has been attributed to incorrect identifications (Nilsson et al. 2006). Efforts to develop DNA databases with vouchered specimens where critical analyses and observations have been performed and referenced (such as whether the methods used were validated and documentation is accessible) will make the use of this data for regulatory diagnostics more reliable (See below discussion on DNA barcoding).

13.5 Serological Diagnostics

Serological assays play a significant role in regulation and certification for plant pathogen diagnostics and provide a common format for screening tests currently developed and deployed (Wu 2006). Prior to immunoassays, serological identification was conducted using methods requiring observation of immunogenic aggregation such as hemagglutination and immunoprecipitation (Barrett et al. 1960; Briner et al. 1959). Because immunogenic aggregation usually relies upon the polymerization of antibodies and antigens for visualization, these tests were not very sensitive compared with more recently developed assays. Serological assays were often used for the specific detection of plant viruses, because some plant viruses could be purified to homogeneity and produced strong immunological reactions when injected into mammals. These tests could be used by regulatory agencies to identify some virus species or to determine virus strains.

Serological assays that detect the specific binding of antibodies without visualizing aggregation provide the basis of very sensitive assays useful to regulation of plant pests. One of the first assays developed was Serologically Specific Electron Microscopy (SSEM). Transmission Electron Microscopy (TEM) can visualize plant viruses and much information can be collected from the physical characteristics of the virus. However, this method is not sufficient for a diagnostic determination. Reaction of antibodies specific to the virus can be detected in TEM if the antibodies are conjugated to metals such as gold, which are opaque to TEM and appear as a large dark particle.

A breakthrough in sensitivity and throughput of serological methods was the development of Radioactive Immunosorbent Assay (RIA) (Berson and Yalow 1959) and the subsequent variant Enzyme-linked Immunosorbert Assay (ELISA). These assays were developed for clinical tests, but the potential for use in plant pest diagnosis was soon realized (Clark and Adams 1977). Antigen immobilization onto polystyrene and the development of enzyme catalyzed colorimetric reactions increased the sensitivity and throughput as well as the quantification capability and repeatability of the assay.

Two generally distinct types of immunoassay have been used in the past: The ELISA and the immunoblot (Western). An ELISA assay consists of an antigen, blocking agent, primary antibody and secondary antibody with an enzyme attached. Many variations of ELISA are now in use for research, clinical, and regulatory purposes (Voller et al. 1978). ELISA has become a primary test method for regulatory identifications, diagnostic determinations and plant germplasm clean stock programmes. Improvements in ELISA technology have led to the ability of rapid detection (lateral flow) and microarray formats. A Western blot assay differs from ELISA in the desired outcome of how a sample will be characterized. Instead of mechanically separating or ignoring non-target material, Western blotting characterizes and identifies target in the presence of other materials by use of denaturing gel electrophoresis (SDS-PAGE) followed by use of blocking, primary and secondary antibodies (MacPhee 2010).

The usefulness of serological assays is based on the specific interaction of antibodies to target molecules derived from the plant pest. This specificity depends on the quality of the antibody produced. Mammals will produce immunological reactions to many foreign molecules, some of which are common to broad groups of organisms. In the past, purification of the target molecules was necessary to provide contaminant-free immunogens for antibody production. This was often possible for plant viruses, because the molecular structure of these pathogens were simple, but was far less common for more complex pest organisms such as fungi, bacteria and insects. Even highly specific polyclonal antibodies produced a range of antibodies with a range of affinities to the target molecule. In addition, the production of polyclonal antibodies was limited by the ability of the host mammal to produce serum, requiring periodic repetition of the process for fresh antibodies. These limitations reduced the ability to standardize protocols for widespread adoption and long-term use of immunological methods by regulatory agencies.

The development of monoclonal antibody production systems has provided several benefits that address the past limitations on the use of immunological diagnostic systems. As the term 'monoclonal' implies, antibodies produced by this system are identical to each other in terms of target specificity and affinity. Since the key step of antibody selection occurs late in the process, the purity of the initial immunogen target molecules at the outset of the process is not as critical as with polyclonal processes. Method developers also have more latitude in choosing the level of specificity for antibody selection. For example, monoclonal antibodies for Potato

Fig. 13.4 Immunostrip test results for *Ralstonia solanacearum*. The two *lines* represent the area on the immunostrip where the positive control reacted (*upper line*) and where the test sample reacted (*lower line*) indicating the presence of *R. solanacearum* in the sample (Image courtesy Laurene Levy, CPHST, APHIS, USDA)

Virus Y (PVY) have been produced for detection of individual virus strains, for detection at the species level, and even for all members of the potyvirus genus.

In some cases, the level of immunological specificity achieved by monoclonal antibody production has not reached the level of specificity required to make unambiguous regulatory determinations. Examples include user-friendly technology like immunochromatographic assays (ICA), also known as lateral flow devices or immunostrips, which can detect to a genus or species level. ICA devices can be used to detect the plant pathogen *Ralstonia solanacearum* (Fig. 13.4) but because of the specificity of the antibody used in the device it cannot detect to the subspecies (Race and biovar) level needed for identification of the regulated Potato Brown Rot pathogen. The assay is specific to this bacterium species, so the lateral flow device is useful for regulatory purposes at this level. In Canada, *R. solanacearum* has not been detected, so positive results may be used for a regulatory response. However, in the USA, the Race 1 subspecies is common in the southern states and is not regulated at the national level. Only the cold-tolerant subspecies Race 3 biovar 2 is regulated, so confirmatory diagnostics require subspecies identification assays for regulatory action to take place. In the USA, ICA devices are useful (in the case of *R. solanacearum*) for preliminary detection surveys, but positive finds must be forwarded for confirmation using additional assays based on PCR and carbohydrate-utilization for USDA confirmation. The immunochromatographic assay device has the advantage of capturing regulated *R. solanaearum* cells that can be shipped to a confirmatory or central lab and used for further DNA testing.

A similar testing situation occurs with regulation of the plant xylem-inhabiting pathogen *Xylella fastidiosa* (Wells et al.). Serological assays that can identify this bacterium to species level are available, but several diseases with *X. fastidiosa* etiology occur in the USA, including Pierce's Disease in grape and Oak Leaf Scorch. However, only the Citrus Variegated Chlorosis (CVC) strain of *X. fastidiosa* is regulated and immunoassays to detect the disease causing strain do not exist. For this example, positive samples detected with a species-level immunoassay have to undergo further tests for confirmation before regulatory action can occur.

In some cases the serological assay can identify organisms to genus-level only. A good example of this are the serological assays used in several national regulatory programmes in the USA and Canada, for example *Phytophthora ramorum* (Werres et al., 2001). In this case, the serological assay can be used to detect *Phytophthora* species and make negative determinations for regulatory purposes. However, positive samples using this assay must be further tested to differentiate regulated *Phytophthora* species from unregulated species that are widespread in the environment.

The limitations of using serological methods in regulatory diagnostics are minor compared with the usefulness they bring for reliable and practical development and deployment for regulatory programmes. The necessary preparation of plant material for analysis using serological methods is usually less arduous than extracting nucleic acids for DNA analysis. With serological assays, once suitable reagents are made and a suitable method is validated for regulatory use, the costs associated with sample integrity and determining proficiency of personnel to perform the assays for regulatory use is less than commonly used DNA analyses. Cost savings can also be achieved by deploying serological assays on a network-wide scale.

In situations where the diagnosis of a high impact regulatory pest needs to be made, combining of serological results with nucleic acid-based assay results is common. Usage of both in-tandem serves as complementary tools for increasing confidence in positive results (Chang et al. 2011). Some assays currently under development actually combine the two methodologies into one hybrid system, which consists of antibodies non-covalently (Holmberg et al. 2005) attached to oligonucleotides using biotin and streptavidin interaction (Darmanis et al. 2011).

13.6 Nucleic Acid Amplification Technologies

Nucleic acid detection technologies like Spot Hybridization or Dot Blot Hybridization were the first to supplement the use of serological methods (Brandsma and Miller 1980; Oglesbee et al. 1986). The sensitivity of these technologies was measured to be similar with most ELISA formats when plant extracts were used directly. However, when nucleic acids were extracted and purified from the complex matrix of plant samples it was often found to be more sensitive than most serological methods. This technology could detect either DNA (southern blot) or RNA (northern blot) and initially used probes that were radioactively labeled (Hull 1988; Owens and Diener 1981). The need to rely on

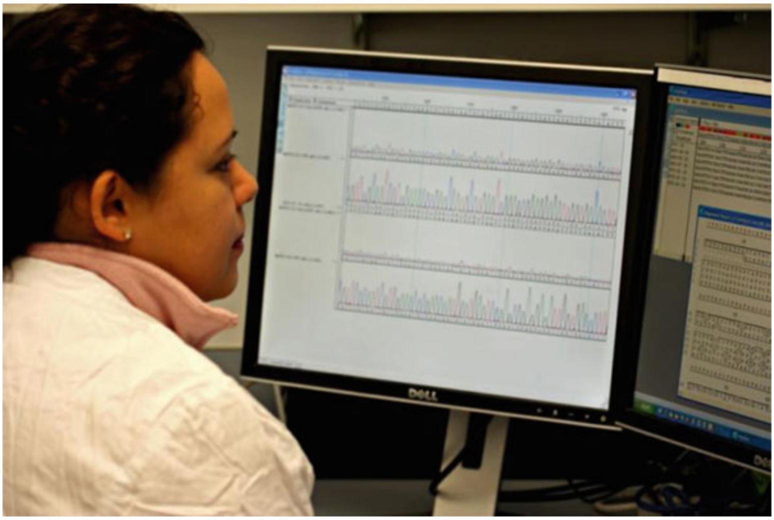

Fig. 13.5 Scientists evaluate the DNA data generated from automated sequencing gene targets. This data is used to inform, clarify, or challenge taxonomic classification, identify gene targets useful for alignment for identification or for the development of rapid diagnostics such as qPCR (Image courtesy Gerard Clover, MAF, New Zealand)

radioactively labeled probes hindered widespread use of this technology for diagnostics. However, a few years later the technique was improved with non-radioactively labeled probes, which made the technology accessible to labs that did not routinely handle radioactivity (Gaudi et al. 1990; Holtke and Kessler 1990). While spot and dot blot hybridization led to increased assay sensitivity, the time and labor required to perform the assay was more intensive than that needed for ELISA. Samples needed extensive preparation or complete nucleic acid extraction, followed by sample application to solid membranes, synthesis and labeling of detection probes, reaction of probes with bound sample and washing of residual probe, followed by the visualization of results by development of exposed films a few days later (especially when using radioactively labeled probes) or the colorimetric visualization a few hours later (when using non-radioactive probes). The increase in sensitivity afforded by this methodology was balanced with a resulting increase by 3–7 days to determine assay results.

Several DNA-based detection methods arose because of the power of DNA sequence data to inform diagnosticians on the identity and taxonomic relatives of most plant pests. There has been an explosion of DNA-based data generated on major taxa of living organisms, most of which are generated by biological researchers from a rather wide range of disciplines. Sizable portions of the DNA data are generated to inform, clarify, or challenge taxonomic classification or phylogeny, and can serve as a basis for targets used for regulatory purposes (Fig. 13.5). However, designing protocols for a specific regulatory programme requires knowledge of the requirements about taxonomic depth, since regulatory action can be taken at the generic, species or subspecies level. Assay developers can use gene regions conserved for a range of taxa as well as those subject to rapid evolutionary change as targets for analysis. DNA-based detection methods are rapidly changing as new technologies allow for cheaper sample preparation and ability to distribute user-friendly molecular diagnostics, methods that are now more accessible outside of the confirmation or reference lab environment to screening labs and first responders in field situations.

The development and implementation of user-friendly DNA diagnostics was driven by medical research, clinical point-of-care diagnostics, and defense biosecurity or anti-terrorism applications after the 9/11 events in the USA. Widespread deployment of DNA-based methods as well as the increasing reliability of data generated from these methods has resulted in almost all regulatory confirmations of high-consequence plant pests now including some form of DNA analysis.

As is the case with many technologies that are informative and powerful, molecular diagnostics can be easily misused resulting in over-interpretation of results, conclusions unsupported by controls, and diagnostic determinations based on assay contamination. These concerns continue to be resolved as methods evolve through the use of quality management practices and increased implementation of advanced technologies within the plant pest diagnostic community. The use of standards in regulatory practice may resolve many of these issues, and may be similar to those now used for admissibility as DNA evidence in court trials.

13.6.1 Nucleic Acid Amplification Detection

Polymerase Chain Reaction (PCR) has emerged as one of the most important diagnostic techniques developed to date. Variations of PCR are applied in nearly every aspect of biological diagnostics, including sequencing (Sanger et al. 1977), reverse transcriptase PCR (RT-PCR), isothermal PCR (Bekele et al. 2011; Tomlinson et al. 2010), real-time PCR or quantitative PCR (Peters et al. 2004), (Fig. 13.6). The development of PCR was a breakthrough in rapid molecular detection improving diagnostic assay turn-around time with superior sensitivity and specificity for detection of targets superior to other assays used in the late 1980s and early 1990s (Mullis et al. 1986; Saiki et al. 1988).

In PCR, a nucleic acid target can be amplified from a few copies to millions of copies. The first generation of PCR technology is often referred to as classic PCR or conventional PCR. Results obtained from conventional PCR are usually visualized using agarose gel electrophoresis, which can be semi-quantitative if run with standards of known quantities. However, for practical and regulatory uses, conventional PCR results are generally considered only in the qualitative sense based on the presence or absence of a band indicating presence or absence of target (Ferre 1992). The most common DNA sequencing reactions are also based on PCR, but in this case only one primer and nucleotide analogue terminators are added to identify nucleotide

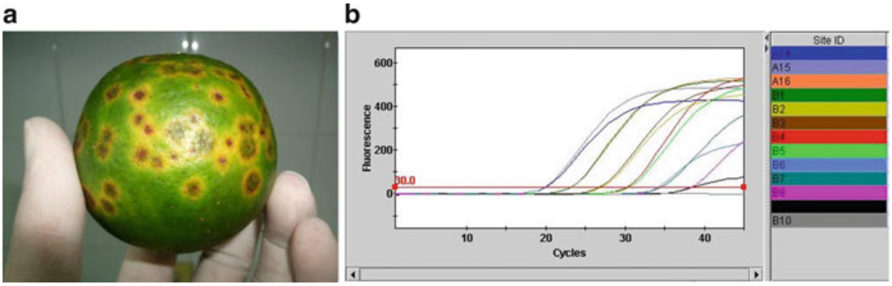

Fig. 13.6 (**a**) Detection of Citrus Leprosis Virus from lesions on citrus fruit using quantitative PCR (qPCR). (**b**) The exponential rise in the curves above the red horizontal cycle threshold line at cycles 20 through 38 indicate a positive detection of the virus. Horizontal lines below the cycle threshold line indicate the absence of the virus (Image of Citrus fruit with Citrus Leprosis Virus courtesy of Abbey S. Guerra)

position. RNA in virus genomes or pathogen gene products can be detected by reverse transcription-PCR (RT-PCR).

Real-time PCR is a detection technology based on conventional PCR. Instead of cumbersome manipulation of amplified products for detection and characterization visualized by gel electrophoresis at the conclusion of the amplification process, real-time PCR uses a probe or nucleic acid concatenating dye added directly to the primary reaction that allows for detection of products as they accumulate during the amplification process. Real-time PCR provides precise quantitative measurements and is also referred to as quantitative PCR (qPCR). The proper use of acronyms is important when referring to real-time PCR that can be easily confused with a different type of PCR, reverse transcription PCR historically abbreviated as RT-PCR. Despite the advantages of qPCR, the detection equipment and purchase of specialized probes incurs additional set-up and on-going costs.

Unlike conventional PCR product detection (known as endpoint detection because the presence of the target is determined at the end of the cycling process) real-time PCR detection can monitor the amplification process during the reaction in real-time. When the real-time process is completed, the threshold (a cut-off that uniformly designates where amplification of the target is occurring) and background noise (the number of cycles where no amplification is expected to be detected regardless of target saturation), can be adjusted to fit the applicable amplification system. (While the threshold and background can be adjusted, at some point these must be established at a permanent setting to maintain reproducible results.) In qPCR, cycles of detection are inversely proportional to the amount of target present and are stated numerically as a quantitative cycle threshold (C_q or C_t) value. The qPCR results for a sample with a C_t value of 25 translates to more targets detected than if a C_t value of 35 had resulted (Heid et al. 1996). Because qPCR is a quantitative assay we can calculate the target genomic copy number from each sample providing standard curves have also been measured.

The use of DNA intercalating dyes can be problematic for real-time amplicon detection due to lack of specificity and potential amplification inhibition. In spite of this, real-time PCR with intercalating dyes (rather than probes) was accepted more quickly by diagnosticians because of its user-friendly operation. Further improvement was realized through the development of complex detection chemistries that utilized the phenomenon of Förster Resonance Energy Transfer (FRET). The most commonly known FRET probes in qPCR are TaqMan® probes. Most qPCR assays developed for regulatory programmes use target-specific oligonucleotide probes labeled at the 5′ and 3′ end with laser excitable fluorescent dye amadites (Lee et al. 1993; Livak et al. 1995). Unbound, the large dyes interact with each other, one acting as a donor (emission) and the other as an acceptor (absorption). During extension of the DNA target during qPCR, the probe binds to its complementary sequence and is cleaved, pulling the dyes apart and allowing the donor to release its photon energy. The use of sequence-specific probes further increased sensitivity and specificity, but also required more information related to the target gene and its variants for design. As a result, additional expenses were incurred to purchase increased throughput real-time PCR and sequencing platforms. However, the increased cost of equipment, reagents and probes was offset by the reduction in size of PCR reaction mix, sample volume and bench space, and reduction in turn-around time.

The advent of probe-specific target detection resulted in the ability for amplification and detection of several gene targets in a single reaction mix. As an example, in 2007 plant virologists used this chemistry to simultaneously detect four potato pathogens in dormant potato tubers (Agindotan et al. 2007). In spite of the clear advantages offered by real-time PCR, it has not completely replaced conventional PCR in the diagnostic community. Several reasons may include the inability for real-time PCR to determine fragment size without further analysis and the required cost and time needed for development. However, all these hurdles can be overcome by recognizing that amplification kinetics rely on fragment size. Gel purification is not needed before sequencing preparation, and the information obtained from a multiplex assay negates need for development of numerous, simplified methods (that often overlap in purpose). This reinforces fit-for-purpose models and reduces the amount of validation required. Challenges were introduced as PCR technology progressed. However, the PCR assay is a flexible system that can be modified with numerous components into derivatives with vastly different and increasingly sophisticated application. We should note that several probe styles have been used in the diagnostics field that utilize FRET, including TaqMan® probes, Beacons, Scorpion probes and Amplifluor primers. Probes can also be chemiluminescent such as those used in Transcription Mediated Assay.

Rapidly evolving diagnostics required increasingly advanced technical visualization components such as introduction of charge-couple device (CCD) cameras. Post amplification processing of PCR product (amplicon), often necessary in diagnostic confirmation studies using various amplification derivatives such as nested PCR or sequencing, increases risk of contamination in future diagnostic assays. Calculations required for analyzing qPCR results continue to increase in complexity as exponentially increasing amounts of data are acquired from a single sample including RNA expression, matrix effects analysis, and target multiplexing.

The unavoidable crux of ensuring integrity of conventional and real-time PCR results in a lack of standardization between networks, organizations, and labs. Two researchers in one lab may label the same primers and/or probes under different names, negative and positive controls representing the sample matrix are often not used, universal internal controls are not easily accessible or known, and quantitative analysis is often done without understanding the uncertainty in the assay. These problems ushered in the need for quality management (quality assurance and quality control) programmes; practices that encourage laboratories to adopt policies in an attempt to reduce the amount of work required to recognize and correct occasional erroneous results. Minimum Information for Publication of Quantitative real-time PCR experiments (MIQE) requires that repeatability and efficiency are documented for each assay performed (Bustin et al. 2009).

New methods allow for amplification of targets that are not classified as PCR and do not require complex instrumentation. Isothermal Amplification (IA) is an example of a new detection format that is being tested and developed rapidly (Bekele et al. 2011). Isothermal Amplification contains all the components of a PCR reaction. The reaction is conducted using one temperature (instead of cycling through 2–3 temperatures used in PCR) in one tube using specialized primers that allow continuous amplification of target DNA. One of those technologies is loop-mediated IA of DNA (LAMP), which amplifies DNA quickly with results that can be easily visualized because of the generation of precipitate. Results can also be read in real-time because the precipitate generates turbidity that can be measured as it is generated. Post-amplification visualization can also be achieved using fluorescent intercalating dyes that target double-stranded DNA. Despite lower sensitivity in most cases, this technology is gaining popularity among diagnosticians because costly real-time PCR machines and probes are not needed.

13.6.2 Nucleic Acid Arrays

Unlike single species DNA-based detection systems that are now routinely used in regulatory plant pest diagnostics, multiplex detection and microarrays (Fig. 13.7) are rarely used compared with other diagnostics methods (Miller et al. 2010). Cost is the main issue limiting microarray development and implementation. Arrays have been designed for viruses (Agindotan 2007), fungi or oomycetes (Lievens and Thomma 2005), bacteria (Fessehaie et al. 2003), and nematodes (Ramanathan et al. 2011). A challenge facing diagnosticians is the development of a single assay to screen for all targets as has been done in the medical field (Palacios et al. 2007). For example, a *Rathayibacter toxicus* Sasaki et al. microarray may

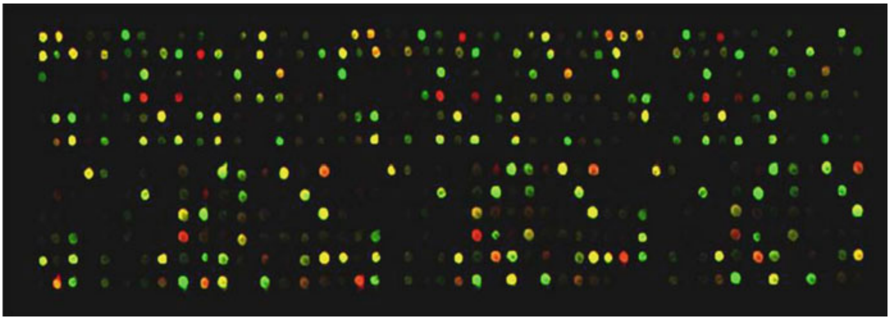

Fig. 13.7 Results image of microarray analysis of DNA. Amplified DNA is usually labeled with two dyes Cy 3 (*red*) and Cy 5 (*green*) and then hybridized to an array of probes on a solid surface such as a glass slide or film often referred to as a "chip". Computer programmes use algorithms to determine the results from the hybridization patterns (Image courtesy Laurene Levy, CPHST, APHIS, USDA)

screen for the host nematode, the bacteria that produces the toxin, and the bacteriophage that is suspected to transfer the toxicity gene (Kathy 1993).

By nature of the matrix involved, assays involving one ampliset (singleplex) amplification reactions are easier to optimize compared with assays using multiplex amplification reactions, due to multiple oligonucleotide (primer) interactions. Likewise, arrays involving multiple singleplex amplification reactions are more difficult to develop than arrays relying on one ampliset because each ampliset requires characterization. However, a multiplex assay can detect and differentiate multiple regulated species or identify one species with increased accuracy.

A microarray can be used to measure RNA transcription from multiple genes in one organism or screen for large amounts of different organisms, and multiplexed arrays compound potential detection capabilities. Microarrays can also mitigate the reduction of sample volumes used in PCR, which reduces sensitivity when the original sample size is large. Otherwise, appropriate statistical analysis of PCR results would be needed (Hart et al. 2005). For some fungal species false negative results increased using DNA arrays compared with fungal growth results from plating (Folta and Kole 2011). For example, cranberry fruit-surface pathogens easily detected by plating at very low concentration resulted in more false negative results than cranberry pathogens infecting the whole fruit. After DNA extraction of whole cranberry fruits, the concentration of the surface pathogens were too low in the extraction aliquot used for PCR to be detected. For quarantine determination, specificity and sensitivity are critical. Approaches to overcome the limits of signal loss due to sampling include target capture (biotinylated probes, bead capture, poly-T hybridization) and sample pooling (Munkvold 2009).

The DNA array developed for most *Pythium* species has been used to detect *Pythium tracheiphilum* Matta in soil and plants (Tambong et al. 2006). This species is regulated in Australia. If an inspector found oomycete oospores on a sample by visual inspection, the sample could be tested on an array system that included specific testing for *P. tracheiphylum* to identify the spores. Ideally, a microarray could be developed similarly for *Phytophthora ramorum* screening which would be able to identify and characterize samples to the species level, giving disease management programmes more information in less time allowing increased accuracy in policy application.

Next generation sequencing is replacing high-density oligonucleotide arrays for functional genomics. Depending on the function needed by the diagnostician, sequencing provides genetic data with less cost than hybridization arrays when validation time is considered. However, high-density amplification arrays are preferential for measuring genomic copies or rapid screening. For low-density macroarrays, which are sufficient for the development of arrays testing for regulated pathogens, the advantages of next generation sequencing over oligonucleotide arrays are less evident. Oligonucleotide macroarrays are fairly inexpensive but can be labor intensive and take 24 h to complete. Some companies developing rapid low-density arrays for medical applications have had difficulty staying in business. Most of these "lab-on-a-chip" platforms were previously too expensive for most uses but this situation is changing. The largest stumbling block in making a decision between use of one or the other is agreement over what constitutes a rapid and cost-effective platform for a given market. Ultimately, the decision involves sample load. For routine qualitative testing of hundreds of samples, the oligonucleotide macroarray approach remains cost effective, but for quantitative sample screening or expression profiles, microarrays provide vastly superior datasets. An essential strategy for regulatory or research diagnostic labs is to ensure nucleic acid arrays can be easily adapted to a wide range of equipment platforms, and easily supported through validation testing components.

13.6.3 Importance of Accurate Genomics

Challenges to diagnostic labs are not just seen in cost and quality management, but also in the ability to access vital genomic information regarding the organism being tested (Fig. 13.8). Reports suggest that within the last decade about 20 % of the DNA sequences in public databases are inaccurate or improperly annotated (Felton 2001; Schena et al. 2008). In general, regions that have been methylated, are G-C rich, subject to degradation, or sRNA are difficult to acquire information from. These challenges have been overcome through technologies such as single molecule real-time sequencing (SMRT) (Pacific Biosciences), Solexa (Illumina), RNeasy kits (Qiagen), and pyrosequencing (sequencing by a synthesis reaction that accurately quantifies sequence variation), respectively. Sequencing of obligate organisms is a challenge due to large genomes and numerous repeat regions. Sequenced genomes provide information about microsatellite regions that can be exploited for strain typing (Abbott 2010; Schena et al. 2008). After a genome is sequenced and annotated, the re-sequencing of additional strains can be compared to the original sequence for reference, ultimately benefiting assay development for strain-specific assays. One example of this is *Escherichia coli* OH157, where the genes responsible for toxin production absent in other innocuous *E. coli* strains were targeted for detection (Cebula et al. 1995; Fratamico and Strobaugh 1998). Mechanisms of infection of plant pathogens require clearer understanding, allowing rapid recognition and response to the emergence of highly pathogenic strains (for example, *Phytophthora infestans* (Montagne) de Bary) which cause severe outbreaks.

Genome sequencing provides a wealth of data for designing species-specific primers, including gene applicability (copy number, nucleotide composition, etc.), sequence integrity, and options related to what is desired from characterization. Single-copy targets in conserved regions related to species specific function enable copy-number assessment, while reducing sensitivity, compared with multi-copy markers that may be more effective for qualitative screening. For example, the mitochondrial genome is rich in sequence data useful for determining specificity but has variation in copy number resulting in difficultly in quantification of organisms in a sample. *Phytophthora* mitochondrial genomes are being sequenced and compared for the purpose of determining sequence integrity and specificity while providing researchers accurate qualitative screening options (Martin et al. 2007).

13.7 New Tools and Technologies

A balance is continually struck between the need for new technology and the cost of development and deployment for regulatory use. The dichotomy of assay development was briefly discussed earlier when describing platform advancements that increased cost, validation time and training while also increasing throughput and characterization abilities. Sometimes a win-win does occur where an improvement on existing techniques can bring about reduced cost and increased accuracy or throughput, diagnostic qualities not mutually exclusive. Often, these applications are slow to be adapted (Oostingh et al. 2011) or approached with great skepticism before ruggedness testing (Percy 2003). Despite uphill challenges and potential validation issues, several assays have been put forward that may change the way diagnostic screens are performed. These methods are based on well-established protocols consisting of PCR, gas chromatography, and nucleic-acid hybridization. In addition, living cells using Cellular Analysis and Notification of Antigen Risks and Yields (CANARY) can now detect antigens in real-time. Such technologies offer potential cost reduction, rapid turn-around time and increased throughput while also possibly reducing the amount of training required by the end-user. Successfully implemented deceptively simplistic assays such as Surface Plasmon Resonance and CANARY may produce defensible results in addition to reducing potential operator error.

13.7.1 DNA Barcoding

Species diagnosis that compares a DNA sequence from a specimen of uncertain taxonomy to a database of DNA sequences derived from expertly identified vouchered specimens representing a wide range of species is known as DNA Barcoding (Hebert and Gregory 2005; Ratnasingham and Hebert 2007). In this methodology, a DNA sequence in the database is referred to as a DNA barcode and the DNA barcodes must be generated from one diagnostically informative gene that is present in many taxonomic lineages. The use of a single gene region for diagnosing many species is possible because genes can include regions of relatively low and high variation. The regions exhibiting lower levels of variation are useful for developing conserved PCR primers that can amplify target DNA from many different species spanning various taxonomic levels (Davison et al. 2009; Folmer et al. 1994). The region located between the two conserved primer sites must be variable between species for DNA barcoding to work as a diagnostic method. Because mutational differences continue to accumulate after a speciation event, the variation within a species (intra-specific variation) is expected to be lower than the variation separating two species (inter-specific variation). When these values are plotted on a graph, we observe a bimodal distribution with a clear separation between the "within-species" distribution and the "between-species" distribution. This separation or gap in the graph is sometimes called a 'barcode gap' (Meyer and Paulay 2005). Demonstration of a barcode gap suggests that the DNA barcode region is diagnostically informative. Other techniques are also available for testing diagnostic utility of DNA barcode regions. Although DNA sequences have been used in previous studies to identify diagnostic characters between species (Brunner et al. 2002; Jacobson et al. 2006; Simon et al. 1994) and analyze systematic relationships among species (Virgilio et al. 2008), the general concept of a DNA barcode has evolved into a more structured identification technology that requires adherence to data standards to facilitate sharing of information and improve overall quality of diagnoses (Ratnasingham and Hebert 2007; Rowley and Shulman 2007).

A truly universal DNA barcode does not exist for all biological species because of the large genetic separation among the major Phyla and Kingdoms (Chase et al. 2005; Seifert 2009). For animals, however, a 650-bp fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) has been proposed as the first formal DNA barcode region (Hebert et al. 2003; Roe and Sperling 2007). This gene fragment has been used to test the utility of DNA barcodes using various animal lineages (Armstrong and Ball 2005; Hajibabaei et al. 2006; Naro-Maciel et al. 2010; Yancy et al. 2008), but many taxonomic groups still remain to be evaluated. The use of the 650-bp COI fragment as a DNA barcode does not preclude the use of other genes or other regions of COI as diagnostic markers (Barr 2009; Glover et al. 2010; Scheffer and Wiegmann 2000; Vences et al. 2005). It does however, provide a useful example for the design of future DNA barcode projects using other regions. Although the gene regions used to barcode plants, fungi, and prokaryotes are still being explored as research in this area continues, the operational and theoretical lessons learned from using COI in animals should be relevant to these other forms of life. In plants (CBOL Plant Working Group et al. 2009) and oomycetes (Robideau et al. 2011) a two-barcode system has been adopted. COI was rejected in fungi while the de facto ITS was shown as the marker of choice following testing guidelines approved by the National Center for Biotechnology Information (NCBI) (Schoch et al. 2012).

A benefit of selecting a gene such as COI for a DNA barcode region is that it has been studied in many taxonomic lineages for intra- and inter-specific variation (Avise et al. 2004; Simon et al. 1994). As a result of these studies, however, we see that COI does not always provide a clear understanding of species limits and in some cases could produce incorrect identifications (Dasmahpatra et al. 2010; McKay and Zink 2010). Therefore, the process of diagnostic tool development and evaluation must be conducted separately from taxonomic revisionary work using that same data set (Packer et al. 2009).

As other molecular diagnostic techniques, DNA barcode technology requires proper sampling of the target species in order to evaluate its diagnostic utility. Although DNA sequence divergence values between relatively distant taxa produce a large 'barcode gap' and should alleviate the need for large sample sizes, this is not true for closely related species (Funk and Omland 2003). Without prior knowledge of genetic diversity estimates from previous taxonomic, geographic, or ecological studies we cannot know the appropriate statistical sample size for the taxa of interest (Weir et al. 1996). Incomplete taxonomic representation also negatively affects DNA barcode performance (DeSalle et al. 2005; Elias et al. 2007; Sundberg et al. 2010). As a result, it is important for diagnostic protocols that use DNA barcodes to (1) explicitly state the species included in the tool, (2) provide rules for interpretation of results, and (3) be evaluated for sampling error by biologists familiar with the organisms.

DNA sequences are easily shared and stored, but the analysis of DNA barcodes is not standardized. The use of DNA sequence databanks such as GenBank (Benson et al. 2011) to perform identifications using the BLAST (Basic Local Alignment Search Tool) algorithm is not recommended because of data quality issues (Sass et al. 2007) and the use of a local-alignment search algorithm like BLAST can be problematic. The Barcode of Life Data System (BOLD; www.barcodinglife.org) provides an alternative search engine for identification that can exclude DNA sequences that do not meet standards for vouchering specimens and DNA data files. Alternatively, a GenBank BLAST using "barcode" as a keyword searches only sequences with proper vouchered specimens, with NCBI-approved barcode markers, and with electropherograms (a plot of results from automated sequencing). The BOLD search engine is one of several programmes that use distance-based analyses to provide identifications (Park et al. 2008; Steinke et al. 2005). Distance-based methods of diagnosis are less prone to misinterpretation when compared to BLAST e-values. However, some authors have criticized them and alternative character-based methods of analysis have been proposed (Cameron et al. 2006; Matz and Nielsen 2005; Meier et al. 2006). The different methods and programmes for DNA barcode analysis each have strengths and weaknesses regarding performance and operational logistics. These aspects must be considered when evaluating how a DNA barcode protocol should be implemented for regulatory programmes.

DNA barcoding is an important technology for biosecurity because it enables a specimen to be compared with many species profiles using a single molecular protocol. Identification to the species-level may not always be possible but in some cases the ability to identify a damaged or immature specimen to genus or species still provides useful information. As mentioned, the technology must always be evaluated for each specific regulatory application. Although the addition of new DNA barcodes to a common database should increase the reliability of the diagnostic tool over time, it will not obviate the need to continually monitor and re-evaluate the growing data set for accuracy. In comparison with other conventional PCR and real-time PCR diagnostics, DNA barcoding is not inexpensive or fast. Until new DNA sequencing technologies or DNA barcode processing centers are developed for streamlined servicing of samples at reduced costs, DNA barcode technology will have a restricted role in routine diagnostics. Currently, DNA barcoding is a useful method for diagnosing samples when the taxonomic scope (e.g., the number of species included in the identification process) of an assay is large.

13.7.2 Portable Gas Spectrometry

Inspectors at Ports of Entry must find a visual sign of a plant pest in the millions of plants and cargo that they inspect each year. Plant diseases, small insects, and pests concealed inside plant material can escape detection and potentially establish in a new country. Recent advances in portable gas spectrometry technology may provide a way to detect plants attacked by exotic pests using their unique volatile signature. Agricultural products are known to produce characteristic compounds that can be detectable through gas chromatography (Loper and Lapioli 1972). Research also has shown that plants attacked by insects produce a distinctive volatile bouquet different from the volatiles emitted by mechanically damaged plants (Engelberth 2011).

Until recently, the most practical and frequently used detection tools were trained dogs. However, dogs are limited in detection for the spectrum of possible threats, cannot be deployed on a 24/7 basis and require periodic retraining. Electronic sensors also have disadvantages, including limited array of odor detection, photo bleaching, complex user interfaces, sensitivity to humidity, and baseline drift (Pearce et al. 2003).

Alternatively, gas chromatography can identify odors based upon their full chemical signature (McFadden et al. 1965). Advances in high-speed gas chromatography now employ technology that can separate chemicals in near real-time so that pattern recognition and trace detection can be performed in seconds (Landberg et al. 2009; Miresmailli et al. 2010). The applicability of one system (Electronic Sensor Technology's "zNose") was critically tested as a high-throughput screening tool. The zNose is a surface acoustic wave (SAW) device that pumps target odor through a heated, meter-long capillary tube to a single sensor. Testing has been completed using the zNose with fruit fly infested citrus (Kendra et al. 2011). Infested fruit with Caribbean Fruit Fly (*Anastrepha suspense* (Loew)) produces a volatile signature different than non-infested and mechanically damaged fruit (Kendra et al. 2010). Key compounds were identified and can be used to detect infested fruit mixed with un-infested fruit.

The zNose portable gas chromatography technology was tested to determine whether volatile signatures could be used to distinguish admissible plant species from prohibited plant species. At USA Ports of Entry, bonsai trees arrive bare-rooted and frequently without leaves due to storage lasting over a month in cargo ships. Citrus and other trees of the Rutaceae could harbor regulated plant pests devastating to the citrus industry and therefore are prohibited. As a result of their condition upon arrival, identification between admissible and prohibited citrus bonsai trees is problematic. The citrus trees and related species of Rutaceae produce a volatile pattern clearly different from the admissible species tested from the families Boraginaceae, Moraceae, Rubiaceae and Ulmaceae. Other compounds were found to be common in many plant families, but occurred in greater quantities in citrus species. Both the citrus-specific compounds and the common odors were used to develop a screening protocol. The protocol was designed to alert the port inspector that the bonsai specimen undergoing inspection might be a prohibited species. In these specific studies, the zNose system not only provided a low detection limit (picograms) but also was also fast, taking only minutes to run one sample. USDA APHIS specialists helped conduct the citrus studies that provided them with hands-on experience using the gas sensors that allowed for immediate feedback on use of the technology. The specialists found that the technology required minimal training, was easy to operate and accurately differentiated prohibited citrus species from the admissible species. After confirming that the technology was applicable for Ports-of-Entry, an SOP was developed and tested at the Hawthorn Plant Inspection Station in California.

Gas sensor technologies work well for a clear, unique odor and the sample can be obtained directly from plant material. (If needed, material can be stored in a small vial to concentrate the odor.) However, to this point gas sensors have not been able to detect variation in levels of the target signature and lack the ability to be reliably calibrated (Leea et al. 2010). Precision and ruggedness testing are critical to develop new gas sensor detection methods. In the Hawthorn Plant Inspection Station study, no shipments of bonsai were received during the 3-month trial period. Screening tools must be developed to detect multiple plant pests to optimize usefulness and cost benefits when applied at Ports of Entry. In the future this technology will be further evaluated to determine its utility for detecting insects, other plant species and plant pathogens.

13.7.3 Surface Plasmon Resonance

Surface Plasmon Resonance (SPR) technology is among the most rapid, sensitive, specific and label-free methods for the detection of microorganisms. First developed in 1984, SPR comprises a biosensor connected to a transducer, usually in close proximity to each other, which converts the binding of an analyte and ligand into measurable signal (Flanagan and Pantell 1984). The biosensor consists of a ligand acting as a capturing molecule (an antibody or oligonucleotide probe for example) to create a reactive surface. A metal substrate such as gold film maximizes the ability to distinguish signal-to-noise based on the density of the analyte-ligand complex, as the sample flows through the microfluidic chamber across the biosensor reactive surface. Since detection is through optical density, SPR is easily transferable to a microarray format (Dorokhin et al. 2011).

Studies have reported the use of SPR for the detection of foodborne bacterial pathogens such as *E. coli* O157:H7, *Salmonella* (Mazumdar and Chen 2008) and *Listeria monocytogenes* (Murray et al. 1926) Pirie 1940 (Nanduri et al. 2007). SPR has also been used to rapidly determine the presence of *Phytophthora infestans* sporangia (Skottrup et al. 2007). All these SPR methods involved the application of antibodies coupled onto sensor surfaces (immunosensors). Antibodies as ligands for detection of pathogens present undeniable disadvantages including degradation, loss of activity, and production cost. Oligonucleotide probes specific for target pathogens however are stable and inexpensive. Sensors coupled with probes can be regenerated and re-used many times. For example, oligonucleotide DNA probes complementary to the 16S rRNA gene sequence of *E. coli* can be re-used, with consistent detection of 2 μg/ml of *E. coli* total cellular RNA (Nelson et al. 2002). An oligonucleotide DNA probe specific for *Fusarium culmorum* (William G. Sm.) Sacc. enabled detection of 0.06 pg fungal DNA in 30 ng of durum wheat DNA (Zezza et al. 2006). Portable SPR devices have been developed and utilized for detection of pathogens and toxins in a field setting. As an example, detection of staphylococcal enterotoxin B was detected in serum and stool samples using antibody-coupled Spreeta sensor chips (Soelberg et al. 2009). Portable SPR devices have also been used to detect small molecules, ricin A chain, Norwalk virus, *Francisella tularensis* Darmstadt-Dieburg LVS and *Bacillus subtilis* (Ehrenberg) Cohn spores with antibody-coupled sensor chips (Chinowsky et al. 2007). Testing by USDA APHIS is being conducted to adapt SPR technology for detection of plant pathogens such as *Ralstonia solanacearum* and *Xyllela fastidiosa* Citrus Variegated Chlorosis strain (CVC).

13.7.4 CANARY

Cellular Analysis and Notification of Antigen Risks and Yields (CANARY) is a promising technology developed by scientists at the Massachusetts Institute of

Technology (MIT) Lincoln Laboratory that has potential to achieve sensitivity equivalent to PCR with ease of use similar to a lateral flow device. Originally developed to detect low levels of bio-warfare agents such as *Yersinia pestis* (Lehmann & Neumann) van Loghem, *Escherichia coli* strain 0157:H7 and Venezuelan Equine Encephalitis (VEE), CANARY consists of genetically engineered mammalian B lymphocytes (B cells) that naturally identify pathogens in organisms with circulating immune systems (Rider et al. 2003). The Lincoln Laboratory engineered a mouse B cell line with an aequorin bioluminescent protein from jellyfish (*Aequoria victoria* (Murbach & Shearer)) and the monoclonal antibody gene sequence of the desired target. When an antigen is cross-linked with surface-expressed antibodies, the resulting transduction cascade triggers calcium expression. Aequorin emits a photon upon an increase in calcium concentration that can be visualized using of a simple luminometer (Tsuji et al. 1986). The B cells used in the assay are prepared (primed) the day before use and once primed they can be stored at room temperature and used over two days. Prepared B cells also can be stored at 5 °C for up to two weeks, and frozen at −80 °C for up to six months. The Lincoln Laboratory re-engineered Several B cell lines have been redundant with longevity genes (anti-apoptosis, etc.) that has further extended their shelf life by up to twofold.

Pathogen-specific B cell lines are created by transfecting antibody light- and heavy-chain constant regions containing variable chain regions specific for the pathogen of interest into the lymphocytes. Transfected B cell lines are then screened for specificity and sensitivity. Pathogen-specific B cell lines have been shown to detect 1,000 CFU of *Bacillus anthracis* Cohn from nasal swabs. B cell lines were developed to detect foot-and-mouth disease virus (FMD) to a single strain, demonstrating pathogens can be detected below species level (Chung and Liao 2003). Since 2007, scientists at the Lincoln Laboratory in cooperation with the USDA APHIS have developed B cell lines for detection of *Ralstonia solanacearum*, *Phytophthora* species, and potyviruses (See Chap. 20). These pathogen-specific B cell lines have been established at USDA APHIS and are being evaluated and applied to detection of these pathogens in environmental samples. Preliminary data has shown that the *R. solanacearum* B cell line has a detection limit of 3 CFU, equivalent to current qPCR methods. Testing for *R. solanacearum* has also been successful from tissue in poor condition that would have generated a false negative with other diagnostic tests. The B cell line targeting *R. solanacearum* is scheduled for additional testing in the UK using environmental samples such as river water.

The assay protocol is simple: An aliquot of the pathogen-specific B cells is placed in the bottom of a microfuge tube followed by adding a small amount of sample on the tube sidewall. The tube is centrifuged briefly to mix the B cells and sample, and immediately placed into a luminometer for visualization. The reaction occurs in 7–10 s, and is read in less than one minute, requiring less than four minutes per sample (Fig. 13.8). In spite of all its strengths, the assay is only as good as the monoclonal antibodies used to develop B cell lines. That being said, CANARY technology carries significant strengths in molecular diagnostics: Speed of results, ease of use, flexibility for development and full validation capability.

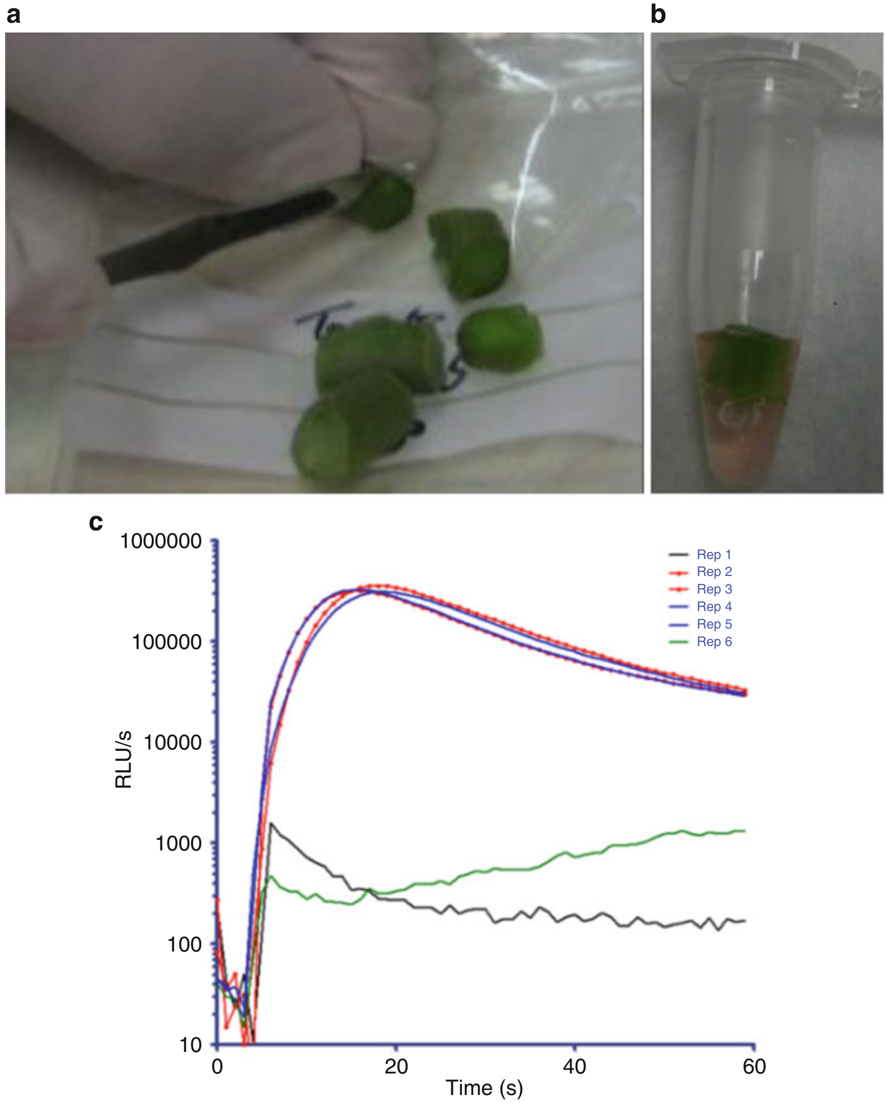

Fig. 13.8 Detection of *Ralstonia solanacearum* in plant samples using CANARY technology. (**a**) Sample stems are cut, chopped and (**b**) soaked in buffer for 5 min before being reacted with *R. solanacearum*-specific B cells. (**c**) A positive reaction, indicated by the raised curves, occurs within 20 s of contact with the B cells. The "flat" lines indicate negative controls (Images courtesy Laurene Levy, CPHST, APHIS, USDA)

13.8 Conclusions

The successes of programmes that seek to provide plant biosecurity hinge on the ability to correctly detect and identify plant pests. The precision and accuracy of identification of relevant pests drives how risk analysis, surveillance and mitigation of outbreaks occur and condition their effectiveness in providing plant biosecurity.

Diagnostic methods and technologies used for identification for any programme are influenced by the circumstances faced by each national plant protection organization. The forms of diagnosis used and the uncertainties in taxonomy and nomenclature for identification can be a subject of disagreement between importing and exporting countries when implementing their safeguarding efforts. While the goal is to protect agriculture and the environment, there is also a responsibility to provide those safeguards while maintaining open trade.

Advances in diagnostic technologies are now being incorporated into national regulatory programmes, beginning with situations in which an advanced diagnostic provides advantages over traditional diagnostic methodologies, and where the value of the commodities being tested is worth the extra costs of method development and deployment of advanced assays. However, as methodologies are adopted, standards for assay deployment and reporting are put into place, and economies-of-scale drive cost-per-test prices lower, the use of these advanced methodologies will proliferate.

Additional requirements for diagnostics used for regulatory purposes must be addressed to direct method development incorporating new technologies. Planning, selection of performance measures and requirements, generation of suitable Work Instructions and deployment of methods to labs providing regulatory services are activities that must occur within each agency so that there is sufficient confidence in the diagnostic technology to satisfy national biosecurity needs and withstand challenges to diagnostic determinations and actions. Some of these activities are addressed by adopting a Quality Management System similar to those used in industrial, human clinical and veterinary laboratories.

Decisions to adapt a previously developed method, further develop a newly published method from the scientific literature, or to develop a new method in-house must carefully take into consideration: (1) origin of the technology or method, (2) data available to assess its fit-for-purpose, (3) cost of development/adaptation, and (4) potential problems in further method development. As validation of the assay is performed and performance measures meet requirements for regulatory use, the Work Instruction must be carefully composed so it is applied as intended by the diagnosticians supporting regulatory determinations based on assay results.

The success of any plant biosecurity programme rests on the effectiveness of diagnosis. Successful identification of a pest relies on the quality of reference collections, materials for exclusion of high-consequence pests and investigation of new taxa. Scientific collections are used as taxonomic references for the identification by visual/microscopic observation and for the development of DNA sequence databases that are used for identification by DNA barcoding or for development of rapid assays for detection of regulatory plant pests.

Several applications of diagnostic tests based on serological reactions have been used for regulatory purposes. Specific reactions of antibodies to plant pests have long been used as evidence for identification and action on various pests, and are particularly useful in identifying plant viruses. Widespread use of serological assays for screening, survey, and detection now occurs using ELISA, which can be customized for a specific regulatory purpose and is often available in standardized formats through commercial sources. Another application of serological reactions for regulatory purposes includes the immunochromatographic (flow) devices that are also commercially available. Several platforms now under development, such as CANARY, show promise in reducing sample processing time and costs and would allow serological assays to be deployed outside the lab environment so that testing can be done at critical regulatory areas such as national Ports of Entry and quarantine inspection stations.

Analysis of nucleic acid is the most rapidly increasing application for making diagnostic determinations of regulatory pests. This technology is similar to the use of nucleic acid information in medical diagnostic, food safety, and forensic fields. Nucleic Acid analysis is now seeing widespread application for making regulatory decisions in plant biosecurity. Nucleic acid amplification technologies (including Polymerase Chain Reaction and DNA sequencing) provide opportunities and challenges when applied to regulatory diagnostics. Genomic data is in high demand due to the diversity of life forms and need for molecular information in species descriptions. However, as standards for identification and diagnostic method developments are proposed and debated, the use of genomics for making regulatory diagnostic determinations must routinely be challenged and scrutinized.

Nucleic acid amplification technologies offer the promise of high sensitivity. Nevertheless, attention continually must be applied to all aspects of sample care to eliminate the threat of sample contamination. Sample integrity is especially critical when diagnostics are used to make high-impact regulatory decisions. Incorrect diagnosis or misidentification may ruin business, generate disputes between trading partners, be challenged in law courts, or otherwise undermine the confidence of the regulatory decision. Often, adoption of a Quality Management System for these diagnostics can provide the confidence needed for reliable results that can withstand challenges from trading partners or courts.

New technologies are finding application in regulatory diagnostics to avoid difficulties in selectivity/sensitivity experienced with current diagnostic methods. We seek new technologies that will allow greater applicability in the field and enable faster, more efficient regulatory decisions. Some new methods improve the turnaround time over older methodologies. For example, consider Surface Plasmon Resonance versus Fluorescence Signaling and CANARY versus ELISA. Diagnostics for plant pest detection will continue to benefit from the investment by other scientific disciplines in development of point-of-care and in vitro diagnostics. PCR was a revolutionary change in the field of diagnostics. The future may see new methods that rely on gas/liquid chromatography and other technologies that detect the signature of diagnostic molecules currently not in our focus. New methods will expand our regulatory toolbox. As with Integrated Pest Management, we should never rely on one approach, no matter what novel technologies are adopted for future regulatory diagnostic programmes. These technologies add diversity for crosschecking and accountability of results when high-consequence regulatory decisions are made.

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Chapter 14
Insect Eradication and Containment of Invasive Alien Species

Ken Bloem, Eckehard G. Brockerhoff, Vic Mastro, Gregory S. Simmons, John Sivinski, and David M. Suckling

14.1 Introduction to Insect Eradication

This chapter provides a brief introduction into the tactics and strategies necessary to achieve eradication of invasive pest insect populations and the requirements needed to mount an effective eradication programme. This chapter also considers pest containment as a component of eradication and as an explicit goal. These response programmes are used mainly against specific organisms that warrant an attempt to mitigate the high management, environmental, or direct impact costs if those pest organisms were allowed to establish and spread. Eradication differs from other management tactics in that the goal is finite. For some pest introductions, the goal from the initiation of any management action has been eradication. In other programmes, where the goal was initially to contain damage or limit pest spread, improvements in management tactics have made it possible to change to an

K. Bloem (✉)
USDA-APHIS-PPQ-Center for Plant Health Science and Technology (CPHST), Raleigh, NC, USA
e-mail: Kenneth.Bloem@aphis.usda.gov

E.G. Brockerhoff
Scion (New Zealand Forest Research Institute), Christchurch, New Zealand

V. Mastro
USDA-APHIS-PPQ-CPHST, Buzzards Bay, MA, USA

G.S. Simmons
USDA-APHIS-PPQ-CPHST, Salinas, CA, USA

J. Sivinski
USDA-ARS-Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA

D.M. Suckling
New Zealand Institute for Plant & Food Research Ltd, Lincoln, New Zealand

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eradication goal. The question then becomes, “Why proceed with eradication or official control?” The main reasons are to maintain or gain market access, or lower the costs of production; other reasons include human health and welfare or environmental impacts.

Eradication programmes have been organized against pest insects (Walters et al. 2008), plant pathogens (Sosnowski et al. 2009), weeds (Panetta and Lawes 2005) and mollusks (Kean et al. 2009; Barker 2002). This chapter’s scope is limited to insect pests. The insect Orders that have been successfully targeted by eradication programmes are Diptera > Lepidoptera > Coleoptera > Hymenoptera. Much less frequently, successful eradications have targeted Hemiptera and Isoptera (Kean et al. 2009). Here, the context is not pest management, although many tactics developed during an eradication programme could be suitable for pest management. More than 800 eradication programmes have been initiated to date (Kean et al. 2009), and the frequency of initiation is increasing exponentially over time. This increase in eradication programmes can be attributed to the increased movement and establishment of organisms in new places due to increased trade and travel (Sect. 1.2).

The global philosophy behind “biosecurity at the speed of commerce”, or without disadvantage to trade, represents one of the reasons the number of new organism introductions is increasing (Knight 2008). This situation must be viewed from the perspective of a dynamic system. New invasive pest arrivals do not replace or displace existing pests from the system. Rather, the system accumulates them. The enlarged pest complex may disrupt or neutralize current management practices and increase long-term pest management costs. Successful eradication offers the possibility of mitigating these long-term costs and impacts. In a similar way, containment of a pest is a tactic to avoid these impacts for as long as possible in areas where the pest is not yet present. These containment programmes are not undertaken lightly. Unfortunately, the outcome of a programme cannot be predicted and the scientific literature is not well developed for recording the progress or execution of these typically government-led programmes. This lack of scientific documentation to guide managers in formulating and executing new programmes highlights the importance of such documentation (reports and publications).

A sequence of decisions and steps are followed when considering whether to eradicate during the early stages of infestation/establishment, after an organism is reported or discovered. The sequence involves: (1) Investigation and data gathering; (2) accumulation of evidence of plausible scenarios that might warrant an intervention from all aspects and perspectives; (3) consideration of options and their likelihood of success and impacts; (4) decision making based on target-setting and available resources; and finally (5) communication and implementation of an operational response (Brockerhoff et al. 2010). Economic considerations are paramount because eradication programmes are complex and expensive (Mumford 2005).

Typically, government appropriations are involved. Cost-benefit analyses are normally developed as quickly as possible, allowing for various scenarios, since it is often difficult to accurately predict the impact of a pest or the exact inputs and the outcome of a programme. As a consequence, cost ranges are normally used in these

Table 14.1 Costs and averted economic impacts from eradications of forest insect pests in New Zealand, amounting to a combined net value of averted losses of $70–700 Million USD (Adapted from Brockerhoff et al. 2010)

Organism	Estimated eradication cost (USD$ million)	Estimated economic impact over 20 years (USD$ million)	Estimated averted costs (economic impact less cost of eradication)
White-spotted Tussock Moth (1996–1998)	9	19–133	$10–124
Gum Leaf Skeletonizer 1 (1997–1998)	3	76–107	$73–104
Painted Apple Moth (1999–2006)	49	44–267	−$5–218
Fall Webworm (2003–2006)	5	14–62	$9–57
Gum Leaf Skeletonizer 2 (2003)	90[a]	76–107	−$14–17[a]
Gypsy Moth (2003–2005)	5	2–218[b]	−$2–214

[a]Eradication not attempted due to an unfavorable cost–benefit analysis; eradication cost estimate (see Brockerhoff et al. 2010 for details)
[b]The value shown for impact over 20 years was calculated according to Harris Consulting (2003)

cases (see Table 14.1 for a comparison of costs and benefits of recent forestry-pest eradication programmes in New Zealand). Nevertheless, the economic, environmental and human impacts likely to be caused by an invader typically exclude various parameters that are difficult to quantify (Holmes et al. 2009), suggesting that the actual benefits (i.e., averted damages) that can be gained from successful eradications are likely to be greater than initial estimates. However, Myers et al. (1998) argue that there is a lack of detailed information on programme operations and outcomes to support this claim and that the benefits of most eradication programmes are overestimated and the costs underestimated.

All eradication programmes include some form of containment as part of the strategy. In some cases, containment may be the only target that can be realistically achieved. Containing or slowing the spread and preserving areas free of an invasive pest will prevent management costs from being incurred, may also permit unrestricted trade and will reduce environmental, health and social impacts.

The human dimension of an eradication programme cannot be ignored. Most eradication programmes encompass private and public areas because of the distribution of hosts and the often polyphagous nature of the targeted pests. If the public is to understand and support these programmes, then there is a strong need for a robust educational effort beyond simple communication of the critical cost-benefit information. This is particularly true early in a programme when the full impact of the pest has not been realized and the operational responses may include inconvenient actions or cause potential non-target impacts (e.g., aerial spraying over urban areas, removal of host plants or establishment of quarantine zones). Failure to work with the people involved can lead to complications or program failure. Examples include attempts to eradicate Citrus Canker in Florida (Kean et al. 2009) (Sect. 18.3) and the

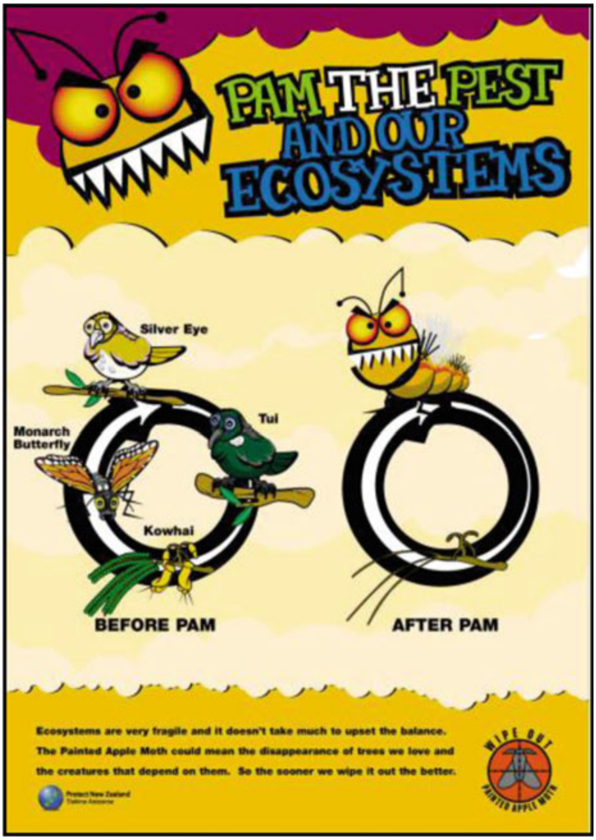

Fig. 14.1 Educational materials highlighting the negative impacts of "PAM the Pest" were used by the New Zealand Ministry of Agriculture and Forestry (MAF) during the Painted Apple Moth (PAM) eradication programme to help focus public sentiment on the moth as the enemy, not the eradication activities or MAF itself (Materials courtesy MAF, Wellington, NZ)

Light Brown Apple Moth, *Epiphyas postvittana* (Walker), in California (Suckling and Brockerhoff 2010). Both programmes were hindered by public resistance largely based on mistrust and misinformation. In the successful eradication of Painted Apple Moth, *Teia anartoides* Walker, in Auckland, New Zealand (Suckling et al. 2007), about 30 % of the budget was spent on research and operations, with the remainder spent on communication and health monitoring to ensure that the health and well-being of 180,000 people were not affected by aerial spray of a bacterial insecticide formulation (Fig. 14.1). Similarly, large investments were made in monitoring human health and non-target impacts in Asian Gypsy Moth, *Lymantria dispar* (Linnaeus), eradication programmes in Washington, Oregon and North Carolina during the early 1990s (Kean et al. 2009). In other cases, host destruction has been the most controversial area (Smith et al. 2009). Above all, outreach and education efforts must utilize the newest social media devices as well as traditional forms of communication (Chap. 8).

We see renewed interest in population ecology to better understand the role of population effects that occur at low density (such as the interaction between density-independent and density-dependent factors), including deliberate intervention tactics and their life-stage targets. Tactics that change the Allee threshold, at which the growth of small founder populations becomes positive, can be very useful, because they help to drive invader populations to extinction (Liebhold and Tobin 2008). Use of combinations of tactics such as the release of pheromones for mating disruption and the Sterile Insect Technique (SIT) can lead to super-additive outcomes through their positive interactions at low pest densities (Suckling et al. 2012). This deployment of combined tactics has helped foster the development of concepts such as "Integrated Pest Eradication" (see Sect. 14.6). Any eradication is must be area-wide and consider the total pest population. Of course, the population must be contained while the programme is conducted. Often this is accomplished by regulatory restrictions to slow the artificial spread and population suppression measures along the spreading/leading edge of the infested area.

14.2 Chemical Controls

Insecticides of one form or another have been used in all insect eradication programmes since the 1800s (Metcalf and Metcalf 1993). Early eradication programmes used inorganic arsenical or other environmentally hazardous and/or persistent materials that are no longer acceptable (Fig. 14.2), including nicotine and organochlorines such as DDT. Today, governments consider the hazardous properties of different options and weigh them against efficacy in a Cost Benefit Analysis. A combination of different insecticides with different properties may be used depending on the target pest and the circumstances of where the infested area is located. For example, less ecotoxic materials (such as insect growth regulators) may be applied near waterways, or biopesticides and pheromones may be acceptable when applied over human populations. Pheromones and other attractants, which are not insecticidal, represent a minimally hazardous class of chemical tools for managing certain insect groups such as moths and beetles (El-Sayed 2011). These are considered elsewhere in this chapter (see Sect. 14.3).

Fig. 14.2 Early Gypsy Moth eradication efforts in the 1920s included burning vegetation and widespread spraying with chemicals such as lead arsenate (Photo courtesy USDA-APHIS-PPQ-CPHST, Buzzards Bay, MA)

14.2.1 Classes of Insecticides

Insecticides operate by different modes of action, including poisoning the insect nervous system or disrupting endocrine processes with man-made analogues of natural products (Metcalf and Luckmann 1994; Metcalf and Metcalf 1993). Some insecticides operate through direct contact (e.g. aerosols) or require ingestion while feeding (*Bacillus thuringiensis* var. *kurstaki* or *Btk*), and a few may be acquired systemically through the plant (neonicotinoids). Some insecticides are very short-lived (e.g. biological insecticides) and could require repeated applications as a compromise for greater environmental safety. These properties determine the types of application tactics that must be considered to achieve eradication and manage environmental risks (Dubey and Patyal 2007; Plapp 1991). Table 14.2 shows examples of different types of insecticides, some of which were recommended for use in California against recent incursions there.

Organophosphates are the oldest class of insecticides still in general use (Metcalf and Metcalf 1993). They disrupt the insect's nervous system by binding to enzymes involved in signal transport (e.g. acetylcholinesterase), usually causing rapid death. Organophosphates are highly effective against insects, but unfortunately also affect a relatively wide range of other organisms, including vertebrates (Metcalf and Metcalf 1993). Similar to organophosphates, carbamates also affect the insect's nervous system, but they generally are less persistent and somewhat less toxic (Metcalf and Luckmann 1994).

Pyrethroids form a major class of insecticides and are based on analogues to natural pyrethrin insecticides extracted from plants (Krieger 2010; Metcalf and Metcalf 1993). The analogues vary enormously in persistence and toxicity depending on their structure, and typically act very quickly to cause "knock-down", although recovery can eventuate. Pyrethroids are more selective towards insects than the insecticide classes listed above, although fish and amphibians are highly sensitive to them.

Insect Growth Regulators (IGRs) *and Ecdysone agonists* represent another major class of insecticides (Krieger 2010; Yu 2008; Metcalf and Metcalf 1993). IGRs tend to be very selective, although some persist in the environment (Krieger 2010; Yu 2008; Metcalf and Metcalf 1993). These compounds target insect endocrine systems, and typically disrupt development, rendering them slower acting than other insecticides. Their selectivity and generally low environmental hazard make them attractive for use in sensitive ecosystems.

Neonicotinoids, recent synthetic analogues of nicotine sulfate insecticides, have a much lower acute mammalian toxicity, greater persistence, and many have systemic properties (Krieger 2010; Yu 2008). This can mean that application to soil, directly as a cover spray, or through injection into the plant leads to uptake and transport throughout the plant's living tissues, which can give excellent foliar coverage and reach cryptic insects.

Other insecticides could be considered on a case-by-case basis, although for eradication programmes only those with excellent efficacy should be considered. Anti-feedants, certain biological insecticides, and other products may not have

Table 14.2 Products representing most insecticide classes available for insect eradication. Suitability and formulations may differ depending on target

Class	Examples of active ingredients	Target life stage	Comment
Avermectin	Emmamectin benzoate	Larvae	Selective to insects
Biological insecticide	*Bacillus thuringiensis*	Larvae	Weakly active for 72 h, very selective
Biological insecticide	*Cydia pomonella* granulovirus	Larvae	Ultra-selective, short-lived
Biological insecticide	Gypsy Moth Nucleopolyhedrosis Virus	Larvae	Ultra-selective, short-lived
Carbamate	Carbaryl	Larvae and adults Very effective	Broad-spectrum, short-lived
Ecdysone agonist	Methoxyfenozide, Tebufenozide	Larvae only Highly effective 28+ days	Moderately persistent
Insect Growth Regulator	Diflubenzuron	Eggs Unknown efficacy	Moderately persistent
Insect Growth Regulator	S-methoprene, Pyriproxyfen	Larvae	Narrow-spectrum
Juvenile Hormone Analogue	Fenoxycarb	Eggs and larvae	Moderately persistent
Neonicotinoid	Acetamiprid, Thiacloprid, Imidacloprid	Larvae Unknown efficacy	Narrow-spectrum
Organophosphate	Chlorpyrifos, Phosmet, Diazinon, Dichlorvos, Malathion, Dimethoate, Trichlorfon	Eggs, larvae, adults Excellent control	Broad-spectrum
Pyrethroid	Deltamethrin, Cypermethrin, Lambda-cyhalothrin	Larvae and adults Highly effective	Broad-spectrum
Phenyl Pyrazole	Fipronil	Larvae	Narrow-spectrum
Spinosyn	Spinosad, Spirotetramat	Larvae + adults Very effective 10–14 days	Low-persistence, selective to insects

sufficient efficacy to contribute to insect eradication and are more appropriate for pest management. However, *Bt* and Spinosad have both been used effectively in Gypsy Moth and fruit fly eradication programmes, respectively (Hajek and Tobin 2009; Burns et al. 2001). Emmamectin benzoate when applied through injection shows excellent efficacy against the Emerald Ash Borer, *Agrilus planipennis* (Fairmaire).

14.2.2 Use of Insecticides

The target organism, the size and density of its population(s), characteristics of its host, and the physical and environmental circumstances will all contribute to the selection of insecticides and the application techniques. Aerial application of insecticides may be necessary when the area to be covered is large or the terrain is inaccessible. For instance, Red Imported Fire Ant (*Solonopsis invicta* Buren) baits were aerially applied over a steep and dangerously unstable hillside in New Zealand, in the interest of worker safety (Kean et al. 2009). Aerial applications of formulations containing *Bacillus thuringienis* (Foray 48B) were used in three recent campaigns in urban New Zealand, against Tussock Moth (Gypsy Moth group). The largest programme covered more than 12,000 ha, with up to 40 aerial applications of Foray 48B (Suckling et al. 2007), supported by spray deposition modeling (Richardson et al. 2005). Individual applications gave 85 % mortality of larvae on sprayed foliage (Charles et al. 2005), and this information was used in a model to predict the impact of multiple applications, and ultimately to declare successful eradication (Kean and Suckling 2005).

In a large successful containment programme for European Gypsy Moth (*Lymantria dispar* L.) ("Slow the Spread") in the USA, *Bt* and mating disruption formulations have been applied to hundreds of thousands of acres annually (Sharov et al. 2002). Aerial application of S-methoprene on salt marsh was critical to the successful eradication of the Southern Salt Marsh Mosquito, *Aedes camptorhynchus* (Thomson), in New Zealand (Yard 2008). Mosquito larvae arriving in used-car tires with water was the likely pathway that led to the need for the eradication of this pest and represents an example of commerce (in used tires) presenting a potentially greater risk than the economic benefit. Aerial application of insecticide bait sprays, such as malathion and NuLure or Spinosad and GF-120, which utilize hydrolyzed proteins as food-based attractants, have traditionally been used to lower fruit fly populations prior to the initiation of aerial releases of sterile insects when combating Mediterranean or other fruit fly incursions in the USA (Kean et al. 2009).

More recently, the use of bait sprays has come under public criticism because of perceived environmental and health-related problems, and lawsuits have been filed to stop application (Dyck et al. 2005; Burns et al. 2001). Indeed, aerial application of any pesticide, pheromone or other pest management materials over residential areas is controversial and the benefits must be explained to the people being affected. As stated earlier, resources focused on public outreach and education and health monitoring may eclipse those expended on direct pest management aspects of the programme.

Ground application is often preferred for small areas, although coverage can be problematic. Ground applications of pesticides seem to be more easily accepted by the public even though in some cases more material is applied per unit area. However, in some cases the insecticide used is the point of contention. The ground application of chlorpyrifos and deltamethrin against Painted Apple Moth in New Zealand was discontinued in response to community pressure, and while undoubtedly more effective, the compounds were replaced by multiple applications of *Btk* (Suckling et al. 2005). Although aerial applications of bait sprays now seldom are used in the USA for fruit fly eradication programmes, key states at risk for exotic fruit fly introductions still regularly use ground applications under Special Local Needs (SLN) permits to treat host plant material (e.g., oviposition, mating and resting sites) immediately around fly finds. In addition, insecticides have been applied as soil drenches under host trees at infested sites to kill larvae or pupae that may have left or fallen from infested fruit. Currently, the only effective chemicals available for this purpose are organophosphates such as diazinon and their use is now greatly restricted and requires emergency crisis exemptions.

Tree injection has been used against a range of recent pest incursions in the USA, where amenity values of urban forests can be high (Kean et al. 2009). Tree injection, with various formulations of imidacloprid, is used regularly in the Asian Longhorn Beetle Programme (*Anoplophora glabripennis* (Motschulsky)) to treat trees near known infested trees. Known infested trees are removed and destroyed, but newly attacked trees are difficult to detect even when examined by experienced tree climbers. Treatment of surrounding trees acts to eliminate any residual population, either by killing the larvae still feeding in the cambium or by killing emerging adults feeding on the leaves of treated trees.

14.3 Pheromones and Other Semiochemicals

Pheromones and other odorants have important roles in intra-specific and inter-specific communication (Howse et al. 1998; El-Sayed 2011). Well-known examples are the use of plant volatiles for host location by plant-feeding insects and the attraction of male moths to female sex pheromones. Because these and other ‘semiochemicals’ are often highly species-specific, they can be very valuable tools for invasive species eradications and associated activities. Such potent attractants are commonly used for detection (i.e., discovering the presence of an invader), delimitation (i.e., determining the geographic distribution), and population monitoring (i.e., assessing the relative abundance), which are all critical elements of eradication programmes. Semiochemicals can also be used directly for population control in techniques such as mass trapping, lure-and-kill, and mating disruption. Although these last-named techniques are not yet mainstream components of eradication programmes, we see considerable potential for their application as ‘greener’ alternatives to conventional control techniques that rely on pesticides (Brockerhoff et al. 2010).

14.3.1 Trapping for Detection, Delimitation, Monitoring

Early detection is an essential prerequisite for successful eradication (Myers and Hosking 2002), while the distribution of an invader is still limited and amenable to area-wide control. Traps baited with pheromones and other attractants can be highly effective for the detection of incipient populations, potentially well before any pest damage becomes apparent. Although some traps rely on other mechanisms (such as attraction to specific colours/wavelengths or flight intercept traps), they are usually less powerful than attractant-baited traps (Howse et al. 1998). Pheromones and other attractants are known for many species (El-Sayed 2011), and they may be available for purchase as synthetic compounds. However, for many other species such attractants remain to be discovered, and suitable long-range attractants for some taxa may not exist. Some of the most extensive detection programmes target the Asian Gypsy Moth, a highly invasive defoliator mainly of oaks and other broadleaved trees. In the USA, in a large, comprehensive, multi-agency containment and exclusion programme (Gypsy Moth Programme Manual – http://www.aphis.usda.gov/plant_health/plant_pest_info/gypsy_moth/index.shtml), approximately 90,000 traps were placed in the Slow-the-Spread area to monitor the expansion of the European strain in 2010. During 2010, an additional 248,500 traps were deployed to survey areas not infested by the European strain, and in ports for detection of introductions of the Asian strain (respectively, pers. comm., Leonard, USDA-FS STS National Programme Manager, and Spaulding, USDA-APHIS-PPQ Gypsy Moth Programme Manager). This large effort to detect new isolated infestations of the European Gypsy Moth in western and southern states has been ongoing since the late 1970s.

APHIS began to monitor ports and other high-risk sites for introductions of the Asian Gypsy Moth in the early 1990s (Mastro pers. comm.). Similar programmes aimed at Gypsy Moth are carried out in Canada and New Zealand (Ross 2005; Régnière et al. 2009). Detection trapping programmes using pheromones and host attractants have also been implemented for wood borers and bark beetles in several countries (Brockerhoff et al. 2006; Rabaglia et al. 2008). Recognizing the importance of early detection, the USDA established a nationwide system to track more than 400 pests of concern. The list of pests is reviewed and reprioritized annually. This Cooperative Agricultural Pest Survey (CAPS) Program is managed cooperatively by USDA-APHIS and state departments of agriculture, with universities, industry groups, and natural resource protection organizations as partners (http://www.aphis.usda.gov/plant_health/plant_pest_info/pest_detection/pestlist.shtml).

When an invasive species has been detected, traps are often used to delimit the affected area and to monitor population trends. For these goals, the number and density of traps are likely to be much greater than for detection trapping. If no known long-range attractant exists, then caged live insects (e.g., female moths) may be used for this purpose. This procedure was employed during eradication of the Painted Apple Moth in NZ (Suckling et al. 2005) and initially in the Slow-the-Spread program for Cactus Moth, *Cactoblastis cactorum* Berg, in the USA until a pheromone-based attractant was identified (Bloem et al. 2003) (Fig. 14.3).

An interesting variation of detection and delimitation trapping programmes was implemented during the eradication campaign against Dutch Elm Disease in Auckland, NZ (Gadgil et al. 2000). Over 200 traps baited with the pheromone of the Elm Bark Beetle, *Scolytus multistriatus* (Marsham), were used to determine the

Fig. 14.3 Wing-type traps with sticky bottom panels initially baited with live females to survey for the invasive Cactus Moth, *C. cactorum*, until a pheromone-based lure was developed. Live females were changed every 3–5 days; the current lure is replaced every 4–6 weeks (Images courtesy of USDA-ARS-CMAVE, Tallahassee, FL)

presence of the fungus (*Ophiostoma ulmi* (Buisman) Nannf.) causing Dutch Elm Disease by isolating fungal spores from this beetle, which is the obligate vector of this fungus. Sometimes, when a long-range attractant is not available, traps are used that depend on host plant odours and/or other behavioural cues. Such is the case in the Emerald Ash Borer programme in the USA, which depends on visually attractive traps baited with host plant volatiles. Even though the trap is not as effective as some pheromone-baited traps, it has allowed the programme to conduct effective detection and delimitation surveys (Crook et al. 2008).

14.3.2 Mass Trapping

The use of mass trapping for pest control is an intriguing concept, and it has received some attention in relation to eradication (El-Sayed et al. 2006). However, important limitations to using mass trapping for population control are founded in the population ecology of species (Howse et al. 1998; Yamanaka 2007). In order to be effective in reducing the population size of an invader, it is likely that 90 % or more of the population should be removed. Because attractants are often only available for males (e.g., male moths responding to female sex pheromones), effects on the ability of females to reproduce are less pronounced, particularly in species where males can mate many times. Consequently, mass trapping may only be effective if the number of traps approaches the number of individuals. Therefore, mass trapping may require the deployment of an unrealistically large number of traps. In unpublished studies with Gypsy Moth, a minimum of 25 traps/ha were required to eliminate mating with simulated low density populations (Schwalbe et al. 1984). However, smaller incipient populations may be controlled by mass trapping, especially in conjunction with other tactics (El-Sayed et al. 2006).

14.3.3 Lure and Kill

The attract- or lure-and-kill tactic involves combining a pheromone or other attractant with a contact insecticide and a viscous carrier material (typically a paste, gel or wax). Here, the goal is to control the target species by attracting it to large droplets of the formulation that are applied to a suitable substrate in the treatment area, causing mortality shortly after contact (Brockerhoff and Suckling 1999; El-Sayed et al. 2009). This tactic resembles mass trapping, but because droplets can easily be applied in large numbers, lure-and-kill is much more suitable for large-scale area-wide control and more cost-effective than mass trapping. Lure-and-kill is now well established for pest control (e.g., in orchards – Suckling and Brockerhoff 1999), but it has not been used often for eradication (El-Sayed et al. 2009). However, its use for eradications of fruit flies (mainly *Bactrocera* spp.) and Boll Weevil (*Anthonomus grandis* Boheman) were considered successful (El-Sayed et al. 2009). Although this tactic involves application of an insecticide, it is more acceptable than many other tactics because few species (other than the target) are likely to come into contact with the formulation, and it is applied at considerably lower rates than other insecticide applications (such as sprays).

Several other related approaches involving insecticides or other treatments have been considered. For example, bark beetles can be attracted to pheromone-baited trap trees that are subsequently treated with an insecticide, debarked or destroyed by burning or chipping, to prevent the emergence of beetle brood. Similarly, bait sprays have been used extensively in fruit fly eradication programmes. However, all lure-and-kill approaches are generally less feasible as eradication treatments when invader populations are large and widespread.

14.3.4 Mating Disruption

Mating disruption is usually considered a tactic for use on low-density populations and is ideal for use after a pest introduction but before populations have increased. Disruption also can be used after a conventional insecticide treatment has reduced the pest insect's density. Synthetic pheromone formulations can be applied to the environment to achieve the effect that male insects are no longer able to locate 'calling' females, thereby preventing fertilization of eggs (Howse et al. 1998). This method requires the release of pheromones such that their aerial concentration makes orientation impossible, because of habituation of pheromone receptors and the central nervous system (Cardé 2007).

An alternative mechanism, often referred to as 'false trails', may also occur under certain circumstance where males follow plumes or trails of pheromone from synthetic sources rather than those emitted by 'calling' females. If the number of pheromone release points (relative to the number of females) is much greater, then the likelihood of a male encountering a female is reduced. Mating disruption involves comparatively small quantities of pheromone (typically non-toxic and highly target-specific) and is considered one of the most environmentally friendly pest control methods. Mating disruption is well established as the method of choice for the control of numerous pests, primarily moths (Cardé and Minks 1995; Cardé 2007).

In recent years the use of insecticides has become increasingly controversial (especially when applied by aircraft), and mating disruption has been increasingly considered an effective alternative. Mating disruption is amenable to large-scale, area-wide application, and is used over very large areas in the eastern and central USA to achieve the localized eradication of European Gypsy Moth along the expanding edge of the infested area. In this ‘Slow-the-Spread’ programme, aerial application of the Gypsy Moth pheromone ‘disparlure’ has become the primary tool since 2000 (Sharov et al. 2002) and by 2010 over 1.4 million hectares will have been treated (USFS 2009). Mating disruption, in combination with other tactics, recently has been used for area-wide eradication of Pink Bollworm, *Pectinophora gossypiella* (Saunders), populations (Walters et al. 2000; Tabashnik et al. 2010).

Recent efforts to eradicate the Light Brown Apple Moth (*Epiphyas postvittana* (Walker) in California explored the use of mating disruption as the primary treatment (Suckling and Brockerhoff 2010). Because of the large area involved, and difficulties with access to some places, aerial pheromone application was the only viable option (Suckling and Brockerhoff 2010). A disadvantage of mating disruption is that for some time after a pheromone application, pheromone traps for monitoring are ineffective. However, traps in Gypsy Moth mating disruption areas provide an indirect measure of mating success. Areas where the treatment has not been totally effective can be defined, even though most male capture is shut down. This is actually an indicator of efficacy, but it worries programme managers accustom to trapping information.

14.4 Sterile Insect Technique (SIT)

SIT plays a significant role in containment and eradication programmes for numerous pests around the world (Klassen and Curtis 2005). SIT is defined by the International Plant Protection Convention (IPPC) as: “. . . a method of pest control using area-wide inundative releases of sterile insects to reduce the fertility of a field population of the same species” (FAO 2005). For SIT to be used as an operational method of pest control, several requirements must be met. These include the application of economic and effective methods of mass production for the target pest, an effective sterilization method and dose, and efficient handling and release methods for sterile insects (Dowell et al. 2005; Hendrichs et al. 2005).

To achieve control of a field population an effective over-flooding ratio of sterile to wild insects must be achieved that reduces the probability of a fertile mating so that with repeated releases over time, no offspring are produced and the local pest population is eliminated (Hendrichs et al. 2005). To achieve this goal, the released sterile insects must compete and mate successfully with their wild counterparts, and pest populations must be low such that effective over-flooding ratios can be achieved with reasonable economic release rates. Because sterile insects must perform well against the wild target pest, a sterile insect quality management system is a critical element of for programme management (Calkins and Parker 2005; Simmons et al. 2010). As with mating disruption, treated areas must be sufficiently large and/or isolated to minimize the effect of immigration from surrounding areas (Barclay et al. 2011).

SIT is not a stand-alone eradication tool. However, the use of SIT as a control tactic has many advantages, including species specificity and compatibility with the use of most other control tactics such as mating disruption, biological control, cultural/mechanical control and the use of pesticides (Klassen 2005; Carpenter 2000). When these methods are used together on an area-wide basis, SIT can form the foundation for a very powerful approach that has been and continues to be used successfully for eradication and long-term suppression (Hendrichs et al. 2007; Pimentel 2007).

14.4.1 Strategies

Four strategies are used in SIT to create a plant protection tool. These strategies are prevention, containment, suppression, and eradication (Hendrichs et al. 2005). All these strategies have been used together with other Integrated Pest Management (IPM) tactics compatible with use of the SIT in an area-wide control programme approach with varying degrees of success (Hendrichs et al. 2005).

Prevention: A Preventative Release Strategy or Programme (PRP) can be employed in a pest-free area at high risk of invasion. For example, consider an agricultural area or environment with suitable conditions for pest establishment and development (Hendrichs et al. 2005). The Los Angeles basin in California is at high risk of invasion by tephritid fruit flies (Barinaga 1991). The climate is warm, many potential host plants are grown in the basin, and it has a very busy port (Long Beach) with a very high volume of international air travelers (LAX) bringing in fruits that may be infested. The cooperative Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann), exclusion programme has been operating in this region since 1996, releasing over 250,000,000 flies per week year round over a 2,155 mile^2 area (CDFA http://www.cdfa.ca.gov/phpps/pdep/prpinfo/index.html, Enkerlin 2005). The benefits of this programme have an estimated annual savings of $1.3–1.9 billion USD in costs that would be required for control, regulatory and quarantine compliance and loss of markets (Enkerlin 2005).

Containment: Containment programmes use release of sterile insects to prevent the spread of an established pest into an uninfested area by providing a barrier or buffer of sterile insects between an infested and an uninfested area (Hendrichs et al. 2005). This tactic may consolidate gains made in an eradication programme (Hendrichs et al. 2005), or prevent a pest coming into an area at risk to invasion. The barrier zone in Panama in the Darien Gap serves as an example. Sterile New World Screwworm Flies are released to prevent reinvasion into the eradicated zone in Panama. This operational eradication programme has now entered a maintenance phase (Hendrichs et al. 2005; Vargas-Teran et al. 2005).

One of the longer running containment programmes has been the release of sterile Pink Bollworm in large cotton production areas in the Central Valley of California for more than 40 years, to stop the spread of Pink Bollworm from the infested cotton production areas in southern California (Hendrichs et al. 2005; Bloem et al. 2005). During the programme's operation, regular interceptions of wild Pink Bollworm on monitoring traps at the southern end of this containment area have demonstrated the effectiveness of using SIT to block the invasion and establishment of this pest (Tabashnik et al. 2010). With the current success of the Pink Bollworm eradication programme, the need for this containment may diminish.

Suppression: For suppression, SIT is used as a control tactic to maintain the pest below an economic threshold. The first SIT efforts were viewed as primarily eradication tactics and were not considered cost effective as regular control tactics. However, improvements in rearing technology, market forces, consumer demand for pesticide-free fruit, and restrictions on the use of certain pesticides, have made the routine use of SIT for pest control a viable economic option (Hendrichs et al. 2005). Many programmes now use SIT in this manner, including: Several programmes for Mediterranean Fruit Fly in the Middle East, South Africa and Spain (Cayol et al. 2004; Hendrichs et al. 2005; Enkerlin 2005); Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel), in Thailand (Orankanok et al. 2007); as well as several programmes for moths such as Codling Moth, *Cydia pomonella* (L.), on apples in British Columbia, Canada (Bloem et al. 2007b); False Codling Moth, *Cryptophlebia leucotreta* (Meyrick), on citrus in South Africa (Carpenter et al. 2007); and Pink Bollworm on cotton in desert valleys of southeastern California (Walters et al. 2000; Bloem et al. 2005). A highly developed rearing and release system has helped to facilitate expansion of the Pink Bollworm programme and a shift of the goal to eradication of this pest from North America (see below).

Eradication: Successful eradication efforts using SIT require the operation of a coordinated area-wide control programme where several compatible technologies are applied, reducing the pest population so that sterile insect rearing and release costs are not prohibitive. Several successful eradication campaigns against plant pests have used SIT as one of the primary tactics (Hendrichs et al. 2005). The New World Screwworm, *Cochliomyia hominivorax* (Coquerel), was eliminated from North America using SIT in combination with careful inspection and treatment of cattle (Vargas-Teran et al. 2005). Several tephritid fruit fly species in diverse agricultural regions in California, Mexico, Florida, and Japan and several moth species also have been successfully eradicated (Kean et al. 2009; Suckling et al. 2007; Hendrichs et al. 2005; Enkerlin 2005; Bloem et al. 2005). A large, 10 year, area-wide campaign against the Pink Bollworm has driven this pest to undetectable levels across the south-western cotton belt in four states and northern Mexico (Fig. 14.4). This programme uses a combination of tactics, including regional widespread planting

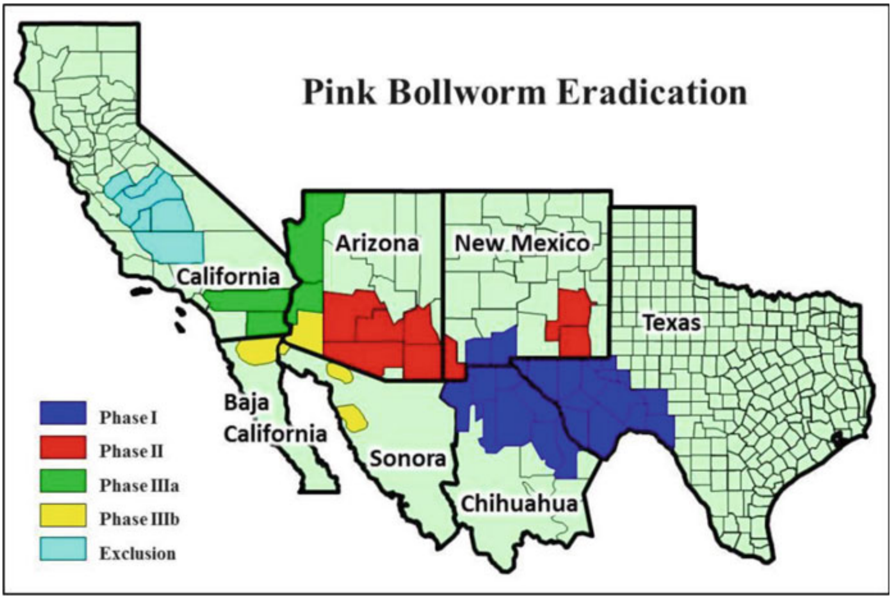

Fig. 14.4 Incremental phases of the international Pink Bollworm eradication programme. This programme uses a combination of tactics, including *Bt*-cotton, sterile insect technique (SIT), mating disruption and cultural control. The SIT component is considered particularly important as a final control measure to achieve eradication. Exclusion activities to prevent establishment of PBW in the San Joaquin Valley of California have been ongoing since 1968. *Phase I* of the eradication programme began in 2001 in the El Paso and Trans Pecos regions of western Texas. Operations in south-central New Mexico and the state of Chihuahua, Mexico, began in 2002. *Phase II* began in 2006 with the addition of cotton growing areas in southern Arizona, as well as southwestern and southeastern New Mexico. *Phase IIIa* began in 2007 with the addition of cotton acreage in western Arizona and southern California. *Phase IIIb* began in 2008 with the addition of Yuma County, AZ, Sonora, Mexico, and San Luis and Mexicali, BC, Mexico. As of December 2011, *Phases I* and *II* were 99% complete (i.e., PBW populations had been reduced by 99 % relative to preprogram levels) and being monitored for eradication, with sterile moth releases continuing throughout much of the area to prevent reestablishment. *Phase III* was approximately 98 % complete, with on-going monitoring and treatments using *Bt*-cotton, mating disruption and SIT where trap captures indicated breeding populations (Map and information updated by Walters 2011, APHIS-PPQ-CPHST, Phoenix, AZ, from El-Lissy 2009, APHIS-PPQ, Riverdale, MD)

of genetically modified cotton expressing the *Bt* toxin, SIT, mating disruption, and cultural control methods (Tabashnik et al. 2010). A similar application of SIT combined with integrated area-wide tactics against a much smaller infestation of the Painted Apple Moth in New Zealand resulted in the eradication of this species after 2 years of programme operation (Suckling et al. 2007).

Eradication campaigns using SIT must be well organized and operated in a coordinated fashion to ensure compatible technologies are used appropriately and the sterile insect resource is used effectively (Hendrichs et al. 2005). As in any operational programme, comprehensive monitoring and data management are critical to ensure that timely information is delivered to programme managers for “decision making” (Brockerhoff et al. 2010).

14.4.2 Choice of Strategy

The direct and indirect benefits of using SIT in control programmes are summarized in Enkerlin (2005) and Hendrichs et al. (2005). These include direct benefits such as increases in fruit or commodity quality and yield by decreased damage, reduction of pesticide use, reduction in production costs, and increased market access with the maintenance of Pest-free Export Zones. The selection of which strategy to use may depend on economics and other factors such as size of the established population, proximity to infested areas, and pest biology (Hendrichs et al. 2005). The most cost-effective option is prevention, compared with the costs of control or eradication of a pest after it becomes established (Hendrichs et al. 2005; Enkerlin 2005). If the pest is already established, then the option of eradication, while expensive, will return the most benefits relative to long-term options of suppression or containment (Enkerlin 2005). Factors such as the costs of operating permanent quarantine and monitoring activities, coupled with the lost opportunity costs of exporting agricultural commodities, must also be considered when choosing a strategy (Enkerlin 2005).

14.5 Biological Control

Two forms of biological control (classical and augmentative/inundative) have potential roles in eradication programmes. *Classical Biological Control* is the deliberate attempt to introduce exotic natural enemies to help reduce the densities of a target pest, usually an invasive species (Hoddle and Syrett 2002). Classical Biological Control programmes represent a process that can take 3–5 years or longer before biological control organisms are released. Therefore, they are not usually considered eradication tools. In fact, for many years biological control was not investigated as a management option for a new exotic pest by regulatory agencies until after eradication efforts were deemed no longer feasible. Conventional wisdom held that an early interest in biological control would send the wrong message to trading partners that regulators were not serious about eradication and that growers were already planning to live with the pest.

14.5.1 Classical Biological Control

Classical Biological Control for eradication attempts has also been dismissed because of the conventional assumption that success would result only in a stable, self-sustaining balance between the new natural enemy and their now less numerous, but not eliminated, target pest. However, introduced arthropod natural enemies sometimes appear to cause local insect and plant extinctions (Murdoch et al. 1985). In these cases the pest persists area-wide because of reintroductions from outside the area of eradication. For instance, two parasitoids were introduced into Nova

Scotia to control the invasive European Winter Moth, *Operophtera brumata* (L.), a defoliator of hardwood trees. Within years of the last introduction, the moth essentially disappeared from hardwood forests but was still present in apple orchards and shade trees. In another case, extensive sampling of the Larch Sawfly, *Pristophora erichsonii* (Hartig), in Manitoba following parasitoid introductions suggested a pattern of local extinction and reinvasion. Perhaps local extinctions effectively could be monitored and expanded as part of an Integrated Pest Eradication Programme. Certainly the inadvertent decimation of species such as the American Chestnut (*Castanea dentata* (Marsh.) Borkh.) By invasive organisms illustrates the potential impact of freely reproducing natural enemies.

Perhaps a more likely role for Classical Biological Control in eradication efforts is simply the suppression of pest populations to the point that other techniques become more practical. For example, the argument has been made repeatedly that lowering tephritid fruit fly populations through the establishment of parasitoids would make future SIT eradication programmes more effective and affordable (Hendrichs et al. 2005). In the most spectacular instance, Hawaiian populations of the Oriental Fruit Fly (*Bactrocera dorsalis* (Hendel) plummeted by ~90% with the introduction of the egg-prepupal braconid parasitoid *Fopius arisanus* (Sonan) (Haramoto and Bess 1970; Newell and Haramoto 1968). Confirmation that the cause of the decrease was indeed the natural enemy was obtained when the pest and parasitoid were recently reunited in Tahiti. Again, fly numbers dropped precipitously (Vargas et al. 2007).

Natural enemy establishment could also help maintain barriers erected with other techniques. For example, there is a multi-national attempt to create an SIT/host-removal barrier to prevent the spread of the invading cactus moth along the southeastern USA coastline into the western cactus-rich states and ultimately into Mexico (Bloem et al. 2007a). If this barrier can be erected, then it would probably be more effective and more cheaply sustained if pest population pressures were lower on its infested borders. Similarly, since 1996, APHIS, State and City cooperators in New York, Illinois, and New Jersey, and the US Forest Service have undertaken eradication activities against the Asian Longhorn Beetle by imposing regulated boundaries, conducting survey and control activities around confirmed sites, removing infested trees, and planting trees to restore areas where trees were removed (USDA APHIS ALB Cooperative Eradication Programme Strategic Plan http://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/index.shtml). Although the programme has deregulated some areas, program activities are expected to continue at least until 2020. Biological control, if available for this insect, might be useful to help minimize spread and possibly reduce the number of trees removed, during a long eradication process.

14.5.2 Augmentative/Inundative Biological Control

Augmentative/Inundative Biological Control (artificially increasing the numbers of a host-specific parasitoid or predator) has been frequently proposed as a viable eradication or area-wide pest-management tool (Knipling 1992), particularly as a method of dealing with pests of high value crops. Augmentation has been used extensively in greenhouses where growers of such crops as cut flowers prefer very low to nonexistent pest numbers. Various models predict the possibility of target eradication and some rely on the idea that since the seasonal growth of natural enemy populations tends to lag behind that of their target, an early-season mass release will inflict higher percentage mortalities than might occur in nature. In this way, low initial pest numbers can be driven even lower, perhaps to the point of extinction (Liebhold and Tobin 2008).

For practical purposes, any attempt at eradication through Augmentative Biological Control should first address several important concerns. First is the vulnerability of the target. If "refugia" habitats exist where the pest is safe from attack, then eradication becomes less likely. For example, tephritid fruit fly larval parasitoids (even those with the longest ovipositors) have difficulty reaching hosts that feed deep in the pulp of large fruits. Releases of parasitoids when and where large fruits are numerous would be less effective than releases into habitats where fruits are small. Fortunately some pest fruit flies are attacked by parasitoids that oviposit into eggs, (e.g., the highly successful *F. arisanus* previously mentioned) and host eggs are generally much closer than larvae to a fruit's surface.

The second concern is to determine natural enemy release rates (Parrella et al. 1992). This is often not easy to accomplish experimentally, particularly under ecologically realistic conditions in the field. Pests and natural enemies housed in large field cages may provide more opportunities to replicate different treatment levels, but these comparisons present difficulties of their own, such as preventing natural enemy dispersal.

A third concern is the expense of rearing, transporting and releasing natural enemies. The cost of mass-reared natural enemies can be high but must be compared with the alternatives. For example, Augmentative Biological Control of the Two-Spotted Spider Mite (*Tetranychus urticae* Koch) in strawberries through releases of *Phytoseilius persimilis* Athias-Henriot was shown to be possible many years before pesticide resistance, as well as the loss of some chemical controls and the rising costs of others, led to its widespread use (Parrella et al. 1992). Augmented releases of tephritid parasitoids such as *Diachasmimorpha longicaudata* (Ashmead) have suppressed Caribbean Fruit Fly, *Anastrepha suspensa* (Loew), by as much as 95 %. However, to be economically practical a parasitoid must be superior to its alternative, a sterile fly, such that the additional rearing costs are justified (Sivinski et al. 1996). In this case, effective release rates for parasitoids may be lower than those used for sterile males. Also, methods can be developed to reduce rearing costs by exploiting sexually dimorphic developmental rates to harvest mass-reared female fly larvae for exposure to parasitoids, simplifying release procedures by irradiating hosts to prevent adult fly eclosion, and perhaps manipulating parasitoid sex ratios with the endosymbiotic bacteria *Wolbachia* so that only female parasitoids are produced.

Augmentative Biological Control can be easily used in conjunction with SIT (Gurr and Kvedaras 2010). For example, the Australian Painted Apple Moth

(*Orgyia anartoides* (Walker) was eradicated from New Zealand through combined applications of the entomopathogen *Bt* and sterile males (Suckling et al. 2007). Arthropod natural enemies and SIT together might be particularly effective as an eradication technique because the control tactics can complement one another (Knipling 1992). This is because the attack rates of insect parasitoids are often positively density dependent. Foraging efficiency is highest when host populations are high, and the efficacy of SIT is negatively density dependent. Overflooding is more easily achieved with low target populations. Thus, the success of parasitoids makes SIT more potent. Models predict that the combination of the two can result in a synergistic effect, i.e., together their capacity to control a pest population is greater than the sum of their individual contributions. Synergism has been demonstrated in a greenhouse experiment comparing the ability of parasitoids, SIT and their combination to control the Onion Leafminer, *Liriomyza trifoli* (Burgess) (Kaspi and Parrella 2006). An economic bonus is bestowed on some combined release programmes because the rearing facilities and means of release for mass-reared parasitoids and sterile males are often similar and do not require separate infrastructures.

Recent interest in SIT + parasitoid augmentation has centered on the suppression of tephritid fruit flies. This is due, in part, to the desire to improve the substantial SIT + insecticide-bait-sprays efforts underway both to eradicate invasive populations and to maintain barriers such as along the Mexican-Guatemalan border which prevents the northward spread of the Mediterranean Fruit Fly. Experiments comparing the efficacy of fruit fly parasitoids + SIT to either technique alone have yielded mixed results.

A pioneering Hawaiian field study combined sterile Mediterranean Fruit Flies with a larval parasitoid, *Diachasmimorpha tryoni* (Cameron). The study suggested that the two techniques were most effective when employed together (Wong et al. 1992). For many years, Mexico has successfully released sterile *Anastrepha* spp. along with *D. longicaudata* in their efforts to create/maintain fly free and low prevalence areas. Mediterranean Fruit Fly populations developing in field-caged coffee (*Coffea arabica* L.) were significantly more suppressed by SIT + *F. arisanus* than SIT alone, although parasitoid augmentation alone was also often more effective than SIT (Rendon et al. 2006). Based on these results, the MOSCAMED fruit fly suppression programme along the Mexican-Guatemalan border now includes augmentative releases of *F. ceratitivorus* Wharton, a biologically similar species that specializes on Mediterranean Fruit Fly (Lopez et al. 2003) and is easier to mass-rear, in hotspot areas and areas where bait sprays are problematic (Fig. 14.5). Alternatively, sterile Melon Flies (*Bactrocera curcurbitae* (Coquillett)) released on a non-crop source of infestation were superior to mass-released larval parasitoids (*Psyttalia fletcheri* (Silvestri)) in suppressing the numbers of adult flies subsequently eclosing. Such diversity of outcomes emphasizes that the natures of the pest, the parasitoid, and the environment are all likely to influence the outcome of any biological control effort, including augmentation.

In addition to tephritid control, encouraging experiments have combined sterile moths and parasitoids. For instance, damage to fruit in cages containing fertile male

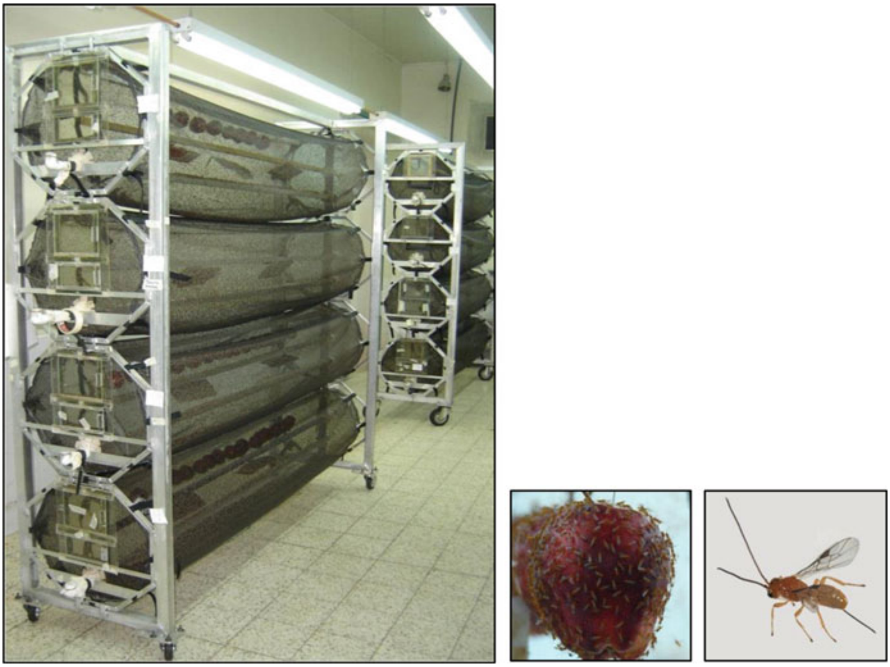

Fig. 14.5 The MOSCAMED programme in Guatemala is mass-rearing the egg parasitoid *F. ceratitivorus* to help combat the Mediterranean Fruit Fly. Apples are first pricked and exposed to Medfly adults for oviposition, and then placed in large sleeve cages for exposure to the parasitoid. In 2011, approximately 1.0–1.5 million parasitoids were released per week in hotspot areas to help eliminate recurrent Medfly detections in the coffee production region of southwest Guatemala (Photos courtesy USDA-APHIS-PPQ-CPHST, Guatemala City, Guatemala)

and female Codling Moths was lowest when sterile males and the egg parasitoid *Trichogramma platneri* Nagarkatti were introduced together (Bloem et al. 1998). In this case, the bisexual release of sterile moths may result in an abundance of sterile eggs being laid, which can then be capitalized upon through the inundative release of egg parasitoids. Field populations of the parasitoid might thus be maintained at more consistent and higher numbers and provide additional control. Similar lab and field cage experiments also were conducted to determine the acceptability and suitability of sterile False Codling Moth eggs to parasitism by *Trichogrammatoidea cryptophlebiae* Nagaraja (Carpenter et al. 2004).

14.6 Integrated Pest Management and Eradication

The United Nations Food and Agriculture Organisation (FAO) says "Integrated Pest Management (IPM) means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified, and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms" (www.fao.org/agriculture/crops/core-themes/theme/pests/ipm/en/). IPM is therefore an ecologically-based pest control strategy that favours methods that are least disruptive to the environment and ecosystem services, by using complementary tactics to reduce natality and increase mortality of pests. Tactics widely used in IPM include selective or narrow-spectrum insecticides, pheromones, biological control (Classical, Inundative or Conservation), host plant resistance, cultural management practices such as habitat manipulation, modelling for prediction and decision support, and other knowledge-based approaches (Maredia et al. 2003). In IPM programmes many pests are often present, although only a few may be key pests.

IPM has emerged as the "best practice" systems approach for managing established pests, while minimizing non-target impacts from interventions. IPM in the field can also be integrated with a post-harvest systems approach to the export of fruits or other affected commodities, by taking into account the reduction in risk of pest prevalence at each stage, from field and harvest through post-harvest handling or quarantine procedures (e.g., Jang 1996), thus enabling market access. These systems approaches are designed to replace the need for fumigation with methyl bromide, which causes ozone layer depletion when released to the atmosphere and is being phased out under the Montreal Protocol (UNEP 2006) (see Chap. 10).

The increase in negotiated acceptance between trading countries of "areas of low prevalence" or "pest free areas" has meant that certified evidence of absence of catch in on-going surveillance (e.g., pheromone or fly trapping programmes with continuous zeros) can enable exports to markets free of certain pests without the need for post-harvest fumigation (Follett and Neven 2006). Alternative tactics for fruit commodities based on this approach include combinations such as low temperature and controlled atmospheres, and irradiation, as well as measures of host range and utilization that can indicate low risk of infestation. These systems are designed to help facilitate trade where eradication is not possible, but add to the on-going costs of pest management. Such on-going pest management costs are taken into account in developing the baseline for a cost-benefit case for supporting eradication.

Where pests have a wide distribution, there may be areas with pest suppression supported by buffer zones to reduce or prevent immigration. In such situations, the containment area may gradually expand where suppression is underway, using the "rolling carpet" approach (Hendrichs et al. 2005). This approach has been used successfully with Screwworm, Pink Bollworm and fruit fly programmes.

The term "Integrated Pest Eradication" (IPE) was proposed (Suckling and Brockerhoff 2010) to build on this well-established philosophical approach and extend it to dealing with the analogous case of invasive species that may be targeted by an eradication programme. An example of this approach might aim to use complementary tactics against different life stages, such as insecticides targeting larvae and SIT or pheromones for mating disruption against the adult stage. Features of individual tactics must be taken into account at the programme level. Features include availability, registration and approvals, costs, reliability and effectiveness, inter-compatibility, social acceptance and scalability. Unfortunately, some low-impact tactics are not available for all Orders of organisms, because of the pest biology. In some cases, tactics can work synergistically, which is ideal. An example is the combination of SIT and augmentative releases of parasitoids, as previously discussed (see Sect. 14.5).

The goal of eradication is implicitly far more challenging than continuing pest management, because it involves preventing the establishment of self-sustaining populations from occurring anywhere in the target zone, no matter what landscape is involved. Allee Effects (Liebhold and Tobin 2008) can contribute usefully to the extinction of pest populations, without necessarily killing the last individual, because very low density populations may go extinct by themselves. Some inversely density-dependent tactics, such as SIT or mating disruption that work better at low pest density, exploit this weakness.

The economic threshold for an eradication or containment response is high because these programmes typically cost many millions of dollars and can last several years. The cost and duration implies a degree of coordination that is usually only achieved at government level. A careful summation of cross-sectoral pest impacts ensures that unwarranted eradications are not undertaken. Host-specific plant pests (including plant pathogens) are less likely to trigger an eradication response on this basis, even when tools are available for surveillance and eradication.

Several basic conditions are essential for success in an eradication campaign. Tools must be available that can be used to monitor and control populations of the target organism, and the distribution of the invader should still be limited, known, and not expanding rapidly. Public support and adequate funding are also important (Myers and Hosking 2002; Brockerhoff et al. 2010).

The tools available for eradication may also be more limited than those for pest management, because pest management is typically applied in agricultural, forest or horticultural production systems where producers are more accepting of pesticides and other interventions than are urban populations. IPM typically is applied on private land, whereas eradications must target pest populations on both public and private lands. Land owners may not be sympathetic to government programmes, even when a cost-benefit analysis indicates economically-favorable outcomes from eradication of unwanted organisms.

Ironically, unwanted organisms are often discovered first in urban areas. Hence societal attitudes can directly affect the feasibility of eradication, particularly in cities or other sensitive ecosystems. Public attitudes to iconic amenity plants and trees also must be considered. Differences in public attitudes to different eradication tactics must be elucidated. More work is needed in this area, as well as in the development of new tools.

14.7 Challenges and Outlook

Significant advances have been made in the development, application and integration of tools available for eradication, and this is apparent in the increased rate of success of recent eradication programmes. As the rate of new organism incursions is accelerating, driven by increased global tourism and trade, a similar trend could be expected in the rate of spread and costs due to high impact pests. Examples include Red Imported Fire Ant and mosquitoes, where it is likely that governments would attempt containment or eradication if possible. This suggests that governments should increase their investment in this area, to avoid the phenomenon discussed as the "ambulance at the bottom of the cliff" during the recent US$45M eradication of an Australian Tussock Moth (called Painted Apple Moth in urban Auckland, New Zealand). Up to 40 aerial applications of *Btk* were applied together with other tactics (Brockerhoff et al. 2010).

The Australian government has attempted to proactively deal with the issues of who benefits and who pays for eradication in the following way. If the pest is only of public interest, then clearly the federal government is the interested party and will pay for the programme. Alternatively, if agriculture has a large interest (presumably because the unwanted organism is a known threat), then the costs may be shared with government according to a formula based on this information. This recognition of shared interest presumably helps to bring pests that might not otherwise be eradicated into the eradication realm. Examples are sector-specific pests with a narrow host range.

Other issues of equity arise during eradication programmes because parties at risk from an organism may not be geographically the same as the public directly affected by treatments where the organism is found. In this case, it is helpful to invest heavily in communications and, if necessary, in socially-acceptable solutions. In New Zealand, publication of the results from regular surveys indicating a high degree of support from affected residents helped the government to deal with vocal opposition from a minority of protestors based outside the affected area during the Painted Apple Moth eradication programme involving 30,000 acres of urban and suburban Auckland.

In some cases, eradication clearly is possible and justifiable as a desirable outcome for governments, affected parties, and the public. The substantial cost, commitment and effort needed for eradication require a substantial cost-benefit analysis, including the expected impacts of an invader and feasibility of eradication (Brockerhoff et al. 2010). Considerable progress has been made in our understanding of costs and benefits of eradication, and many recent programmes have provided significant financial benefits despite weak consideration of non-market values, such as impacts on amenity values. Nevertheless, constraints include increasing public interest and occasional strong opposition to such programmes. New media, such as internet-hosted libraries of videos from interested or affected parties, are being used by opponents of such programmes and present a major challenge to governments to present their case in support of intervention.

Many eradication programmes face the challenge of developing cost-effective treatments for large areas, often best achieved using aircraft. However, the aerial application of broad-spectrum insecticides used in previous decades against fruit flies in California (Barinaga 1991) is generally no longer acceptable. The aerial application of pheromones has been used for many years to slow the spread of Gypsy Moth in the eastern USA (http://da.ento.vt.edu), but even this tactic was withdrawn by the Governor of California after public opposition mounted to use of microencapsulated pheromone in a recent eradication programme for the Light Brown Apple Moth (www.cdfa.ca.gov – Light Brown Apple Moth Project, CEQA Mandated Findings 03/22/10). This example suggests that a change in public attitudes may be occurring, possibly because of increasing urbanization and reduced societal understanding of the need for pest management in food production. Alternatively, when the public understands the impact of a pest and the risks and benefits of aerial application of pesticides (e.g., *Btk* for Gypsy Moth suppression in eastern USA) the tactic may be accepted and controversy withdrawn when funding the programme.

We must increase the awareness and understanding among the public of the threat posed by some invasive species, and that a decision not to eradicate also can affect the public negatively. Unfortunately, the lack of effective socially-acceptable tools for eradication is often a significant limitation. More research is needed into tools with fewer non-target impacts to fill this gap. A critical requirement for the future is an improved understanding of the ecology and management of invasions, including modelling and other decision-support tools, as well as an expanded tool kit of options for cost-effective surveillance and eradication. The strong trend, supported by general cost-benefit analyses for certain types of high profile organisms (see Table 14.1), is a greater need for activity and knowledge in this area of applied ecology. Hence, the outlook for employment of new graduates interested in regulatory issues is arguably excellent as new invasive organism problems unfold. Further, we cannot underestimate the policy challenges faced by governments dealing with an increasing demand for resources just to maintain the *status quo*.

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Chapter 15
Invasive Insects in Plant Biosecurity: Case Study – Mediterranean Fruit Fly

D.R. Lance, W.M. Woods, and M. Stefan

15.1 Introduction

Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) ("Medfly"), is one of agriculture's most destructive and infamous insect pests (Jackson and Lee 1985). Economic analyses suggest that the establishment of Medfly in California alone could cost the state more than $1 billion USD annually (Siebert and Cooper 1995). Medflies are polyphagous, with over 200 types of fruit reported as hosts (Liquido et al. 1991). They readily infest diverse commodities in major cropping systems, including citrus, pome fruits and stone fruits. The list includes numerous tropical fruits that are traded in lower volume than those mentioned above, but are still of regulatory concern. Examples include papaya (*Carica papaya* Linnaeus), loquat (*Eriobotrya japonica* Lindl.), guava (*Psidium* spp.), and litchi (*Litchi chinensis* Sonn. Mill.) among many others. Coffee (*Coffea arabica* Linnaeus) is considered as a possible ancestral host of the fly (Prokopy et al. 1997), and Medfly can build to very high numbers in coffee-growing areas. Still, the insect is not considered a major coffee pest because larvae feed primarily on the pulp of the fruit rather than the bean.

The current geographic range of the medfly includes Africa, southern Europe, Central America, Hawaii, portions of South America, and Western Australia (Diamantidis et al. 2008). The species may have evolved in Sub-Saharan Africa and spread through the remainder of its range within the past two centuries, largely

D.R. Lance (✉)
USDA-APHIS-PPQ-CPHST Otis Laboratory, 1398 W. Truck Rd., Buzzards Bay, MA 02542, USA
e-mail: David.R.Lance@aphis.usda.gov

W.M. Woods
Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth, WA 6151, Australia

M. Stefan (Retd)
USDA-APHIS-PPQ-CPHST, 1730 Varsity Dr., Raleigh, NC 27606, USA

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Fig. 15.1 Mating pairs of the Mediterranean fruit fly "medfly," *Ceratitis capitata* (Image courtesy David Lance CPHST, APHIS, USDA)

via human transport of fruit (Bonizzoni et al. 2004; Malacrida et al. 1998). Because of this demonstrated ability to colonize new areas, medfly is a serious agricultural pest in its own right and a regulatory pest that is quarantined by many uninfested countries such as China, Japan and the USA (Bergsten et al. 1999). These countries regularly intercept incoming medflies, as well as other pest tephritids (Li et al. 2009; Liebhold et al. 2006). Despite these efforts, incipient populations of Medfly periodically appear and extensive programmes are required to ensure that populations are detected quickly and eradicated effectively (Bergsten et al. 1999).

Female medflies deposit eggs within fruit, and the larvae (maggots) feed on the fruit, creating tunneling damage and promoting rotting. Mature larvae leave the fruit to pupate in the litter. The adult flies, slightly smaller than a typical housefly, are multi-coloured with patterned wings (Fig. 15.1). The entire lifecycle can be completed in less than a month depending on temperature and available host plants. Medfly is a member of the Family Tephritidae (true fruit flies), which includes many other serious agricultural pests such as Oriental Fruit Fly (*Bactrocera dorsalis* (Hendel)), Mexican Fruit Fly (*Anastrepha ludens* Loew), and Apple Maggot (*Rhagoletis pomonella* (Walsh)). Medfly cannot survive harsh winters and consequently is restricted to tropical through warmer temperate regions (De Meyer et al. 2008; Papadopoulos et al. 2001a).

Fig. 15.2 Male Medfly releasing sex/aggregation pheromone from the tip of its abdomen (Image courtesy David Lance CPHST, APHIS, USDA)

15.2 Behaviour and Chemical Ecology

Adult Medflies exhibit a rich behavioural repertoire, with several traits that have lent themselves to the development of management tools. Those traits involve the flies' methods of locating mates, food, and egg-laying (oviposition) sites, and have been exploited in the development of monitoring tools (e.g., traps and lures), chemical control methods (bait sprays and other attract-and-kill methods) and the Sterile Insect Technique (SIT).

15.2.1 Mating Behaviour

Mating behaviour of Mediterranean Fruit Flies has been studied extensively, largely in relation to the SIT (discussed in detail below). Briefly, male medflies roost, typically on undersides of leaves, in small, loose aggregations that many authors have equated to the lek behaviour of some vertebrates (Prokopy and Hendrichs 1979). Males produce and release an odour (Jang et al. 1989) that functions as a sex-attractant pheromone (for virgin females) and presumably an aggregation pheromone (for males) (Fig. 15.2). When a female approaches a male, the male initiates a complex courtship ritual that involves rapid "head shaking" and pulsed wing-fanning in addition to continued release of pheromone. If the female remains attentive, then the male attempts mounting and may or may not subsequently succeed in mating. Females, then, actively choose mates and (more often than not) reject suitors by leaving at some point during the courtship process

(Lance et al. 2000). Following mating, females become relatively unresponsive to males and shift their focus to finding fruit for egg-laying (Jang et al. 1999).

Pheromone-related behaviour of medflies remains poorly understood, which is perhaps surprising given the volume of research on this species and the theoretical potential to use a male-produced sex attractant to suppress populations by trapping females. The complexity of the male-produced odour, which includes over 100 compounds (Jang et al. 1989; Mavraganis et al. 2008), has been a deterrent to fully characterizing the pheromone components and their behavioural effects. Blends of multiple components will attract females in an olfactometre (Landolt et al. 1992; Light et al. 1999) and show some ability to enhance trap catch (Baker et al. 1990; Heath et al. 1991); extracts of males have been shown to enhance capture of females and males in traps (Mavraganis et al. 2008). Practical applications of the pheromone, however, have not been developed.

15.2.2 Feeding and Food-Related Attractants

Adult Medflies require food for energy and egg production, and feed on a variety of substrates such as fruit juices, bird droppings, and other materials on leaf surfaces (Hendrichs and Hendrichs 1990; Hendrichs et al. 1991). Proteinaceous liquids such as nulure (a corn hydrolysate) and suspensions of torula yeast have long been known to attract medflies as well as many other tephritids (Heath et al. 1994), presumably because they approximate odours from potential sources of adult food. The attractive qualities of proteinaceous liquids have been exploited by using them to bait traps and by mixing them with insecticides to form “bait sprays”. While much of the feeding attractant work has been based on odours from adult food (Heath et al. 1994, 1995b), medflies also appear to respond to visual and odour cues of larval food (i.e., egg-laying sites). Mated females will seek fruit models that emanate fruit odours (Jang et al. 1999). Responses to food and host odours can be influenced by colour, with yellows and greens preferred to blues and white (Epsky et al. 1996; Katsoyannos 1987). Odours of coffee berries, especially crushed berries, attract female medflies, though it could be argued that they provide a potential source of adult, as well as larval, food (Prokopy et al. 1997; Warthen et al. 1997b).

15.2.3 Male Attractants

In some groups of tephritids flies, males can be attracted using specific compounds that, at least when discovered, had no obvious relation to the insect’s biology. More specifically, these compounds were not produced by the insect and were not obviously related to food or oviposition hosts, although some are plant-derived or structurally similar to plant-derived compounds (Metcalf 1990). These attractants have been referred to as "parapheromones" although some authors reserve the term to refer to compounds that are strictly anthropogenic (i.e., do not occur in nature) and, in many cases, are analogs of components of the insect's actual pheromone (Renou and Guerrero 2000). Among tephritids, several male lures now appear to be pheromone components, precursors of pheromone components, or otherwise involved in enhancing mating competitiveness of males (Hee and Tan 1998; Nishida et al. 1997; Shelly 1999, 2001).

Known male attractants for Medfly include Trimedlure and related compounds, and α-copaene and similar compounds (Flath et al. 1994a, b). Trimedlure, tert-butyl 4- (and 5-) chloro-trans-2-methylcyclohexane-1-carboxylate, remains the standard attractant for medfly-specific (actually, *Ceratitis*-specific) trapping (Warthen et al. 1995). An iodo-analog, ceralure, is a more potent attractant but cost-effective methods for producing it have not been forthcoming (Avery et al. 1994; Leonhardt et al. 1996). The sesquiterpene α-copaene is also highly attractive but is very difficult (expensive) to synthesize, and it is more practical to obtain it from natural sources such as essential oils from ginger or *Angelica* (Nishida et al. 2000; Shelly 2001; Warthen and McInnis 1989).

Behaviours elicited by the various males' lures can differ somewhat; for example, male medflies will form aggregations and become relatively sedentary at sources of α-copaene, a behaviour that is not elicited by trimedlure (Shelly and Villalobos 2004). An increasing body of evidence also suggests that these or similar compounds may play a role in medfly mating, as the ability of males to acquire mates increases after males are exposed to either trimedlure or α-copaene (Shelly 2001; Shelly et al. 1996).

15.3 Management Tools

Activities aimed at protecting an area from incursions of an exotic invasive pest can be categorised, as elsewhere in this text, as pre-border (Chap. 5), border (Chap. 6), and post-border (Chap. 7), or functionally, as exclusion (typically pre-border and border), detection, and mitigation.

15.3.1 Exclusion Tools

Human transport of infested fruit is the most common manner by which tephritid flies are carried to areas outside of their geographic range (Reid and Malumphy 2009). Infestations of tephritids in fruit are not always apparent, so a common strategy for uninfested countries is simply to forbid importation of any host materials from infested areas (Chap. 6). Because this would block international trade of many commodities, several methods for ensuring that these materials are pest-free (or nearly so) have been developed (reviewed by Follett and Neven 2006, and Chap. 5). In the case of fruit pests, the most basic is simply treating fruit before shipment to eliminate any pests that may be present. Approval of treatments for elimination of fruit flies has historically required post-treatment survival rates of 3.2×10^{-5} (Probit 9) or less, but this requirement can be relaxed under specific conditions (see *Systems approaches*, below).

Several treatments are used and approved, in different settings, to kill immature stages of medfly in fruit. For example, the USA specifies, depending on the type and origin of the fruit, fumigation with Methyl Bromide, heat treatment (water immersion, forced hot air, or vapour), cold treatment, a combination of cold and fumigation, and/or radiation (USDA-APHIS-PPQ 2010). In most cases, treatment schedules specify the required duration of a treatment at given intensity (e.g., temperature, concentration of fumigant) for specific combinations of commodity and pest. Other treatments are more generic; for example, 150 Gy of ionizing radiation is an acceptable treatment for any tephritid in any commodity. Acceptable treatments will not only be lethal to medfly, but also must not substantially degrade the quality of the fruit (Armstrong 1990; Obenland et al. 1999; Schirra et al. 2006). Research continues to provide treatment schedules for additional commodities and other treatment methods involving alternate fumigants, heating mechanisms (e.g., microwave, radiofrequency waves), or other physiological mechanisms such as modified atmosphere (Alonso et al. 2005; Armstrong and Follett 2007; Powell 2003; Torres-Rivera and Hallman 2007).

Regulations that forbid importation of untreated fruit are typically enforced through border checks and inspections. These efforts may be augmented through several off-shore or post-entry measures. In addition, new technologies in electronic imaging and chemical sensing are being adapted to detect regulated materials in baggage and cargo. Old "technologies" including dogs are also increasing in use. Quarantine and inspection efforts are a front line in the defense against medfly introductions, but not discussed here as they are not specific to medfly.

15.3.2 Detection and Survey Tools

Attractant-baited traps for adult flies are the most commonly used tools to survey medfly populations. Programmes at times will sample fruit, either by systematically cutting fruit to look for larvae and eggs or by holding fruit to allow larvae to mature, exit, and pupate (USDA-APHIS-PPQ 2003). Fruit sampling may be implemented for confirming the presence of locally breeding populations or as an adjunct sampling method. However, compared with trapping, sampling is a much less sensitive and more labour-intensive method. Several types of traps are used for medfly.

Traps based on male lures. The most commonly used trap and lure for medfly detection is a small trimedlure-baited "delta"-type trap known as a Jackson Trap (Fig. 15.3a). The trap is made of plastic-coated cardboard with a wire hanger; it is inexpensive and easy to deploy (FAO/IAEA 2003; USDA-APHIS-PPQ 2003).

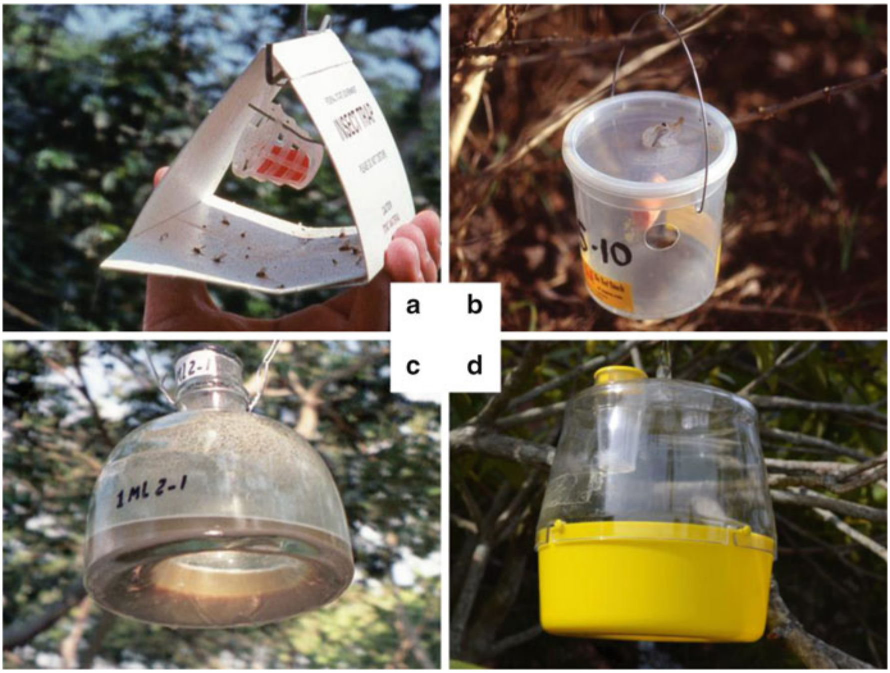

Fig. 15.3 Examples of medfly traps (**a**) *Jackson Trap*, with sticky bottom insert and basket containing a polymer plug that releases the attractant trimedlure. (**b**) *Bucket Trap*. These traps can vary regionally as to their size and shape as well as the number and diametre of entry holes. In some models, entry holes are invaginated to impede flies from leaving. (**c**) *McPhail Trap*, hand-blown glass with an invaginated bottom, used with proteinaceous liquids that act as food-odour lures. (**d**) *Multi-Lure Trap*, one of several plastic versions of the McPhail that can be used with synthetic as well as traditional food lures. A variety of other traps are also used for medfly (FAO/IAEA 2003) (Image courtesy David Lance CPHST, APHIS, USDA)

Trimedlure (typically 2 g) is often formulated into a polymer plug that is hung in a plastic basket inside the trap; some programmes may still use Trimedlure-soaked cotton dental wicks as lures. Flies enter the trap and are caught on the sticky surface of the replaceable insert that covers the trap bottom.

Other traps are also used in conjunction with male lures. Bucket Traps (Fig. 15.3b) can perform at least comparably to Jackson Traps and are preferable for monitoring high-density populations where the sticky surfaces of Jackson Traps can become saturated with flies (Cowley et al. 1990; Katsoyannos 1994). These traps are typically made from plastic containers (with lids) and have several holes around their upper perimetres; various versions have been referred to as Nadel, Lynfield, or Harris Traps, among other names. Another style is the Steiner Trap – a horizontal plastic cylinder with entry ports in the caps at both ends (Nakagawa et al. 1978). Flies that enter these types of traps are killed by an insecticide with fumigant action (typically DDVP or Naled (trade names Bromex, Dibrom). Tephri Traps incorporate both the side ports of the bucket traps and the invaginated bottom of McPhail Traps (see *Traps with food-based lures*) and can be used with either an insecticide killing agent or a liquid to entrap and drown flies (FAO/IAEA 2003). Yellow panels can also be used – they can be more efficient than the Jackson Trap but are somewhat more difficult to handle and tend to catch more non-target organisms. In simplest form, a panel trap is a sheet of plastic-coated cardboard that comes folded in half with sticky material between the halves. For deployment, the trap is folded back along the crease to form a panel with sticky material on both sides. For years, Trimedlure was mixed directly into the stickem, but, more recently, the cardboard panel has been perforated and a wafer containing the attractant is placed between the two sides of the trap ("ChamP" Trap). Panel traps with other configurations have also been evaluated, such as the "C&C" trap, which incorporates two sticky panels on either side of a large polymer-panel release device (FAO/IAEA 2003; Leonhardt et al. 1994; Warthen et al. 1997a).

Traps with food-based lures. Food-related odours have been used extensively for trapping tephritid flies including medfly. Historically, food-odour baits have been proteinaceous liquids that are held in the reservoir of McPhail Traps (Nakagawa et al. 1971). These are bell-shaped traps with an invaginated opening in the bottom (Fig. 15.3c). The original McPhail Traps are hand-blown glass; plastic facsimiles are also available (USDA-APHIS-PPQ 2003). Flies that are drawn to the lure enter the opening and drown in the liquid. McPhail Traps, including the glass version baited with liquid, are still used in many programmes for general survey of tephritids and in some programmes are the primary trap type used for detection of *Anastrepha* spp. For Medfly, they have the advantage of attracting females as well as males. The most commonly used bait liquids are Nulure (or PIB-7; protein insect bait no. 7, a corn product) and a suspension of torula yeast (FAO/IAEA 2003). The yeast baits typically come as large pellets that are added, along with water, directly to traps (USDA-APHIS-PPQ 2003). These pellets also contain borax to improve field life of the liquid (and of captured flies) and to maintain appropriate pH, which can have a pronounced effect of attractiveness of proteinaceous baits (Heath et al. 1994). Several other commercial hydrolysates, with trade names such as Buminal, Pinnacle, and Solbait, have also been used (Fabre et al. 2003).

During the past two decades, active components in proteinaceous baits have been isolated and identified, allowing the development of synthetic food-based lures (Epsky et al. 1999; Heath et al. 1995a). These lures have typically included ammonium acetate and putrescine; a third component, trimethylamine, may be added and typically enhances medfly capture (Epsky et al. 1999). Overall, the relative effectiveness of the two- versus three-component lure, as well as the synthetic lures versus proteinaceous liquids, has varied from test to test and among tephritids species. The synthetic lures are used in 2-piece, plastic McPhail-like traps (Fig. 15.3c), Tephri Traps, or open-bottom traps with sticky inserts (Epsky et al. 1999; Heath et al. 1996; FAO/IAEA 2003). The resulting trap/lure systems are easier to handle in the field and more standardised in terms of attractant release than the standard liquid-baited McPhail Trap. Depending on the programme, the traps may be deployed dry, using a controlled-release strip with DDVP to kill flies, or wet, where flies drown in an aqueous solution. The solutions typically include a few drops of a non-foaming detergent as a wetting agent plus low-toxicity antifreeze (propylene glycol; typically

5–10 %), which retards both evaporation and decomposition of captured insects. Food-lure-baited traps, in most cases, capture fewer total medflies over time when compared to Trimedlure Traps, but the relative effectiveness of the two trapping methods for various purposes remains a subject of debate (e.g., (Broughton and de Lima 2002; Papadopoulos et al. 2001b).

15.3.3 Suppression Tools

Many suppression tools are available to regulatory programmes, including:

Chemical control methods. Historically, the most commonly used method of suppressing medfly populations has been bait sprays. A liquid feeding attractant, typically one of the protein hydrolysates discussed in *Traps with food-bait lures*, is combined with insecticide and often with feeding stimulants (e.g., sugars) and other appropriate adjuvants. When the material is sprayed on foliage, flies are attracted to and feed on the dried droplets, making the sprays efficacious even when very low application rates of active ingredient are used. Organophosphates (Malathion in particular) were used in bait sprays for several decades, but in recent years environmentally "softer" insecticides, such as Spinosads, are becoming the active ingredients of choice (Mangan et al. 2006; Vargas et al. 2001). Bait sprays are applied using conventional spray equipment ranging from hand-held sprayers to spray planes and have been key components of both IPM and regulatory (eradicative) Medfly management efforts (Braham et al. 2007; Jackson and Lee 1985; Leza et al. 2008; McQuate et al. 2005; Vargas 2004).

In medfly management areas with high population pressure, more conventional insecticide sprays, often referred to as "cover sprays" may be used. To date, these sprays have typically consisted of organophosphate insecticides (discussed in *The Medfly in Australia*, below).

In eradication projects, soil drenches are sometimes used as a supplemental control method (USDA-APHIS-PPQ 2003). Areas under host trees are sprayed, from tree base to drip line, with a broad-spectrum and relatively persistent insecticide such as diazinon. These treatments are typically applied only in the immediate vicinity of areas where medflies were known to occur and, in particular, under trees that held fruit in which immature medflies were found.

Fruit stripping. The sanitation method of fruit stripping is sometimes used during eradication to eliminate egg and larval stage medflies in the immediate areas surrounding locations where detections occurred (USDA-APHIS-PPQ 2003). Cutting all or a portion of this fruit to look for immature medflies can be used to supplement trapping for delimiting or monitoring the population. Areas stripped are typically limited in size due to concerns that wider-area fruit stripping will encourage female flies to disperse long distances in search of oviposition sites. Stripped fruit is fumigated and/or deeply buried (USDA-APHIS-PPQ 2003).

Attract-and-kill methods. Several mass-trapping and bait station methods have been considered and evaluated for medfly suppression. This strategy is similar to bait sprays in that flies are eliminated from the population when they come to sources of attractant; the distinction between the two is that bait sprays are broadcast applications whereas attract-and-kill methods rely on fewer but more concentrated sources of attractant that are distributed throughout the environment individually. When male lures are used, the technique is typically called “male annihilation” (MA) and functions by reducing mating rather than directly reducing the population. Because one male can mate with many females, success of MA requires that very high proportions of males are removed by the time of, or soon after reaching, sexual maturity. MA has been used successfully against *Bactrocera* flies (especially *B. dorsalis* – the Oriental Fruit Fly) since the 1960s (Knight 2003; Steiner et al. 1965). The effectiveness is due to methyl eugenol’s potency as an attractant for *B. dorsalis* males, which become responsive before reaching sexual maturity (Steiner and Lee 1955). In contrast, while MA has been suggested as a possible medfly control tactic (Avery et al. 1994), it has not been shown to be effective enough for use for population suppression or eradication.

The availability of improved food-based attractants has led to development of attract-and-kill methods that target both sexes of medfly. Mass-trapping has most commonly involved traps and lures described, or similar to those described, in *Traps with food-based lures* (Navarro-Llopis et al. 2008). Population suppression has been sufficient to allow incorporation of mass-trapping into IPM schemes for medfly in the Mediterranean region (Cohen and Yuval 2000; Leza et al. 2008), although supplemental control may be needed in areas with high pressure of flies emigrating from outside of managed areas (McQuate et al. 2005). An alternative approach to simply trapping flies is to lure them to sources of Insect Growth Regulators such as Lufenuron, which is safe for vertebrates but functions as an insect chemosterilant (Bachrouch et al. 2008; Navarro-Llopis et al. 2007). To date, food-lure-based attract-and-kill methods apparently have not been evaluated for potential use in medfly eradication.

Sterile Insect Technique (SIT). The Sterile Insect Technique involves the release of large numbers of reproductively sterile insects such that the released males will mate with, and thus block reproduction of, wild females in the target population. SIT is probably the most complex insect suppression method in use today, because it involves large-scale rearing of insects, sterilization, and release methods, as well as monitoring of insect quality and programme effectiveness. Components of insect behaviour, physiology, genetics, and ecology come into play, along with a variety of additional disciplines as diverse as microbiology, food technology, nuclear physics, avionics, and public relations (Dyck et al. 2005). As a control method, however, it has become increasingly favored for area-wide Medfly programmes as it is species-specific and does not involve use of toxins or biological control agents that could have potential non-target effects. Currently, SIT programmes targeting medfly probably exceed, in scope and resources expended, those directed at any other insect.

Sterile medflies are produced in large, factory-like facilities. Eggs or neonate larvae are infested onto trays of a semi-solid diet that typically consists of a plant-based bulking agent (e.g., sugar cane bagasse, wheat middlings or corn cob grits)

Fig. 15.4 Mass-rearing medflies Facility manager Stuart Stein (*left*) and USDA-ARS Entomologist Eric Jang (*right*) discuss a tray of larval diet at the former Hawaii Fruit Fly Rearing Facility in Waimanalo (Image courtesy Scott Bauer ARS USDA)

mixed with nutrient sources (e.g., yeasts, sugars, supplemental ingredients), water, and agents to adjust pH and control microbial growth. Mature larvae migrate from the diet in 7–11 days and are placed in a container with sand, fine vermiculite, or other medium that encourages pupation. A few days before adult emergence, the pupae are sifted from the medium; at 1–2 days before emergence, they are irradiated to make them reproductively sterile. Before irradiation, pupae are treated with Day-Glo powder.

Higher dipterans use their ptilinum (a fluid-filled sack on their head) to break out of their puparium. The ptilinum retracts back into the head following emergence, and carries with it some of the Day-Glo powder to create a permanent mark that is used to distinguish sterile flies from wild flies. During irradiation and subsequent shipment to the emergence facility, pupae are kept under hypoxic conditions. Hypoxia is achieved by back-flushing their holding containers with nitrogen and/or sealing the flies in air-tight containers such as plastic bags. Hypoxia helps improve sterile fly quality by minimizing damage to somatic tissues during radiation, and it delays development so that flies don't emerge en route to the emergence facility, which may be hundreds or thousands of kilometres from the rearing factory (Bakri et al. 2005).

Irradiation is accomplished using isotopic sources (^{60}Co or ^{137}Cs) at most SIT facilities. Low-energy x-ray systems are being developed for this purpose (Mehta 2008; ISO/ASTM 2013). Doses for sterilizing medflies have typically been 145–160 Gy and expressed as a minimum required or central target (e.g., median) dose, with the former being the standard (see ISO/ASTM 2013). When using genetic sexing strains (and depending on the level of security required) somewhat lower doses may be used to improve sterile insect performance (Robinson 2002) (Fig. 15.4).

At emergence and release facilities, sterile insects are prepared for distribution into the field. A simple release method involves placing containers of pupae throughout the treatment area and allowing flies to emerge directly into the environment. More often, sterile medflies emerge at a central facility and are then released as adults. Pupae are distributed into emergence containers, such as PARC boxes (pupa-adult rearing container), or stacks of screen trays and provided with a source of moisture and sugar (Shelly et al. 2006b). When most adult flies are 2 days old, they are immobilized (chilled) and transferred to release devices. Most larger-scale programmes release flies from aircraft: The adults are put into refrigerated boxes from which they are metred out of the plane using a conveyor or auger system. Similar systems can also be truck-mounted for ground release. For aerial releases, GPS tracking systems are used to guide flights, record flight data, and monitor release of flies.

In earlier medfly SIT projects, flies of both sexes were released, but the development of genetic sexing strains in the 1990s now allows the release of only males. There are several advantages to males-only releases. First, field trial data indicate that males-only releases of sterile medflies are more effective than releasing the same number of males in bisexual releases (McInnis et al. 1994; Rendón et al. 2004). Second, sterile females don't produce eggs but will still "sting" fruit, creating blemishes and possible routes of entry for pathogens (Hendrichs et al. 1995). Third, males-only should be, potentially at least, more cost-effective, as fewer insects need to be reared, irradiated, shipped, and released. The current world standard for genetic sexing of medflies is a sex-linked temperature-sensitive lethal ("tsl") trait that allows facilities to kill off females by incubating eggs at 34°C for 24 h before they are seeded onto diet (Franz 2005; Hendrichs et al. 1995). A specialised colony maintenance procedure, known as a "filter," had to be worked out to maintain the sex-linked *tsl* trait under pressures of mass rearing (Caceres 2002; Fisher and Caceres 2000) (Fig. 15.5).

For a successful SIT effort, the sterile male flies must compete for mates against the wild flies in target populations. The factory environment and artificial diets can result in insects that differ behaviourally and physiologically from wild insects, both through direct effects on phenotype and due to genetic changes in the colony as it adapts to the factory setting (Briceño and Eberhard 2000; Lance and McInnis 2005). In addition, sterilization, shipping, and release procedures can degrade the quality of sterile insects. As a result, sterile medflies are routinely monitored for such traits as size, percent emergence, survival, flight ability, and ability to mate, including mating with wild-type females (Calkins and Parker 2005; IAEA 2003).

The issue of mating competitiveness is especially critical for sterile medflies because of the combination of a female-choice mating system and their complex male mating behaviour: Wild females could potentially tend to reject sterile males if their mating behaviour becomes altered even slightly by production processes (Hendrichs et al. 2002). Indeed, wild female medflies have typically been found to accept sterile males several-fold less readily than they accept males from their own population, which reduces effectiveness of releases (Lance et al. 2000; Rendón et al. 2004). In one instance, the ability of wild medfly females to select wild over sterile males led to the evolution of resistance to SIT following an extended period of releases (McInnis et al. 1996).

Fig. 15.5 Mass-rearing medflies Medfly larvae are reared in large diet-filled trays that are stacked on carts or trolleys (*on right*) and held in large environmentally controlled rooms. Mature larvae migrate from the diet (some are visible on the sides of trays at *left*) and, in most rearing systems, drop into water. The water stops migration and stalls development, which improves synchronization of pupation following harvest (Image courtesy David Lance CPHST, APHIS, USDA)

In practise, medfly SIT programmes often target "overflooding" (sterile: wild) ratios of 100:1 or greater (Jackson and Lee 1985), but that still may not be sufficient to induce high levels of sterility into the wild population under some conditions (Rendón et al. 2004). Along with a switch to males-only strains, several approaches have been tried in an effort to improve competitiveness of sterile medflies, including improved adult diets (Shelly et al. 2006a; Yuval et al. 2007), altered microbial associations (Niyazi et al. 2004), selective breeding of flies with high mating competitiveness or survival (McInnis et al. 2002), and exposing males to attractants (or "aromatherapy") (Shelly 1999, 2001). Of those, aromatherapy, especially with oils containing α-copaene, has consistently enhanced mating success and has been incorporated into medfly SIT programmes (Shelly et al. 2007).

15.4 Management Strategies for Regulatory Programmes

The destructive nature of medfly has prompted uninfested countries to develop a diversity of programmes designed to exclude the insect. Most typically, quarantines are imposed on host fruit from infested areas, and regulatory agencies enforce the quarantines by inspecting incoming materials such as cargo and passenger baggage for medfly host material (See Sect. 5.2). Additional programmes are often put in place to detect and (typically) eradicate incipient populations that can occasionally arise when infested fruit make their way past agricultural inspection. Diverse strategies and tactics are used in the delivery and execution of these regulatory programmes.

15.4.1 Reactive (Detect and Eradicate) Strategies

Reactive programmes are based on extensive detection trapping efforts (see *Design of medfly trapping programmes*, below). Capture of a medfly leads to intensified "delimitation" trapping in the area of initial captures to confirm the presence of a population and determine its extent and size. If one or more "triggers" are met, then the area is considered infested and regulatory and eradication measures are started (USDA-APHIS-PPQ 2003). Typical triggers include the discovery of a mated female or immature stages breeding in the environment, or multiple captures of males within a specified time and area. The mitigation (typically eradication) methods employed will depend on several factors, including availability, funding, environmental constraints, and size of the population at detection, as well as social, political, and land-use characteristics in the programme area. Due to public concerns about the use of insecticides and other chemicals, mitigation programmes have become increasingly reliant on the use of sterile insects over the past two decades.

Regulatory measures are imposed to reduce the risk of local spread of the population through movement of infested fruit and to stop infested fruit from entering intra- and international commerce. Typically, quarantines are put in place to forbid transport of untreated host fruit out of the programme area. In the USA, fruit wholesalers, retailers, and processors are put under compliance agreements that require them to take measures to safeguard against marketing or moving infested items (USDA-APHIS-PPQ 2003).

15.4.2 Preventative Release Programs (PRP)

In areas at high risk of introduction and establishment of medfly, a Preventative Release Program (PRP) is an alternative to reactive detect-and-eradicate programs. PRP is an area-wide tactic in which sterile flies are released continuously over the at-risk area during periods when adult flies could be active (in most cases, year-around). The theory is that newly introduced female medflies will mate with the sterile males that are present, squelching most infestations before they reach a size where they are likely to be detected (Dowell et al. 2000; Dowell et al. 1999). Release rates (sterile flies per unit area) under PRP are typically half or less compared with those used in conventional SIT eradication efforts. PRP has been used for medfly

Fig. 15.6 Reproductive sterilization by radiation Bags of medfly pupae are being loaded into the "drawer" of a self-contained dry-storage irradiator in Guatemala. The drawer lowers to the center of an annular array of vertical rods that contain Cobalt-60, which exposes pupae to a highly uniform dose of gamma radiation. The irradiator weighs >4 metric tons because of the lead shielding required to protect operators from radiation. The red color of the pupae (bags on cart at *left*) is from Day-Glo powder used to mark sterile flies (Image courtesy David Lance CPHST, APHIS, USDA)

management in the U.S. (California and Florida) and in South Australia (Dowell et al. 1999; Shelly et al. 2006c; Smallridge and Hopkins 2004) (Fig. 15.6).

Historical evidence suggests that occasional medfly infestations will still arise under PRP, but the number and scale of eradication efforts will be far less than what would be experienced in the same area under a reactive management strategy (Dowell et al. 1999). Infestations that are detected under PRP can typically be eliminated with a temporary increase in sterile fly release rate around the infestation. The SIT in these cases is often augmented with highly localized use of bait sprays at the points of detection.

The decision to switch from a reactive to proactive (PRP) strategy is based at least in part on economics. When medfly detections occur frequently, the reduction in eradication efforts under PRP can be substantial enough to make the strategy less expensive over time than a conventional reactive medfly program, despite the cost of continuously rearing, releasing, and identifying (in trap catch) the sterile flies. Policy makers and politicians tend to appreciate the reduced eradication activity with PRP because large-scale eradication programs are becoming less popular with the public in many areas. In addition, frequent or ongoing eradication can erode the confidence of trading partners and the public in the effectiveness of the overall biosecurity program. Management of rearing facilities is also simplified under PRP, as the demand for flies is relatively constant in comparison with reactive detect-and-eradicate programs, where production is intermittently scaled up and down (along with labour forces, supply needs, etc.) to meet the broad changes in demand for sterile insects.

15.5 Design of Medfly Trapping Programs

Regulatory medfly trapping programs are tailored to several different goals which may include: (1) Detecting incipient populations in an area that is not known to be infested, (2) delimiting (determining extent and size of) a newly detected population, (3) monitoring wild or sterile fly populations, (4) "confirming" eradication, and/or (5) providing data in support of a systems or fly-free-zone approach to quarantine security.

15.5.1 Detection

Detection trapping is conducted in at-risk areas where medfly is not known to occur and includes some of the most extensive and expensive of all insect surveillance efforts. Detection programs should be designed to find an incipient population when it is at a size that allows the program to carry out the desired mitigation measures. In the case of medfly, the desired mitigation often is eradication. Theoretically, as more effort (=cost) is put into detection, incipient populations will be discovered at an earlier time and smaller stage of development. This will make them easier and less expensive to eradicate. However, highly intensive trapping programs, like large eradication programs, are very expensive. Ideally, we strive to balance costs of detection trapping against the expected average annual cost of eradicating these populations such that the combined overall program costs are minimised. Detection trapping is probabilistic, meaning that the actual size of a population at detection can vary quite a bit just due to chance (Lance and Gates 1994). Medfly detection systems, then, should also be designed so that, even at the maximum expected size of a population at detection, eradication will still be a feasible and prudent option. The likelihood that a population will spread to additional sites prior to detection (including by human transport) is another consideration.

Many factors influence the balance of costs between detection trapping and the resulting mitigation efforts. For example, the risk (frequency) of a population being introduced and establishing will directly affect the average annual cost of eradication. With many invasive species, risk of introduction and initial establishment is difficult to estimate because these events are relatively rare. However, reasonably accurate figures can be developed for medfly, at least for higher-risk areas using historic program data.

Tools that are available for suppressing a medfly population also influence the cost and feasibility of eradication. Medflies typically are moved into uninfested areas by people transporting fruit, and, accordingly, most detections of incipient populations occur in areas with high human population densities (cities and suburbs). Due to public concerns, programs in urbanized areas have been relying increasingly on SIT rather than insecticides as the primary eradication tactic. This places an additional premium on detecting the population at an early stage. As a result, medfly detection programs often deploy traps at 2–4 sites per km^2 in urbanized areas (FAO/IAEA 2003).

The traps usually include a combination of male-lure- (typically Trimedlure) and food-lure-baited traps (USDA-APHIS-PPQ 2003). Risk of medfly establishment is relatively lower in areas where medfly hosts are grown commercially, and detection traps in those areas may be as sparse as one per several km^2 (USDA-APHIS-PPQ 2006b). This at first may seem counter-intuitive (commercial production is what the program is ultimately protecting), but, in rural agricultural areas, introduction rates of medfly will be low (fewer people to carry them in) and use of tactics for knocking down higher-density medfly populations, such as aerial application of bait sprays, may still be feasible.

In practise, detection programs typically distribute traps more-or-less uniformly throughout an area to be surveyed. For example, in urban areas of California each 2.6 km^2 block (1 mile by 1 mile) is divided into five sections of equal area. For areas under PRP, a trimedlure-baited trap is deployed in each section. This strategy ensures that no areas in the landscape are more than several hundred metres from a trap. The probability of catching a fly in a trap is highly dependent on the distance between the fly and the trap. Populations that are centred hundreds of metres from traps can potentially build to large sizes before being detected (i.e., one or more flies are trapped) (Lance and Gates 1994).

Fruit fly detection programs can also minimise the maximum distance from fly population to trap over time by relocating traps on a scheduled basis (e.g., every 6 weeks). Computer simulations of detection trapping grids indicate that relocation does not affect the likelihood of detecting very small populations, but does reduce the chances that populations will grow to unacceptably large sizes before being discovered (DRL, unpublished). Relocation also provides the programs with an opportunity to keep traps in trees with ripe fruit whenever possible, which will keep traps in areas that flies are likely to frequent.

15.5.2 Delimitation

After a fly is captured in a detection trap, a much denser grid is deployed in the surrounding area to confirm the presence of an infestation, and to provide the higher-resolution information needed to determine the population's size and the area it occupies. This information is critical for effectively employing mitigation measures. In areas within ca 1 km of the initial find, delimitation, traps are typically placed at 20–50 traps per km^2 (FAO/IAEA 2003; USDA-APHIS-PPQ 2003).

Arrays as dense as 400 traps per km^2 have been used (Lance and Gates 1994). Delimitation trapping typically continues for several km beyond the core area, often decreasing in density with increasing distance, to help ensure that the initial capture was not a fly that had dispersed or been carried a mile or more from its source population (USDA-APHIS-PPQ 2003). Following eradication efforts, grids comparable in density to delimitation protocols are often deployed for several generations to help assure that no breeding population remains in the program area.

15.5.3 Systems Approach to Medfly Exclusion

A Systems Approach to quarantine security consists of a series of steps designed to safeguard against movement of a pest to an uninfested area. None of those steps individually may provide adequate protection, but, taken together, their cumulative effect is to provide a high degree of assurance that the pest will be excluded. A Systems Approach typically is developed through cooperation between exporting and importing countries.

An example of a Systems Approach to Medfly exclusion is the agreement between Spain and the USA allowing commerce in clementines (a variety of mandarin orange, *Citrus reticulata* Blanco) and summarized by Livingston et al. 2008. Spanish growers who wish to export their crop to the USA must trap for medfly according to a specified protocol, starting at least 6 weeks before harvest. If medfly catch exceeds an established threshold, then bait spray treatments are required for the orchard. Before shipment, a USDA-APHIS inspector randomly samples several hundred clementines based on hypergeometric sampling and size of the shipment for the presence of live medfly (all life stages), and discovery of a single insect will cause rejection of a shipment. During transit to the USA, shipments receive a cold treatment that will kill most Medflies that may have survived and gone undetected to that point. In the USA, APHIS inspectors examine the cold treatment data to confirm that specified time and temperature criteria were met and also re-sample fruit for the presence of live medfly life stages. A final level of security results from the fly's bisexual mode of reproduction – given that the numbers of live medflies in the fruit should be minimal, it would be very unlikely that two would survive and emerge near enough in space and time to mate and reproduce.

15.5.4 Fly-Free zones

Countries with established medfly populations may choose to keep one or more agricultural areas free of medfly, allowing fruit from that area to be exported freely, at least with regard to medfly. This strategy is arguably best suited for countries such as Chile or Australia (Gonzalez and Troncoso 2007); also see *Mediterranean Fruit Fly in Australia*, below), where specific production areas are geographically

Fig. 15.7 "El Pino" rearing facility The El Pino facility (Barbarena, Guatemala) is the world's largest fruit fly rearing facility. The four original rearing modules (at *left* in the picture) and two additional two-story modules provide ~10,000 m^2 of rearing area and capacity to produce >3 billion sterile male medflies per week (Image courtesy David Lance CPHST, APHIS, USDA)

remote from medfly-infested regions and/or are ecologically isolated by wide tracts of desert or other habitat unsuitable to the insect. The programs to keep the areas medfly-free will typically be similar to national programs of medfly-free countries, with quarantines, detection trapping, and protocols and systems in place to respond rapidly to detections in the pest-free zone. Reactive and/or preventative-release strategies may be employed (Gonzalez and Troncoso 2007).

15.5.5 Offshore Risk-Reduction Efforts

Uninfested countries, at times, will work proactively with exporting and/or neighbouring countries to reduce the risk of medfly introductions. A significant example is the Moscamed program, which has been a joint effort of the USA, Mexico, and Guatemala for over 30 years (USDA-APHIS-PPQ 2006a). The ultimate stated goal of the program is to eradicate medfly from Mexico and Guatemala, though shorter-term operational goals have been to reduce medfly populations in Guatemala and stop their spread northward into Mexico. Currently, the program conducts trapping to monitor populations in the Mexico-Guatemala border area and to detect populations in other parts of Mexico. The program's control efforts rely primarily on sterile insects; bait sprays and biological controls have also been used. Moscamed operates the El Pino (Guatemala) rearing facility, which is currently the world's largest fruit fly factory with a capacity of several billion pupae per week (USDA-APHIS-PPQ 2006a) (Fig. 15.5). El Pino provides sterile flies for Moscamed's control efforts and the PRP and emergency programs in the USA, among other users (USDA-APHIS-PPQ 2006a) (Fig. 15.7).

15.6 Mediterranean Fruit Fly Programs in the USA

15.6.1 History of Medfly in the USA

With the exception of Hawaii, the USA is not generally infested with medfly. However, numerous medfly infestations have been discovered in the continental USA since 1929. The resulting programs to keep the country medfly-free have led to eradication projects in Florida, Texas, and California. These programs have typically been operated jointly by the USDA and state agencies – in cases detailed here, either Florida Department of Agriculture and Consumer Services or California Department of Food and Agriculture (CDFA). These programs have included some very large, expensive, and contentious efforts, and have evolved over the years in response to changes in pest risk (introduction rates), available technology, environmental regulations, public attitude toward large-scale insect control projects, and experience gained in the previous eradication programs.

15.6.2 Florida 1929–1930

In April of 1929, the presence of maggots in grapefruit led to the discovery of a very large medfly infestation in central Florida. The ensuing eradication program eventually covered four million hectares and employed approximately 6,000 people (Ayers 1957; Clark and Weems 1989). Control actions consisted primarily of an effort to eliminate all fruit, even uprooting some plantings, within 1 mile of known infested areas in combination with crude bait sprays that were applied using ground-based equipment. The most commonly used bait was mixture of brown sugar, molasses, water, and lead arsenate – an insecticide that was originally developed for use against another invasive insect pest, the Gypsy Moth. Over 135,000 kgs of lead arsenate were reportedly applied in this effort (Clark and Weems 1989). McPhail traps (>12,000) were used along with fruit sampling to detect infested areas and to monitor program progress. The traps were baited with kerosene, which attracted male medflies (Clark and Weems 1989). In addition, regulatory measures, including roadblocks manned by the National Guard, were put in place to stop movement of fruit out of the program area (Rohwer 1958). The program concluded in late 1930 at a cost of over $7 million USD, and a medfly population wasn't seen again in Florida until 27 years later (Ayers 1957; Clark and Weems 1989).

15.6.3 Florida 1956–1958

The discovery of the second incursion of medfly in Florida occurred in the Miami area in April of 1956. By that time, technology for survey and control of the pest had advanced substantially – and continued to advance during the program. Arsenate insecticides were supplanted by organophosphates (in particular, malathion), which were relatively more effective despite their lower application rates. Protein hydrolysates replaced less attractive molasses and sugar, and the baits were applied primarily by aircraft, which delivered them much more efficiently than ground-based equipment. In all, over 300,000 ha were treated – most multiple times – leading to a total aggregate treatment of ~2.5 million hectares (Clark and Weems 1989; Rohwer 1958). Along with this, the role of fruit removal was downplayed in comparison to the 1929 program (Ayers 1957; Clark and Weems 1989). Plastic "Steiner" traps largely replaced McPhails early in the program (Steiner et al. 1961). These were initially baited with *Angelica* seed oil, later shown to contain the male lure α-copaene (Jacobson et al. 1987), and subsequently with a synthetic attractant named "Siglure," which formed the basis of later structure-activity studies that led to the development of Trimedlure and Ceralure (Beroza et al. 1961; Warthen et al. 1994).

The second Florida medfly eradication project ended in February 1958 at a cost of ~$11 million USD (Clark and Weems 1989). Although the 1956 program was smaller in area than the 1929 effort, it was still massive by modern standards. From 1956 to 1958, the program's 54,000 traps caught nearly 12,000 medflies (Clark and Weems 1989) – a number far greater than total number of wild medflies captured in all of California's medfly programs (Carey 1996; K. Hoffman, CDFA, personal communication). One outcome of the program – and the reason that subsequent eradication programs have been smaller – is that intensive trapping programs were put in place to detect incipient populations of exotic fruit flies in Florida and other at-risk portions of the USA (Rohwer 1958).

15.6.4 California 1975–1982

Medfly was first detected in California during 1975, and the ensuing eradication effort marked the first time that sterile flies were used in medfly eradication (Cunningham et al. 1980). The program also included bait-sprays of host trees using ground-based equipment and limited fruit-stripping, but SIT was considered the primary control method (Jackson and Lee 1985). A total of 77 wild flies were captured in 1975, with no additional captures until 5 June 1980, when another, similar, eradication effort was launched against a small infestation (total of five flies captured) in southern California (Carey 1991).

Also on 5 June 1980, two male medflies were discovered in a trap in Santa Clara County, just south of San Francisco Bay (Jackson and Lee 1985). This detection led to the most contentious and controversial insect program in California's history. Trapping in the area had been minimal, and the program was slow to start. Control efforts consisted of SIT, ground-based bait sprays, soil drenches, and some fruit stripping, but weren't sufficient to get ahead of the population. By December 1980, the USDA had begun pressuring California's governor (Jerry Brown) to begin aerial bait spray applications (Jackson and Lee 1985). The idea of aerially applied

Malathion was extremely unpopular with the residents of Santa Clara County, and alternate strategies such as systematic fruit-stripping were substituted until June 1981. At that point, detections – which had been down early in the year – suddenly increased. Several scientists believed that the increase resulted from the release of a batch of Peruvian "sterile" flies that had not been properly irradiated (Marshall 1981) – a claim that was never conclusively proven or refuted. Regardless, on July 10, Governor Brown accepted the USDA's recommendation to allow aerial spraying. The detection trapping system was also substantially upgraded at that time, resulting in detections that pushed the area under regulation to almost 10,000 km^2 across seven counties at the peak of the program – with nearly 4,000 km^2 being treated with regularly scheduled bait sprays. The spraying phased down in the fall of 1981 but continued at some level until June 1982; the last area came out from under regulation that September. The entire program, including smaller eradication efforts in 1980 and 1981–82 in Los Angeles County, cost ~$100 million USD (Jackson and Lee 1985).

The Central California medfly program brought about several changes. Legislation was passed to improve the USDA's ability to respond to emergencies rapidly and to fund emergency activities. In addition, changes to the Federal Plant Pest Act made it possible for the Secretary to invoke emergency powers regarding regulation of intrastate movement of commodities, initiating eradication programs, and entering private property, though in practise these powers have rarely been used. The density of traps in detection grids in high-risk areas (i.e., residential neighbourhoods) was increased from 0.4 (or fewer) up to 2–4 traps per km^2 in hopes of catching incipient populations while they were small enough to easily and quickly remove (Jackson and Lee 1985).

15.6.5 California 1987 to Mid-1990s

After 1981, single medflies were captured in 1982, 1984, and 1986. However, starting in 1987, infestations cropped up annually, usually at multiple foci, in southern California. Each infestation was met with an eradication program, based increasingly on SIT with supplemental ground-based bait sprays and localized soil drenches under known infested trees. Despite this effort, the pattern and frequency of medfly discoveries led to controversy over their source: Was each infestation the result of a separate introduction, as proposed by USDA and CDFA, or was southern California generally infested with a medfly population that would occasionally increase in one area or another to the point where detection was likely? Either way, the resulting programs were expensive, and their extent and frequency was eroding confidence of the public, the states, and USA trading partners in the federal regulatory pest programs. In 1996, the medfly program in southern California switched from reactive to a proactive Preventative Release Program (Dowell et al. 1999). The program has reduced the number of infestations detected from several per year to <1 per year (many of which have been outside the PRP zone) and reduced overall program costs (Dowell et al. 1999; Dowell et al. 2000).

15.6.6 Current Medfly Programs in the USA

The USA has active programs to keep all susceptible areas, with the exception of Hawaii, free of medfly. Exclusion efforts rely primarily on the country's broader phytosanitary program, which operates through a combination of regulations, inspections, pre-clearance measures, and penalties to either exclude host fruit or certify it as pest-free based on various measures (see Chaps. 5 and 6). In addition, several programs specific to excluding medfly, such as Moscamed in Guatemala and Mexico, and the Spanish Clementine program are detailed above.

The USDA continues to operate, in conjunction with state and, in some cases, county agencies, extensive trapping programs to detect incipient populations of medfly (USDA-APHIS-PPQ 2003, 2006b). Allocation of trapping effort is risk-based with 2–4 traps per km^2 being deployed in high-risk (residential and urbanized) areas. Captures of flies are followed by specific delimitation trapping protocols, and, if triggers are met, by an eradication effort. Most detections occur in populated areas, and eradication in those areas currently rely primarily on releases of sterile flies (USDA-APHIS-PPQ 2003, 2006a). The SIT efforts are supplemented by ground-based application of bait sprays near fly finds, and, in some instances, by soil drenches of insecticides under trees suspected of harbouring immature medflies. The organophosphates in the bait sprays have been replaced by "softer" insecticides, with Spinosad being the toxin of choice since the early 2000s.

As noted above, a large portion of Los Angeles County (~5,400 km^2) was put under a Preventative Release Program in 1996, and releases of sterile insects have continued to the time of this writing. The PRP area was increased to >6,400 km^2 in 2000 to cover portions of Orange and Riverside Counties where infestations occurred in 1998 (K. Hoffman, CDFA, personal communication). California has had approximately a dozen medfly eradication programs since 1996, with almost half of those resulting from finds within the PRP zone. Still, this represents a substantial drop in the frequency of eradication programs since the late 1980s and early 1990s (Dowell et al. 1999). The recent programs have typically been quite small; in all cases, fewer than 30 wild flies were captured, with the exception of Riverside County in 1998 (75), which was not in the PRP zone at the time.

In Florida, medflies were found in the Tampa area during 1998, and the subsequent delimitation determined that the population had grown undesirably large, both in area and in numbers of flies, before detection. Following an extensive eradication project, the infrastructure that was developed for emerging and releasing sterile flies was left in place (though it has subsequently been moved), and the project transitioned to a PRP program. Portions of South Florida (Miami area) were also put under PRP. Medflies were not detected again in Florida until 2010 in Boca Raton.

The South Florida PRP has since been expanded northward to incorporate that program area and other high-risk portions of Dade, Broward and Palm Beach counties. Other medfly-susceptible areas in the USA, including parts of Texas, Arizona and several other southern states, along with lower-risk portions of California and Florida, remain under reactive medfly programs.

15.6.7 Is Medfly Established in California?

During 1991, Dr. James Carey, an entomologist at University of California at Davis, published a paper in the journal *Science* proposing that medfly was established through much of the greater Los Angeles (LA) area (Carey 1991, 1996). This flew in the face of assertions by USDA and CDFA that each new infestation was being successfully eradicated. Carey's hypothesis was based on several factors: (1) Medfly populations had been detected annually since 1986 in southern California, usually at multiple locations; (2) the detections tended to be centred around, and spread outward from, locations where flies had been detected in previous years; (3) an examination of data on pests intercepted by USDA in measurable pathways (such as airline baggage) purportedly indicated that introduction rates were too low to account for the number of infestations found; (4) detections of Medfly in the LA area appeared disproportionally higher than in other parts of the USA with suitable Medfly habitat (as well as being disproportionately higher than those of other exotic tephritids); and (5) an assumption that populations could exist for extended periods at a level below the ability of the trapping system to detect them. The controversy spilled over into the popular press, reducing the public's already-eroding confidence in California's medfly program and causing consternation among the state's trading partners. At least two nations that imported California produce responded by sending staff to review U.S. fruit fly programs.

Since the publication of Carey's 1991 and 1996 papers, DNA analyses have provided evidence for multiple introductions of the insect into California (Meixner et al. 2002), and subsequent examinations of pest interception data suggest that Carey substantially underestimated the rate at which Medflies are being introduced into the USA (Liebhold et al. 2006). These realizations don't rule out the possibility of an established Medfly population in California, but they do leave the situation open to alternate explanations. In addition, data on the sensitivity of the Medfly detection trapping system (Lance and Gates 1994) make it difficult to envision the existence of numerous sub-populations that are large enough to be viable yet completely escape detection for multiple consecutive generations across southern California (for example, no Medflies were caught in California in 2000, 2003 or 2006; K. Hoffman, CDFA, personal communication). A more parsimonious explanation for observed patterns of Medfly discovery in California may be that commercial fruit smuggling plays a central role, given the levels of wholesale agricultural products that are being intercepted coming into the U.S. illegally (USDA-APHIS-PPQ 2006c). Regardless, the controversy continues (Carey 2010; Liebhold et al. 2010).

15.7 Mediterranean Fruit Fly in Australia

15.7.1 Origin and Spread of Medfly in Australia

Medfly established in Western Australia (WA) during the late 1890s (Sproul 2001), probably through infested citrus fruit. Around the same time medfly was found near Sydney and Tasmania where it was eradicated. In New South Wales (NSW), medfly established and remained an important pest until it died out during the 1940s, possibly as a result of the expansion in range of Queensland Fruit Fly (*Bactrocera tryoni* (Froggatt)) (Hely et al. 1982; Waterhouse and Sands 2001). Medfly also established in Victoria with the last outbreak in 1953 in Melbourne (R. Mapson, personal communication). Outbreaks at Alice Springs in the Northern Territory during 1976 and 1982 were eradicated (Allwood et al. 1979). In South Australia, which has a similar Mediterranean climate to WA, infestations have been detected every 2–3 years (Madge et al. 1997), and, during the last 10 years, outbreaks have occurred in Adelaide in 2000, 2002, 2006 and 2010. Medfly populations have not been reported in other states during the last 50 years. Medfly has not established in Queensland, possibly due to competition from the large endemic fruit fly fauna.

From the initial infestation in Perth, medfly spread on infested fruit and is now established in towns and growing areas from Esperance on the south coast to Derby in the subtropical north. Medfly is most pestiferous in growing areas surrounding the capital city Perth, where large populations develop in urban areas. In the colder Manjimup region, it is only a minor pest, and an area free of medfly is maintained in the Ord River Irrigation area near Kununurra in the far north. That area has an extreme tropical climate, with high temperatures and humidity in the wet season, which is unfavourable for Medfly survival.

In most of the WA bushland, there are no native or feral medfly hosts. A few feral hosts survive along river systems in the southwest while some native hosts grow in the tropical north but disjunctions in fruiting phenology means that medfly is unlikely to survive away from human habitation. The desert areas between western and eastern Australia form a natural barrier to its spread eastward.

Medfly is the only species of *Ceratitis* established in Australia. Medfly adults are readily distinguished from other pestiferous Australian tephritids, which are primarily *Bactrocera* species (White and Elson-Harris 1992). Larval keys are available (Dadour et al. 1992) and allozyme methods (M. Adams: personal communication) can be used to separate medfly larvae from those of Queensland Fruit Fly.

15.7.2 Host Range in Australia

In reviewing the WA literature, Sproul (2001) reported 69 hosts while (Hancock et al. 2000) listed 53 species in 23 plant families as hosts. Key commercial hosts include citrus, stone fruit, and pome fruit. Citrus is the main over-wintering host.

In urban areas loquats and kumquats (*Citrus, sensu lato*) are important hosts while table grapes (*Vitis vinifera* L.) and olives (*Olea europaea* L.) can also be attacked. At Carnarvon, 1,000 km north of Perth, medfly attacks citrus, mangoes (*Mangifera indica* L.) and infests overripe capsicums (*Capsicum anuum* L.) left after harvest. The host range in the town of Broome, 2,000 km north of Perth has been studied with fruits of 18 plants found to be hosts (Woods et al. 2005). Based on abundance, fruiting phenology and host suitability, the most important hosts were mango, kumquat, Barbados cherry (*Malpighia glabra* L.), orange jessamine (*Murraya paniculata* (L.) Jack), guava, Pacific almond (*Terminalia catappa* L.), "blackberry tree" (species not confirmed) and yellow oleander (*Cascabela thevetia* (L.) Lippold).

15.7.3 Eradication Programs in Australia

In 2001, the Department of Agriculture in Western Australia commissioned a benefit-cost analysis on the eradication of medfly from Australia (Mumford 2005). The analysis found that eradication over 6 years, using baiting followed by release of sterile insects, would cost US$35 million, had a high probability of success, and if the area planted for horticulture doubled over 20 years then net benefits at present value were likely to be positive.

15.7.4 Tools for Managing Medfly in Endemic Areas

Bait sprays. Before the advent of systemic organophosphate cover sprays in the late 1950s, baiting combined with good orchard hygiene was the mainstay of fruit fly control. Baiting is still an important control tool, especially in citrus orchards where weekly baiting can maintain good control. In high pressure areas with susceptible crops such as stone fruit, baiting must be supplemented by cover sprays.

Twice weekly bait applications are required if fly pressure is high. Protein hydrolysate baits are most widely used in combination with the insecticide Malathion (known in Australia as "Maldison") or Trichlorfon. Spinosad is an organically compatible alternative but is higher priced and rarely used except by organic growers and in some community baiting schemes in urban areas. In the latter cases, Spinosad's low toxicity and public relations benefits can justify the extra cost, although some community baiting schemes still rely on protein bait/organophosphate mixtures.

Community baiting schemes involve application of bait mixture to all fruiting trees in a town or adjacent horticulture area for 6–12 months of the year. If well managed, they can maintain Medfly populations at non-damaging levels. Ratepayers are generally levied fees based on the number of trees per property and shire involvement is essential for long-term sustainability. Maintaining these schemes over several years has proved a difficult task, with many schemes terminating due to funding or staffing issues.

Cover sprays. The introduction of the systemic cover sprays containing the organophosphate insecticides fenthion and dimethoate revolutionized fruit fly control in Western Australia (WA). They are still widely used, especially in high-susceptibility crops in high-pressure areas. These areas often abut urban areas where fly populations can reach very high levels. If maggots infest fruit, cover spraying is the only effective option left for growers. Restrictions on the use of these insecticides are likely in the near future, which may make fruit production uneconomical in some areas.

Mass trapping. As noted above, effective female lures have been available for at least 10 years (Heath et al. 1996), but growers in Australia have only recently begun experimenting with mass trapping. Mass trapping alone probably will not give effective control, especially in high-pressure areas where many small orchards are adjacent to urban areas or orchards with unmaintained fruit trees.

Biological Control. Parasitism of field-collected larvae in WA is very low with only the native *Bactrocera* parasitoid *Diachasmimorpha kraussii* (Fullaway) reared (I. Lacey: personal communication). Attempts to establish other parasites during the 1950s were not successful (Waterhouse and Sands 2001). *Fopius arisanus* (Sonan) is established in Queensland on *Bactrocera* species and could possibly be used for inundative release in conjunction with SIT, because it causes significant mortality to medfly in Hawaii (Rousse et al. 2005). *Fopius ceratitivorus* Wharton is the most promising candidate for classical biological control (Lopez et al. 2003) but must undergo specificity testing against native tephritids before approval would be given for its release into Australia.

Sterile Insect Technique (SIT). SIT has not been used for field control in WA. Orchards are generally small, may contain mixed varieties of fruit, and often abut urban areas, making area-wide management using SIT difficult. SIT, however, is a critical part of programs to keep South Australia free of medfly (see below).

Interstate movement. The Medfly Code of Practise (Anon 2008) describes responsibilities and procedures that apply to the management of medfly and lists phytosanitary requirements for trade between Australian states. The draft document is under constant review and treatments may differ from international standards. The Code lists requirements for pest free areas, areas of low pest prevalence, pest free places of production and infested areas. Surveillance procedures for establishment of area freedom (e.g. trap type, trap number; trap location and frequency of inspection) are listed. Risk management in terms of buffer zones, movement restrictions, and treatment of susceptible hosts is discussed. Thresholds for suspension and suspension areas are listed. Annexes list susceptibly of produce, approved disinfestation treatments, and re-instatement dates after loss of area freedom.

Quarantine barriers. Only one major paved road connects Perth with Adelaide in South Australia, and a 24-h quarantine checkpoint operates at Ceduna, which is near the South Australia border. All vehicles are stopped, manifests checked, and private vehicles inspected for fruit fly hosts.

Interstate Certification Assurance (ICA). This is a system of plant health certification based on quality management principles and is a national scheme administered by all Australian states and territories (Anon 2013). To be accredited, a business must demonstrate it has effective in-house procedures in place that ensure produce consigned to intra- or interstate markets meets specified plant quarantine requirements. The ICA scheme seeks to provide a harmonized approach to the audit and accreditation of businesses throughout Australia. Under ICA protocols businesses can issue a plant health certificate enabling interstate trade rather than involving government officials at far greater cost. Businesses issuing ICA's are regularly audited by government authorities. ICA's of relevance to medfly include ICA-04: Fumigating with methyl bromide, ICA-07: Cold treatment, ICA-16: Certification of mature green condition of bananas, ICA-23: Area or property freedom, and ICA-30: Hard condition of avocado for Mediterranean Fruit Flies.

Disinfestation. The code of practise lists over 100 species that require disinfestation treatment to enter the other states. Fifteen of these are in the Family Rutaceae, e.g. *Citrus* spp., and 13 in the Family Roseaceae, *e.g. Prunus* spp. Avocado (*Persea americana* Mill.), banana (*Musa acuminata* Colla), lime (*Citrus* spp.), olives, papaya and strawberry (*Fragaria* spp.) must be treated unless harvested mature green. Durian (*Durio zibethinus* Murr.), lychee (also called litchi), mangosteen (*Garcinia mangostana* L.), passionfruit (*Passiflora edulis* f. *flavicarpa*), pomegranate (*Punica granatum* L.), and rambutan (*Nephelium lappaceum* L.) do not require treatment if the skin is unbroken. Of the solanaceous vegetables listed as hosts, capsicums can be attacked when overripe, tomatoes (*Lycopersicum esculentum* L.) are very rarely attacked even when overripe, and the host status for eggplant (*Solanum melongena* L.) needs to be confirmed.

The list of some of the produce from WA that requires disinfestation treatment for medfly includes: Apple (*Malus domestica* L.), apricot (*Prunus armeniaca* L.), blackberry (*Rubus fruticosus* L.), blueberry (*Vaccinium corymbosum* L.), calamondin (×*Citrofortunella mitis* (Bunge) Wijnands), capsicum, carambola (*Averrhoa carambola* L.), cherry (*Prunus avium* L.), chili (*Capsicum* spp.), citron (*Citrus medica* L.), eggplant, feijoa (*Acca sellowiana* (O. Berg) Burret), fig (*Ficus carica* L.), grape, grapefruit (*Citrus* × *paradisi* Macfad.), kiwifruit (*Actinidia* spp.), kumquat, lemon (*Citrus limon* (L.) Burm.), lime, loquat (*Eriobotrya japonica* (Thunberg) Lindl.), lychee, mandarin (*Citrus reticulata* Blanco), mango, mulberry (*Morus spp.*), nashi (*Pyrus pyrifolia* (Burm. Nak.)), nectarine (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C. K. Schneid), olive, orange (*Citrus* × *sinensis* (L.) Osbeck), papaya, peach (*Prunus persica* (L.) Batsch), pear (*Pyrus* spp.), persimmon (*Diospyros* spp.), plum (*Prunus domestica* L.), pomegranate, quince (*Cydonia oblonga* Mill.), raspberry (*Rubus* spp.), strawberry, Tamarillo, tangelo (*Citrus* × *tangelo* J. Ingram & H. E. Moore), and tomato.

Disinfestation methods. Despite the recognition of generic radiation dose for fruit fly disinfestation, there are no commercial irradiators in WA so this option is not available. Also, hot water treatment, high temperature forced air, and vapour heat are not used for medfly disinfestation in WA. Cold treatment is the most common disinfestation treatment and is widely used on temperate crops. Its effectiveness at different temperature on a wide range of commodities has been demonstrated in Western Australia (F. DeLima, personal communication). Fumigation with Methyl Bromide remains the treatment of last resort and is still widely used despite the possibility of deregistration in the long term. Post-harvest dipping or flood spraying with insecticide was once widely used, but these treatments are likely to be withdrawn because of residue concerns.

Area-wide management and Systems Approaches. Currently, no Systems Approaches are certified for export of produce potentially infested with medfly (Jessup et al. 2007). A draft ICA for Systems Approaches is being developed. Some areas of Western Australia with low pest pressure due to unfavourable climate and/or lack of hosts (e.g., west Midlands, Manjimup) may be able to export fruit to eastern Australia under a protocol combining areas of low pest prevalence with pest free places of production. As yet no officially recognised areas of low pest prevalence exist in Western Australia.

15.7.4.1 Fly-Free Zones

Area freedom in Western Australia. In WA area freedom from medfly is maintained in the Ord River Irrigation Area (ORIA) in the north of the state. Approximately one hundred traps on a 400 m grid in the town of Kununurra and a 1 km grid in the growing area are checked weekly (April-October) when outbreaks are likely and fortnightly at other times. Traps are bucket-type Lynfield Traps (Wijesuriya and De Lima 1995) with dental wicks containing Capilure® (a commercial mixture of Trimedlure with extenders to increase field life (Hill 1987) and a small piece of Dichlorvos Pest Strip (1 cm^2) in the traps to kill the flies and pests such as ants. Traps are re-lured and pest strips changed every 2–3 months. Outbreaks in the past have been eradicated using a combination of bait spraying, fruit stripping and cover spraying. Future eradications are likely to use SIT with bait spraying and limited fruit removal.

Area freedom in South Australia. The state of South Australia is free of fruit flies and supports a large horticultural industry with export of citrus fruit to the USA from the inland Riverland region. Medfly outbreaks regularly occur in Adelaide and are eradicated. The eradication programs have evolved from those involving primarily fruit stripping, then organophosphate insecticide baits and cover sprays, to (since 2001 an integrated approach involving baiting with organic insecticide, minimal fruit stripping, and release of sterile flies.

Lynfield Traps (Cowley et al. 1990) are used to detect incursions with Capilure® and dichlorvos added to the dental wicks. These are placed on 400-m grids in urban areas and 1-km grids in growing areas. Traps are checked weekly in the season when outbreaks are likely and fortnightly at other times. Despite the intensive trapping, medfly infestations in this area are often found by the public reporting larvae in fruit before flies are found in traps (D. Cartwright, personal communication). Reporting of any maggot-infested fruit is encouraged through regular media campaigns. When an outbreak has been detected, supplementary traps are deployed. Both male and female traps are used. The female traps are McPhail-type using the three-component synthetic food lures (Broughton and de Lima 2002).

When an outbreak has been declared, release of sterile flies follows an initial 2–4 week period of baiting with Spinosad to lower fly numbers to negligible levels. Sterile fly release typically continues for 10 weeks after the baiting ceases. However, if winter weather encroaches into this period then sterile release may be suspended and resumed in spring.

Sterile fruit flies produced at the Department of Agriculture and Food Western Australia factory at South Perth, Western Australia are used to eradicate outbreaks in South Australia. Flies must meet or exceed international quality standards (IAEA 2003). The Vienna 7/99 "mix" strain has been in continuous production since 1999, with production levels maintained above 1.5 million per week and to up to 10 million per week during outbreaks. Vienna 7/99 is a *tsl* genetic sexing strain, and a filter rearing system is used to ensure that the level of deleterious recombinants are minimised (Fisher and Caceres 2000). Flies are reared on a bran-based diet using boiling water to minimise bacterial contamination. Larvae are collected into water, spread on vermiculite for pupation, and hand-sieved to maximize pupal quality and flight-ability. Two days before emergence, pupae are irradiated at a mid-point dose of 160 Gy in a Gamma cell 220 irradiator. Pupae are flushed with nitrogen for 10 min before irradiation and during irradiation. A radiation-sensitive indicator (Sterin badge) is placed on each canister to provide visual confirmation that the canister was irradiated (ISO/ASTM 2013).

Irradiated pupae are dyed, heat sealed in plastic bags and placed in foam vegetable boxes with Techni ice® for overnight shipment to South Australia. In South Australia 14.5 g of pupae are placed into 5 l cardboard buckets and held in an emergence room for 5 days. The buckets have two screen inserts in the side to allow room exposure to ginger root oil (GRO, a source of α-copaene) which improves mating competiveness (Shelly et al. 2007). On day five the flies are moved to a separate cool room for exposure to GRO from wicks hung from the roof using fans to circulate the air. Flies are released from a purpose-built release pod on the back of a utility vehicle; one bucket is released approximately every 100 m. Initial release rate is higher before dropping to a lower maintenance level once sufficient fly numbers have been reached in the area. Identification of wild or sterile flies is accomplished with an electro-florescent microscope and confirmation is established by dissection of the ptilinum.

Suspension zones These are based on analysis of outbreak data from South Australia (Meats et al. 2003). If a gravid female or larvae are found then an outbreak is declared with a 7.5 km radius from the outbreak centre. If one male is caught, then no further action is required. If two males are caught then a larval search is required and 16 supplementary male traps may be deployed within the 200 m zone. If supplementary traps are not deployed and three males are caught within 1 km and 14 days, then an outbreak must be declared. If supplementary traps are deployed the trigger moves to five flies before an outbreak is declared. However despite scientific evidence for a 7.5 km zone, Queensland only accepts a minimum 15 km zone and Tasmania a 80 km zone.

Reinstatement of pest freedom The pest free status of a suspended area, which is subject to eradication following a Medfly incursion can be reinstated providing no wild Medfly is trapped in the surveillance program for a generation and 28 days, or 12 weeks after the last larva or wild Medfly is captured in the traps, whichever is the longer. Reinstatement dates for several centres and dates have been calculated from Australian government metrological data and are tabulated in an annex to the code of practise (Anon 2008).

15.8 Concluding Remarks

The Mediterranean Fruit Fly remains a feared agricultural pest throughout tropical to warmer temperate areas around the globe. While it can produce substantial in-field losses of many fruit crops, the insect's ability to move though commerce to uninfested areas has led to most of its infamy. The medfly is one of the most studied insects in the world, and efforts to detect and control the pest have led to broader innovations in areas such as insect attractants and detection, control methods, and regulatory procedures. The development of programs to combat and contain this insect has mirrored the broader development of regulatory programs worldwide, making medfly an excellent case study in regulatory entomology.

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Chapter 16
Case Study. Invasive Insects in Plant Biosecurity: The Asian Longhorned Beetle Eradication Program

Michael Stefan, Christine Markham, Robert Benjamin, and Jeffrey Coath

16.1 Introduction

Asian Longhorned Beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera; Cerambycidae), is an invasive beetle that attacks and kills living and stressed host trees. Throughout the USA, ALB poses a serious economic and environmental threat to city, urban, rural and forested areas. ALB has an impact on the people and industries that depend on these natural resources and the environment in which they live. The woodborer attacks 13 genera of hardwood tree species in the USA. Preferred hosts include *Acer* spp. (maple, boxelder), *Betula* spp. (birch), *Ulmus* spp. (elm), *Salix* spp. (willow), and *Aesculus* spp. (horsechestnut, buckeye).

In 2009, the United States Forest Service (USFS) estimated that throughout the USA about 169 million acres of forested habitat with maple species is vulnerable to attack from ALB. The estimated maximum potential national urban impact of ALB is a loss of 34.9 % of total canopy cover, 30.3 % tree mortality (1.2 billion trees) and value loss of $669 billion USD (Nowak et al. 2001). Given the range of affected species, other potential adverse impacts include the forest products industry (lumber and furniture), nursery stock industry, maple syrup production, and fall-foliage tourism. Loss of trees also decreases property values, causes aesthetic damage, and lessens the environmental benefits of trees. Potential environmental impacts would be wide-ranging and not restricted to the loss of host trees. Negative impacts to soil and water quality will occur, as well as impacts to fish and wildlife, including threatened and endangered species that depend on host trees. The loss of ecological function related to host-tree loss would also impact natural resource management activities and add an additional stressor to forests that are already impacted due to

M. Stefan • C. Markham (✉)
USDA APHIS PPQ, 920 Main Campus Drive, Raleigh, NC 27606, USA
e-mail: btlebopper@gmail.com; ckm9220@gmail.com

R. Benjamin • J. Coath
USDA APHIS PPQ, 2300 E. Devon Avenue, Suite 122, Des Plaines, IL 60018, USA

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other manmade and natural factors. The beetle potentially can alter the ecological diversity of the natural forests in North America. (http://www.fs.fed.us/foresthealth/technology/invasives_anoplophoraglabripennis_riskmaps.shtml)

In 2010, USDA APHIS began a multiyear cooperative agreement with Pennsylvania State University and the Cary Institute of Ecosystem Studies to determine the potential ecological and economic impacts of ALB invasions in the northeast USA Forests, providing no eradication or management actions occur. Results show that 45 % of the adult trees in eastern USA forests are at risk for ALB infestation (current USA ALB host list), including maples (sugar and red maples), birches, poplar, and elms. Red maple and sugar maple are the two most abundant tree species in the eastern USA (all states from Minnesota south to Louisiana and eastward). Sugar maple is an ecologically important species in late successional and old growth forests. Red maple occurs across a range of environments from ridge tops to swamps. Both species are distributed throughout the eastern USA.

Virtually all individuals of preferred host trees are expected to die within 10–20 years following ALB invasion of a stand. Loss of preferred tree species triggers replacement by non-preferred tree species. However, in many regions of the USA, species that are expected to replace the species at risk from ALB are themselves threatened by other introduced pests and/or pathogens, such as Beech Bark Disease and Hemlock Woolly Adelgid (*Adelges tsugae* (Annand)). The net effect is the significant reduction of the merchantable volume of timber in eastern forests over at least the next 50 years. Based on the assumption that the outbreak occurs everywhere at the same time, the apex of the outbreak is estimated at year 25 when over 71 billion board-feet are anticipated to be killed by ALB throughout the study region. Over 100 years, about 850 billion feet are expected to be killed by ALB in the region. Reduction in timber volume is mirrored in reductions in carbon storage and represents a significant reduction in the strength of the sink for CO_2 in eastern forests. (Canham, Jacobson, 2012, personal communication).

On several occasions during the 1980s and 1990s, ALB was intercepted at ports of entry in solid wood packing materials (SWPM) as part of international commerce. The first established population of ALB was discovered in New York City (1996) and then in Chicago (1998). Subsequently, ALB has been found in New Jersey near New York City (2002), Middlesex/Union Counties New Jersey (2004), Worcester, Massachusetts (2008), and Boston, MA (2010), and recently in Bethel, OH (2011). On March 9, 1999, the US Secretary of Agriculture officially declared the discovery of ALB an emergency, paving the way to obtain funding to address the SWPM pathway and contain and control the outbreaks, and eradicate the ALB wherever it may be found in the USA.

Potential losses from an unchecked outbreak are high. However, eradication is possible given the biology of ALB, current extent of infestation, and available treatment options. Compared with other forest insect pests, such as Emerald Ash Borer (*Agrilus planipennis* Fairmaire), ALB has a low reproduction rate and does not naturally disperse long distances. Current infestation areas in the USA are also comparatively small, and effective control techniques are available. However, they are costly and involve destruction of infested trees and chemical treatment of exposed host trees.

Eradication efforts have been successful in Illinois and New Jersey and certain areas of New York. Similar results are expected within several years in other areas of New York where USDA APHIS eradication efforts are underway. Successful eradication depends on many factors including strong stakeholder support and sufficient resources to carry out effective eradication efforts. Here we report on the ALB cooperative eradication national program with a focus on the key elements in the successful eradication program that centered in Chicago, Illinois during 1998 to 2008. Eradication efforts continue to make progress in New York, Massachusetts and Ohio.

16.2 Biology

ALB is a large-bodied cerambycid beetle endemic to eastern Asia, primarily China and South Korea (Yan 1985; Peng and Liu 1992; Wu and Jiang 1998; Lingafelter and Hoebeke 2002). The beetle is distinctive in its large size (2–3 cm long), coloration (predominantly black with irregular white spots), and elongate antennae that are banded with white and black (to 10 cm long) (Fig. 16.1a, b). The elongated feet are black with a whitish blue upper surface on young adults. The female adult beetle chews a depression in tree bark of branches and along the main trunk of host trees and deposits one egg per oviposition site (Fig. 16.2a, b). Eggs hatch within 1–2 weeks and the young larvae feed on phloem just under the bark. Typically, females lay 25–40 eggs during their lifetime (Haack et al. 1997; Becker 2000; Smith 2000). Females lay eggs and larvae thrive on healthy or stressed host trees of all ages. The first three larval instars tunnel through the phloem and cambial tissue of the tree disrupting growth and the circulatory system. The late third and early fourth instars bore into the xylem weakening the physical structure of the tree (Fig. 16.5). ALB can overwinter as an egg, as a larva developed within an egg, as a larva, or as a pupae. Newly developed adult beetles chew through the bark and emerge from round exit holes, ~1.5 cm in diameter, on trunks and branches (Figs. 16.7 and 16.8). Adults typically are active from May to October and feed on bark and cambium of twigs and petioles and veins of leaves. As the number of beetles attacking the tree increases, the tree eventually dies.

16.2.1 Life Cycle

Beetle holometabolous, undergoing egg, larva, pupa and adult stages.

Egg Stage (Fig. 16.3): Eggs off-white, oblong and 5–7 mm long; both ends are slightly concave (Peng and Liu 1992). *Larval Stage* (Fig. 16.4): Mature larvae 50 mm long. Prothorax with brown mark; front of mark lacking a brown margin (Peng and Liu 1992). *Pupal Stage* (Fig. 16.6)*:* Pupae off-white, 30–33 mm long and 11 mm wide; eighth segment of abdomen has a protruding structure (Peng and Liu

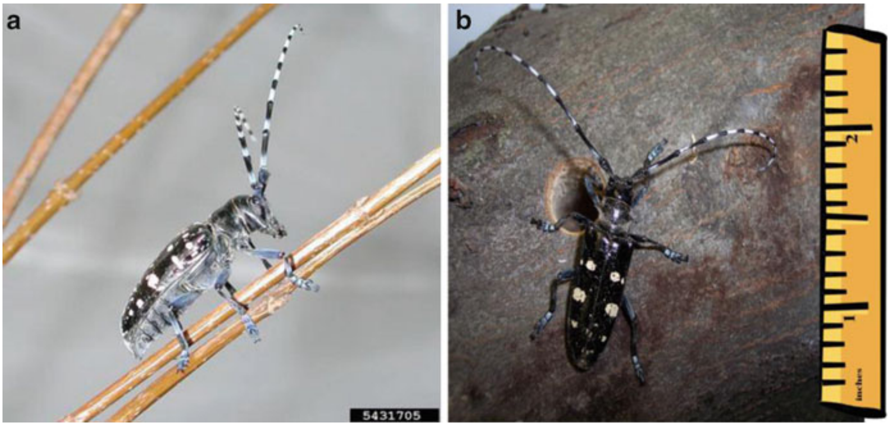

Fig. 16.1 (**a**) ALB adult (Image courtesy M. Keena, USFS); (**b**) Newly emerged female ALB adult and exit hole (Image courtesy A. J. Sawyer, APHIS USDA)

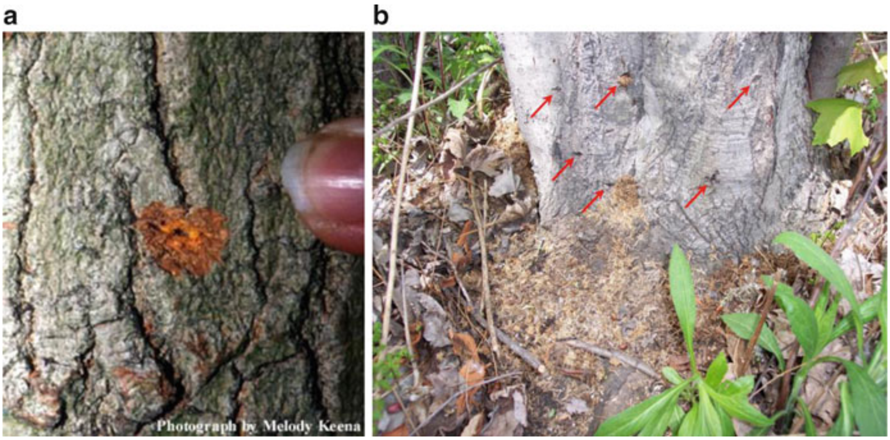

Fig. 16.2 (**a**) (*left*) Oviposition site on *Sugar Maple* (Image courtesy M. Keena, USFS); (**b**) (*right*) ALB oviposition sites on *Red Maple* (Linden, NJ). Small larvae eject frass from some sites, which collects on the ground (Image courtesy A. J. Sawyer, APHIS USDA)

1992). *Adult Stage* (Fig. 16.1a, b)*:* Adults are 20–35 mm long, 7–12 mm wide and jet black with white specks. Antennae have 11 segments with base of the antenna whitish with a blue-black colouration. Antennae of males are 2.5 times their body length; the antennae of females are 1.3 times their body length. The bases of the elytra do not have a granular appearance. Each elytron has about 20 white spots (Peng and Liu 1992).

Fig. 16.3 ALB eggs under bark (Image courtesy M. Keena, USFS)

Fig. 16.4 Post-eclosion from egg, ALB larvae bore into tree and feed (Image courtesy, A. J. Sawyer, APHIS USDA)

Fig. 16.5 Damaged limb and cross-sections of *Red Maple* at Linden, New Jersey (Image courtesy A. J. Sawyer, APHIS USDA)

Fig. 16.6 ALB pupa (Image courtesy M. Keena, USFS)

Fig. 16.7 Round, dime-size exit holes from which beetles emerge (Image courtesy A. J. Sawyer, APHIS USDA)

Fig. 16.8 Host tree with multiple exit holes (Image courtesy A. J. Sawyer, APHIS USDA)

16.2.2 Biological Aspects Important for Successful Management

ALB has three aspects in its biology that work to the advantage of successfully regulating and eradicating this pest: (1) A limited natural dispersal rate, (2) a year long egg-to-adult development interval, and (3) a large showy appearance that facilitates easy identification by the public. The natural spread of ALB is relatively slow. Researchers estimate that dispersal is typically less than 1.5 miles per year depending upon the landscape and density of hosts (Sawyer, Smith, 2000, personal communication). ALB requires at least 1 year for completion of a full life cycle. Research by the USFS has shown that complete life cycle development is temperature dependent on certain stages of the ALB. If the required degree-day accumulation is not met within a given year, the ALB life cycle development may take more than 1 year to complete (Keena et al. 2010). However, this extended development has not been directly observed in outbreak areas of the USA. As of 2012, an effective trap-and-attractant is not available to detect ALB. For many years, scientists have been researching, developing and refining a lure for ALB adults and testing potential lures and traps in China and within ALB infested areas of the USA. The results show promise and USDA APHIS is working with the USFS to determine efficacy of a trap and lure for operational use in the ALB eradication program. The large size, distinctive coloration, and long antennae, however, catch the attention of both children and adults. Each of the outbreak areas in the USA was first reported by an alert, curious citizen.

16.3 Development of the National ALB Cooperative Eradication Program

Following a Declaration of Emergency by the Secretary of Agriculture, USDA APHIS established two goals: (1) to prevent further introductions and outbreaks of ALB by closing the pathways of introduction, and (2) to eradicate outbreaks of ALB in the USA.

16.3.1 Exclusion

Efforts to achieve eradication would be fruitless without taking steps to prevent additional introductions of ALB into the USA. On December 17, 1998, USDA APHIS amended regulations for importing logs, lumber, and other unmanufactured wood articles by adding treatment and documentation requirements for SWPM imported from China, including Hong Kong (USDA APHIS 1998) This change required that wooden pallets, crating, dunnage, and other SWPM imported into the

USA from China must be heat-treated, fumigated, or treated with preservatives and certified by the Chinese government before departure from China and entry into the USA.

Although the interception of invasive species in SWPM from China and Hong Kong decreased drastically after the promulgation of this regulation, interceptions of harmful invasive pests and pathogens from other parts of the world through infested SWPM continued to rise. Coping with the pest risks associated with introduction of these pests of SWPM became an increasingly important issue with the expansion of international trade and potential impacts to the environment (USDA, APHIS 1994). It became clear that the USA had to do something further to diminish the threat.

USDA APHIS adopted the phytosanitary standards contained in the International Plant Protection Convention's (IPPC) "Guidelines for Regulating Wood Packaging Material in International Trade" (IPPC 2002). The IPPC is an international treaty on plant health to secure action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control (Chap. 2). Currently 177 signatories are on the agreement. IPPC Guidelines provided effective, equitable, and uniform standards (prescribed treatments, certification procedures, and standardized markings) that all nations could use to mitigate risk from SWPM. The International Standards for Phytosanitary Measures "Guidelines for Regulating Wood Packaging Material in International Trade" (ISPM15) is one of several International Standards for Phytosanitary Measures adopted by the IPPC. ISPM15 is a standard upon which many countries Wood Packing Material regulations are based.

On July 5, 2006, full enforcement began on all SWPM entering the USA and North America. Shipments containing noncompliant regulated SWPM are not allowed to enter the USA (USDA APHIS 2004). When noncompliant material is identified, it is immediately quarantined and promptly shipped back to the exporting country until compliance has been met. If infested packing material is found, then in most cases the infested material must be separated from the imported products and immediately destroyed.

16.3.2 National Survey and Eradication

Early detection is essential to successful and efficient eradication of an exotic pest. Discovering the pest when the infestation is small in size allows managers more flexibility when choosing tactics and control methods, and provides a greater chance of success for eradication. Upon discovery, immediate and aggressive actions to eliminate the pest result in shorter and less expensive eradication programs. In 2000, USDA APHIS implemented a national survey for ALB at high-risk importing establishments throughout the USA to determine whether there were any other incipient infestations of ALB or other exotic wood borers. The Exotic Wood Borer/Bark Beetle National Survey, as it is known today, aims to: (1) Conduct pathway analyses, inspections, and trapping activities in high-risk areas; (2) stress the importance of the submission of timely and accurate reports; and (3) make the public aware of wood pests.

At the time when the first infestations were detected in the USA, little was known about the biology and life cycle of ALB and strategies to eradicate this invasive species. Initial efforts by federal, state and city officials in Chicago and New York City were focused on visual inspection of host trees, removing infested trees, and preventing additional spread by establishing quarantines surrounding the infestations and regulating movement of the pest or host material out of the infested area. The initial regulated areas and response were focused immediately surrounding the infestation since little was known about the dispersal potential of the insect.

USDA APHIS recognized the need to establish a national management structure to coordinate field activities, obtain resources to implement the program and develop the strategies for eradicating the ALB from USA based on scientific principles. In April 2000, the New Pest Response Guidelines, Asian Longhorned Beetle, *Anoplophora glabripennis*, was developed to provide consistent and uniform procedures and actions to take when addressing an outbreak of ALB. The document includes the technical and general information needed to implement any component of an ALB eradication program. The procedures described in the New Pest Response Guidelines were developed by consulting with USDA APHIS, State Plant Regulatory Officials and scientists directly involved in ALB eradication. In August, 2008, the New Pest Response Guidelines for the ALB were updated to reflect refinements, efficiencies, and procedures to the eradication response (USDA APHIS PPQ 2008).

During September 2000, the Strategic Plan for Eradication of ALB from New York and Illinois was completed and implemented. The Plan was written with input from the primary collaborators: USDA APHIS, USFS, New York State Department of Agriculture and Markets, New York City Department of Parks and Recreation, City of Chicago, Department of Streets and Sanitation, Bureau of Forestry (BoF), Illinois Department of Agriculture (IDA), and New York State Department of Environmental Conservation. The goal was to eliminate ALB from the USA. To do so, the Plan uses an area-wide integrated pest management strategy for eradication that integrates the following activities: (1) Regulatory activities to prevent the pest's spread; (2) Visual survey; (3) Control through host tree removal and chemical treatment; (4) Public outreach; (5) Replanting of removed trees with non-host species; and (6) Research. The Plan defined roles, set requirements for deregulation and defined eradication, provided a time line and actions to accomplish eradication in each outbreak area, and established performance measures. The ALB Cooperative Eradication Strategic Plan provided cooperators, stakeholders, and the public a clear blueprint to follow to achieve ALB eradication. With the help of the Plan, USDA APHIS was able to obtain $49 million in emergency funding to implement the program. The Strategic Plan was updated in December 2005 to include eradication plans for the New Jersey outbreaks and update the actions and timelines for eradication in New York and Illinois (USDA APHIS PPQ 2005).

16.3.3 Current Status of ALB Infestations in the USA

As is often the case with new invasive pests, little information was known or available about this invader. USDA APHIS scientists immediately started working with Chinese collaborators to understand its biology and the means to detect and manage the pest. Several chemicals and methods of application were evaluated and successfully implemented into the USDA APHIS response program. Through the efforts of program managers and the scientists working closely together, ALB has been eradicated from Illinois and New Jersey, and the Islip area of New York. Treatments are completed and final confirmation surveys are underway to achieve eradication in additional areas of the New York outbreak, including Manhattan and Staten Island. The outbreak in Boston Massachusetts was detected very early as a result of public outreach and an observant and diligent individual who detected the infestation with only six host trees affected by ALB infestation. Three consecutive years of area wide chemical treatments have been completed surrounding the infested trees and no additional infested trees have been detected since the initial discovery in July 2010. The most recent larger outbreak areas in Worcester, Massachusetts and Clermont County, Ohio are in the delimitation, containment and suppression stage.

16.4 Components of an Emergency Program

The ALB Cooperative Eradication program, similar to most eradication programs, consists of several components, including regulatory to contain the pest to prevent spread, survey to determine where the pest is present to guide management activities, and control to suppress and eliminate the pest population. The ALB program also addresses recovery of the affected area where appropriate by replanting trees that are not host to the ALB in areas directly impacted by host removals to mitigate tree loss from these areas. Public outreach is delivered to the impacted communities to encourage support of eradication efforts and to educate communities to search for and report suspect infestations. Research is a vital component of the eradication strategies to develop and enhance the tools for successful eradication and improve efficiencies of operations.

16.4.1 Regulatory

To prevent established infestations from spreading through human assisted transport, domestic quarantines are enacted to control regulated materials from moving outside of quarantined areas. The regulated boundary is typically set at least a 1½ mile radius around an infestation. This boundary was determined based on research studies into the natural dispersal distance of ALB (Sawyer 2006; Smith et al. 2004). However, this boundary may be adjusted dependent on the size and scope of the infestation and the host density and distribution in the area (Sawyer and Panagakos 2009).

Federal Quarantines for ALB include 7 CFR 301.51 for eradication programs and 7 CFR319.40 for solid wood packing material. However, under these regulations, USDA APHIS cannot quarantine a geographical area smaller than an entire state. As a result, the State Plant Regulatory Agency from the impacted state must enact an interior state quarantine for ALB to facilitate regulatory activities in a geographical area within the state.

ALB regulated articles include the following: (a) all life stages of ALB; (b) firewood (all hardwood species), green lumber and other material living, dead, cut, or fallen, inclusive of nursery stock, logs, stumps, roots, branches, and debris of half an inch or more in diameter of the following genera: *Acer* (maple), *Aesculus* (horse chestnut), *Albizia* (mimosa), *Betula* (birch), *Celtis* (hackberry), *Cercidiphyllum* (katsura), *Fraxinus* (ash), *Koelreuteria* (golden rain tree), *Platanus* (sycamore), *Populus* (poplar), *Salix* (willow), *Sorbus* (mountain ash), and *Ulmus* (elm); (c) any other article, product, or means of conveyance not covered by (a) if an inspector determines that it presents a risk of spreading ALB. If additional genera or species are found to support complete life cycle development of ALB in the wild within the USA or within China, then these genera or species will be evaluated by USDA APHIS for possible addition to the USA Host List: (http://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/hostlist.pdf.)

To facilitate compliance, USDA APHIS and the State Plant Regulatory Officials enter into compliance agreements with establishments handling regulated material. This entails educating the establishments on ALB biology and symptoms of damage, and the procedures that must be followed to prevent transport of host material outside regulated areas.

16.4.2 Survey/Detection

Detection activities have several purposes in the program. Initially, an intensive visual inspection of all hosts is completed to delimit the size and scope of the pest outbreak. This information is vital for determining the best tactics and developing the strategy to eliminate the pest. Ongoing monitoring surveys during the life of the program are conducted to measure progress and identify any changes in the distribution and abundance of the pest. Last, intensive confirmation surveys are completed to verify the pest no longer exists in an area.

As of 2012, there are no traps, chemical attractants, or pheromones available for the program to use to find ALB. Surveys are completed through visual inspections on all host trees in quarantined areas; however, light infestations are difficult to detect, especially when surveying trees from the ground. The program incorporates "bucket" trucks and tree climbers when needed to better ascertain the infestation status of a tree or tree. Along with surveying the host trees surrounding the infestation, official surveys are conducted outside the infested area around locations of businesses that work with regulated material (e.g. tree care companies, landscapers) in the infested areas. Human assisted transport of infested material may occur and satellite infestations from the core infestation may result if regulations are not followed.

16.4.3 Control

Control activities consist of host removal and chemical treatment conducted individually or in combination. Tactics are typically applied to at least a 0.5 mile (0.80 km) radius (Sawyer 2006; Smith et al. 2004) from an infested tree but this distance may vary dependent on the density, level of infestation, and distribution of host trees in the area and the purpose of the application (Sawyer et al. 2011). Known infested trees are always removed because chemical treatments do not reach the late larval stages developing in the wood (xylem) of the tree. Host trees near infested trees are considered high risk and are often removed to reduce the likelihood of missing light infestations due to imperfect survey efforts. Host removal is the most certain tactic to eliminate a pest from an area. It can be used effectively as an initial "knockdown" of the total pest population to limit immediate spread and improve the success of subsequent chemical treatments which are strategically used on host trees not known to be infested but may be exposed to infestation due to their proximity to the outbreak.

Depending on the level of infestation and other factors, large-scale high-risk removal may be carried out. Trees within the area requiring control that are not removed are treated with a preventative treatment using imidacloprid, a systemic insecticide, injected into the soil at the base of the tree for uptake by the roots, or directly into the tree trunk within 12 in. of the soil line, just beneath the bark, where active transport takes place (Wang et al. 2001). Treatments are applied annually in the spring. A minimum of three consecutive annual treatments are needed to optimize control. Additional treatments may be required if survey results are positive.

16.4.4 Outreach

Outreach is critical to the success of the program. Local public officials are notified immediately of detections and possible actions to foster cooperation and support for the program and help them address citizens' concerns. Since activities are carried out on public and private property, the general public will be directly impacted. Outreach activities focus on increasing public awareness on the potential impact of the beetle, how to spot and report sightings of the beetle or symptoms, and what the public can do to keep it from spreading. A good outreach campaign results in public support for, acceptance of, and permissions to carry out the ongoing program activities on their property. The initial find in each state was reported to the USDA APHIS by a member of the general public (USDA APHIS PPQ 2012).

16.4.5 Replanting

The program plants non-host trees to mitigate the impact of tree removal. In general, only landscape and street trees are eligible to be replaced; however, it is not done on a one-to-one basis. A replanting program facilitates continued community support. The USFS along with the state forestry departments take the lead in this effort, working with the local communities and organizations.

16.4.6 Research

The ALB program is science based. The program's new pest response guidelines and area-wide strategy are based on initial and ongoing research and methods development. Continued research is required to continue to improve program delivery. New information on population dynamics, dispersal, survey and treatment methods, and other topics allow the program to be more effective and efficient.

16.4.7 Performance Measurement, Quality Assurance, and Data Management

The ALB program uses quality assurance activities, data management and geographic information systems (GIS) to monitor program effectiveness and progress towards eradication. Along with supporting day-to-day operations planning and implementation and contract development and monitoring, the collection of both positive and negative survey and treatment data allows long term strategic planning and budget formulation using various tactical and funding scenarios.

16.5 Illinois: Lessons Learned and Keys to Success

ALB was declared eradicated in Illinois in April 2008, 10 years after discovery. At its peak, 35 mile^2 were regulated for this invasive insect. ALB was first detected in the Ravenswood neighborhood of Chicago during July 1998. Subsequently, the beetle was detected in several areas: Loyola, Kilbourn Park and Oz Park within Chicago, and the suburban Chicago areas of Summit, Addison, Park Ridge and Bensenville (near O'Hare International Airport, Fig. 16.9). Cooperative efforts between USDA APHIS, IDA, City of Chicago BoF and other affected municipalities, resulted in lifting regulation of all areas and eradication declared in 2008. Early cooperation among Federal, State, and local agencies made the program a success.

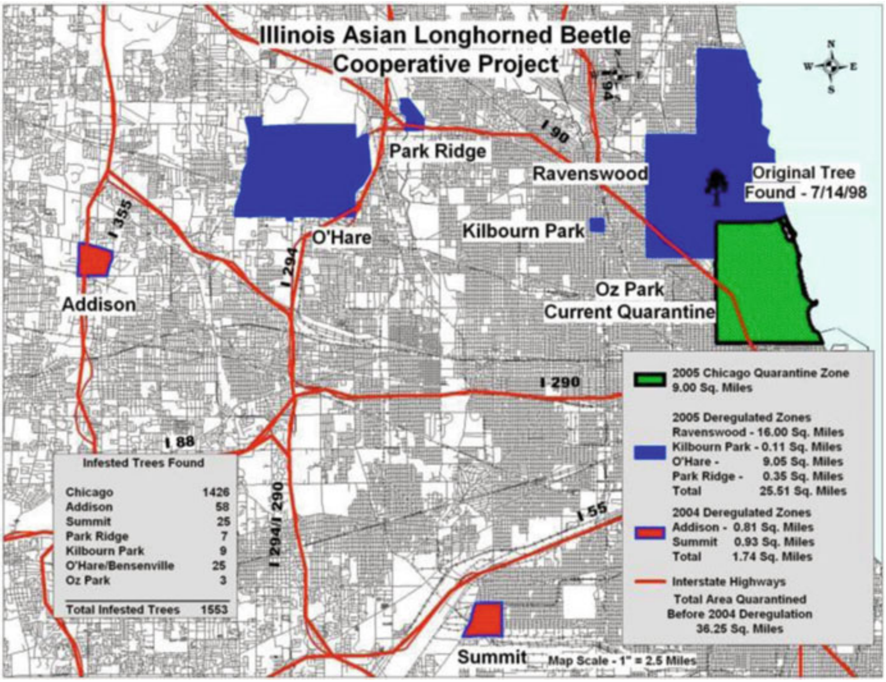

Fig. 16.9 Illinois regulation history through 2005 (Image courtesy APHIS USDA)

16.5.1 Strong Committed Leadership at All Levels

The relatively quick and successful program to eradicate the ALB from Illinois can be attributed in a great part to the formation of a dedicated, full-time management organization in which all levels of government (federal, state and city) played significant roles in the implementation of program activities. The USDA APHIS provided a full-time national director to implement the program, a full-time national coordinator to obtain resources and support, and an onsite manager to perform daily liaison with State and local officials and run daily operations. The State provided: (1) high-level technical support, (2) authority to remove and chemically treat host trees and (3) dedicated personnel to conduct regulatory activities. The USFS assisted with survey efforts by making available and coordinating the temporary assignment of smokejumpers to the program operations for surveying trees by climbing.

The USFS also provided a local and national public information officer in support of ALB eradication efforts. Leadership of the City of Chicago was fully committed to the eradication of the ALB. The City, under the strong interest and direction of the Mayor, provided a BoF Senior City Forester as a counterpart to USDA APHIS and State managers, utility vehicles and personnel to aid survey, and several proactive regulatory policies to address compliance by tree-care professionals and landscapers.

The City shared database records outlining tree locations and maintenance history and provided GIS support. The City also shared contact information on contractors, arborists and green industry professionals who had been working within and near the infested areas. This enabled USDA APHIS and state managers to quickly identify and regulate companies that worked with host material within the infested area. In addition, the City took the lead in building a positive relationship with the media. These key administrators and managers at each level of government were empowered to make decisions, develop a plan of action, and assign roles, resources, and personnel to the program.

Even though a dedicated organization was formed to respond to the outbreak, ALB leadership recognized the need for a strategic plan to elucidate the goals and objectives and how to achieve them. USDA APHIS took the lead because of its experience with other plant pest eradication programs. Subject matter experts and scientists were consulted, tactics were devised based on the best available science, and a long-term plan was developed based on an area-wide integrated pest management approach, which is the integration of several different control tactics to manage the total pest population within a delimited area (Hendrichs et al. 2007). Before the plan, response activities in Chicago and New York were reactive and applied only to specific locations where the pest had been found and not where the pest was likely to still exist. In addition to providing onsite managers clear guidance on where, when and how to apply various tactics, the plan also provided an easy-to-understand document to explain and "market" the program to high-level Agency Administrators, the public and their political representatives who provided funding for the program, the media, and other interested organizations.

Most often, government leaders and managers are responsible for addressing several projects and programs simultaneously. Having a dedicated organization allowed ALB managers to focus 100 % of their time delivering the program and, also, to be held accountable for its success or failure. The strategic plan projected a timeframe to achieve program goals and objectives and set performance measures. Many of the tactics were new and had limited technical data to support them such as the natural dispersal distance from which survey, treatment, and regulatory boundaries were set and the effectiveness of the chemical treatments. To their credit, leaders were open to new ideas and willing to take risks. To monitor the progress and ensure success, leadership required extensive data collection, including host information (tree size measured in diameter at breast height (d.b.h.), tree species, and tree address/location), records on all surveys and treatments completed on host trees, records on all infested trees detected and host trees removed, records on all companies working with regulated material and quality assurance activities that checked and verified that the work completed was accurate.

Broad measures such as "number of infested trees detected and removed" and "size of area regulated" were used to measure overall program progress (Figs. 16.10 and 16.11). Data collected during surveys and treatments also allowed managers to monitor progress towards meeting annual objectives, make operational adjustments, and ensure the requirements to declare eradication were met (Figs. 16.12 and 16.13).

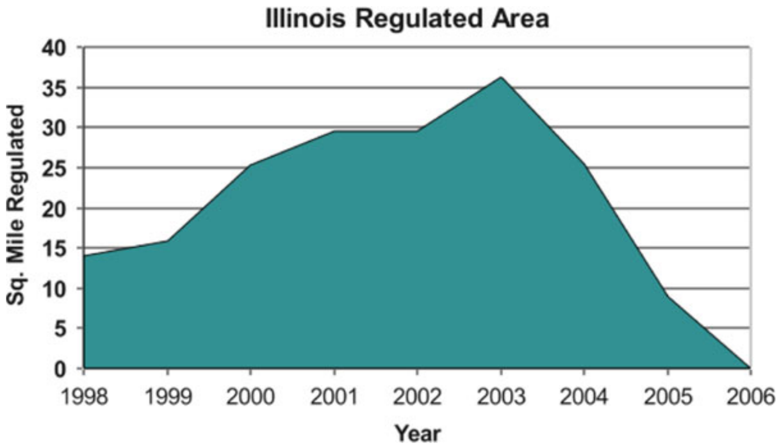

Fig. 16.10 Illinois ALB regulated area in square miles (1998–2006)

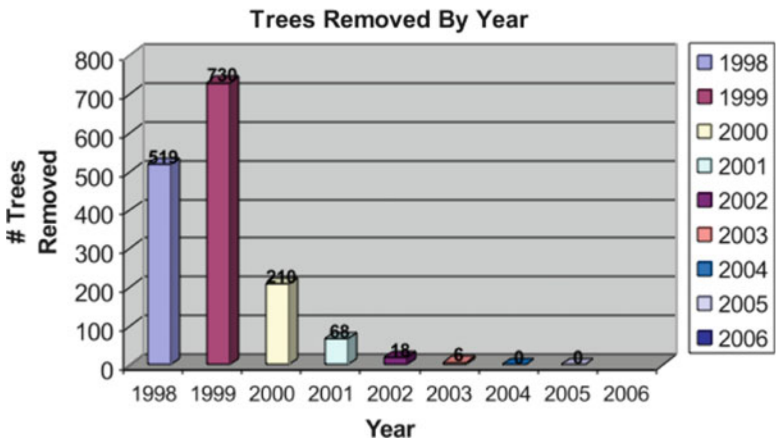

Fig. 16.11 Number of host trees removed by year in Illinois

16.5.2 Communications

Collaboration and cooperation among partners were absolutely critical for the success of the eradication program. From the initial discovery of ALB, Federal, State and local government officials were committed to the eradication of the pest. Through continual communication, they fostered a work environment of cooperation, trust, and mutual respect that allowed the leadership team to overcome minor obstacles and disputes. Decision-making was inclusive. Onsite co-managers were collocated facilitating day-to-day decision-making and streamlining communications between the Agencies. Efforts were made to blur Agency affiliation and present a unified ALB

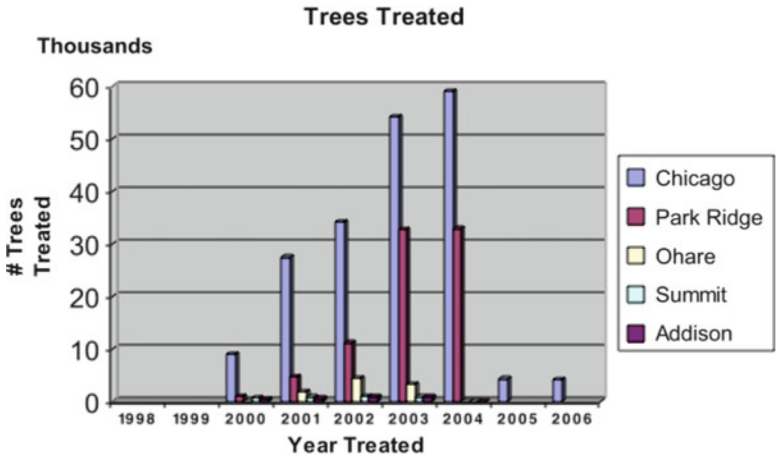

Fig. 16.12 Number of trees treated by infested area by year

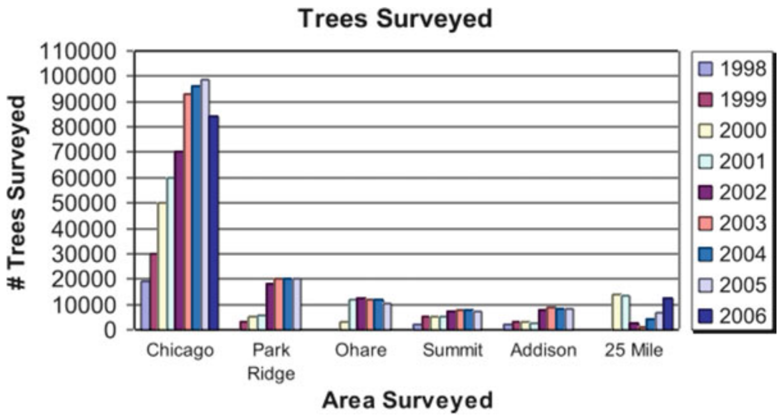

Fig. 16.13 Number of host trees surveyed within each infested area by year

Cooperative Eradication Program team to the media and the public. Leadership from each of the government agencies participated in periodic scheduled meetings or conference calls to address complex issues and resolve high-level differences. The common goal of achieving eradication facilitated the successful resolution to the issues at hand. The national response guidelines and the strategic plan although drafted by USDA APHIS involved numerous discussions with the principle cooperators, partners such as USFS, and elected officials to ensure operational feasibility and political and social acceptance.

Communications with the media and the public were carefully planned and monitored. All levels of government have public relations staff eager to promote their employers and "control" the message. Early in the program, the ALB leadership decided to present a unified front. All three principle cooperators would participate in TV and radio interviews when possible. Talking points were mutually crafted and reviewed. Press briefings and newspaper stories and editorials were reviewed by all before release. It was understood by all and agreed that the City should take the lead in working with the local media. It was thought that the citizens of Chicago were more comfortable obtaining and more likely to believe information coming from their local elected officials. Also, the Mayor and local political officials who were strongly supporting the eradication effort were directly accountable to their constituents. The local media maintained an open line of communication with the ALB Program and was willing to run stories on treatments, quarantine revisions, survey progress, and other program related topics to help inform the public.

With the media generating focused and positive messages, the ALB program team was able to capitalize on an educated and supportive public community. Also, public meetings were held in impacted areas periodically and prior to major program actions, such as chemical treatments, to increase awareness and gain support. At each public meeting, the program asked the local elected officials to open and facilitate the meeting to demonstrate that local officials were involved in the process and the public had a voice. These meetings actively solicited public participation and suggestions. As a result of these efforts, the ALB quickly became the common enemy of everyone. Whether citizen, renter, homeowner, business owner, reporter, City worker, elected official, religious leader or volunteer, the ALB was the enemy and the all-encompassing "WE" were going to work together to rid the City of Chicago from this invader. Obtaining access to private property, a necessary task to complete both survey and treatment activity, rarely presented a problem. Property owners were extremely aware of the ALB situation and would readily offer access to host trees located in secure private property. Survey crews and treatment contractors were able to gain access to nearly all ALB host trees located on private property. Access to these sites was critical because leaving a single tree un-surveyed could lead to an unknown infestation.

The partnerships developed among the ALB cooperators and stakeholders through good communications matured as the program evolved. From program operations to media relations, the cooperators who had been involved with the eradication efforts continued a strong commitment and involvement in program developments. The media continued to follow and report ALB issues up to and including the formal eradication ceremony, at which point the media continued to deliver a message of awareness around ALB, and other invasive species on the horizon. Many of the partnerships developed during the ALB response in Chicago are still benefiting USDA APHIS, especially as the agency continues to manage other invasive pests in the area. Some of these invasive pests require different management strategies, but often require the same strategies for developing working relationships among the units of local government.

16.5.3 Strong Regulatory Program

The regulatory program for the ALB Program in Chicago was cooperative from the onset. The USDA APHIS authority to regulate for a plant pest is limited to interstate movement of regulated articles, essentially quarantining the entire State where the pest is found. However, federal regulations can be applied to a lesser portion of the State if State intrastate regulations are equivalent to the Federal regulations. In 1998, USDA APHIS, IDA and the City's BoF worked together to establish mutually agreed upon regulations and regulated boundaries encompassing only the areas in proximity to where ALB infested trees were found. This area was determined based on scientific data on the natural dispersal of ALB.

Cooperation between USDA APHIS staff, IDA inspectors and BoF Senior Foresters was essential for the success achieved in monitoring and regulating companies that worked and/or were located within the regulated areas. These businesses included tree companies, landscapers, construction companies, waste management companies, roofers, wood recycling companies, firewood dealers, retail nurseries, and municipalities. Regulated material could not be removed from an ALB quarantine zone unless chipped to less than 1 in. (25.4 mm) in two dimensions (Chipping to this size ensured that larvae feeding on trees would not complete their development (Wang et al. 2000)).

To reduce the burden on industry, many companies working within the regulated area entered into compliance agreement with USDA APHIS and IDA. In order for a company to be granted a compliance agreement, federal and state inspectors conducted a site visit of the company to ascertain whether a company was able to institute measures in compliance with the ALB regulations. Compliance agreements were aggressively sought from all companies that worked with in or near a quarantined area, but due to IDA policy, these agreements had to be considered voluntary in nature. The compliance agreement was a written agreement between the ALB program and a person engaged in growing, handling, or moving regulated articles in which the person agrees to comply with the provisions of the regulations. Persons signing an agreement could move regulated material outside the regulated area without waiting for inspection, providing the ALB regulations were followed. Companies working under compliance agreement were subject to periodic, unannounced inspections by federal and state officials to verify compliance. In total, 248 compliance agreements were issued.

The cooperation between USDA APHIS staff, IDA inspectors and the City's BoF Senior Foresters was essential to the success achieved in monitoring and regulating companies that conducted tree work. The City worked closely with USDA APHIS regulatory staff to promote regulatory awareness and compliance among a large network of tree care professionals and arborists. The City took several unique, proactive measures to address regulatory compliance. City landscape and maintenance crews were given work assignments that kept them segregated either completely inside or outside the quarantine zone on a daily basis. The City also initiated a tree planting policy that excluded the planting of

Fig. 16.14 City of Chicago Tub Grinder (Image courtesy APHIS USDA)

ALB host trees in any community that was included in or adjacent to a quarantine boundary. Additionally, the City reviewed the planting schedule for all contractors and required them to either schedule work in the quarantine area for a full workday or remain outside for the entire day.

Management of the significant quantity of woody debris moving out of the regulated area quickly became an issue for the ALB regulatory program. Many of the entities working within the area were small companies or individuals who could not meet the requirement to chip woody material before leaving the regulated area. In June 1999, USDA APHIS and the City of Chicago through a cooperative agreement purchased a tub grinder for ALB program operations that was stationed within the regulated area (Fig. 16.14). City staff agreed to operate the tub grinder and manage its use. All unmitigated, regulated wood material that was removed by the City and private tree companies within the regulated area was taken to this tub grinder and chipped free of charge. Regulated entities eagerly complied to avoid disposal fees at the landfill. The woody debris disposal program facilitated compliance and prevented companies from hauling regulated material for disposal outside of the regulated area thereby preventing the human assisted spread of this invasive pest. Part of the success of the containment of the ALB infestation in Chicago can be attributed to this woody material disposal program. Having a convenient disposal option for the green industry was a critical element in obtaining compliance.

With the tub grinder in place and operational, the City required that all companies performing tree work in the quarantine area obtain a "quarantine certificate".

This City-issued quarantine certificate stated the company name, address of the work site, tree species and size, and whether the tree work was on City or private property. If the tree work was on City property, then the City required an additional City of Chicago permit. To dump logs at the ALB Program tub grinder facility, tree companies were required to give these certificates to the City staff located on site at the tub grinder facility. These certificates and permits were entered into both the City database and an ALB Program regulatory database.

The suburban quarantine zones also had wood disposal procedures. Because the quarantine in Park Ridge was confined primarily to a nonresidential area where tree work was unlikely, any company conducting tree work was required to chip all host tree wood on site. A similar policy was enacted for suburban Addison. Tree companies working in the Addison quarantine were required to chip everything on site because no wood disposal site had been established. The Villages of Summit, and Bensenville established log dumpsites at their public works facilities, where companies working in the respective quarantine zones could dump host tree material free of charge.

Regulatory work for the Chicago ALB Program involved considerable surveillance in and around the quarantined areas. Regulatory officers canvassing the quarantine zones kept detailed records of companies performing tree work in the area. Officers spoke with workers and crew-leaders, distributed ALB information, issued Compliance Agreements and notified the City about host wood requiring removal and proper disposal.

Federal and state officials prefer voluntary compliance and typically achieve adherence to the regulations through educating companies of the serious risks associated with spread of an ALB infestation. Occasionally companies did not follow the regulations and fines were issued. The first official quarantine violation was issued in July 2001 to a tree company for removing host tree logs from the Ravenswood quarantine zone. In total, four official violations were issued. Three of the violations were issued to tree companies for removing host tree logs from a quarantine zone and one was issued to a wood recycling company for accepting host tree logs originating from a quarantined area. The issuance of violations, though not a preferred method for compliance, does have a positive effect of achieving adherence to regulations by all companies working with regulated material by demonstrating the seriousness of infractions against the federal and/or state regulations.

16.5.4 Public Involvement

The ALB adult is a large, showy beetle that leaves very visible holes in the trunks and branches after the adult emerges from the wood. As a result, people notice the beetle or its presence on their trees or property while working in their yards or walking in their neighborhoods. In Illinois as well as New York, New Jersey, Massachusetts and Ohio outbreaks, a curious or concerned citizen was the first person to observe and notify authorities of its presence. ALB program leadership

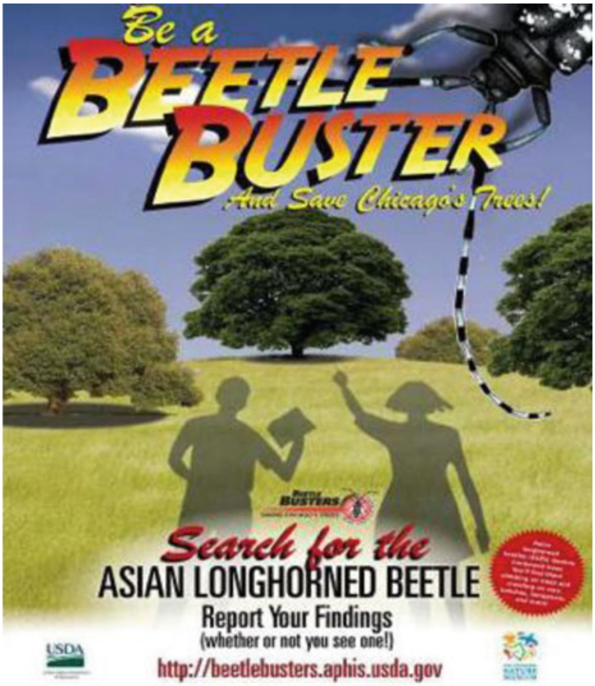

Fig. 16.15 ALB beetle buster poster (Image courtesy APHIS USDA)

recognized the opportunity to enlist the public to help find the beetle and augment and focus the ongoing systematic surveys conducted by the program.

A general outreach campaign was launched focusing on increasing awareness and how to spot and report the beetle. Newspaper advertisements, TV and radio announcements, posters, car bumper stickers, community meetings, brochures, pamphlets, door hangers, and all media releases included a message reminding the public to look for and report the beetle (Fig. 16.15).

In the summer of 2004, USDA APHIS contracted with a company to organize and manage a volunteer survey program enlisting volunteers from local “green” organizations and concerned citizens to survey host trees for ALB infestation. Participants were trained, provided information about the beetle to give to people they met, and assigned an area of their choice within targeted locations identified by program operations to periodically look for presence of the beetle. For safety, they were instructed to stay on sidewalks while observing trees and they were requested to complete a simple report to document their activities. For their efforts, participants received a T-shirt and other ALB outreach material such as kitchen magnets, litter bags, and key chains. With a large area to survey, program inspectors only surveyed some areas every other year. The volunteer efforts, although not considered in meeting the requirements of survey to declare eradication, did provide managers a degree of comfort and, when prioritizing program survey assignments, helped direct focus to areas that were not covered by volunteers.

ALB program managers wanted to involve children to help find the beetle and introduce the concept of invasive species at a young age. A consulting firm with subject matter expertise in school curriculum development was contracted to develop lesson plans on invasive species using ALB as the primary example. Part of the plans required children to engage their parents in a discussion of ALB and help the child survey the trees in their yard and trees within their neighborhood. The contractor obtained the required approvals from the school board to use the lesson plan in the Chicago school system and provided the lesson plans and materials to interested teachers. In addition, portions of the plan were provided to summer camp counselors at Chicago neighborhood parks to engage the children in learning about invasive species by surveying trees in the park. With success of the Chicago school system curriculum, USDA APHIS subsequently contracted and developed a national ALB curriculum that is available to schools and anyone interested throughout the country. The ALB curriculum is available through the USDA APHIS developed ALB web site at http://asianlonghornedbeetle.com.

The ALB program also contracted a marketing firm to develop the "Beetle Busters" website www.beetlebusters.info (updated in 2013 to http://asianlonghornedbeetle.com), to provide a centralized location on ALB information in order for the public to obtain the most up to date information about the eradication efforts in the USA. This website includes information on beetle biology, signs and symptoms of ALB damage, status of eradication efforts, and instructions on how to spot the presence of the beetle and report it (Fig. 16.16).

Another opportunity to engage citizens as part of routine program delivery came through day-to-day contact with the public by ALB field staff. Survey and regulatory site visits often evolved into information-sharing sessions with the public, whose desire to contribute to ALB search efforts was considerable. The ALB program's bucket truck survey team in Chicago was an extremely visible unit on city streets. Homeowners came to expect the survey crews canvassing their neighborhoods multiple times per year. Each survey crew was well stocked with printed outreach material to distribute to an ever curious public. Some of the common questions fielded included status of the survey, newly documented finds and chemical treatment plans. Survey crews were encouraged to spend time interacting with homeowners in the field and these interactions often led to citizens reporting their own findings after a survey crew had left the area. Every suspect citizen report was addressed and followed up upon as necessary.

Building on Chicago's efforts, USDA APHIS maintains a comprehensive advertising effort to address the need for public information and education around the ALB. The Agency continues to include a citizen "call to action" to find the beetle as ALB infestations are fought in Massachusetts, New York and Ohio. The overall campaign goal is to urge people to recognize the ALB and report signs of infestation. The plans include "getting the word out" through movie theater spots, transit advertising, Public Service Announcements (PSA) on radio, paid PSA placements on cable stations, mobile billboards, roadside billboards, newspaper ads, a central ALB website, email, texting of information and internet advertising.

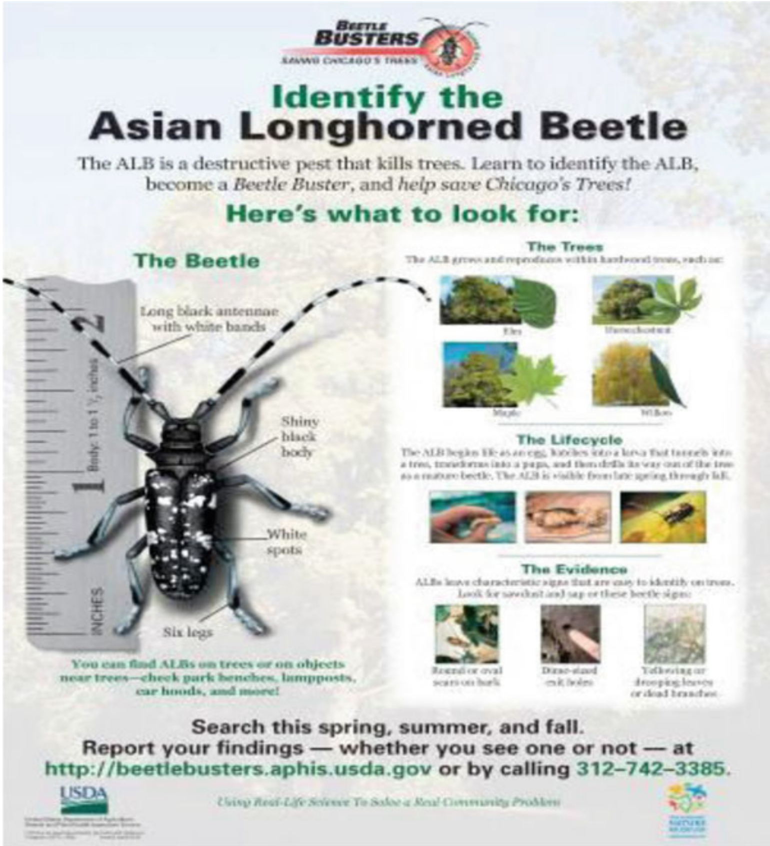

Fig. 16.16 ALB beetle buster poster (Image courtesy APHIS USDA)

16.5.5 Replanting

Tree removal and destruction is the only control method for trees infested with ALB. Within the core of the infested area of Chicago, many streets lost all of their host trees to ALB infestation leaving the once tree-lined streets bare (Figs. 16.17 and 16.18). In Illinois, 1,551 host trees were removed for the eradication of ALB. In order to mitigate the loss of trees within the urban landscape, the City replanted 2,645 trees that are non-host to ALB. The Village of Addison received 37 non-host trees. The replanting of 2-in. caliper trees in the landscape, though not comparable to mature shade trees lost to ALB, does offer closure and relief to communities (Fig. 16.19). Many people have emotional attachment to trees, signifying the birth or passing of loved ones, providing serenity, homes to wildlife, or simply energy reduction through cooling shade.

Fig. 16.17 ALB host tree removals in progress (Wolcott Avenue, Chicago, IL) (Image courtesy City of Chicago Bureau of Forestry)

Fig. 16.18 Wolcott avenue (Chicago, IL) before (*left*) and after (*right*) ALB infested tree removal (Images courtesy APHIS USDA)

16.5.6 Innovation

The ALB program leadership was open to new ideas and willing to take calculated risks to potentially move the program forward. Examples already mentioned include

Fig. 16.19 Chicago city street after replanting (Image courtesy APHIS USDA)

the purchase and operation of the tub grinder to improve regulatory compliance and the development of volunteer surveyors and school curricula to augment survey and increase awareness of invasive species. The Chicago program took the lead in implementation or helped develop several other significant innovations that are incorporated today in the remaining existing outbreak programs.

Initially, program surveyors stood on the ground with binoculars to visually inspect each tree for signs of ALB. The ability to detect ALB in trees was significantly improved when utility trucks with a crane and attachment (bucket) were introduced into the Chicago program (Fig. 16.20). Surveyors then were able to inspect trees at the canopy level instead of from the ground. The concept of better positioning the surveyor to improve detection led to the incorporation of tree climbers trained to recognize signs of ALB: Oviposition sites, exit holes, and frass (Fig. 16.21). Tree climbers were able to move about throughout the canopy and view the trunk and branches from most sides. Studies by USDA APHIS scientists determined that surveys by ground inspection were about 30 % effective in detecting lightly infested trees. Survey by climbing increased efficacy of detecting light infestations to about 60 % (Sawyer, 2000, personal communication). Ground surveys continued to have a role in program operations, particularly when examining smaller host trees that can be thoroughly examined from the ground and

Fig. 16.20 ALB bucket truck survey, Chicago Illinois (Image courtesy APHIS USDA)

Fig. 16.21 Frass ejected from an oviposition site (Image courtesy A.J. Sawyer, APHIS USDA)

detecting infested trees with high levels of infestation that are easily observed from ground level. Ground surveys are also the most cost-effective survey method, enabling quicker surveillance throughout an area to determine the general boundaries of the core of the infestation.

Most survey in Chicago involved urban or suburban settings, but a Forest Preserve located within the regulated area required modifications to survey protocols due to the high density of host trees. This was the first time that the program was faced with this environment and realized that existing guidelines were not applicable. Chicago personnel worked with USDA APHIS leadership and scientists to develop and refine guidelines for surveying forested areas.

Chicago personnel were also involved with program leadership in developing procedures and guidelines for conducting quality assurance activities on survey and treatment activities. To estimate the effectiveness of surveys and survey methods, pseudo exit-holes and oviposition sites were created by a team of climbers in random trees ahead of program surveyors. Chicago and USDA APHIS leadership and scientists analyzed the data and established guidelines and procedures for quality control that were based on sound information and operationally feasible. The cooperation between Chicago operations and USDA APHIS leadership and scientists was repeated for other program activities including: (1) chemical residue sampling from treated trees to measure the effectiveness of the treatment, (2) sampling of pesticide tank mixtures to verify application rates, (3) sampling schemes to meet environmental monitoring requirements for measuring potential impacts to humans and the environment from the program's chemical treatment applications, and (4) guidelines for monitoring survey and treatment quality. As deficiencies were detected in operations, immediate feedback was provided to the field inspector who performed the work. These quality assurance inspections served as training tools, resulting in improving the technical abilities of the field inspectors.

ALB program leadership relied heavily on USDA APHIS scientists to provide the science upon which to base program activities. Little technical information was available at the start of the program. Studies were immediately initiated in China where high populations of ALB were available. These studies complemented considerable work conducted in the USA. As previously mentioned, the estimates of dispersal distance that determined the survey, regulatory and treatment boundaries were primarily drawn from analysis of Chicago infested tree data (Sawyer 2006). The development and implementation of a chemical treatment for ALB (Wang et al. 2001, 2003, 2005a; Lewis et al. 2005) may, however, be the most significant innovation in which the Chicago program played a role.

Chemical treatments were added to the ALB National Response Guidelines and became an integral component of the ALB eradication strategy during 2000. Under these guidelines, the chemical imidacloprid was injected into the tree base by drilling a hole through the bark and into the vascular system. A low-pressure capsule was then inserted into the hole. The number of capsules required to achieve the dosage depended on the size of the tree. The initial chemical treatments were intended for New York City. However, New York City officials were concerned about public acceptance and the impact of the application method on their trees and declined the treatment. Officials in Chicago, however, believed that removal of host trees over large areas of Chicago was unacceptable and embraced the opportunity to use chemicals to eliminate the pest. The first treatments were conducted in a limited area of Chicago during the spring of 2000.

Because the capsules at the base of the trees were obvious and the capsules remained on the tree for 4 h for maximum chemical absorption by the tree, program managers and officials were concerned that children or pets would disturb the capsules. As part of the treatment, to ensure public safety, the program contracted for “watchers”. A “watcher” was assigned to watch all the treated trees within line-of-site to prevent curious people and pets from possibly handling the capsule and pesticide. The treatment was expensive due to these complications and as a result treatment areas were limited in size on an annual basis, attributed to both cost and logistics. Even with these limitations, New York adopted chemical treatments into their eradication efforts during 2001.

Additional challenges were immediately realized. National leadership quickly realized the urgency for simplifying treatment applications and strongly urged USDA APHIS scientists to develop application methods that were “city” friendly. Improved treatment technologies were developed, including direct basal soil injection (Lewis 2006) and direct low-pressure trunk injection (Wang et al. 2005b) that eliminated the need for “watchers” and reduced the cost of treatment applications. The willingness of ALB leadership to take a risk and make it work ultimately provided a tool that was socially and politically acceptable and could be used on an area-wide basis opening the door to eradication.

16.5.7 Data Management

The strategic plan provided a clear blueprint for managers to follow. The plan set “triggers” to deregulate areas and declare eradication. A specific number of years of annual treatments of all hosts and complete host survey with negative results were required to reach the goal. Collection of the number and location of positive trees and the area surveyed by program personnel colored-in on a wall map was no longer adequate to measure effectiveness or efficiency. To implement an area-wide integrated pest management system over several years, a robust data management system, including both positive and negative survey results, was needed to enable managers to measure progress and performance and better manage the program.

Complete development of the system occurred over several years and included modules to manage survey, treatment, tree removal, regulatory, public outreach, and quality assurance activities. A relational database underpins the system tying the data from separate modules together. Control and survey history on all host trees and host properties was necessary to verify that area wide activities were completed with no misses or voids that could harbor infestation and compromise eradication success. Although primarily designed to directly support day-to-day operations, the system also provides summary information for use by high level management for short and long term planning and program status and justification. USDA APHIS scientists also found the wealth of information stored in the system over time valuable in analyzing ALB host density, distribution and preference, and ALB dispersal and population dynamics. Through development and testing of the national system, the ALB program was forced to thoroughly examine their procedures and processes. They used the opportunity to introduce novel ways and business practices to better use program data to operate the program and ensure success.

16.6 Summary

Through all the advances and improvements to operational technologies, USDA APHIS and state and city cooperators, successfully eradicated the ALB infestations from Illinois and New Jersey. The successes learned in Chicago were incorporated and improved upon in New York and subsequent infestations that were detected within the USA – New Jersey, Massachusetts and most recently in Ohio. Area-wide host removal was first executed surrounding the infestations in New Jersey and quickly eliminated the infestation from that state. Within 2 years from initial discovery, no additional infested trees were detected as a result of this aggressive control measure. There are, however, limitations where area-wide host removals can be applied. Environmental, economic and human impacts must be determined and eradication strategies tailored to particular environments are necessary. Continued improvements to eradication strategies and new discoveries to advance eradication success are needed, especially when resources are limited.

Even with the advancements made to ALB eradication strategies in the USA, many years of program execution are still needed to realize success. Early detection of ALB infestations would minimize the resources necessary for eradication. However early detection is difficult since surveys are restricted to visual inspections and it takes 3–5 years for population levels to build in host trees before populations becomes readily apparent with visual inspections. Studies continue to improve survey technologies. USDA APHIS is training and testing ALB detector dogs to detect ALB infestation throughout an area instead of tree-by-tree surveys. USFS and Pennsylvania State University Scientists are collaborating to develop and refine a lure for ALB that is used in detection traps. These are area-wide survey tools and if successful may eliminate or reduce tree-by-tree surveys to detect infestations and confirm eradication.

Eradication of ALB from the USA continues to be a priority. The environmental and economic impact should ALB disperse throughout the country would be devastating. Even with the more efficient technology that has been developed to date, eradicating ALB continues to be a time and resource intensive process. Continued support for resource allocation is needed to successfully eradicate ALB. This requires strong support by Federal, State, and local governments, stakeholders, and the general public.

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For More Information on Asian Longhorned Beetle, Visit

http://asianlonghornedbeetle.com http://www.aphis.usda.gov/newsroom/hot_issues/alb/alb.shtml http://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/index.shtml http://na.fs.fed.us/fhp/alb/ http://www.mortonarb.org/tree-plant-advice/article/758/asian-longhorned-beetle-anoplophora-glabripennis.html

Chapter 17
Phytoparasitic Nematodes: Risks and Regulations

Eric L. Davis and David K. Nendick

17.1 Introduction

Nematodes (invertebrate 'roundworms') are the most numerous multi-cellular animals on earth (Poinar 1983), and second to insects (arthropods) in the number of described species. Estimates declare only about 3 % of all nematode species have been identified and studied. Nematodes are often overlooked and relatively unknown when compared with insects because of their small to microscopic size and tendency to live within inconspicuous habitats. Most nematodes average less than 1 millimeter (mm) in length while some animal parasites range up to 8 m long! One cubic foot of soil may contain millions of individual nematodes belonging to many taxonomic groups. Different nematode species feed on algae, bacteria, fungi, and higher plants, as well as invertebrates and vertebrate animals (including humans). Nematodes have broad stress tolerances and they have successfully exploited diverse habitats including every conceivable terrestrial and aquatic environment on earth ranging from temperate to tropical soils, arid deserts, salt and fresh water, hot springs, and polar regions (Nickle 1991; Shurtleff and Averre 2000).

All nematodes belong to the Phylum Nematoda (Decraemer and Hunt 2006) and all nematodes are aquatic (as they must live in association with liquids in an abiotic environment or inside a biotic host substrate). In body form they are triploblastic (derived from three embryonic tissue layers), unsegmented, non-coelomate (pseudocoelomate), bilaterally symmetrical roundworms (Fig. 17.1). All nematodes undergo embryonic development within an egg and become worm-shaped (vermiform). However, a few species achieve swollen (pyroform) and contoured body shapes later in adult life stages (Fig. 17.2). Nematodes have a "tube within a tube"

E.L. Davis
Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA

D.K. Nendick (✉)
Biosecurity and Environment Group, Plants, Food and Environment Directorate,
Ministry for Primary Industries, Wellington, New Zealand
e-mail: dave.nendick@mpi.govt.nz

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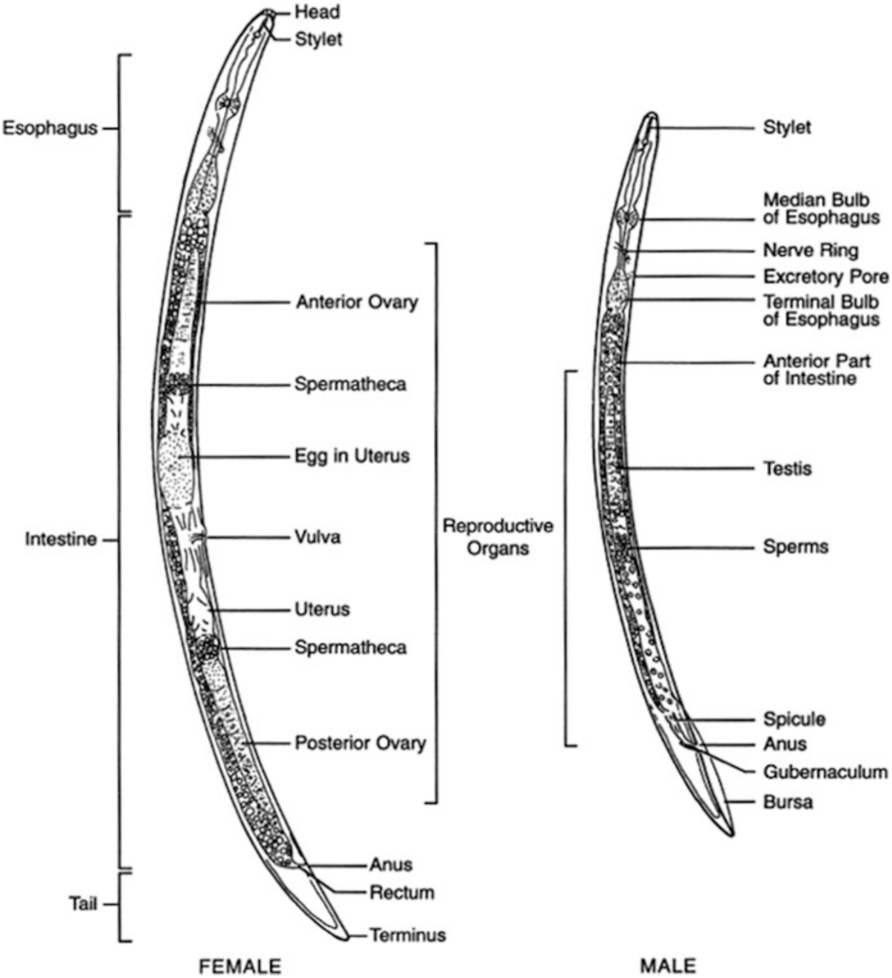

Fig. 17.1 Line drawings of generic phytoparasitic nematodes. Note the stylet used for feeding from plant cells, details of the esophagus including the median bulb (metacorpus), female ovaries and vulva, and male testis and spicules (Reproduced with permission from Shurtleff and Averre 2000)

body plan (Hirschmann 1971) that consists of an outer body wall and an inner digestive system, and the body shape is maintained through pressure of the pseudocoelmic fluid against the flexible body wall (forming a hydrostatic exoskeleton). In all phytoparasitic (plant-parasitic) nematodes (but few other nematode species), the mouth of the digestive system contains a “stylet” – a hypodermic needle-like structure used to pierce plant cell walls for feeding (Agrios 2005; Shurtleff and Averre 2000). All nematodes molt their outer (collagenous) cuticle four times during juvenile growth and maturation to reproductive adult. These four juvenile stages are sometimes termed “larvae”. All nematodes have a skeletal,

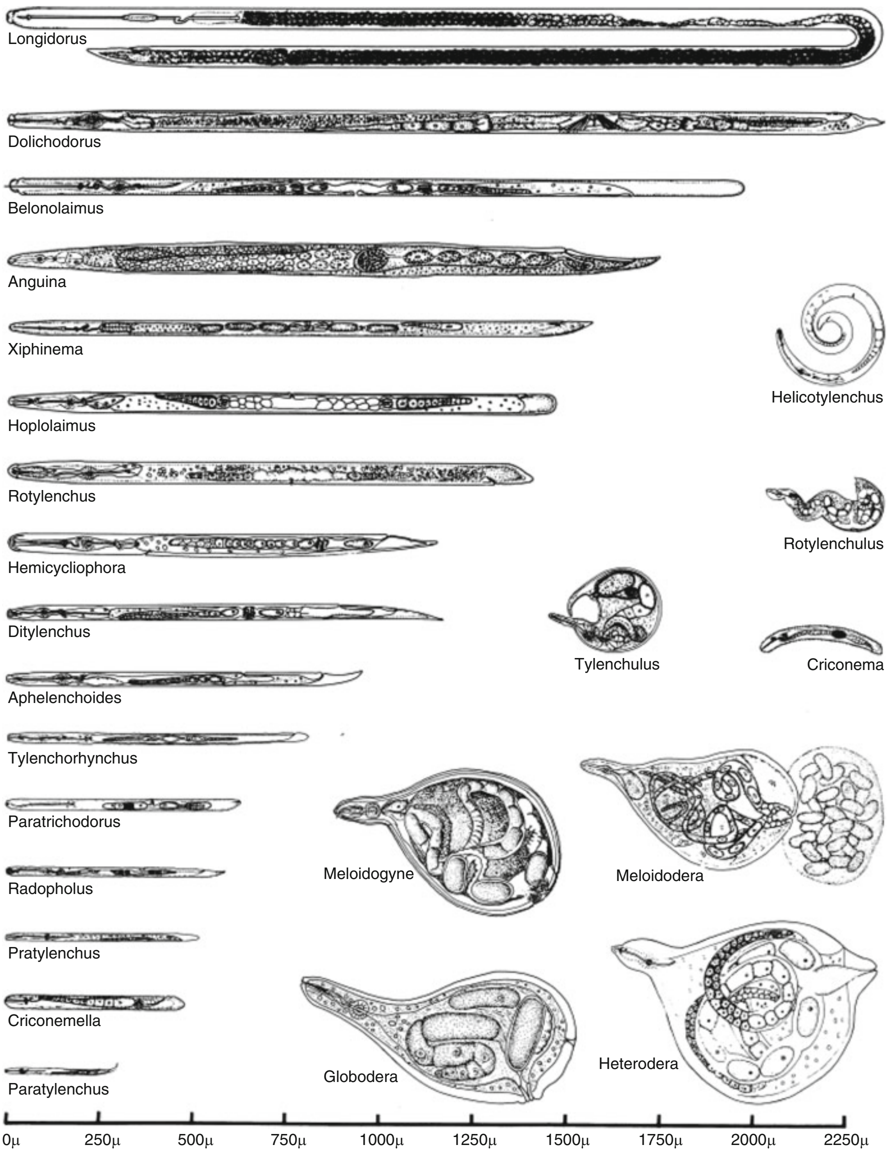

Fig. 17.2 Line drawings of some major Genera of phytoparasitic nematodes that indicate relative size and shape. Genera of nematodes that remain worm-shaped (vermiform) in all life stages are compared on the *left*, and Genera with swollen (pyroform) reproductive adult life stages are shown on the *right* (Reproduced with permission from Shurtleff and Averre 2000)

muscular, excretory, digestive, nervous, and reproductive organ system, but they lack circulatory or respiratory systems. Typically, nematodes are transparent and colourless and usually are dioecious (separate male and female adults that mate).

Microscopy is used to determine nematode body shape, size, and structure of internal organ systems. Microscopy has been the traditional technology used to identify and classify nematodes taxonomically (Mai et al. 1996; Shurtleff and Averre 2000). More recent taxonomy for the Nematoda is based upon DNA sequence data (De Ley and Blaxter 2004). The adoption of molecular technologies for practical identifications of nematodes for diagnostics remains limited (Subbotin and Moens 2006). However, recent technology advances have provided promising new approaches that include direct extraction of total DNA (See Sect. 13.5) from soil samples and subsequent use of species-specific real-time Polymerase Chain Reaction (PCR) primers in multiplex assays to identify different phytoparasitic nematode species within a single sample (Ophel-Keller et al. 2008). Not all nematodes in a soil sample (or any abiotic nematode habitat) are parasitic. Most nematode species are not parasitic on animals or plants. Roughly 50 % of all nematodes live in marine environments as zooplankton and there are relatively few parasitic species among them. About 25 % of all nematodes live in freshwater in lakes, rivers, soils, and streams. Among this group of nematodes most species are microbial feeders that play an intricate role in ecosystem food webs and nutrient turnover (Poinar 1983). An example of bacterial-feeding soil nematode is *Caenorhabditis elegans* Maupas which has become a premier biological model for the study of animal behaviour, development, genetics, genomics and pharmacology (Bird et al. 1999).

The remaining 25 % of nematodes are the parasitic species. About 15 % of these species are parasites of animals (Poinar 1983). Some of the invertebrate parasites (of arthropods) have been exploited to develop commercial control for insect pests in agriculture (Nickle 1991). The remaining 10 % of the parasitic nematodes include about 4,000 species within about 200 genera (Nickle 1991) that are parasitic on plants. Annual global crop yield loss to phytoparasitic nematodes is estimated to be around $USD 100 billion (Chitwood 2003). This figure is probably underestimated because their impact is probably greater since plant symptoms of nematode damage are often non-specific and unrecognised as nematode damage. Economic, environmental and practical considerations dictate the level of management that farmers can use to reduce damage from phytoparasitic nematodes including use of chemical control (nematicides), resistant crop cultivars, or rotations from hosts to non-host crops. Nematode diseases that damage plants that grow in areas of human development, forests, or prairies present an even greater management problem to reduce phytoparasitic nematode populations to non-damaging levels. After a phytoparasitic nematode infestation has occurred, the nematode is rarely eradicated from the infested area. Exclusion and sanitation remain the single best management strategies to prevent phytoparasitic nematodes from becoming established or introduced to new areas (Hockland et al. 2006).

This chapter summarizes some of the more damaging species of phytoparasitic nematodes and those of international regulatory concern. For purposes of this chapter we group phytoparasitic nematodes based upon their parasitic habit (Agrios 2005; Hussey and Grundler 1998; Perry and Moens 2006) as follows: (1) *Ectoparasites* that feed with their stylets from outside of host plant tissues; (2) *Endoparasites* (Fig. 17.3a) that penetrate and feed within host plant tissues; (3) *Migratory*

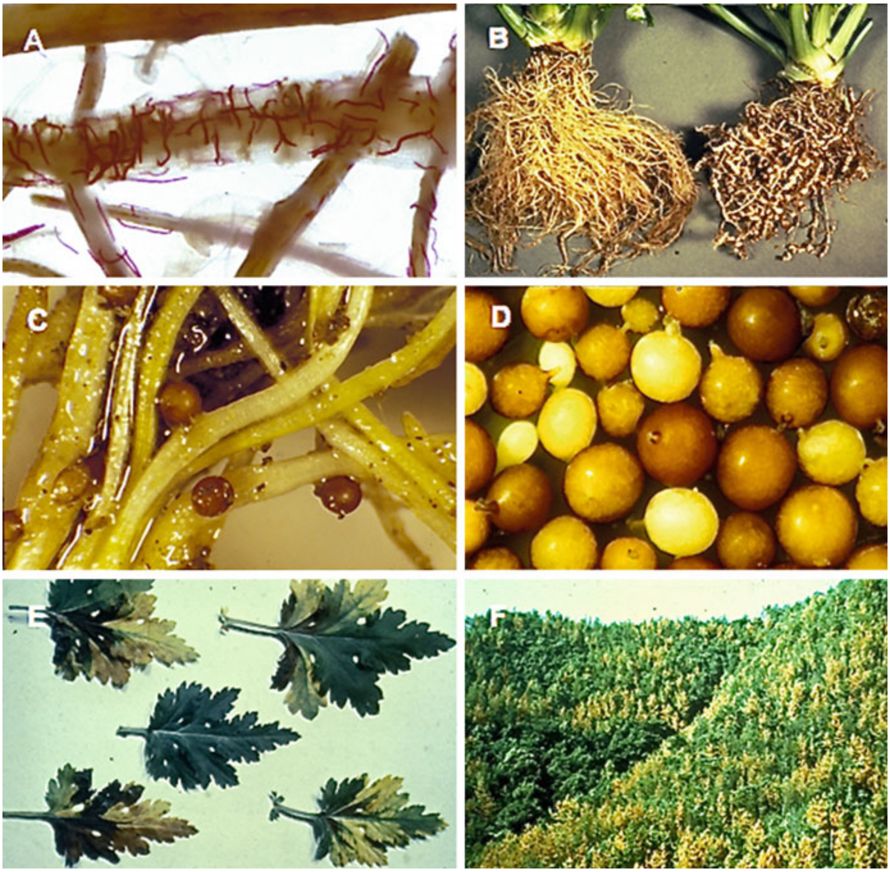

Fig. 17.3 (**a**) A micrograph (100×) of multiple individuals of the migratory endoparasitic lesion nematode (*Pratylenchus* spp.) stained red with acid fuchsin inside a corn root; (**b**) a healthy, fibrous root system of celery on the *left* and a severely galled celery root system infected with many root-knot nematodes (*Meloidogyne* spp.) on the *right*; (**c**) a micrograph (40×) of several brown, round females of the tobacco cyst nematode (*Globodera tabacum*) on roots of tobacco; (**d**) A micrograph (100×) of females of *G. tabacum* extracted from host roots demonstrates different stages of cyst maturation from *white* (young female) to *yellow* to *brown* (mature cyst); (**e**) leaves of chrysanthemum infected with foliar nematodes (*Aphelenchoides* spp.) display characteristic symptoms of interveinal chlorosis, necrosis and angular lesions; (**f**) extensive damage to a pine forest in Japan from infestation with the pinewood nematode, *Bursaphelenchus xylophilus*

nematodes that feed from a host cell for a relatively short period of time and move to another host cell; and (4) *Sedentary* nematodes that establish prolonged feeding (1 day or longer) from a single site in host plant tissues. Most phytoparasitic nematode species are soil-dwelling pests and feed from the roots of a host plant. Several significant phytoparasitic nematode species, however, do infest and feed in the upper parts of plants in flowers, seeds or shoot tissues (Nickle 1991; Pirone 1978).

17.2 Ectoparasitic Nematodes

*17.2.1 Dagger Nematodes (*Xiphinema *spp.)*

Dagger nematodes primarily are ectoparasites of woody plants (small trees and tree fruits) but can damage annual crops like corn, soybean, some cereals and herbaceous perennials (Decraemer and Geraert 2006). For instance, *Xiphinema* is an important pest in all grape-growing regions of the world because it can also introduce and spread (vector) Grapevine Fanleaf (GVFL) Virus. *Xiphinema* also vectors other NEPO viruses (Comoviridae) such as Prunus Necrotic Ringspot Virus on peach in the northeast USA. Dagger nematodes also cause direct damage by feeding at or near root tips where they often cause slight galling and necrosis. These are relatively large phytoparasitic nematodes (1.5–4.5 mm long) with an odontostylet that has an odontophore with three basal flanges, a distinct 'guiding ring', and a two-part esophagus (no metacorpus). Females have one or two ovaries depending upon vulval position; the female's tail shape can range from conoid to more rounded with a small, distinct peg-like projection (Mucro) at the tip. Males are rare in some species and have a similar body shape with stout spicules and no bursae. Of the approximately 60 species described, the three most agriculturally-important species include: (1) *X. americanum* Cobb – one of the more common nematodes in USA, probably a "species complex"; (2) *X. index* Thorne and Allen – mainly found associated with its natural host – grapevine; and (3) *X. diversicaudatum* Micoletsky – the largest species (4.3 mm long) which is very damaging to strawberry.

Xiphinema species differ in their selection of feeding sites; most feed ectoparasitically at the tips of roots and produce small galls on certain plants. Dagger nematodes have very long stylets that allow them to feed deep into root tissue. They may feed from the same site for relatively long periods of time, and therefore may also be considered as sedentary ectoparasites. Feeding can result in cessation of root growth and swelling (galls) of root tips within 12 h of feeding with transmission of plant viruses often occurring within this period. Necrotic cells are often surrounded by the hypertrophied (over-sized) cells with multiple nuclei at the feeding site that serve as the food source for the nematode's sedentary feeding.

*17.2.2 Sting Nematodes (*Belonolaimus *spp.)*

Sting nematodes can be one of the most devastating nematode pathogens (Smart and Nguyen 1991) due to their severe effects. Hosts include field crops (corn, cotton, peanut, soybean), fruits, grasses and trees. Sting nematodes are primarily found in the southern USA and some Caribbean islands. Sting nematodes also prefer sandy soils, like the coastal plains of the southeast USA. In Florida, sting nematodes are one of the most important pests of turf, and they have relatively recently been detected in California. The most important agricultural species is *B. longicaudatus* Rau. Sting nematodes are relatively long compared with other phytoparasitic nematodes (>2 mm), with an offset, bulbous head, very long stylet with small knobs, moderate annulation and the esophagus slightly overlaps ventrally. The female has two ovaries and a rounded tail, and males have a pointed tail with long, tapering bursae.

Sting nematodes feed as migratory ectoparasites on root tips causing extensive and severe root damage. Their long stylets penetrate deep into cortical and sometimes into vascular tissue. This type of feeding causes cell necrosis in the root apical meristem and can often result in a stunted, "coarse root system". Root tips cease to grow and lesions and necrosis may occur as well. Very few sting nematodes (<10 per 100 cc of soil) can cause severe damage to plants, especially seedlings and grasses. The above-ground symptoms include severe stunting, leaf chlorosis and even seedling death can occur.

17.2.3 *Stubby-Root Nematodes (*Trichodorus *and* Paratrichodorus *spp.)*

The feeding activity of Stubby-root nematodes destroys root tips and results in a shortened, sparse, "stubby root" system (Decraemer 1991). Stubby-root nematodes are distributed globally and prefer sandy, well-drained soils. They have a wide host-range that includes crops like clover, corn, potatoes, turf and many vegetable crops. Stubby-root nematodes cause direct plant damage and can also vector the NETU plant viruses (Tobraviruses). One of the stubby root-vectored viruses causes "corky ringspot" of potato tubers. Both *Trichodorus* and *Paratrichodorus* species have cigar-shaped bodies about 0.5 mm in length, a dorsally-curved onchiostylet without basal knobs or flanges, and they have a two-part esophagus. This type of stylet is very unusual in that it resembles a tooth and it is grooved rather than being hollow! Females of both genera have two amphidelphic ovaries. The genus *Paratrichodorus* includes about 20 species and *Trichodorus* contains about 50 species; the most important agronomic species is *Paratrichodorus minor* (Colbran) Siddiqi.

Stubby-root nematodes feed as migratory ectoparasites over the entire root system, but they mainly feed on epidermal cells at the root tips. Stylet penetration of a plant cell and ingestion can occur in a few minutes but this rapid feeding still allows time for the transfer of plant viruses. Lateral root initials can be produced at feeding sites by the host plant as a response to feeding; other stubby root nematodes, which produce the stunted, branched look of the root system can immediately attack these initials. Above ground symptoms can result in nutrient stress, severe stunting, water stress, and in extreme cases this can lead to plant death.

17.3 Endoparasitic Nematodes

*17.3.1 Root-Knot Nematodes (***Meloidogyne** *spp.)*

Root-knot nematodes cause more damage worldwide than any other genus of phytoparasitic nematodes (Eisenback and Triantaphyllou 1991). They are a distributed among all agricultural regions of the world and collectively parasitise over 1,700 different plant species (Agrios 2005). The second-stage juveniles (J2) of root-knot nematodes penetrate plant roots completely and establish elaborate feeding sites. Upon establishment of the feeding site, the J2 begins feeding, becomes sedentary and swells through its molts to the adult stage. Conspicuous galls (knots) form on the roots (Fig. 17.3b) at the site of infection as the result of nematode feeding. The J2 of root-knot nematodes are 0.4–0.6 mm long and have a short, weak stylet, subventral overlapping esophageal gland cells, and a blunt, rounded tail with a hyaline area in the anal region. The rounded adult females may be 1.0 mm in diameter. The pattern of cuticular striations surrounding the female vulval and anal openings (with their distinctive perineal pattern) has traditionally been used for species identification. The four most important species of root-knot nematodes found around the world (Sasser 1980) include *M. arenaria* Chitwood*, M. hapla* Chitwood*, M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood. These species of *Meloidogyne* collectively cause about $10 billion in annual crop losses (Chitwood 2003) although their existing worldwide distribution mostly reduces them to minimal regulatory concern. However, *Meloidogyne* species of more localized distribution such as *M. chitwoodi* Golden, O'Bannon, Santo & Finley and *M. fallax* Karssen in potato, or *M. exigua* Goeldi in coffee are of regulatory concern and are managed accordingly.

Individual Root-knot Nematode females lay several hundred eggs in a gelatinous mass on the surface of the root-galls that they induce. As with all phytoparasitic nematodes, the first-stage juvenile (J1) molts within the egg and the J2 hatches into the soil matrix. Root-knot nematode J2s migrate in soil and penetrate host plant roots in the zone of elongation just behind the root tip (Hussey and Grundler 1998). The J2 migrates intercellularly (between cells) within the roots using stylet thrusts and they secrete enzymes that loosen the cell wall matrix until it reaches the root vascular tissue. Effector parasitism proteins from secretory cells in the nematode esophagus are then secreted through the stylet into selected root vascular cells to transform them into elaborate feeding cells called "giant-cells" (Davis et al. 2008).

Giant-cells can be 100-times the size of normal plant cells, they also have multiple nuclei, dense cytoplasm, and reinforced cell walls (Hussey and Grundler 1998). Localized root cell division occurs around the feeding site to produce the intercalary root galls (knots) that are the defining visible feature of *Meloidogyne* species infections. The feeding infective juvenile stages undergo three more molts as they become more swollen (pyroform) and rounded to the reproductive adult stage. The pyroform adult female remains embedded within the gall and it is ultimately positioned to lay its eggs in a gelatinous mass on the outside surface of the root gall. One plant may be host to many hundreds or thousands of root-knot nematodes and consequently the parasitic load on the plant and the damage to the root vascular system results in nutrient and water deficiencies for the host plant, including stunting, wilting, and a greatly reduced crop yield.

*17.3.2 Cyst Nematodes (***Globodera** *and* **Heterodera** *spp.)*

Cyst nematodes are characterized by the hardening and colour change (tanning) of the mature pyroform female body wall after death to form a protective "cyst" (Fig. 17.3c, d) that encloses the eggs (Baldwin and Mundo-Ocampo 1991). Unlike root-knot nematodes, no root galls are formed by cyst nematodes and the swollen adult female is primarily exposed on the outside of the plant's root. Cyst nematodes only have their heads buried within the root to feed. Cyst nematodes are probably the second most economically-important nematode pathogen of major crops after root-knot nematodes. Unlike root-knot species, cyst nematode species have a much more limited plant host-range. However, their effects on successive generations of host plants can be extensive because the eggs contained within the protective cysts can remain viable for a several years in soil. Cyst nematodes are very important nematode pests of (colder) temperate agriculture (for example, potato and winter cereals), but species have also been found on major crops (for example, sugar cane, rice) in warm climates. The J2s look similar to those of root-knot nematodes, but they are slightly larger (0.6–0.8 mm long) with a distinctly more robust stylet (25 μm long) with 'heavier' knobs. While the subfamily Heteroderinae contains seventeen genera, the two most agriculturally-important genera are *Globodera* (having round cysts) (Fig. 17.3c, d) and *Heterodera* (having lemon-shaped cysts).

Important species of cyst nematodes include Beet Cyst Nematode (*H. schachtii* A. Schmidt), Cereal Cyst Nematode (*H. avenae* Wollenweber), Potato Cyst Nematodes (where the two main species include Pale Cyst Nematode (*G. pallida* (Stone) Behrens) and Golden Nematode (*G. rostochiensis* (Wollenweber) Behrens), Soybean Cyst Nematode (*H. glycines* Ichinohe), and Tobacco Cyst Nematode (*G. tabacum* Lownsbery & Lownsbery). Most of these significant cyst nematode species cause considerable economic damage on their respective host crop. However, they are relatively limited in distribution compared with the root-knot nematodes and are subject to strict international regulatory activities and certification requirements.

The cyst nematode life-cycle is similar to that of a root-knot nematode, with some notable differences. Many cyst nematode species require their eggs to be near host-plant roots because egg hatching is stimulated by specific host root exudates. When the J2s penetrate plant roots, they migrate intracellularly (through plant cells) to the root vascular tissue, destroying root cells along their migratory path (Hussey and Grundler 1998). Cyst nematodes also secrete effectors to induce formation of a multinucleate feeding site called a "syncytium" by coordinated cell wall dissolution between neighbouring root vascular cells (Davis et al. 2008). As with root-knot nematodes, after the feeding site is formed the J2s swell, become sedentary and molt three more times until the adult stage has developed. No root gall is formed, and the swollen female cyst nematode protrudes almost completely from the root and retains most eggs within her body (which eventually falls off the root and becomes the protective cyst). As with root-knot, root damage and parasitic load from hundreds or thousands of nematodes causes extensive disease to the host plant resulting in crop loss.

*17.3.3 Reniform Nematode (*Rotylenchulus reniformis *Linford & Oliveira)*

Reniform Nematode is widely distributed in tropical and subtropical areas (Jatala 1991). This pathogen has a fairly wide host range of at least 140 plant species that span 30 plant families. Primary hosts in the tropics include banana, citrus, coffee, ginger, pineapple, and tea; primary hosts in the subtropics include cotton, cowpea, sweet potato, and soybean. This nematode has become increasingly important during the past two decades (Robinson 2007), and appears to be expanding its distribution in warmer climates. Reniform Nematode is distributed in many areas in the USA, especially the southeast where concerns for root-knot and cyst nematodes have left the Reniform Nematode relatively unmanaged. Pathogenicity of different reniform populations varies, and sometimes high population densities can cause relatively little damage. In Hawaii, reniform populations are extremely aggressive and survival of the pineapple industry is dependent on maintaining adequate Reniform Nematode control. The J2 of Reniform Nematodes are relatively small (about 0.5 mm long) with a rounded sclerotized lip region and pointed tail similar to other members of the Hoplolaimidae. Reniform Nematode juveniles undergo superimposed molts through J3/J4 stage in soil without feeding; the emerging immature female nematode penetrates the root and forms a syncytium along the endodermis of the root vascular system. The developing female feeds from the syncytium as a sedentary endoparasite and swells to become a kidney-shaped (renale) female protruding from the infected root that is characteristic of this species. Although *R. reniformis* is not presently subject to specific regulatory action (other than generic phytosanitary controls), it's rapidly increasing geographic distribution, relatively high soil population densities, and plant damage potential combined with a wide plant host-range should make it a phytoparasitic nematode species of concern.

*17.3.4 False Root-Knot Nematode (*Naccobus aberrans *(Thorne) Thorne & Allen)*

False root-knot nematode, *Naccobus aberrans*, is a sedentary endoparasitic nematode that also forms a feeding site in infected roots (Jatala 1991). This nematode is generally limited in distribution and global damage in agriculture, but it has been a problem in potatoes grown in Central and South America. The nematodes can infect potato tubers and be very destructive in these crops. However, its current limited distribution has made this species subject to strict regulatory practises. *Naccobus aberrans* was originally isolated in Utah, and is primarily found in the Americas. It has a wide host range including chenopods, crucifers, cucurbits, leguminous and solanaceous plants. The nematode forms similar galls as *Meloidegyne* spp. on roots of host plants giving it the name "false root-knot nematode". *Naccobus aberrans* is a member of the Family Pratylenchidae. Juveniles are relatively small (0.4–0.5 mm long) with a flat sclerotized lip region, strong stylet, and rounded tail. The J2, J3, and J4 stages penetrate plant roots and feed as migratory endoparasites of roots until the immature female forms a syncytium (not giant-cells) and feeds as a sedentary endoparasite. The female swells to become rounded and a gall forms around the developing female, but the gall forms laterally from the root (on one side only), as compared with the root-knot nematode gall that extends from all sides of the root.

*17.3.5 Lesion Nematodes (*Pratylenchus *spp.)*

This group is considered the third most economically important Genus of phytoparasitic nematodes (behind root-knot and cyst nematodes) because of its global distribution, wide host-range, and very destructive parasitic habits (Duncan and Moens 2006). For example, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans, is considered as the most economically important nematode in the northeastern USA. About 70 species are identified in *Pratylenchus*; they vary and overlap geographically and in plant host preference (Loof 1991). All life stages are vermiform, 0.3–0.9 mm long, and display a flat sclerotized lip region, rounded tail, and esophageal glands that overlap ventrally. All species and life stages are migratory endoparasites of subterranean plant parts (roots, pods, tubers etc.). They cause extensive plant tissue necrosis (lesions) by their feeding activity and intracellular migration. More important species include: (1) *P. penetrans* – very wide host-range (>400 plant host species from row crops to fruit trees); (2) *P. vulnus* Allen & Jensen – important crop hosts include fruit trees and ornamentals; (3) *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven – important crop hosts include peanut and soybean; (4) *P. zeae* Graham – the most important crop host is corn; (5) *P. coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven – important crop hosts include banana, citrus, coffee and strawberry; and (6) *P. scribneri* Steiner – may be the most widely distributed lesion nematode in the USA. The wide global geographic distribution of lesion nematodes makes them subject to relatively limited regulatory activity. However, lesion nematodes are extremely destructive species in warmer climates. Species such as *P. coffeae* are of considerable concern for further geographic spread.

*17.3.6 Burrowing Nematode (***Radopholus similis** *(Cobb) Thorne)*

Burrowing nematodes are taxonomically related to and very similar to lesion nematodes in morphology and parasitic habit as migratory endoparasites of underground plant parts (Duncan and Moens 2006). Dorsal overlap of esophageal gland cells and strong sexual dimorphism of males are key features that distinguish *Radopholus* from *Pratylenchus* (Loof 1991). Burrowing nematodes are an important pest causing "spreading decline" of citrus plantations in Florida, "toppling disease" of banana in Central and South America, and "pepper yellows" disease in Indonesia. *Radopholus similis* is present in tropical Africa, parts of Central and South America, Australia, within some ornamental plant industries in Europe, in the Indonesian archipelago and it is found in Florida and Hawaii in the USA. Strict quarantine regulation has helped limit the spread of burrowing nematodes within countries where it is found and around the world. However, the wide range of host plant species (ca 250) for *R. similis* and its immensely destructive capabilities make it an important pest in its range and a constant threat for spread into new areas (Duncan and Moens 2006; Hockland et al. 2006).

17.4 Shoot Parasites

This group of phytoparasitic nematodes feeds on above-ground plant tissues including buds, floral primordia, leaves, seeds and stems (Agrios 2005; Horst and Nelson 1997; Pirone 1978). Some species may also feed on below-ground plant tissue; notably, most shoot parasites can also use their stylets to alternatively feed on fungi. This group of nematodes are mainly migratory endoparasitic but some feed ectoparasitically during parts of their life cycles. The number of shoot parasitic nematode species is relatively limited and may be due to the relatively fluctuating and potentially adverse environmental conditions above ground. Most shoot parasites have evolved extreme adaptations for surviving unfavourable environmental conditions, most notably the ability to "dry-down" (in a process called anhydrobiosis) into a long-term (which can extend for several years to decades!) survival stage (sometimes called "dauer") that can be revived with the addition of water.

*17.4.1 Stem and Bulb Nematodes (***Ditylenchus** *spp.)*

Ditylenchus species exist in many ecological niches (Sturhan and Brzeski 1991). *Ditylenchus* is among the genera of phytoparasitic nematodes with the greatest variety of feeding habits. Species feed upon fungi, roots, bulbs, and stems. These nematodes have a global distribution but are most damaging in temperate regions. Male and female bodies are fairly long (1–2 mm) and attenuated, the flat lip region is not sclerotized or offset, includes a fine, short stylet with small knobs, esophageal glands form a non-overlapping bulb, and females have one ovary. Species feed mainly as migratory endoparasites within shoots; they also infect below-ground plant parts and can migrate to feed in the shoots. The most important economic species include: (1) *D. dipsaci* (Kühn) Filipjev – a name that comprises about 80 species that form a "species complex." They are among the nematodes of greatest economic impact globally. Taxa under this name parasitise over 500 plant host species and feed on flowers, leaves and stems. This species complex has excessive intra-specific variation (with about 20 host races including differential chromosome numbers) that in heavy infestations can cause crop losses of 60–80 %; (2) *D. destructor* Thorne – causes Potato Dry Rot. This nematode is a migratory endoparasite on underground plant parts and can feed on fungal mycelia. Fortunately, *D. destructor* is limited in distribution within the USA and other temperate regions; and (3) *D. myceliophagus* Goodey – a fungivoral pest of the mushroom industry that feeds upon mycelia in commercial mushroom beds.

The fourth-stage juvenile (J4) of *Ditylenchus* is the survival and infective stage (Sturhan and Brzeski 1991). *Ditylenchus dipsaci* migrates and feeds within parenchymous tissues of the stem and can move below ground to invade host roots and storage organs such as bulbs, rendering all infected organs malformed, necrotic and twisted. After several generations of reproduction, the J4 form a dried mass of thousands of juveniles called "nema wool" on infected plant parts (mainly in or on storage organs like bulbs). The extensive lesions of dry rot caused on potato by *D. destructor* also contain dried aggregations of J4 nematodes. A sanitation program to reduce *D. destructor* infestation in USA growing regions had been relatively successful until recently… *D. destructor* appears to be on the increase again in some USA growing regions (Hafez et al. 2010). The regional/world-wide distribution and the destructive and survival ability of *D. dipsaci* and *D. destructor* keep them permanently under international regulatory control.

*17.4.2 Wheat (Cereal) Gall Nematodes (*Anguina *spp.)*

Anguina species have similar above-ground (but not below-ground) parasitic habits as *Ditylenchus*, but mainly feed on or in developing buds and floral primordia, and colonize cereal seed heads where they reproduce in large numbers within infected seeds (Krall 1991). *Anguina tritici* (Steinbuch) Filipjev (Wheat Gall Nematode) forms darkened "cockles" in place of wheat seeds that may contain thousands of anhydrobiotic nematodes that may remain viable for 30 years! A sanitation program to remove infected cockles within sown wheat seed has been largely effective to virtually eliminate *A. tritici* as a threat in major wheat growing regions; it remains under regulatory phytosanitary control in wheat growing countries around the world. *Anguina funesta* Price, Fisher & Kerr and other *Anguina* species (e.g., *A. agrostis* (Steinbuch) Filipjev in earlier reports) attack seedheads of rye and other cereal crops. In Australia, these *Anguina* species vector a bacterium, *Rathayibacter toxicus* Sasaki, Chijimatsu & Suzuki (Riley & Ophel) (syn. *Clavibacter toxicus*, *Corynebaterium toxicus*) that can be infected with a bacteriophage. This entire disease complex produces a powerful neurotoxin (corynetoxin) in developing seedheads that causes a major disease of grazing livestock called "annual ryegrass toxicity" that often produces fatal poisoning. This disease complex is also subject to international phytosanitary regulation.

*17.4.3 Foliar Nematodes (***Aphelenchoides** *spp.)*

Members of this Genus are primarily endoparasites of foliar tissue and buds; some minor species are fungivores (Duncan and Moens 2006; Nickle and Hooper 1991). They are limited in their distribution, and primarily prefer temperate climates (USA and Europe). They can be a serious economic threat when infesting ornamental plant production nurseries. The most important species are (1) *A. fragariae* (Ritzema Bos) Christie which causes "Spring crimp" of strawberry and has at least 250 host plant species including ornamentals like *Begonia* spp., ferns, and *Hosta* spp.; (2) *A. ritzemabosi* (Schwartz) Steiner & Buhrer which is a primary pest of plants in the Compositae such as *Chrysanthemum* sp., *Fragaria* sp. (strawberry) and many other host species; and (3) *A. besseyi* Christie which prefers warmer climates and causes "summer crimp" of strawberry and "white tip" disease of rice. All 180+ species of *Aphelenchoides* have a prominent rectangular metacorpus; they are long (1 mm), slender nematodes with a short, fine stylet with small knobs, raised lip region, and dorsal overlapping esophageal glands.

Foliar nematodes often overwinter within decomposing foliage on the ground and unusually do not infest soil (Agrios 2005; Duncan and Moens 2006). They can infect emerging shoots of germinating plants and can feed ectoparasitically on leaf primordia. They climb the shoots of plants in a film of water and usually enter leaves through the stomata. Foliar nematodes feed on parenchymous mesophyll tissue, destroying cells and resulting in leaf blotches (Fig. 17.3e). The infected leaves turn brown, then black, and the discolourations are often delineated by the primary leaf veins (angular appearance). Interveinal necrosis is common and severe infections can lead to extensive defoliation and poor plant growth and appearance. The foliar nematode life cycle is relatively short (2 weeks), promoting multiple generations and as many as 15,000 nematodes can occur in a single leaf! Since plant symptoms often lag behind substantial nematode infestation, foliar nematode spread has been on the increase, especially within the ornamental plant industry (McCuiston et al. 2007).

*17.4.4 Pinewood Nematode (***Bursaphelenchus xylophilus** *(Steiner & Buhrer) Nickle)*

Bursaphelenchus xylophilus is the primary economically important member of the Genus (Duncan and Moens 2006). It is one of two important phytoparasitic nematode species that are unique in that they are vectored by insects and feed on both plant cells and associated fungi in host trees. They were originally reported to be the causal agent of "pine wilt" disease, but several associated organisms and plant toxins are believed to act in association with *B. xylophilus* to induce "pinewilt disease complex" (PWDC). The nematode may have been imported into the USA from Japan where it is a devastating pathogen of endemic genotypes of pine trees (Fig. 17.3f). The predominant species of pines and other tree hosts in the USA, however, are not as susceptible to PWDC as the Japanese pines. (For example, it does not produce epidemics, but some localized problems do occur in the USA). Pinewood nematodes also have a prominent metacorpus and they are long (0.4–1.5 mm), slender nematodes with a high, offset lip region, short, fine stylet with small knobs, excretory pore posterior to metacorpus, and the esophageal glands are weakly developed and slender (Nickle and Hooper 1991).

The pinewood nematodes are vectored by cerambycid beetles (*Monochamus* sp.) and thus have a life cycle closely associated with the life cycle of the beetle. The nematodes feed on plant cells and perhaps mainly on fungi. A blue-stain fungus (*Ceratocystis* spp.) is almost always associated with this nematode infection in trees, and other fungi such as *Botrytis cinerea* (De Bary) Whetzel are occasionally found and serve as a food source for the nematode. Essentially, two life cycles of *B. xylophilus* are observed: (1) During active feeding of "newly infected" trees the nematodes feed on epithelial cells of the xylem and cortex and the life cycle can be as short as 4.5 days (life span 15 days, produces 80 eggs) and produce many generations in the tree. The infected tree foliage becomes discoloured (grayish) and often the needles are retained (where they normally would drop). The pattern of discolouration and wilting is irregular (patchy) among the tree at first, and at this point growth the symptoms induced by the blue-stain fungus are obvious. Also the tree produces phytotoxins (as a defense response) that may become systemic and actually harm the tree itself. Eventually the entire tree becomes discoloured, wilted, and eventually dies. (2) The dispersal cycle occurs in dead or dying wood, perhaps after overwintering, where the nematode feeds primarily on associated fungi. The nematodes molt to become resistant third-stage dauer-larvae with a thickened cuticle and stored food (lipids and glycogen) reserves. The third-stage dauer-larvae migrate to the pupal chambers of beetles that have invaded the same tree. The nematodes molt to become fourth-stage dauer-larvae, and then enter the respiratory system (spiracles) of developed, young beetle adults.

An average of 15,000–20,000 dauer-larvae can be vectored (this does not hurt the insect) by one emerging adult beetle and transferred to healthy trees by the feeding of the beetle. The presence of the nematodes in both the trees and beetles compounds potential spread and regulatory concerns. The regulatory concern extends to all wood products derived from infected trees, including packing materials for shipping. The development of the International Standards for Phytosanitary Measure No. 15 (ISPM 15) by the IPPC occurred due to concerns about the introduction of forestry pests via international trade in wooden articles (https://www.ippc.int/). This international regulatory standard (developed in 2006 and modified in 2010) has led to a significant increase in import protection via the use of heat–treatment and pre-shipment fumigation to disinfest logs, lumber and wooden products to eliminate pest such as the Pinewood Nematode and wood boring insects.

A related species, *Bursaphelenchus cocophilus* (Cobb) Baujard (syn. *Radinaphelenchus cocophilus*) has a similar disease cycle (Duncan and Moens 2006) in palm trees. This nematode is vectored by a Palm Weevil (*Rhynchophorus*) and is an important pathogen of coconut and ornamental palms in Central America, South America and the Caribbean. The nematode infects the foliage and eventually the stem (trunk) of palms and forms the characteristic "red ring" disease that can be seen in the cross-sections of infected tree trunks. Again, problems associated with eventual disruption of vascular transport will eventually kill the tree and the nematode is vectored to new trees by the weevils.

17.5 Detection and Management of Phytoparasitic Nematodes

Efficient sampling is a critical component of any pest detection scheme, and phytoparasitic nematodes are no different (Barker and Davis 1996; Been and Schomaker 2006). Regulatory management of nematodes also will be discussed in the section below. A brief discussion on sampling, detection, and identification of phytoparasitic nematodes for grower use is presented here. Sampling for diagnostic purposes should include soil and/or plant (roots or shoots, where appropriate) samples taken at the margins of the diseased area since the nematodes will migrate from dead tissues to infect living plant tissues. Fields with a history of nematode infestation and sampled for advisory purposes should collect samples in a systematic pattern to obtain an average representation of the nematode population density across the entire growing area. Samples should be submitted to a certified nematode assay lab in a timely manner for extraction of nematodes from samples and subsequent nematode identification and quantification.

The type (public or private) and availability of nematode assay labs differs across geographic regions, and even among different states within the USA (Barker and Davis 1996). Various flotation methods are used to separate nematodes from soil samples (Barker and Davis 1996; Shurtleff and Averre 2000), with the floating nematodes subsequently captured on fine-mesh sieves (25–50 μm openings). Extraction of nematodes from plant samples (and sometimes soil samples) can include methods to stimulate nematodes to emigrate from plant tissues or simply to macerate the host tissues to release the nematodes and capture the nematodes on sieves. Nematode assay labs then depend upon highly-trained personnel to identify (usually only to Genus-level) and quantify the nematodes present in a sample using microscopy and make nematode management recommendations as appropriate (Shurtleff and Averre 2000). As indicated earlier, molecular tools may soon be more widely adapted to assist in accurate and practical identification of phytoparasitic nematodes (to Species-level) for diagnostic and advisory purposes (Ophel-Keller et al. 2008; Subbotin and Moens 2006).

Available management options to reduce potential phytoparasitic nematode damage depend upon several factors, most importantly the type and population densities of nematodes present, crop history and future cropping systems, and predicted cost-efficiency of an appropriate management tactic. Nematicides have been a staple for nematode management in high-value crops for decades, but the high cost, inherent toxicity, and potential environmental damage of many nematicides have limited their use (Haydock et al. 2006). Soil fumigants such as Methyl Bromide, 1, 3 dichloropropene (Telone II), methylisothiocyanate (MITC) emitters (Vapam), dazomet (Basamid), and chloropicrin (tear gas) greatly reduce pre-plant levels of nematodes to provide crop protection. The relatively recent listing of methyl bromide as an ozone-depleting agent, however, has virtually resulted in its elimination from commercial use globally except for official pre-shipment or quarantine use and where exemptions apply. (http://ozone.unep.org/Publications/MP_Handbook/Section_1.1_The_Montreal_Protocol/Article_2H.shtml; Schneider et al. 2003).

Granular and liquid non-fumigant nematicides are generally nerve toxins (acetylcholinesterase inhibitors), such as the organophosphates fenamiphos (Nemacur), terbufos (Counter), and ethoprop (Mocap) or carbamates such as aldicarb (Temik), oxamyl (Vydate), and carbofuran (Furadan). These non-fumigant compounds can be applied post-plant and usually provide some systemic activity in plants. However their high costs, groundwater concerns, and mammalian toxicity have greatly restricted their authorized use.

A viable alternative to nematicides is the use of crop cultivars that are genetically resistant to phytoparasitic nematodes. Cultivars of several major crop species such as cotton, soybean, tomato, and others have been bred for resistance to specific species of phytoparasitic nematodes and provide excellent crop protection and yields (Starr et al. 2002). The risk of selection of resistance-breaking races within a nematode species and the lack of identified sources of resistance to other nematode species and for other major crop species, however, suggests the urgent need for the development of new nematode-resistant crop cultivars.

Rotation to a non-host crop species presents an effective cultural practise to reduce phytoparasitic nematode population densities to levels that are below economic damage thresholds (Noe 1998). This technique works well for infestations of nematodes with a limited crop host range and lack of long-term survival strategies with the availability of practical and economically-viable rotation crops. Incorporation of nematode-resistant cultivars into a crop rotation scheme can provide the potential for long-term sustainability to manage some nematode infestations.

17.6 International Biosecurity Regulations of Phytoparasitic Nematodes

17.6.1 Introduction

We provide a basic outline of biosecurity (quarantine/plant health) related to the management or regulation of phytoparasitic nematodes in international trade. This introduction to biosecurity gives examples of historical events, international agreements, legislation, phytosanitary requirements and techniques that prevent or limit the spread of nematodes via trade in plants, plant products and commerce. Effective regulation is essential because nematodes are often difficult to detect and easily overlooked. Indeed, many people are unaware of their existence.

The importance of nematodes as pests in agriculture and horticulture is relatively poorly known to the general public, compared with other pests such as insects. Nematodes often go undetected because they are typically microscopic and tend to be located in plant roots or in soil where they are virtually impossible to see without knowledge of nematode symptoms, detection techniques and/or training. In addition to being found in soil, roots, and underground plant organs, nematodes can also spread in the upper parts of plants such as stems, floral organs, seeds, and wood (Agrios 2005; Duncan and Moens 2006; Hockland et al. 2006; Shurtleff and Averre 2000; Taylor 1971).

17.6.2 Historical Spread of Nematodes via Trade

Without effective biosecurity regulation, phytoparasitic nematodes can easily spread to new areas/regions/countries via trade in plants and plant products. Nematodes also are spread in association with farm/garden tools, machinery or shipping containers that are contaminated by plant material or soil (Agrios 2005; MAF Biosecurity New Zealand (MAFBNZ) 2009a; Norton and Niblack 1991; Shurtleff and Averre 2000). Introduction of nematodes in this manner has occurred frequently with serious agricultural consequences. Spread is only stopped or restricted by effective biosecurity regulation that may include specific phytosanitary activities being implemented before importation or after detection in new areas (Cotten and Van Riel 1993; Hockland et al. 2006).

Distribution and establishment of phytoparasitic nematodes can also be influenced strongly by climatic conditions. For example, tropical nematode species will usually not do well in temperate regions and vice-versa. Nematodes with a narrow host range may also struggle to establish in the global distribution of their hosts species (Noe and Sikora 1990). Early examples of phytoparasitic nematodes reported in the literature include: Needham's initial observations (1743) of the Wheat Weed Gall Nematode; Schmidt's identification of *Heterodera schachtii* (1850) from "exhausted" sugar beet fields in Europe; reports by Cobb (1891) of nematodes associated with plants from Fiji; Treub's identification of *Meloidogyne javanica* from Indonesia in 1885, and Prayer's description (1901) of a nematode disease of banana in Egypt (El-Sharif 1997; Luc et al. 1990; Thorne 1961).

Discovery of the Golden Nematode (*Globodera rostochiensis*) in 1941 in New York State was reportedly associated with military equipment and vehicles returning to the USA after World War 1. This infestation led to millions of dollars being spent on the Golden Nematode Control Project by the USDA to prevent further spread. It also resulted in a tightening of import regulation for plant material entering the USA (Brodie and Mai 1989; Cotten and Van Riel 1993; Hockland et al. 2006). Despite tight regulations and surveillance, Golden Nematodes have been detected in other areas of North America.

The enforcement of strict Potato Cyst Nematode quarantine regulation delayed detection of *G. pallida* (Pale Potato Cyst Nematode) until 2006 when eight fields in Idaho were found infested (Skantar et al. 2007). Similar finds of Potato Cyst Nematodes in New Zealand (1983) and Australia (1986) emphasizes that very stringent international biosecurity regulations may only slow spread before other regulatory measures must be implemented (Cotten and Van Riel 1993; Marshall 1998; Stanton 1986; Wood et al. 1983).

17.6.3 The International Basis for Phytosanitary Agreements and Regulation of Nematodes

Exclusion via effective biosecurity regulation is the most important tool or strategy to prevent phytoparasitic nematodes from becoming established or introduced to new areas. When introduced into a new area or country, phytoparasitic nematodes are rarely eradicated because initial infestations may often go unnoticed or undiagnosed for considerable periods of time. This offers time and opportunity for nematodes to spread further from the first point of introduction via movement of infested machinery, plant material, soil, tools, vehicle tire treads or by water and wind. Incidentally, exclusion via biosecurity (quarantine) regulation of plant products in international trade is certainly the most effective way of limiting the movement of phytoparasitic nematodes (Barker 1997; Hockland et al. 2006).

Regulation is chiefly conducted cooperatively under the auspices of the IPPC (See Sect. 2.2). As stated the IPPC was formed in 1951 to promote and manage international cooperation in managing plant pests (Cotten and Van Riel 1993; https://www.ippc.int/). The IPPC is managed by the Commission on Phytosanitary Measures (CPM), which promotes cooperation between member nations in order to protect the world's crop plants and natural plant environments from the introduction and spread of plant pests, while aiming to minimise interference with usual international trade and the movement of people (Chap. 2). A major revision of the IPPC occurred in 1997; currently 177 signatory nations agree to the timely exchange of phytosanitary information and use standardised phytosanitary terms. We recognise 34 International Standards for Phytosanitary Measures (ISPMs) that include export and import phytosanitary guidelines. For example, ISPM No. 1 is the guidance document for "*Phytosanitary Principles for the Protection of Plants and the Application of Phytosanitary Measures in International Trade*" (http://www.ippc.int/) (Chap. 2). Additionally, Regional Plant Protection Organisations (RPPOs) operate cooperatively in specific regions of the globe. For example, the Pacific Plant Protection Organisation was formed in 1994, includes 27 Pacific member countries//territories, and is recognised by the IPPC (https://www.ippc.int/index.php?id=pppo).

17.6.4 Country or Region Specific Regulation of Nematodes

Typically, regulated pathogens or pests do not occur in the country imposing the regulatory importation requirements. Biosecurity regulation of phytoparasitic nematodes is conducted similar to ways used for plant pathogens (bacteria, fungi, viruses etc.). Regulated (biosecurity status) nematodes are designated by the IPPC as being “a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled” (https://www.ippc.int/). Biosecurity-status nematodes (like other pests and pathogens) of concern are identified or determined by a National Plant Protection Organisation (NPPO) or equivalent regulatory agency after conducting risk analysis or risk assessment (Chap. 5). For example, this work is conducted by the NPPO of a country such as MAFBNZ in NZ, DAFF-AQIS in Australia or USDA-APHIS in the USA. In addition, RPPOs may also develop pest lists and biosecurity strategies for wider areas, such as the European Plant Protection Organisation (EPPO) for the European and Mediterranean region. Using IPPC guidelines, country or regional organisations determine and impose biosecurity requirements on the importation pathways to prevent introduction of pathogens and pests of concern (Cotten and Van Riel 1993; Hockland et al. 2006; Mathys and Smith 1984; https://ippc.int/). For our purposes here, the regulatory efforts of MAFBNZ will serve as an example of similar efforts among other participating countries.

17.6.5 Development of Phytosanitary Requirements for Import Regulation of Nematodes

In NZ, MAFBNZ conducts risk analyses and develops Import Health Standards (IHSs) for importing plants and plant products that hold specific pest lists, and also hold generic and targeted import requirements (as per IPPC guidelines). IHSs usually undergo regular revision and are periodically modified to reflect new pest risks and the discovery of new pests that become established (in addition to reporting new pests as is required under IPPC guidelines). The IHS that applies most particularly for regulating most nematodes is the one for propagable plants and plant parts – IHS for Nursery Stock: 155.02.06 Importation of Nursery Stock (MAF Biosecurity New Zealand 2010a; http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf). MAFBNZ also publishes an Unwanted Organisms Register on its website (MAF Biosecurity New Zealand 2010b; http://www.biosecurity.govt.nz/pests/registers/uor).

This register holds a list of 368 nematodes that NZ considers to be unwanted biosecurity organisms and having likelihood to cause serious economic or environmental harm. Examples of some of the world’s most serious phytoparasitic nematodes are listed and include: *Aphelenchoides besseyi* (Rice White-tip Nematode), *Aphelenchoides bicaudatus* ((Imamura) Filipjev & Schuurmans Stekhoven) (Foliar Nematode), *Belonolaimus longicaudatus* (Sting Nematode and one other species), *Bursaphelenchus xylophilus* (Pine Wilt Nematode), *Ditylenchus dipsaci* [stem and bulb nematode strains not occurring in NZ], *Heterodera cruciferae* Franklin (Cabbage Cyst Nematode), *H. glycines* (Soybean Cyst Nematode), *Hoplolaimus columbus* Sher (Columbia Lance Nematode) *H. galeatus* (Cobb) (Crown-headed Lance Nematode), *Longidorus* spp. (Needle Nematodes and 25 other species), *Meloidogyne chitwoodi*, (Columbia Root-knot Nematode and another 14 RKN species), *Naccobus aberrans* (False Root Knot Nematode), *Paralongidorus* spp., (virus-vectoring and stunt nematodes, three species in total), *Paratrichodorus* spp. (Stubby Root Nematodes, four species in total), *Pratylenchus brachyurus* (Root Lesion Nematode), *P. indicus* Siddiqi (Root Lesion Nematode) *P. scribneri* Steiner (Scribner's Root Lesion Nematode and another 12 species), *Radopholus similis* (Burrowing Nematode and another three species), *Rotylenchulus reniformis* (Reniform Nematode and another three species), *Trichodorus* spp. (Stubby Root Nematodes, eight species in total), *Tylenchorhynchus* spp. (Stunt Nematodes, 16 species in total) and *Xiphinema index* (Dagger Nematode and another 34 species).

Like most NPPOs, MAFBNZ requires that imported plants and plant parts are accompanied by a Phytosanitary Certificate (PC) that officially certifies that the plants and plant material has been inspected and/or treated in the exporting country in accordance with appropriate official procedures and found to be free of any visually detectable regulated pests, and conforms with NZ's import requirements. For whole plants (with roots) the following additional declaration must also to be written on the PC: (1) "The plants were raised from seed/cuttings in soil-less rooting media in containers maintained out of contact with the soil"; or (2) "The roots of the plants have been dipped in fenamiphos (Note this is an organophosphate nematicide) at 1.6 g active ingredient per litre of water for 30 min)".

In addition to the requirement for official certification after inspection, MAFBNZ also specifies particular pesticide treatment requirements for regulating nematodes on plants and plant parts. These requirements are applied generically across multiple imported host plant species. For dormant bulbs both fumigation or hot water treatment and dipping in fenamiphos are required for nematode control (the methods of treatment must be written on the PC in the Treatment Section) as follows:

(1) Methyl Bromide fumigation: Fumigation for 2 h at atmospheric pressure using one of the following combinations of rate (g/m^3) and temperature (°C):

Rate (g/m^3)	Temperature (°C)
48	10–15
40	16–20
32	21–27
28	28–32

OR Hot water treatment: Immersion in hot water at a constant temperature of 24 °C for 2 h, followed by immersion in hot water at a constant temperature of 45 °C for 4 h (period required at the stated temperatures excluding warm-up times), AND (2) Chemical treatment: Immersion in fenamiphos (1 g active ingredient per litre of dip) for 1 h.

By comparison, the USDA maintains a Regulated Pest List associated with imported plants and plant products. This list was developed by using pest data obtained from the US Code of Federal Regulations (7 CFR 300–399), biosecurity data on pests found on imported goods at the USA border, data on biosecurity pests determined by USDA – Animal and Plant Health Inspection Service (APHIS) and by stakeholders in the USA as having potential to cause serious economic or environmental damage (http://www.aphis.usda.gov/import_export/plants/plant_imports/regulated_pest_list.shtml; United States National Archives & Records Administration 2008).

This list includes the Potato Cysts Nematodes, *Globodera pallida* and *G. rostochiensis* which are strictly regulated by the USDA from entering the USA and from spreading internally in the USA from areas where they have established. Information on US Federal Domestic Quarantine requirements are located at the following address: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&rgn=div5&view=text&node=7:5.1.1.1.2&idno=7. In particular, information relating to the US domestic quarantine requirements for *G. pallida* and *G. rostochiensis* are specified here also. These Potato Cyst Nematode species are very good examples of serious economic pests that have spread widely from their origin in the Andes of South America to the potato growing regions of the world. Due to the importance of potatoes in world agricultural trade potatoes, these pathogens are currently managed extensively and strictly regulated. Presently *G. pallida* and *G. rostochiensis* are specifically biosecurity regulated by 55 and 106 countries respectively (Hockland et al. 2006; Jones and Kempston 1982; Skantar et al. 2007).

The importation requirements for *Solanum tuberosum* L. (potato) nursery stock into NZ are quite restrictive in that only plants in tissue culture are permitted to be imported by people or organisations that are granted import permits. The tissue culture can be imported in two ways: (1) Imported from facilities accredited by MAFBNZ as meeting certain production and pest free/sanitary standards. The cultures must be accompanied by official certification attesting to freedom from designated pathogens/pests, visually detectable pathogens/pests. Following inspection (on entry to NZ) the tissue cultures are not required to be held in post-entry quarantine; and (2) imported from facilities that are not accredited by MAFBNZ. As above, they must be accompanied by official certification attesting to freedom from designated pathogens and pests, visually detectable pathogens pests and once inspected (on entry to NZ) the tissue cultures are required to be held in post-entry quarantine for a minimum of 3 months (MAF Biosecurity New Zealand 2010a; http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf). Although nematodes are not specifically mentioned in the pest list for *S. tuberosum* (as the potato cyst nematodes and *Ditylenchus destructor* occur in NZ and are not subject to complete biosecurity movement control) it is unlikely that other nematodes that could be associated would escape the testing and inspection scrutiny associated with the tissue culture pathway.

17.6.6 Phytosanitary Management of Exported Plants and Plant Products

As a major agricultural and horticultural exporting nation, NZ is also required to meet an importing country's phytosanitary requirements for plants and plant products. MAFBNZ is tasked with preventing the entry of new pathogens and pests into NZ and also with preventing the spread of pests found in NZ to other countries on exported products. One example of an export certification program (ECP) relating to potato exports is aimed at phytoparasitic nematodes. The program is run by MAFBNZ. MAFBNZ provides a rigorous ECP for the export of table potato exports (for human consumption) to Taiwan (these requirements may also cover exports other countries). Details of this program may be found at http://www.biosecurity.govt.nz/files/regs/exports/plants/potatoes/potato-pcn-wart.pdf. The program was developed in cooperation with the NZ Potato Export Access Committee and is aimed at meeting Taiwan's phytosanitary requirements for entry of potatoes where freedom from *G. pallida* and *G. rostochiensis* (and Potato Wart Fungus – *Synchytrium endobioticum* (Schilbersky) Percival) is required. The specification listed in the ECP defines MAFBNZ's operational requirements for exporters, growers, Independent Verification Agencies (IVAs as approved by MAFBNZ to carry out export certification activities), packing facility operators and storage facility operators. Requirements for this program are extensive so they are summarized as follows:

1. Production Requirements: Producers of intended export crops must:
   - Agree to the terms and conditions of the ECP by signing a compliance agreement;
   - Only use production sites that have a history of freedom from the Potato Cyst Nematodes (and Potato Wart Fungus);
   - Have official approval and registration for the site (by MAFBNZ via an IVA);
   - Use certified seed potatoes (proof required);
   - Maintain production records;
   - Manage movement and cleanliness of equipment and machinery into production sites to prevent introduction of Potato Cyst Nematodes; and
   - Apply a sprout inhibitor at harvest or ensure this is done during post-harvest processing.
2. Packing Facility and Storage Facility Requirements: These facilities must:
   - Be registered by MAFBNZ via IVA;
   - Have specialists staff who are aware of the ECP requirements;
   - Ensure potatoes are identified with specific MAFBNZ code numbers;
   - Ensure potatoes from Taiwan are segregated and sorted/packed separately from other lines; and
   - Notify MAFBNZ immediately if specific export pests are identified.
3. Exporter Requirements: Exporters must:
   - Be registered with Horticulture NZ;
   - Be registered with MAFBNZ, and provide traceability and post-inspection security; and

- Ensure where the potatoes are subject to MAFBNZ endpoint inspection, an authorized IVA or MAFBNZ approved organisation provides traceability and post-inspection security.

All MAFBNZ approved organisations involved in the ECP must be registered and meet the requirements of applicable MAFBNZ standards. The ECP and other standards form part of the MAFBNZ export phytosanitary certification system. These standards provide delegation of authority by MAFBNZ to authorised IVAs and approved organisations to carry out certification services and activities that contribute to the issuance of MAF phytosanitary certificates. For example, Potato Cyst Nematode sampling must be conducted by MAFBNZ-approved persons using approved sampling methods and techniques. The soil/plant samples may only be processed for identification by MAFBNZ approved labs that must meet the requirements of MAFBNZ Standard: Plants Export Operations Pest Identification Requirements (MAF Biosecurity New Zealand 2009b; http://www.biosecurity.govt.nz/exports/plants/certification/peo-pir.htm). These labs must follow a written (MAFBNZ approved) lab management system for the identification of specific nematodes. Finally, MAFBNZ periodically assesses the lab management systems for nematode identification using MAFBNZ auditors and a nematologist as a technical audit expert. MAFBNZ is extremely careful to ensure the ECP requirements are met in full in order to meet Taiwan's (and other countries) import requirements and prevent further spread of the potato cyst nematodes.

17.6.7 Phytosanitary Methods for Detecting, Excluding, and Treating Nematodes

As nematodes are most commonly found in/on plant tissues or in soil associated with plants, the main emphasis of phytosanitary regulatory management is logically focused on plants (plant nursery stock, propagative parts, some seeds) and soil. Importation requirements for nursery stock plants and plant propagative parts may involve a range of possible phytosanitary activities. These activities could be conducted in the country of origin (such as specific sampling or operating an ECP) or upon importation in the destination country. Detection of nematodes for regulatory purposes (to meet import export or phytosanitary requirements) most often relies on sampling. Usually this includes looking for symptoms of nematode damage in the field or place of production and soil and/or plant samples (roots or shoots depending on the type of nematodes) are taken for analysis.

Knowledge of nematode life cycles and habits in plant parts and soil is very important in determining the best time to conduct sampling. Soil samples should be taken when soil is moist, not dry or wet, and sampling is usually conducted systematically across fields. Where possible, sampling should be focused near host plants or where they were grown as nematodes are usually highly clustered around those areas in fields. However, the success of sampling may also depend on a number of different factors including the host plant, type of nematodes, sampling depth and soil type (Barker and Davis 1996; Been and Schomaker 2006; Fortuner 1991; Shurtleff and Averre 2000).

A significant problem can occur when nematodes may have been recently introduced to an area and/or occur at levels below the usual level of detection for some particular reason. Here there is the possibility of falsely attributing freedom from target species in these areas or crops even where plant and soil samples have been taken systematically and appropriate analysis and identification processes have been conducted. Timeliness of sending samples to appropriate nematology labs is also important as samples can degrade by drying out and reducing the survival of nematodes. There is a great variation in facilities that deal with extraction and identification of nematodes around the world and those that have official government certification and/or meet international standards are preferable (Barker and Davis 1996; Luc et al. 1990; MAF Biosecurity New Zealand 2009).

Several standardised lab methods are used for the extraction of nematodes from soil samples (Barker and Davis 1996; Fortuner 1991; Shurtleff and Averre 2000). Extraction of nematodes from plant samples (mostly roots) also varies widely. Methods have been developed to stimulate nematodes to leave plant tissue samples. For example, extracted plant material can be placed on funnels above beakers in mist chambers to stimulate the gradual movement of the nematodes outwards. Another approach is to coarsely grind the plant material to release the nematodes and capture them on fine mesh sieves. Nematode analysis and identification labs then rely on specialist nematology professionals (scientists and technicians) to identify to genus level and quantify the nematodes extracted in a sample using microscopy.

Reports on nematodes that are identified are then used for biosecurity or regulatory decisions as required (Cotten and Van Riel 1993; Fortuner 1991; Shurtleff and Averre 2000). In addition, the ongoing development of molecular tools may become further refined to help with the accurate and rapid identification of such nematodes (to species level) for regulatory and phytosanitary purposes (Ophel-Keller et al. 2008; Subbotin and Moens 2006).

When phytoparasitic nematodes are identified as being problematic in certain areas or in certain plants or plant materials, other techniques can be used for eradication. These techniques may include the use of designated biosecurity treatments on bulbs, rootstocks, other plant parts and seeds including dipping in nematicides, the use of fumigation at specific rates and temperatures, and hot water treatment (Bridge et al. 1990; Cotten and Van Riel 1993; Hockland et al. 2006; MAF Biosecurity New Zealand 2010). Other commonly used strategies for excluding nematodes by managing imported materials include post-harvest or pre-export inspection, banning the importation of plant host material or specific types of host material, banning the importation of plant bedding material and soil, and requiring that imported plants are certified as being grown in artificial planting media (Barker 1997; Cotten and Van Riel 1993; Hockland et al. 2006). These strategies often involve the additional use of phytosanitary treatments and may also require post-entry quarantine for important or high value planting material.

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Chapter 18
Invasive Pathogens in Plant Biosecurity. Case Study: Citrus Biosecurity

P. (Broadbent) Barkley, T. Schubert, G.C. Schutte, K. Godfrey, V. Hattingh, G. Telford, G.A.C. Beattie, and K. Hoffman

18.1 Introduction

Citrus (*Rutaceae*) The Family Rutaceae (Order Sapindales) is widely distributed with centres of diversity in southern Africa and Australia (Bayer et al. 2009). The most widely used taxonomic systems for classifying citrus are Swingle and Reece (1967) and Tanaka (1977). Many species of *citrus* still exist in nature as wild plants or as little-altered land races. These wild species have given rise to the diversity of *citrus* cultivars known today. The taxonomy of *citrus*, and particularly cultivated

P. Barkley (✉)
Citrus Australia Ltd, PO Box 46, Mulgoa, NSW 2745, Australia
e-mail: pat.barkley@bigpond.com

T. Schubert
Florida Department of Agriculture & Consumer Services, Division of Plant Industry, PO Box 147100, Gainesville, FL 32614-7100, USA

G.C. Schutte
Citrus Research International, PO Box 28, Nelspruit 1200, South Africa

K. Godfrey
Contained Research Facility, University of California-Davis, 555 Hopkins Road, Davis, CA 95616, USA

V. Hattingh
Citrus Research International, Department of Conservation Ecology and Entomology, Stellenbosch University, PO Box 2201, Matieland 7602, South Africa

G. Telford
Department of Agriculture, Fisheries & Forestry, Biosecurity Queensland, PO Box 1241, Oxley, QLD 4075, Australia

G.A.C. Beattie
University of Western Sydney, Locked Bag 1797, Penrith, New South Wales 2751, Australia

K. Hoffman
California Department of Food and Agriculture, Pest Detection and Emergency Projects, 1220 N Street, Sacramento, CA 95814, USA

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forms (Bayer et al. 2009), is complicated by a long history of cultivation and wide cross-compatibility among species (Mabberley 2004). Recent work suggests that *citrus* comprises about 25 species (Mabberley 2004) including reunification of *Eremocitrus, Fortunella*, *Microcitrus* and *Poncirus* with *citrus* (Mabberley 1998). In a molecular analysis (Bayer et al. 2009), *citrus* was broadened to include *Oxanthera* Montrouz and *Feroniella* Swingle. Unless stated otherwise, classification of plants in this chapter is based on Mabberley (1997, 1998, 2004, 2008), Scott et al. (2000), Samuel et al. (2001) and Bayer et al. (2009).

Commercial citrus are derived from wild species indigenous to the sub-Himalayan tract, China and western Malesia. For a history of the establishment of the major citrus industries see Tolkowsky 1938; Webber et al. 1967; Ramón-Laca 2003.

Current world citrus production is approximately 120 million tonnes: 58 % is consumed locally, 30 % is processed and 12 % exported (Imbert 2010). Statistics on the citrus industries in each country are available at http://faostat.fao.org/site/339/default.aspx or for production, supply and distribution for selected countries see the USDA citrus: World Markets and Trade at http://www.fas.usda.gov/htp/2011_Jan_Citrus.pdf.

18.2 International Movement of Citrus Pathogens

A combination of luck in the early days and judicious selection or quarantine in later years successfully separated many of the co-evolved natural enemies (pests and pathogens) from crop plants. This increased plant fitness to such an extent that most major production areas were outside their centres of origin, and free from pest and disease pressures. Nevertheless, progressive globalisation during the past 150 years has meant that co-evolved pests and diseases have been reunited with their host plants, often with disastrous socio-economic consequences (Evans and Waller 2010). This risk has been accentuated by growing citrus as a monoculture (Arora 2000). Additionally, some ‘new-encounter’ pathogens have not co-evolved with citrus. These include pathogens of South American citrus (citrus leprosis virus causing leprosis and *xylella fastidiosa* causing citrus variegated chlorosis), *spiroplasma citri,* the causal agent of stubborn (which is limited to parts of the Mediterranean Basin and California), mal secco (*phoma tracheiphila*) in some Mediterranean countries and viroids causing exocortis and cachexia, two diseases which were not known in varieties of Chinese origin.

Inevitably, movement of germplasm involves a risk of accidentally introducing pests of biosecurity concern with host plant material (Chap. 13). Some pathogens (e.g. graft-transmissible viruses) are symptomless in some hosts and pose a special risk. To minimize this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of quarantine pests (Sect. 13.5). The FAO/IBPGR Technical Guidelines for Safe Movement of Citrus Germplasm (see http://ecoport.org/Resources/Refs/IPGRI/citrus.pdf) provide general recommendations on accessing germplasm, collecting and treating citrus seed and budwood, therapy procedures and indexing strategies.

Countries have introduced programs that handle introduction, pathogen testing and distribution of budwood. For example in the USA consider the University of California Citrus Clonal Protection Program, the Citrus Germplasm Introduction Program (State of Florida) and the Citrus Budwood Registration Program (State of Florida). Arizona and Texas acquire pathogen tested citrus budwood from these programs and maintain, re-test and distribute budwood to their industries via distribution-certification programs (Krueger et al. 2010). In Australia, the Australian Department of Agriculture, Fisheries and Forestry (DAFF) undertakes post-entry quarantine for imported citrus budwood, while the Australian Citrus Propagation Association Inc. (trading as Auscitrus) is responsible for supply of citrus budwood and seed (http://www.auscitrus.com.au).

Citrus pathogens have spread internationally through various means of dispersal. Precautions notwithstanding, individuals still seek to gain advantage through new or improved varieties by illegally importing citrus propagative material and circumventing the biosecurity system (Chap. 6). Rarely, pests have also been introduced on plant material that has been inadequately tested, treated or inspected. An example is the introduction of fresh curry plant (*Bergera koenigii* L.) leaves to California from Hawaii with the Asiatic Citrus Psyllid (ACP) (*Diaphorina citri* Kuwayama) (Wilkinson 2007). Passive transport of insects in aircraft and ACP through air movements (e.g., cyclonic and jet streams, Gottwald et al. 2007; Sakamaki 2005; Aubert 1987a; Beattie and Barkley 2009) have been proposed as a method of intercontinental spread. The movement of fresh cut foliage or non-commercial fruit, especially in border communities such as along the USA – Mexican border is a pathway for introducing new pests. Bulk shipments of fruit (with or without leaves attached, Halbert et al. 2008, 2010), moving between areas within a country or region could be a method of dispersal of insect vectors (e.g. ACP) or pathogens such as citrus canker (*Xanthomonas citri* subsp. *citri,* Schaad et al.). E-commerce sale of citrus and orange jasmine (*Murraya exotica* L.) plants has been a potential transport route for citrus from Florida (with canker and the Asiatic Citrus Psyllid) to other areas of the USA. Doubt remains concerning seed transmission of Huanglongbing ('*Candidatus* Liberibacter' spp. Hartung et al. 2010; Albrecht and Bowman 2009), but other citrus pathogens are occasionally seed transmitted e.g. Citrus Leaf Blotch Virus (Guerri et al. 2004), *Xylella fastidiosa* subsp. *pauca* subsp. nov (*Xf*) Schaad et al. causing citrus variegated chlorosis (Li et al. 2003), Citrus Tatter Leaf Virus (Tanner et al. 2011). While there are no reports of transmission of Citrus Exocortis Viroid (CEV) through citrus seed, CEV is transmitted through seed of *Impatiens walleriana* Hook.f. and *Verbena* x hybrid (Singh et al. 2009).

18.2.1 Phytophthora

The spread of pests and pathogens of citrus has often had devastating consequences, which have changed citrus production techniques. For example, the *Phytophthora* epidemics of the nineteenth century and the tristeza epidemics beginning in the 1930s, led to interlinked cultural changes. The first record of citrus gummosis (1832) (undoubtedly due to *Phytophthora*) was from the Azores. The disease spread from the Azores to Mediterranean countries during the mid 1800s; subsequently, it appeared in NSW, Australia (1867), California (1875) and Florida (1876) (Fawcett 1936; Fraser 1949). These worldwide epidemics of phytophthora root rot caused a change in culture from growing citrus on their own roots or on rootstocks such as rough lemon and sweet orange, to the use of *Phytophthora*-tolerant sour orange stocks. But the subsequent introduction of Citrus Tristeza Virus (CTV) caused the death of oranges and mandarins on sour orange stocks ('quick decline') giving rise to the name "sad disease" or tristeza.

18.2.2 Tristeza

CTV probably appeared first in the areas of origin of commercial citrus and was dispersed at the end of the nineteenth century in infected plants to other continents, where it interacted with new host varieties and stock/scion combinations under different climatic and environmental conditions (Moreno et al. 2008). Some CTV strains cause quick decline while others cause stem pitting. Since the early twentieth century, extensive commercial movement of CTV-infected nursery trees or budwood (sometimes with aphid vectors including *Toxoptera citricida* (Kirkaldy) has occurred between countries and continents, e.g. from South Africa to Argentina in 1930 (Wallace 1956), from Australia to Argentina in 1933 (Fraser and Broadbent 1979), and from Japan to Peru during the 1970s and early 1980s (Roistacher 1988). The most destructive epidemics of tristeza are described in Roistacher (2004) and Moreno et al. (2008). In some countries, citrus production has only been possible through the use of CTV tolerant rootstocks and mild strain cross protection (Moreno et al. 2008).

18.2.3 Leprosis and Citrus Variegated Chlorosis

Examples of new-encounter pathogens that have spread with devastating impacts include leprosis, and citrus variegated chlorosis (CVC). Leprosis caused by two viruses (CiLV-C, CiLV-N) and transmitted by tenuipalpid mites of the genus *Brevipalpus,* occurs in citrus-growing countries in South America and is spreading northward through Central America and Mexico. Spread most likely occurs via virus-infected plants or viruliferous mites present on plant material. Additionally, the mite is dispersed by wind. As CiLV is localized in lesions, transmission by infected budwood is very unlikely. Determining the source of introduction is difficult for many regions; researchers hypothesise that the disease has occurred unnoticed in these areas for long periods of time. Many countries have tried to eradicate leprosis without success as the efforts commenced when the symptoms were identified, but by this stage the disease was already widespread (Bastianel et al. 2010).

CVC is caused by *Xylella fastidiosa* subsp. *pauca* subsp. nov (*Xf*). The Asiatic origins of the major commercial citrus cultivars suggest the recent evolution of CVC in South America, as *Xf* is a New World organism (Chen et al. 2002). CVC strains are genetically highly homogeneous. CVC is endemic in São Paulo State in Brazil, and occurs also in Argentina, Paraguay and Costa Rica. CVC was first observed in 1987 in Brazil, and by 2005 it had infected 43 % of the 200 million sweet orange trees in São Paulo state and was present in all citrus growing regions of the country (Bové and Ayres 2007). Phylogenetic relationships among this group of *Xf* strains support the idea that CVC-associated bacteria have evolved directly from *Xf* strains that cause coffee leaf scorch (CLS) (Li et al. 2001). Most areas in Brazil in which citrus orchards are presently cultivated were previously dedicated to coffee plantations and there have been reports of CVC vectors feeding on coffee trees (Li et al. 2001). Control strategies to manage CVC include nursery production under vector-proof screens, use of CVC-free budwood and seed, and pruning of symptomatic parts of trees to remove inoculum. Control of the sharpshooter vectors is very difficult. In 2003, the São Paulo State banned commerce and transport of citrus rootstocks and citrus plants produced in field nurseries (Gonçalves et al. 2011) in an effort to control the spread of this serious disease.

Xf can be transmitted through citrus seed to seedlings (Li et al. 2003). Strains of *Xf* isolated from diseased citrus and coffee in Brazil have produced symptoms of pierce's disease after mechanical inoculation into seven commercial grape varieties. Thus, any introduction of the CVC strains of *Xf* would also pose a threat to grape industries (Li et al. 2002). To prevent the introduction of CVC into the USA, regulations have been enacted that govern the importation of seed of several Rutaceae genera from countries where CVC is present (Federal Register: April 6, 2010 Vol 075, No. 65, pp 17289–17295). In Australia, imported citrus seed from countries with *Xf* is treated by immersion in hot water or the seed is grown in a post-entry quarantine facility for a minimum of 2 years, with testing of progeny by PCR or ELISA for *Xf* (all strains) (see the ICON database http://www.aqis.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=8867801&intCommodityId=3321&Types=none&WhichQuery=Go+to+full+text&intSearch=1&LogSessionID=0).

18.2.4 Mal Secco

This pathogen occurs in some countries around the Mediterranean Basin, and is a threat to lemons worldwide. The causal fungus *Phoma tracheiphila* (Petri) Kantschaveli & Gikachvili (syn. *Deuterophoma tracheiphila* Petri) does not occur throughout the Mediterranean citrus-growing areas, possibly due to the severe restrictions on movement of citrus propagating material mainly in relation to virus diseases (OEPP/EPPO 2007). No obvious climatic or cultural factor limits potential establishment of mal secco in uninfested areas (Migheli et al. 2009). Infected fruits usually fall to the ground before harvest. While seed coats can become infected, treatment of contaminated seeds with water at 50 °C for 10 min is effective against *P. tracheiphila* (Ippolito et al. 1987). Consequently the likelihood of infected fruit serving as a source of disease spread is low.

18.2.5 Huanglongbing and Citrus Psyllids

Huanglongbing (HLB) is thought to be caused by the phloem-limited bacterium '*Candidatus* Liberibacter' spp. and is a highly destructive disease of citrus. Early records of citrus maladies suggest that HLB may have been present in India during the mid 1700s (Capoor 1963) while other reports suggest the disease was present in other regions of India during the 1800s and early 1900s (Beattie et al. 2008). Husain and Nath (1927) first described symptoms indicative of HLB. Records of observations in India and southern China suggest eastward movement, directly or indirectly, of the disease and its psyllid vector in Asia from India to China during the late 1920s or early 1930s (Beattie et al. 2008). For further information on the spread of HLB and ACP through Asia, see Beattie and Barkley (2009). HLB, transmitted by *Trioza erytreae* (del Guercio), was first reported in South Africa in 1928/9 and was spread with infected planting material (le Roux et al. 2006a).

HLB losses are enhanced by growing citrus as a monoculture, principally due to the activities of the ACP vector. Aubert (1988) noted that *D. citri* in Asia, in contrast to the African citrus psyllid, *T. erytreae*, is not able to build up massively on a wide range of alternative rutaceous hosts. Several alternative hosts to '*Ca.* L. africanus' have been identified in Africa (van den Berg et al. 1991–1992; Korsten et al. 1996; Moran 1968).

ACP is the only identified vector of HLB in Asia, although other psylloids feeding on Rutaceae (including citrus) have been described (Mathur 1975; Yang and Li 1984; Inoue et al. 2006; Fang and Yang 1986; Lahiri and Biswas 1980; Osman and Lim 1990). Recently, the presence of '*Ca.* L. asiaticus' was confirmed in the Black Psyllid, *Diaphorina communis* Mathur collected in Bhutan (Donovan et al. 2011), and in *Cacophylla (Psylla) citrisuga* (Yang & Li) in China (Cen et al. 2012). However, we do not know whether these psyllids can transmit '*Ca.* Liberibacter' spp.

ACP has been in Brazil since about 1940 (Halbert and Núñez 2004); in 2004 both '*Ca.* L. americanus' and '*Ca.* L. asiaticus' were first discovered at the same location in orchards in Brazil (Lopes et al. 2008). '*Ca.* L. americanus' was initially the prevalent species in citrus, but has been replaced by '*Ca.* L. asiaticus', presumably due to its higher acquisition and transmission rates (Gasparoto et al. 2010). By contrast, ACP was first found in Florida during 1998 (Halbert and Núñez 2004) and '*Ca.* L. asiaticus' was found during 2005 (Halbert et al. 2008). Phylogeographic studies suggest that *D. citri* populations did not invade North America from South America (de Leon et al. 2011).

The potential for populations of *D. citri* to exist and flourish declines with increasing altitude and latitude (< 800 m asl depending on region, with a preference for lower altitudes) and high saturation deficits (warm to very hot climates with low relative humidity) preferred to high altitudes and low saturation deficits (warm to hot climates with high relative humidity) (Aubert 1987b, 1990; Yang et al. 2006; Beattie and Barkley 2009). ACP tolerates low ambient temperatures (Xie et al. 1988) and temperatures as high as 50 °C (Husain and Nath 1927). By contrast, *T. erytreae* cannot establish in hot and dry areas. However, adults are highly tolerant of weather extremes (Samways 1990; Aubert 1987b). *Trioza erytreae* could colonize Mediterranean coastal areas, with egg laying and larval development periods during spring, and adults surviving during other seasons. Larvae of this psyllid live in galls on the underside of leaves and their transport over long distances could occur on rooted planting material. Then adults, with fairly good flight capability, will spread the species in newly contaminated territories (Aubert 2009).

'*Ca*. L. africanus' is not as aggressive as '*Ca*. L. asiaticus' and symptoms of African HLB, are less severe. The two forms can be distinguished on the basis of temperature tolerance (le Roux et al. 2006a; Schwarz 1968; Schwarz and Green 1972). Other differences between '*Ca*. L. americanus' and '*Ca*. L. asiaticus' include their sensitivity to high temperatures (Lopes et al. 2009b) and transmissibility (Lopes et al. 2009a; Barbosa et al. 2011). These factors may explain the shift in prevalence from '*Ca*. L. americanus' to '*Ca*. L. asiaticus' in Brazil.

Spread of ACP in USA. ACP quickly spread throughout urban and citrus production areas in Florida and Texas through natural spread and the movement of infested nursery plants, particularly orange jasmine (*Murraya exotica,* commonly cited as *M. paniculata* (L.) Jack (see Lopes et al. 2010; French et al. 2001; Manjunath et al. 2008) and into South Carolina, Georgia, Alabama, Mississippi, and Louisiana (Bech 2008; NAPIS 2008). ACP arrived in Tijuana in June 2008, and was first found in Southern California in August 2008 and in Arizona near the Mexican border in 2009 (Bech 2009).

Response to detection of HLB or ACP. Interstate movement of citrus and other rutaceous nursery stock was prohibited from areas in the USA quarantined for HLB and ACP (April 2011), unless moved in accordance with a protocol given at http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus/downloads/interstate-mvmnet-protocol.pdf.

In response to detections of ACP in Tijuana, Mexico during 2008, "*D. citri* Detection, Delimitation and Treatment Guidelines" were drafted to include detection and intensified survey protocols in California (http://www.cdfa.ca.gov/plant/pdep/Insect_Trapping_Guide/itg_sections_acp-am.pdf).

Densities of the psyllid must be kept as low as possible to reduce the numbers of ACP available to colonize new areas and/or acquire HLB from an undetected HLB-infected tree. Provisional treatment guidelines for citrus in quarantine areas in California are given at http://www.ipm.ucdavis.edu/EXOTIC/diaphorinacitri.html.

On January 13, 2012, USDA confirmed the presence of '*Ca*. L. asiaticus' in tissue samples collected from a symptomatic tree in a commercial citrus grove in Texas. The Texas Department of Agriculture imposed a temporary emergency quarantine (5 mile/8 km). Information on quarantine areas in Texas can be found at http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/index.shtml.

In March 2012, a sample of three adult ACPs collected by CDFA in Los Angeles County tested positive for '*Ca*. L. asiaticus'. A citrus tree on the same property was also found infected and was removed. The CDFA embarked on a survey and eradication program. The ACP treatment radius was 800 m, and a quarantine zone (radius 5 miles/8 km) was implemented. More information is available at http://www.cdfa.ca.gov/plant/acp/index.html. A public outreach program was implemented to create awareness and cooperation with a focus on homeowners and public officials in infested areas, retail nurseries and big-box outlets, master gardeners, and traditional and social media.

Eradication. ACP was recorded in the Northern Territory, Australia (1915) during an incursion of citrus canker and is assumed to have resulted from the introduction of citrus plants from Asia. ACP was eradicated by chance during the 1916–1922 eradication campaign for citrus canker (Bellis et al. 2005). This is the only recorded eradication of ACP.

No instances of HLB eradication can be cited although effective control measures from the 1960s in South Africa have resulted in the incidence of infected trees decreasing from 38 % to 1 % in 2006 (Buitendag and von Broembsen 1993; le Roux et al. 2006b). Ke and Xu (1990) reported successful control of HLB and ACP in the coastal region of subtropical southern China. In Brazil, HLB has been successfully controlled on individual farms by managing many factors (Belasque et al. 2010). Despite all attempts at control, HLB continues to spread in Florida and Brazil (Belasque et al. 2010, http://cssrc.us/publications.aspx?id=7878). An analysis of successful programs around the globe has shown that effective management of HLB can be achieved by implementing several measures (Beattie and Barkley 2009).

18.3 Citrus Canker in Florida

18.3.1 Overview

Several reviews of citrus canker outbreaks in Florida have been published (Stall and Seymour 1983; Civerolo 1984; Schubert et al. 2001; Brown 2001; Gottwald et al. 2002a; Graham et al. 2004). This text reflects on dealing with canker over the last century in Florida, USA. Following the historical account is a collection of observations, assertions, questions, conclusions and concepts that have emerged from the canker experiences in Florida.

Asian strain of citrus canker successfully established in Florida on at least three occasions. The first was during 1910, which resulted in nearly statewide presence in Florida and throughout the citrus growing regions of southeastern USA. The second was during 1986 and the most recent during 1995. The latter two events commenced in residential citrus and eventually spread to infect commercial citrus. Each of these major events was discovered by routine surveillance of residential and commercial citrus. In each case the disease apparently had been active in the local area for some time, perhaps years, though the spread was still manageable. The pathways of introductions of the pathogen were never ascertained with certainty in the most recent cases, but diseased imported nursery rootstock was very strongly implicated for the original outbreak (Berger 1914). Illegal entry of propagation material by non-commercial horticulturists is suspected for the other two because oldest infections manifested in residential landscapes. The first two introductions were successfully eradicated, with eradication discontinued on the last outbreak during January 2006. Citrus canker is now endemic in Florida, with the disease present at many locations in the lower two-thirds of the state. Citrus canker infests the same geographic range as Huanglongbing.

Citrus canker caused enormous damage in the humid subtropical climate of Florida early in the twentieth century. Consequently, any incursion of citrus canker in Florida was worthy of eradication. Canker is a good candidate for eradication because:

1. High food and economic value of the crop;
2. Moderate rate of natural spread;
3. Absence of a vector;
4. Easily recognizable symptoms of the disease;
5. The pathogen's restricted host range in the Rutaceae;
6. The pathogen's poor survival potential when the host is absent.

Citrus canker remains one of the few examples of a major plant disease that clearly meets all of the major scientific criteria to justify attempted eradication (IPPC 1998). The virulence and aggressiveness of canker, along with a few other contemporaneous pests and pathogens such as Mediterranean Fruit Fly (Chap. 15), inspired the first complementary federal and state plant protection laws in the USA (Loucks 1934; Dopson 1964, USDA Plant Quarantine Act of 1912 at http://www.archive.org/details/plantquarantinea00unit).

As the Florida citrus industry developed, demand for rootstocks quickly exceeded supply. Around 1910, trifoliate orange seedlings were introduced from Japan to meet that demand. At some locations where the rootstock seedlings were delivered, a debilitating scab-like disease appeared that was later determined to be asian citrus canker. Several years passed before growers came to the consensus that canker and profitable citriculture could not co-exist in Florida. Subsequently, the grower association asked state and federal agriculture departments for assistance to undertake eradication. By 1927, the disease was finally eliminated from Florida by systematic inspection and destruction of infected citrus (Dopson 1964). After extensive clean-up work on residual infections on non-commercial citrus in Louisiana and Texas, canker was determined to be eradicated from the entire Southeastern USA by the late 1940s.

In 1984, a canker-like *Xanthomonas*-induced disease appeared in several Central Florida citrus nurseries causing significant damage to the rapidly growing rootstock seedlings and budded plants. The syndrome resembled asian citrus canker, but the community of causal bacteria lacked the aggressiveness and damage potential to be a problem on mature citrus in an orchard. The disease is now known as citrus bacterial spot (caused by *Xanthomonas alfalfae* subsp. *citrumelonis*) (Gottwald et al. 1988; Graham and Gottwald 1990).

While a regulatory response to citrus bacterial spot was underway, an outbreak of asian citrus canker was discovered in 1986. Residential infections had spread into nearby commercial citrus. Using periodic inspections, coupled with removal of infected citrus and removal or 'hatracking' of exposed citrus within 125 ft (38.1 m), steady progress toward eradication was achieved. The exposure radius had been empirically determined in Argentina (Stall et al. 1980). The 'hatracking' procedure (cutting exposed trees back to brown, mature wood) was based on the assumption that the pathogen cannot infect older citrus tissues. Although 'hatracked' trees occasionally become infected with canker, the practice was successful and useful on a limited scale. Canker was reduced to non-detectable status in about 7 years, and declared eradicated 2 years later.

Canker appeared for the third time in 1995. It was discovered as well-established infections in citrus in a residential neighborhood near the Miami airport. Molecular evidence indicated a different genotype from the 1985 pathogen. This discovery reinforced the need for broad, continuous surveillance for early disease detection, even in residential areas and is vital to successful regulatory plant pest management.

When the disease was first detected in 1995, the option of removing all citrus within the 14 sq mi (36.3 sq km) infested area was considered. This option was rejected from a public relations perspective as being too extreme and politically ill advised, with the potential to endanger all future regulatory activities. In retrospect, strictly from a biological perspective, such an action might have been successful. However, the general public reacted strongly to the regulatory action and procured a legal injunction of unspecified duration. Without public support the program enjoyed little success.

Infected trees were removed as soon as practical (Fig. 18.1). Exposed hosts within 125 ft (38.1 m) were initially 'hatracked'. Over the next 18 months, control activities did not proceed systematically and uniformly over large areas due to property access limitations and new growth on 'hatracked' trees was frequently infected within a short time span. In addition, a new exotic citrus pest, the citrus leafminer (*Phyllocnistis citrella* Stainton), first detected in Florida in 1993, was also enhancing canker. In early 1997 the removal of exposed trees was adopted universally, and the practice of 'hatracking' was discontinued.

The next year of the program was spent attempting to stop the progression of the disease into new areas. By February 1998, the Commissioner of Agriculture declared a year-long moratorium on cutting exposed trees to assuage mounting public disillusionment with the program. At that time the program was only impacting residential citrus and commercial citrus growers were strongly in favor of the protective efforts.

Fig. 18.1 Canker infected and exposed citrus in residential settings was removed with chain saws, moved to curbside, and chipped into a closed truck bed for disposal at a sanitary landfill or electrical co-generation plant to be burned as fuel. Residential eradication activities were conducted only in dry weather (Image courtesy Florida Department of Agriculture and Consumer Services-Division of Plant Industry FDACS-DPI)

During this moratorium, regulators had an opportunity to study canker epidemiology under urban Florida environmental conditions. A large and skilled workforce, now dismissed from eradication activities, was freed to undertake much of the intense preliminary survey work and data gathering in the designated study areas. A proposal to perform a geo-referenced spatiotemporal analysis of advancing canker in urban Southeast Florida was quickly devised, and resources to undertake the massive investigation were provided by the USDA and State of Florida (Sect. 11.4)

Investigation revealed that citrus canker could establish at a much greater distance from known infections than had been previously used to guide program policy (Gottwald et al. 1992; Gottwald et al. 2002). This additional information indicated a significant increase in the exposure radius for tree removal from 125 ft (38.1 m) to 1,900 ft (579.1 m) to capture all new infections on the disease front in a 30–60 day time period about 95–99 % of the time. Additional major findings from the study were:

1. Citrus leafminer wounds are very conducive to establishment of new infections;
2. Citrus leafminer is responsible for a reduction in the degree of aggregation of new infections (Christiano et al. 2007);

3. A distance to encompass a set percentage of new infections over a set time-period can be derived from combined spatiotemporal data collected from a population of previously unexposed citrus;
4. The "exposed" population within the radius around an infected citrus tree contains infected plants that competent visual inspection cannot reliably detect;
5. Cryptically infected trees eventually are identified by inspection;
6. No universal exposure-radius exists for a canker eradication effort, but by combining continuing regular surveys with a well-chosen exposure radius promptly applied for host removal, one has an excellent chance at eventual eradication.

Based on these findings, new eradication criteria were devised and put into action in early 2000. By this time, the absolute size of the infested area dictated some strategic program implementation to concentrate on the advancing edges of the infestation, with the intent of later returning to those core areas already heavily infected.

The revised eradication guidelines met with mixed success over the next several years. Residential property owners were notified about survey activities in the area. As mapping techniques became more sophisticated, residents were informed exactly where infection had been identified and where exposure zone boundaries fell. If properties fell partly in the exposure zone, the property was left under surveillance with no tree removal. All diagnostics were performed in a central lab until sample volume and shipping costs necessitated opening a satellite diagnostic lab in the heavily infested area to help sort and share the diagnostic load. Lab techniques included basic disease recognition with microscopy, pathogenicity testing, and use of microbiological, serological, and molecular techniques (Sects. 13.6, 13.7, and 13.8).

In residential areas, infected and exposed trees were removed and the residue chipped. Chips were collected and transported securely to a landfill where all waste was covered. Initially herbicides were used on stumps to prevent sprouting but public sensitivities prompted switching to mechanical stump grinding. Commercial tree removal involved uprooting trees, forming them into piles and burning on location (Fig. 18.2).

Quarantines were lifted as follow-up surveys revealed no canker activity over an 18–24 month period. The standard 24 month host-free period was usually mandated to ensure all citrus in the regulated area had been discovered and either removed if within 1,900 ft (579 m) of the positive tree, or repeated surveys were completed and negative within the quarantine zone. Quarantine zone boundaries usually extended 1 mile (1.61 km) in all directions from the known infections and followed conveniently identifiable boundaries on the map (roads, canals, etc.). In southeast Florida, continuing delays and high rates of disease movement resulted in entire counties falling under quarantine, and little to no progress in stopping the spread of the disease. In commercial and residential areas, where implementation was not hindered by litigation and injunctions and infestations were smaller, significant and steady progress toward eradication was made.

Fig. 18.2 Citrus canker infected or exposed commercial citrus trees were removed using a root rake on a tractor, piled and burned on site, sometimes with the aid of an air blower to improve and accelerate combustion (Image courtesy of Florida Department of Agriculture and Consumer Services-Division of Plant Industry (FDACS-DPI))

Several significant tropical storm events created problems for the program (1995–2004), but each setback was manageable. Good fortune ran out in 2004–2005 when historically unprecedented and recurring severe tropical weather events repeatedly occurred in south and central Florida. Post-storm analysis of probable inoculum dispersal led to the disheartening conclusion that eradication of canker from Florida was no longer feasible (Irey et al. 2006; Gottwald and Irey 2007). The program was criticized for not taking into account the likelihood of inoculum dispersal by catastrophic storms. In fact, the eradication program made progress for about 7 years unaffected by catastrophic storm events, but litigation and injunctions impacted the progress. A retrospective case study of the Florida eradication program suggested several ideas that could inform future regulatory actions attempting to eradicate high-impact invasive exotic pathogens (Centner and Ferreira 2012). Two significant suggestions were: (1) Anticipate likely impediments and determine in advance what, where and when likely stopping points in the eradication effort might arise; be prepared to shift to alternative strategies and tactics; and (2) use an array of strategic public information efforts to inform the public about the basis for the effort, thereby generating sufficient public support.

Citrus canker continues to spread from multiple infection centers at a rate of about 15 miles (24.14 km) per year. Distances for dispersal are beginning to overlap, thus making interpretation difficult. Canker spread is most intense in the south and central portions of the state, while the more northern citrus production areas suffer less from the disease. Since discontinuing the eradication program in 2006, over 1,045 additional square mile (2,706 sq km) sections of residential and citrus production areas have active canker infections. In many southeastern communities, residential citriculture has diminished from a high of 30–40 % of households having citrus on the property in the mid 1980s to 5 % now.

Advanced planning and organization benefited the program. A Citrus Canker Action Plan was prepared in the early 1980s and revised on a regular basis as new information about the disease became available. The Incident Command System approach to program organization and execution facilitated the assembly of a work force and assignment of specific tasks including survey, mapping, lab diagnostics, workspace and equipment procurement, transportation, hiring, supervision, payroll, daily dispatch, record keeping and reporting, training, mapping, etc.

http://www.fema.gov/emergency/nims/IncidentCommandSystem.shtm

The criteria incorporated into a typical risk assessment for a residential or commercial outbreak of canker in Florida took into account the following attributes: Property type (commercial or residential), citrus cultivar, cultivar susceptibility, tree size and age, size of block, tree spacing, horticultural condition, tree distribution, tree density, windbreaks, plant and people movement factors, infection (lesion) age, lesion distribution on host, disease incidence, infection on leaf/stem/fruit tissues, strain of the pathogen, presence/extent of Asian citrus leafminer damage, other predisposing wounds, program resources, ease of access for survey, level of compliance, control action timeliness, security of property, horticultural and pest management practices at the location, disinfection/decontamination practices, presence of other properties nearby with hosts, other properties nearby with infected hosts, chronological data, maps, and verbal/written communications with owner.

Preparation of a regulatory pest response program entails more than just a well-developed action plan and Incident Command System to execute the plan. Finances must be immediately available to undertake a swift response.

18.3.2 Lessons Learned

Future eradication activities must intrude into private and community affairs with extreme care: Avoid being too intrusive, yet sufficiently aggressive to move steadily toward the ultimate goal of eradication with minimal loss of citrus hosts on private property. Commercial citrus growers accept the concept of managing pests regionally, whereas the general community tends to approach pest problems on an individual tree basis.

The citrus canker program raised a significant question: Where does a regulatory agency go to procure the necessary scientific information needed to conduct a rational, coherent and consistent emergency program?

This attempted eradication showed that any regulatory exercise as large and extensive as this canker program needs built-in flexibility. Program officials should eliminate outmoded policies to avoid litigation on the basis of inconsistent application. Program activities that ignore new scientific information constitute an even greater legal exposure.

Clearly, conducting any regulatory program in a residential area poses difficulties for which solutions are limited. Early intervention based on regular residential pest surveys is one good solution because responses can be limited in scope, but these surveys are more complicated and costly than similar efforts in commercial citrus. Detecting exotic citrus pests first in urban areas has become a regular occurrence in Florida with citrus black spot (*Phyllosticta citricarpa* Kiely) being the only pest first detected in commercial citrus. Pest surveillance in residential areas is a high priority.

Public engagement is important and pest specialists should advise decision-makers carefully and often to avoid misunderstandings. Cooperation with implementation of the eradication program was very good in commercial citrus. Cooperation was generally good, but implementation was more expensive, challenging and patchy in residential citrus due to limited access and legal impediments. Program opponents sometimes asserted that eradication was impossible and therefore ill-advised in the first place, even though several examples of successful eradication existed in Florida, South Africa, New Zealand and Australia. Opponents, even when few in number, can make eradication efforts less effective or even cause them to fail (Simberloff 2003).

Numerous hurdles were encountered in undertaking the eradication program. The property taken in the process of eradicating the pest was considered non-compensable by the regulatory agency because of its diseased or exposed status. However, class-action litigation in several counties recently ruled that exposed tree removals constitute a legal taking for which compensation is due.

The exposure concept is hard to comprehend or unpalatable for the layperson, and perceived as destroying trees that have a healthy future. The question then becomes how to define the exposed area, not whether it exists. Eradicating citrus canker is impossible in any setting (nursery, orchard or residential) without the removal of exposed individual hosts. The advancing edge of infection is impossible to see; it can only be approximated based on empirical epidemiological data. The most recent Florida canker eradication programs considered exposed trees as essentially lost and their removal was vital to the success of the eradication effort.

Significant and prolonged delays were imposed by Circuit courts in the form of injunctions in response to residential plaintiff's lawsuits. Their main complaint was essentially an unwillingness or refusal to accept the necessity of removing exposed trees to stop the spread of the disease. Injunctions in one form or another prevailed from November 2000 through April 2004, subjecting the eradication efforts to eventual failure and significantly increasing program costs. Two other major impediments to the program were: Search warrants to go onto private property for agricultural inspections were deemed mandatory by some judges (for the first time in Florida history). Necessary warrants were not always willingly or timely granted by the courts. This constituted the second major delay in the eradication program, preceded by injunctions. As a countermeasure to secure warrants, the program redoubled efforts at obtaining waivers from property owners, allowing about 75–80 % properties to be inspected and regulatory actions taken as the program felt necessary. Many property owners never responded to requests for a waiver, so no action could be taken on those properties. Citizen suspicions and unfounded allegations of incorrect diagnoses, prompted one judge to decree that each sample diagnosed as canker would require a positive pathogenicity test before regulatory action could proceed. This action resulted in greater expense and more time.

Starting and stopping a massive eradication program while courts consider legal issues is extremely burdensome, time-consuming, and costly.

18.3.3 Compensation

Diminution in real estate value has been accepted by the courts as a good basis upon which to compensate for losses. However, professional property appraisers report that removal of a citrus tree (regardless of its health) has no influence on the value of the affected property. At damage trials in the county courts of southeast Florida, tree values for compensation were calculated by evaluating each tree by its height and condition, then searching nursery inventories for large containerized citrus to serve as replacements. There was no formal discount factor for future tree health or fitness in any individual tree evaluation by plaintiffs. Payments to residents who have lost trees to the eradication program should be characterized such that they are an incentive to encourage cooperation with program objectives, not compensation for plant material removed. Diseased and exposed plant material has no economic value, and threatens all other host material in the vicinity. Eradication can move into a containment program where the program retains significant public value even though the original goal of eradication may no longer be achievable.

18.3.4 Impact of Canker

Canker reduces yield, degrades fresh-fruit quality and causes cosmetic flaws that seriously reduce the value for fresh fruit. Typically, the value of fresh fruit is 7–40 times greater than processed fruit. Production costs are higher for fresh fruit due to higher appearance standards. The grapefruit-growing portion of the citrus industry is shrinking because grapefruit is more susceptible than other commercial citrus. Historically, most grapefruit has gone into the fresh fruit market where peel blemishes are a serious detriment; about 95 % of oranges are processed. This is a major reason for the decline in citrus acreage in Florida. Further, the extreme susceptibility of grapefruit makes it difficult to contain spillover inoculum onto less susceptible citrus growing nearby. To the nurseryman, canker can cause severe bud failure in liners.

Canker can cause significant yield reduction, but considerable economic impact is due to the loss of markets because of a perceived risk of transmitting the disease onto fresh fruit. The concern over transmitting canker by this route essentially can be eliminated by practicing a few relatively easy and affordable steps (Gottwald et al. 2009). Regulations pertaining to fresh fruit movement in commerce began with the presumption that fresh fruit should not be permitted to enter trade channels into areas free of canker for fear of spreading the disease. Fruit are distributed for consumption, not propagation; this is the first consideration in the risk assessment process. Further, complete elimination of pathogen inoculum is not biologically necessary to prevent new disease initiation by natural means. An inoculum dose of 100–1,000 cfu/ml (Goto 1962) is required to cause infection, even with a fresh wound present to aid the penetration process. Grading fruit to reduce canker-blemished fruit is the first step. Another level of protection is provided by surface sanitising fruit. After treatment, fruit is coated with an approved wax and exposed to a hot air drying process at 58°C for 60–150 s to dry the wax; these temperatures are lethal to exposed bacteria.

Based on transmission reports from Bonn et al. (2010) and Schubert and Bonn (2010) the USDA has lifted prohibitions against fresh fruit from canker endemic production areas effective October 2010.

The costs of canker eradication ultimately were passed to the taxpayer and citrus consumer. The costs to the commercial grower of allowing canker to become endemic in Florida were calculated to be $107–181 M per year for early, mid-season and Valencia processed oranges and fresh grapefruit (Muraro et al. 2001; Florida Agricultural Statistics 2002). Estimates of yearly commercial grower costs of abandoning the eradication program came to $342 M per year when all citrus were considered (Keck 2001). For comparison, during 1997–2003 the costs of conducting the eradication campaign ranged from $8 to $80 M per year, with costs split between Florida and USDA.

http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/cs/2000-01/cs0001.pdf.

18.3.5 Conclusions

Simberloff (2003) identifies five essential features for a successful eradication program: (1) Resources and commitment must be adequate; (2) clear lines of authority must be established; (3) biology of the pest must be well-characterized and appropriate for eradication; (4) the pest must be detectable at low levels; and (5) subsequent intensive management of the system (such as a restoration plan) may be required. The Florida eradication plan for citrus canker met all these requirements to the extent that eradication should have been possible. Two deficiencies contributed to its abandonment. First, although resources were adequate, they were not always timely, especially as the program grew in scope. Delays in the program as a result of prolonged litigation on issues centered on defining, identifying and eliminating the exposed population, were revisited by the courts. Some of those issues remain unresolved to this day. The delays lasted long enough for natural disease spread, aided by tropical storm events, to doom the project. Retrospective assessment of the program by Centner and Ferreira (2012) advising the garnering of public support through educational programs and the identification in advance of stopping points due to impediments are certainly helpful to a degree. However, in practice, there is still a lack of useful techniques for identifying how much public support is necessary for success, for accurately assessing the magnitude of impediments, or for predicting the timing of their possible resolution while a program is underway.

Three options exist for dealing with the incursion of an exotic pest: Eradication, containment, or no response (Sosnowski et al. 2009). The option for eradication for any future exotic pest incursion in Florida is now clouded by the latest unsuccessful canker eradication program. In many respects, the option of a decisive eradication approach to a new pest has been practically removed from the list of possible regulatory responses.

18.4 Citrus Canker in Australia

18.4.1 Citrus Canker Eradication in Emerald, Australia

Citrus canker is exotic to Australia and its detection in an area may result in a loss of domestic and export markets, loss of production, and the destruction of infested trees and fruit. The first major infestation of citrus canker in a large citrus production region in Australia was reported in June 2004 on navel orange trees in a citrus orchard at Emerald, Queensland (QLD). The Emerald citrus production region is relatively isolated and approximately 500 km from the nearest major citrus production area to the south. Emerald produced 8.9 % of QLD citrus production in 2004/5, with mandarins the principal variety and 35 % exported. QLD production constituted 14 % of Australian citrus production (Australian Citrus Growers Annual Report 2005).

18.4.2 Australia's Exotic Plant Pest Response Framework

Australia has in place an *Emergency Plant Pest Response Deed* (Deed) to ensure timely and effective responses to emergency (exotic) plant pests that could adversely impact on Australia's plant industries. Although the Deed had not been ratified at the time of the canker detection, an in-principle agreement was made to manage the response within the general guidelines of the Deed, with the exception of provision of owner reimbursement (compensation). The response to the detection of citrus canker was modelled on PLANTPLAN, the Australian Emergency Plant Pest Response Plan (http://www.planthealthaustralia.com.au). In accordance with PLANTPLAN, a national Consultative Committee for Emergency Plant Pests (CCEPP) and a National Management Group (NMG) were established to provide the lead response agency, Queensland Department of Primary Industries and Fisheries (DPI&F), with a strategic decision-making and consultative framework in order to appropriately respond to the pest detection. CCEPP established a national Scientific Advisory Panel (SAP) of technical experts to support the decision-making framework and advise the CCEPP on response, control and eradication options.

The Draft Contingency Plan for Citrus Canker (2004) provided the initial course of action for control and eradication of canker until such time as a response plan specific to the incursion was endorsed by the CCEPP and NMG.

18.4.3 Cost Benefit Analysis for Eradication Attempt

On the basis of a cost/benefit analysis it was determined by the stakeholders involved that the benefits associated with eradication substantially exceeded the costs, on the assumptions that canker was not present outside the quarantine area and that eradication would prevent its spread to other regions of Australia. Assuming 100 % of Emerald's citrus orchards were destroyed, the net benefit of successful eradication was estimated at A$100.5 million in net present value. The estimated cost associated with eradication included the direct costs of government eradication and surveillance programs and the lost value of production resulting from the destruction of orchards assuming replanting with citrus after 12 months. The cost of destroying orchards was assumed to be A$4,000/ha and surveillance costs were assumed to be A$500/ha. If 50 % of orchards were destroyed and ongoing surveillance was conducted over 5 years, then direct costs would be around A$3 million and the net benefit of successful eradication was estimated at A$104.5 million (Beare et al. 2005).

18.4.4 Determination of Areas at Risk

In June 2004, on suspicion that the disease was present, a property (IP-1) was quarantined. Within a week of the reporting of suspect symptoms, the initial diagnosis of citrus canker was confirmed by two additional independent labs as *Xanthomonas citri* subsp. *citri* (Xcc), the Asiatic strain of citrus canker. The confirmation allowed broader containment and control measures to be established with the declaration of a Pest Quarantine Area (PQA) in July 2004 that encompassed three shires within 50 km of the property (Fig. 18.3).

During July 2004 officials decided that Queensland would be split into three quarantine-risk zones for the purpose of implementing movement restrictions and carrying out delimiting surveillance to confirm pest free area (PFA) status: The Emerald Pest Quarantine Area (PQA), the Gayndah Mundubbera Management Zone (GMMZ) and the Rest of Queensland Zone (RoQ). These zones were

Fig. 18.3 A key tool used to engage the general community in understanding the requirement to not transport citrus was the use of large roadside signs (Image courtesy of the Queensland Department of Agriculture, Fisheries and Forestry)

established in recognition of different risk profiles that applied to each area. The GMMZ comprised the area where most of QLD's citrus production occurs, and included commercial citrus production nurseries. The GMMZ was recognised as a higher-risk zone to the RoQ because tracing investigations identified movements of citrus propagation material from a number of commercial citrus properties in the PQA in previous years to citrus nurseries that distributed plants to 42 properties in this zone. Later inspection of those nurseries did not find evidence of citrus canker, somewhat mitigating the risk of further spread through that pathway. Tracing investigations did not identify any movement of host plant material or other potential pathways of canker between properties in the PQA and the RoQ.

A zoning system was established to deal with the risks associated with the movement and spread of canker within and outside the PQA and comprised the following five zones:

1. **Destruction Zone** – 600 m radius around confirmed detection of canker. All host plants destroyed. Restrictions on movement of equipment. Intensive surveillance of re-growth.
2. **Quarantine Zone** –3.2 km radius around a Destruction Zone. Movement of all host plants and fruit prohibited. Restrictions on movement of equipment. Intensive surveillance of all host plants.
3. **Buffer Zone** – 3.2 km radius around a Quarantine Zone. Movement of all host plants prohibited. Restrictions on movement of fruit and equipment. Intensive surveillance of all commercial citrus and a proportion of residential and native citrus.

Fig. 18.4 Surveillance team prepares to inspect orchard for citrus canker in Gayndah, Queensland as part of delineation surveys to confirm absence of citrus canker outside of the PQA (Image courtesy of the Queensland Department of Agriculture, Fisheries and Forestry)

4. **Restricted Zone** – Remainder of PQA. Movement of all host plants prohibited. Restrictions on movement of fruit and equipment.
5. **Control Zone** – Areas of Queensland outside the PQA (the non-quarantined area comprising the GMMZ and RoQ). Restrictions applied to movement of host plants deemed 'natural hosts' of canker including their fruit. Surveillance of all properties linked to the PQA through movement of propagation material, all citrus production nurseries, and a proportion of commercial citrus properties, residential and native citrus (Fig. 18.4).

In addition to the movement restrictions placed on host plants, fruit, equipment etc., the *Plant Protection (Canker) Quarantine Notice 2004* required landowners within the PQA to (a) immediately spray host plants within the Destruction Zone with a copper based chemical and burn them, (b) immediately treat any citrus re-growth within a Destruction Zone (c) treat all hosts within 500 m of the Destruction Zone with a copper based spray fortnightly and (d) not replant a host plant without an inspector's approval.

The decision to destroy host plants within 600 m of an infected plant was based on the "1,900-ft rule" (≈ 600 m) put into practice during late 1999 in Florida. Gottwald et al. (2001) and Gottwald et al. (2002) had found that 579 m represents a common distance of citrus canker spread during a 30-day period.

Treatment with copper was initially imposed in the PQA to prevent spread until infected trees were destroyed, but was later removed as a requirement as the copper deposit hindered symptom observation on trees within 500 m of the Destruction Zone.

Additional provisions in July 2004 gave the authorities responsibility for treatment and destruction of plants within the Destruction Zone and applied additional restrictions on the movement of hosts within all areas of QLD outside the PQA. In August 2004, the PQA was decreased to a smaller area encompassing all commercial citrus properties in the Emerald district, the Emerald town and a buffer around those sites of a least 10 km. The revised PQA covered an area of 3,146 km^2, which remained in place until eradication was declared for canker in February 2009.

18.4.5 Delimiting Spread of the Disease

To establish the extent of the infestation, delimiting surveillance was initially carried out in July 2004 in the PQA at the rate of 600 trees per 10 ha block (achieving 95 % confidence of detecting 1 % disease prevalence, assuming surveillance sensitivity of 50 %). A ‘survey block’ was defined as: “A group of trees managed contiguously, of the same variety of citrus, and may have some minor or artificial barriers with the area”. Surveys commenced in blocks where canker infection had been confirmed, moving outward from the confirmed detection sites until all areas of the property were surveyed. Those areas that remained outside the 600 m Destruction Zones were subsequently resurveyed at a higher level of intensity.

By the end of August 2004, infestation had been confirmed on greater than 80 % of the production blocks of IP-1 and all host plants on the property had been encapsulated within a 600 m destruction zone. Prior to destruction only a small number (about 2 %) of the trees on the property were inspected.

A limited epidemiological study, directed at determining the age and source of infection on the property, was conducted on three infected blocks within the property. The study indicated that the disease had been present on the property before 2004 and possibly as early as 2002. The disease was also detected in the nursery on the property (Gambley et al. 2009). Investigations into practices employed on the property indicated the potential for spraying, fruit picking, hedging and topping to spread the disease further. Splash dispersal results in inoculum dispersal within individual trees and among trees in close proximity to one another (Serizawa et al. 1969).

Delimiting surveillance was also carried out in the urban areas of Emerald and on selected rural properties, primarily targeting residential properties that were linked to infected properties through the movement of personnel.

Botanists identified all species of rutaceous plants that occurred in the PQA. A list of hosts identified that the desert lime (*Citrus glauca* (Lindl.) Burkill), native to the Emerald area, is a host of citrus canker. Peltier & Frederich (1920) had reported that desert lime (cited as *Eremocitrus glauca*) was susceptible under field conditions in Alabama. Subsequently desert lime and other potential hosts growing in the Emerald area were evaluated for their susceptibility to the Emerald isolate of *Xcc* (Hailstones et al. 2005).

Restriction on movement. On 7 July, a complete prohibition on the trade of host plants and citrus fruit from QLD was imposed by all Australian states and territories. To facilitate trade while delimiting surveillance was completed and pest free area (PFA) status confirmed for each quarantine risk zone, protocols were negotiated with stakeholders for intra- and interstate movement of citrus material from outside the PQA. On 19 July 2004, all states and territories agreed to lift movement restrictions on rutaceous plants grown outside the PQA with the exception of those genera/species classified as 'natural hosts' of citrus canker. These hosts were permitted movement within and out of the non-quarantined area under agreed conditions including property freedom, treatment, inspection and certification. Through an amendment to the *Plant Protection (Canker) Quarantine Notice 2004*, QLD implemented restrictions on the movement of host plants and host fruit in the non-quarantined area of the state. Restrictions on host fruit included treatment, inspection and certification under the supervision of an inspector or property freedom certification, prior to movement within QLD.

On 21 July 2004, citrus fruit grown in the PQA outside a 6.4 km zone around a confirmed detection of canker was allowed to move to the Port of Brisbane for export to some international markets. Movement occurred under an agreed protocol of property freedom, treatment with SOPP or chlorine, inspection, certification, secure transport via an approved route to an accredited freight handler, secure storage and direct export to a market not sensitive to canker. These conditions were maintained in 2005 except that the 6.4 km exclusion zone was dropped. Organically grown fruit was subject to the same protocol using a mixture of peroxyacetic acid and hydrogen peroxide in lieu of SOPP or chlorine.

On 23 July 2004, following completion of initial delimiting surveys on all 45 linked properties and in other production areas of QLD, all states and territories, except South Australia (SA), agreed to lift their prohibitions on access of citrus fruit grown in the GMMZ and RoQ, subject to consignments being inspected, treated and certified in accordance with agreed protocols. SA maintained a prohibition on the movement of citrus fruit from QLD into the citrus growing Riverland district until February 2006.

Surveys confirmed PFA status for the RoQ which was declared in December 2004 and all restrictions on citrus movement (except to SA) were lifted by mid-January 2005. In the GMMZ, a second survey (6 months after the first survey) was conducted on all citrus blocks that had received budwood or trees with traceability to the PQA. No evidence of canker resulted in PFA status being confirmed for the GMMZ in February 2005. QLD lifted its restrictions on movement in February 2005 and all other states, except SA, removed restrictions on citrus movement by July 2005.

All Australian citrus growers were required to undergo stricter quarantine checks before exporting fruit to New Zealand pending quarantine surveys to establish state, area or property freedom from citrus canker. While the European Union stopped importing citrus from Australia until PFA status was established for the entire country, export to other markets, particularly canker infected markets in Asia, did not encounter restriction.

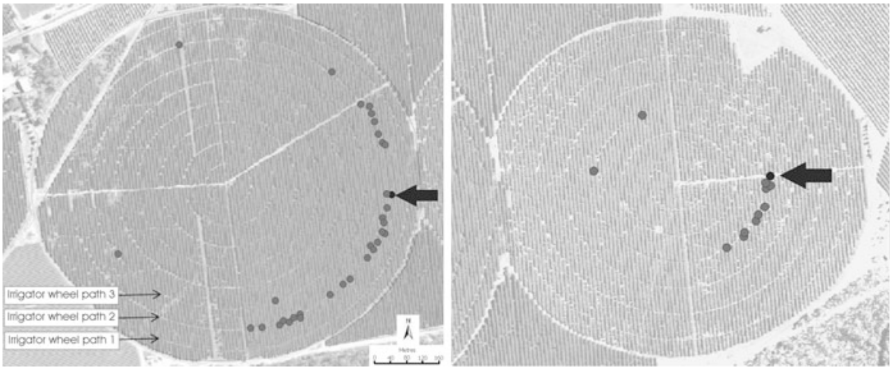


Fig. 18.5 Distribution of canker-infected trees along the pivot irrigator's tracks in blocks 7 and 8 on IP-2 (Taken from Gambley et al. 2009. Courtesy of Queensland Department of Agriculture, Fisheries and Forestry)

Compliance monitoring activities directed at preventing the introduction and movement of host plants in the Emerald PQA resulted in the detection, seizure and destruction of approximately 200 kg of fruit and two host plants that had been moved into the PQA in contravention of the Act.

Detection of citrus canker on a second commercial orchard. In October 2004, about 3 months after completion of delimiting surveys for the detection of canker on IP-1, canker was confirmed in a pivot-irrigated citrus block (watered every 48 h) on a second commercial (IP-2) orchard in the PQA. The orchard was located about 9 km to the north of the IP-1. The detection, by the orchard owner, occurred about 1 month after the removal of the last citrus tree from IP-1, in September 2004.

In the absence of clear evidence of human assisted spread, transfer of inoculum by a significant weather event early in 2004 (autumn) was considered as a possible source of introduction (Gambley et al. 2009) based on the research published by Dalla Pria et al. (2006), Gottwald et al. (2002), Serizawa et al. (1969) and Graham et al. (1992a, b).

Debate arose on whether drainage from IP-1 and downstream uptake into the unfiltered pivot irrigation system could have occurred. Doidge (1918) had reported that floods in South Africa carried the disease from an infected orchard to a farm downstream where partially submerged trees became infected.

The surveillance strategy employed in the pivot blocks was tree-by-tree surveys along the pivot-irrigator's traveller tracks (Fig. 18.5). On each pivot-irrigated block, single trees were identified as the disease foci and statistical analyses supported the conclusion that disease was spread mechanically from these points by the pivot irrigator machinery (Gambley et al. 2009).

The distances and directional nature of intra-block disease spread within some blocks on IP-2 suggested infection could be attributed to wind-driven rain. In contrast, disease spread on another IP-2 block was mostly along the rows, predominantly to one section of the canopy and relatively evenly distributed on both sides of the rows. This, in combination with the protrusion of tree canopies into the inter-row spaces, suggests that disease transfer was probably from equipment moving along the rows (Gambley et al. 2009).

During April 2005, QLD recommended that domestic market access for Emerald citrus fruit should be restored, on the condition of certification of property freedom, inspection and approved fruit treatment and the continuance of the National Citrus Canker Eradication Program. This recommendation was not supported by other Australian governments.

After the detection of citrus canker at IP-2, more intensive surveillance was undertaken in December 2004 to provide a higher degree of confidence of disease detection at lower pest prevalence within survey areas. Due to the large number of citrus trees still present in the PQA and the time it would take to inspect each tree, the approach was to conduct statistically based sampling to identify areas where the disease was clearly present, and remove all trees within 600 m of the infestation. More intensive surveillance was then undertaken on the smaller number of remaining trees.

This approach mirrored the approach taken on IP-1. All trees outside a 600 m destruction zone in a block that fell within a 1,200 m radius of a confirmed detection of canker were surveyed. Elsewhere the sub-area determined for on-going delimiting surveillance was revised to an intensity of 600 trees to be inspected per 5 ha sub-area, i.e. at least 1 in every 5 trees was inspected. Inspection of trees for canker by surveillance staff typically involved, one person on either side for large trees (>2 m high), whereas smaller trees (<2 m high) were inspected on all sides by a single person. The sensitivity of detection of canker was calculated to be 42 % on large trees, 55 % on medium to large trees (1.5–2 m in height) and 75 % on small trees for a person trained and competent in detecting citrus canker. No aerial platforms were used, as utilised in Brazil for citrus canker and HLB detection.

Delimiting surveys following the IP-2 detection were completed on all commercial and non-commercial citrus in the PQA during October 2004 to April 2005. Four other citrus blocks on IP-2 were found to be infested with canker during these surveys. Based on the spread of the disease within the property, it was agreed that the small number of remaining host plants not encompassed within a destruction zone on IP-2 be deemed to be infected and destroyed.

Detection of citrus canker on a third commercial citrus orchard. A third round of more intensive surveillance (100 % inspection) commenced on the remaining commercial citrus properties in the PQA in May 2005 and a third property was found infected (IP-3). All four disease establishment points on IP-2 and IP-3 appear to have developed during autumn 2004 and were all 9–11 km north to north-west from IP-1 suggesting one mechanism of dispersal (Gambley et al. 2009).

A decision for the area-wide destruction of host plants. In June 2005, officials agreed that all commercial and non-commercial citrus within the PQA, and all native citrus within 600 m of the commercial citrus orchards and the Emerald town be destroyed. Officials also agreed that further delimiting surveillance cease on commercial citrus concentrating future surveillance efforts on non-commercial host plants and native citrus in the PQA (Fig. 18.6).

This was not the first time this approach had been considered. After the first disease detection on IP-2 in early October 2004, the owner of that property put forward a proposal to destroy all commercial citrus in the PQA. The core of this

Fig. 18.6 Removal of backyard citrus trees as part of the Emerald citrus canker eradication. Trees were cut down to ground level and stumps treated with herbicide to prevent regrowth (Image courtesy of Queensland Department of Agriculture, Fisheries and Forestry)

proposal was for the Government to pay the grower A$16 m (A$50 payment for destruction of each tree) to destroy all remaining commercial citrus trees in the PQA. The citrus industry considered that this would reduce canker spread and then allow the area to be re-planted after 2 years. The rationale behind the industry plan was that if the current eradication program failed to eliminate the disease completely from the region, on-going outbreaks would lock growers out of markets for long periods of time. If implemented, the pre-emptive destruction proposal would provide growers with certainty about orchard re-establishment and market access. It was proposed that after the destruction, the growers would fallow the land for the next 2 years, replant and return to full production in 5 to 6 years. This proposal received local and national citrus industry support, but was rejected by government authorities because the proposal was made prior to the completion of delimiting surveillance throughout QLD, could not be supported on a legislative basis, did not cover the destruction of non-commercial or native hosts in the PQA, or on-going surveillance activities to delimit the outbreak to support a subsequent declaration of eradication and re-instatement of PFA status.

A second round of delimiting surveys was conducted on all non-commercial citrus in the PQA prior to its destruction. One round of delimiting surveys was conducted on all native citrus within an 1,800 m radius of the destroyed citrus blocks on IP-1, 1,200 m radius of the destroyed citrus blocks on IP-2, 600 m radius of the remaining citrus orchards, and 600 m radius of non-commercial citrus located within the Emerald township. About 350,000 native citrus were identified and inspected during this round with no citrus canker detected.

In July 2005, the *Plant Protection Regulation 2002* was amended to give provision to inspectors to destroy all relevant host plants of canker in the PQA (*Plant Protection Amendment Regulation No. 4 2004*). Prior to this, destruction was directed only at host plants deemed infected under the Act. The revised legislation also made it an offence to possess or plant a host of canker in the PQA without an inspector's approval. Destruction was directed towards five categories of host plants: Commercial citrus, non-commercial citrus and other non-commercial host plants, native host plants within areas buffering high-risk areas within the PQA, regrowth of destroyed host plants and host plant material found entering the PQA in contravention of legislative requirements.

On-site destruction of all commercial citrus was completed on IP-1 in September 2004, IP-2 in early August 2005 and IP-3 in late August 2005. Citrus trees on the remaining commercial citrus orchards were destroyed prior to October 2005. Inspection, removal and destruction of 4,235 non-commercial host plants in the PQA were completed by March 2006. Symptoms of canker were not detected on any non-commercial host plant prior to its destruction.

Between May and November 2005, 346,734 native desert lime plants located within an 1,800 m radius of IP-1, within a 1,200 m of IP-2, and within 600 m of IP-3 and the remaining commercial orchards and the Emerald township were inspected for possible disease symptoms. No canker was found on desert lime anywhere in the PQA. Regardless, spot spraying of herbicide was used to destroy native desert lime plants within a radius of 1,200 m from IP-1 and within 600 m of IP-2 and IP-3. Approximately 95 % were small, spiny plants, less than 40 cm in height growing as suckers from the root network within each clump. Destruction of an additional 30,000 native citrus plants identified within the lower-risk areas of 600 m radius around the remaining four non-infested commercial citrus properties was also completed. Further, between July 2006 and June 2007, areas surrounding IP-1 were burned to remove any residual desert lime plants that may have survived the herbicide treatment. The extensive surveys undertaken on native citrus and non-commercial citrus in the PQA were deemed to be sufficient to justify not requiring destruction of native citrus plants within 600 m of the Emerald Township.

Eighteen month host-free period. During the host-free period (January 2007–June 2008), inspections continued on IP-1, IP-2 and IP-3 and on all properties where non-commercial host plants were destroyed, for re-growth of host plants at 90-day intervals. Prior to the host-free period, inspection for re-growth of a destroyed host plant continued on all properties until September 2007, and then was directed at any previously infected commercial property and any residential property that had not achieved three consecutive clear surveys with no regrowth detected. The last detection of re-growth on a commercial property was in March 2008 and the last detection of re-growth on a non-commercial property was during June 2008. A total of 10,715 re-growth plants were detected in the PQA. Of these 5,065 re-growth plants were detected and destroyed prior to the host-free period with the remaining 5,650 plants detected after the commencement of the host-free period.

Continued inspections of all native citrus plants located in lower risk areas outside of high-risk destruction zones were undertaken from January 2006 –

September 2007. Each plant located within 600 m from the boundary of a native citrus destruction zone around the IP-1 and IP-2 was inspected for anomalous marks or lesions. A total of 499,550 inspections were conducted at 90-day intervals during this time, providing evidence of disease absence in those areas.

Eighteen month pest-free verification period. Following the 18-month host-free period, commercial citrus trees were replanted in the PQA with an Inspector's approval. This required inspection for symptoms of canker within 90 days prior to their movement into the PQA. All trees replanted in the PQA were inspected every 90 days over an 18-month surveillance period to provide evidence of disease absence.

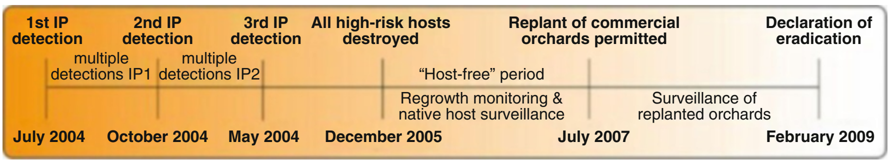
Evidence from other countries has shown that the canker bacterium can only survive in soil at population levels sufficient for infection for a few weeks (Goto 1992; Leite and Mohan 1984; Graham et al. 1989). *Xcc* can survive on several grasses for at least several months while the longevity and level of epiphytic survival of *Xcc* associated with non-citrus plants may depend on the type of plant (Goto 1992).

18.4.6 Declaration of Eradication

No citrus canker was detected on the replanted citrus during the 18-month surveillance period and eradication was declared on 23 January 2009.

The cost of the eradication program, nationally cost-shared between state and federal governments, was A$17.8 million, which included all surveillance, destruction and compliance costs. An additional A$9 million was paid *ex gratia* by the Australian and QLD Governments to assist affected growers. Alam and Rolfe (2006) estimated that the annual loss of revenue to growers if all citrus trees were removed from Emerald would be $31.95 m/year. Approximately 495,000 commercial citrus trees planted over 1,100 ha were destroyed, along with 4,235 citrus trees on 1,283 residential properties. About 150,000 native desert lime plants were also destroyed. The economic benefits of averting a national outbreak of citrus canker, was estimated by Alam and Rolfe (2006) to be A$410 million.

In summary, eradication is a worthy choice compared to living with citrus canker, providing the disease is identified early and legislative provisions and resources are sufficient to allow action to be taken swiftly and decisively. The following timeline summarises the chronology of events:



18.4.7 Citrus Fruit as a Pathway for the Introduction of Citrus Canker

As discussed above, one of the most serious biosecurity threats to citrus around the globe is canker caused by *Xcc*. Peltier and Frederick (1926) stated that citrus canker can develop in all citrus regions of the world sometime in the growing season. Rising temperatures and increased rainfall provide conditions that stimulate rapid host growth and increase susceptibility favouring canker development. The disease is most severe at sites with the greatest number of months with mean temperatures of 27 °C or above. Dalla Pria et al. (2006) found that the most severe canker occurred at 24 h of leaf wetness, with 4 h of wetness being the minimum duration sufficient to cause 100 % incidence at optimal temperatures of 25–35 °C. Borchert et al. (2007) agreed that *Xcc* could become established wherever citrus is grown, but the potential disease intensity would be affected by the frequency of spread events, temperatures, timing of precipitation, host susceptibility and the occurrence of citrus leafminer (Hall et al. 2010).

The main pathway for the long distance dissemination of *Xcc* is on nursery plants or budwood. Concerns remain on whether *Xcc* on commercially traded fruit has the capacity to survive post-harvest treatments and be transmitted to susceptible host material. The USDA has published an updated pest risk assessment (USDA 2009a) of fruit as a pathway for the introduction of citrus canker and a supplemental risk management analysis (USDA 2009b). In those assessments, which were based on Gottwald et al. (2009); and Shiotani et al. (2009), USDA concluded that commercially packed, disinfected fruit is not an epidemiologically significant pathway for transmitting and establishing citrus canker.

The European Commission Scientific Panel on Plant Health concluded that the movement of citrus fruit, particularly latently infected fruit and fruit that shows no external symptoms at harvest, does constitute a pathway for entry of *Xcc* into a canker-free area (EFSA 2006). EU countries are free of citrus canker and according to EU legislation (Anonymous 2000), citrus fruit from countries with canker must be disinfected. In 2011, the Panel on Plant Health delivered a scientific opinion on risk analysis documents provided by USDA in support of the request for the withdrawal of the current EU requirement that citrus fruit imported into the EU be sourced from groves where no symptoms of citrus canker have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation. The Panel concluded that such a change would increase the probability of introduction of *Xcc* into new areas (EFSA 2011).

Viable *Xcc* has been isolated from lesions observed on fresh fruits commercially traded internationally with phytosanitary certification of postharvest bacteriocide treatments (Golmohammadi et al. 2007; Scuderi et al. 2010; Al-Saleh and Ibrahim 2010; Bonn et al. 2010). Studies of post-harvest treatments in removing *Xcc* populations have had variable results with the efficacy of disinfectant treatments dependent on several factors including pH, disinfectant concentration, presence of organic matter, and frequency of renewal of the disinfectant solution (Verdier et al. 2008; Gottwald et al. 2009; Canteros et al. 2000; Stapleton 1986; Dychdala

1983; Brown and Schubert 1987; Al-Saleh and Ibrahim 2010; Schubert et al. 2000). Culling fruits in the packing line may not eliminate all infected fruit because invasion through wounds and multiplication of *Xcc* may be independent of the development of external evidences of canker (Fulton and Bowman 1929), symptoms of late infections of fruits are not always typical (Koizumi 1972), the incubation period of mature fruit is too long for symptoms to occur before harvest (Graham et al. 1992b, 2010) and *Xcc* is buried in a thick matrix of extracellular polysaccharides (Goto 1992; Cubero et al. 2011) or biofilm (Rigano et al. 2007).

The Opinion of the Scientific Panel on Plant Health (EFSA 2006) states *"Even if the fraction of infected fruit shipped to a suitable habitat is small, the inoculum level may be epidemiologically significant"*. Graham et al. (2008) indicated that >5 % infected fruit in a block is an unacceptable risk for export to the EU market and Ritenour et al. (2008) itemized a number of issues essential for shipping fresh fruit in the presence of citrus canker. Despite claims that the risk of spread and establishment is low, Gottwald et al. (2009) provided evidence of the potential for spread of *Xcc* from infected fruit to healthy material, when a plant became infected downwind from a cull pile of non-packing line processed fruit, and *Xcc* was recovered from splash from suspended infected fruit. By contrast, Schubert and Bonn (2010) failed to transmit *Xcc* from infected commercially packed grapefruits to adjacent susceptible grapefruit saplings.

The Australian government Department of Agriculture, Fisheries and Forestry, in their comment on Docket APHIS-2009-0023 Citrus Canker: Movement of Fruit from Quarantined Areas (http://www.regulations.gov/#!docketDetail;rpp=10;po=40;D=APHIS-2009-0023) stated that "*In our view, the cull pile transmission experiments conducted by Gottwald et al. (2009) do not provide conclusive evidence that the risk of fruit-to-tree transmission is insignificant*".

From an epidemiological point of view, epidemics of *Xcc* are composed of a series of discontinuous pulses of inoculum that first introduce *Xcc* to the host population, with a combination of multiple meteorological and mechanical events that further disperse inoculum and exacerbate the epidemic. However, dispersal events vary greatly in distance and quantity of inoculum dispersed (Irey et al. 2006). Smith et al. (1997) state that there is no authenticated record for diseased fruit playing a role in the epidemiology of citrus canker disease. Until there is substantial scientific evidence that *Xcc* on fruit after post-harvest treatment can establish an infection, the risks in importing fruit from countries with canker will remain controversial. *Xcc* remains a quarantinable pest for many countries.

18.5 Citrus Black Spot

18.5.1 Causal Organism and Its Identification

Citrus black spot (CBS) is a fruit and foliar disease of citrus. The latent or endophytic nature of the pathogen causing black spot was recognized by Cobb (1897) and the asexual form of the fungus was described by McAlpine (1899) as *Phoma citricarpa* McAlpine from symptomatic citrus fruit in Australia. *Phyllosticta citricarpa* (McAlpine) van der Aa is currently the accepted name of the anamorph (Van der Aa 1973; Van der Aa and Vanev 2002). The teleomorph was described by Kiely (1948b) as *Guignardia citricarpa* Kiely from citrus leaf litter in Australia. The spermatial state or synanamorph is a *Leptodothiorella* and the species has not been described (Van der Aa 1973; Baayen et al. 2002).

Considerable confusion existed about the geographic distribution, the endophytic nature and host range of *G. citricarpa* (Kotzé 1981; Kiely 1948b). The confusion was thought to have been resolved by the identification of two morphologically similar (McOnie 1964a, c), but genetically distinct species (Meyer et al. 2001; Baayen et al. 2002, Everett and Rees-George 2006). One species (*G. citricarpa*) caused CBS on citrus and the other, (*G. mangiferae* A. J. Roy [anamorph *Phyllosticta capitalensis*]), was a cosmopolitan species associated with a wide range of hosts, but not causing typical CBS symptoms. However, recent studies (Glienke et al. 2011; Wang et al. 2011) support the conclusion that the teleomorph of *P. capitalensis*, which is the common endophytic species in *citrus*, is not *G. mangiferae*. Both *Phyllosticta* spp. may simultaneously colonize the same citrus tissue, being either symptomatic or symptomless on citrus leaves, twigs or fruit (McOnie 1964a, c; Baayen et al. 2002; Bonants et al. 2003) and have been reported to co-exist in a single black spot lesion (Baldassari et al. 2008). Wulandari et al. (2009) described a new species, *Phyllosticta citriasiana* Wulandari, Crous & Gruyter, in association with "citrus tan spot", only on pomelo fruit from Asia. *Phyllosticta citrichinaensis* X.H. Wang, K.D. Hyde & H.Y. Li, associated with minor irregular spots, was isolated from leaves and fruit of four *citrus* spp. in China (Wang et al. 2011). Further studies are required to establish host ranges, geographic distribution, disease status and biosecurity significance. Using 2009 OEPP/EPPO diagnostic PCR protocols, *P. citricarpa* could not be distinguished from *P. citriasiana* or *P. citrichinaensis* (Wang et al. 2011). *Phyllosticta citribraziliensis* is newly described as an endophytic species occurring on citrus in Brazil (Glienke et al. 2011).

The host range of *G. citricarpa* is limited to *citrus* spp. (Meyer et al. 2001; Baayen et al. 2002). Lemons are especially susceptible, but all commercially grown *citrus* spp. are susceptible with the exception of sour orange, its hybrids and rough lemon (Wager 1952; Kotzé 1981; Baldassari et al. 2008). Although Tahiti lime remains symptomless, both *G. citricarpa* and *G. mangiferae* can co-exist on this host in leaves and fruit and both are capable of producing viable ascospores on decomposing Tahiti lime leaves in Brazil (Baldassari et al. 2008). When CBS is first found in a citrus producing area, it is usually observed on lemons before other citrus types are affected (Kiely 1948b). In South Africa, studies show that CBS took 5–30 years to reach epidemic proportions (Kotzé 1981).

CBS originated in South East Asia (Smith et al. 1997), but symptoms were first described from infected sweet orange fruit originating from coastal areas of NSW, Australia (Benson 1895). The first record of CBS in South Africa was in 1929 from areas around Pietermaritzburg (Doidge 1929). CBS is restricted globally to regions that experience summer rainfall and has to date failed to establish in any winter-rainfall region (Baayen et al. 2002; Paul et al. 2005). Consequently certain major citrus producing regions in South Africa and Australia with hot summers and winter rainfall remain CBS-free. CBS has not been recorded in citrus producing regions in Europe, Central America, the Caribbean region, Chile, Japan, New Zealand and most of USA (European Union 2000; Baayen et al. 2002; Paul et al. 2005). Recently it has been detected in Florida, USA (Schubert et al. 2010, 2012).

The pathway for introduction and initial establishment of CBS in Australia was not reported (Benson 1895), but in South Africa it is strongly suspected that the distribution of nursery trees carrying latent infections were the source for spread from 1930 (Wager 1952). Infected twigs can be a source of inoculum (Kiely 1948b; McOnie 1964b; Whiteside 1967) and latent infections of the fungus can be transmitted to a healthy plant through infected grafts (Sueda 1941; Schuepp 1961). Marchionatto (1928) described the spread of CBS on lemon budwood imported to Argentina from Australia.

18.5.2 Phytosanitary Regulations

Delimiting the distribution of a pest is a pre-requisite for implementation of domestic regulations aimed at preventing the spread of a disease and maintaining the pest-free status of production areas, which in turn facilitates export when importing countries impose restrictions on imports from regions where CBS occurs (IPPC 2006). The distribution of CBS within South Africa and Australia has been established through extensive field surveying over many years, making it possible to officially recognize parts of the country as CBS pest-free areas (le Roux et al. 2007; Carstens et al. 2012; Broadbent 1995).

There are no restrictions on the movement of citrus fruit within South Africa, including the movement of fruit from parts of the country where CBS occurs to CBS-free parts of the country. The absence of domestic regulations restricting the movement of citrus fruit reflects the regulatory view that citrus fruit trade does not constitute a pathway for spread of the disease. In contrast to fruit movement, citrus propagation material may not be moved within South Africa from parts of the country where CBS occurs to CBS-free areas and the area of low pest prevalence. Notably, no restrictions existed between the time of first recording CBS in South Africa in 1929 and 1983. CBS has failed to become established in certain parts of South Africa, supporting the contention that climate is an important constraint to the distribution of the organism (Paul et al. 2005).

In Australia, the movement of citrus fruit and propagation material is not specifically restricted on the basis of CBS (Plant Health Australia 2009). Commercial fruit entering South Australia must be asymptomatic for CBS, while non-commercial fruit is banned. Despite the absence of official domestic control of CBS within Australia, the distribution of the organism has remained restricted to some Australian production areas, whereas others have remained CBS-free.

The discovery of *G. citricarpa* in parts of Florida, USA in 2010 (Schubert et al. 2012), gave rise to issuance of an Emergency Action Notification and the subsequent Domestic Quarantine Order DA-2911-03 (APHIS 2010). Portions of two counties in Florida were designated as quarantine and regulated areas. As a condition for interstate movement of citrus fruit from these areas, the fruit must be packed in commercial packinghouses operating under compliance agreements with APHIS. Packinghouse procedures include washing, disinfestation, treatment and waxing. Furthermore fruit must be free of leaves or other vegetative material and covered by an official permit. Movement to a processing facility within an affected State requires the vehicles to be suitably enclosed, with suitable cleaning of the vehicle and equipment after delivery. The processor receiving such fruit must operate under an APHIS compliance agreement and debris must be disinfected. However, given the recent nature of the first discovery of CBS in Florida, and reports that black spot becomes established in orchards years before symptoms appear on fruit (Kiely 1949), it is unlikely that these initial regulations will persist in their present form. Furthermore, reports suggest that APHIS has developed a draft pest risk assessment, which concludes that fruit is not a pathway for CBS to spread to new areas (Ritenour and Dewdney 2010). In the event that this finding is upheld in a finalized PRA, a relaxation of these regulations can be expected.

The USA has maintained restrictive CBS import requirements for citrus fruit. Citrus fruit imported into the USA must either be from CBS-free countries or CBS-free regions within a country where CBS occurs. The establishment of such pest-free regions requires the undertaking of officially controlled surveys to demonstrate pest freedom. In addition, the USDA has conducted field visits to such areas before accepting the results of such surveys. This has enabled the establishment of federal regulation amendments to permit the import of fruit from pest free areas. Only fruit from CBS-free areas in Australia (Riverland, Sunraysia, Riverina) and South Africa (Northern Cape Province, southwestern Western Cape Province and parts of the North West and Free State Provinces) can be exported to the USA (APHIS 2010). In the case of export of citrus fruit from South Africa to USA, South Africa has furthermore been compelled to apply officially regulated protection of such areas, whereby the movement of citrus propagation material from areas where CBS occurs into such CBS-free areas is prohibited and there is continuous verification that these areas remain free of CBS.

In August 2007, APHIS published a draft PRA that evaluated the risk of lemons imported from northwest Argentina. The assessment concluded that the pest risk potential of *G. citricarpa* was medium, "based on worst-case assumptions, but all are severely limited by the low probability of introduction. Although a very low incidence of disease may enter on occasional symptomatic fruit, the evidence indicates that it is highly unlikely that disease could establish via fruit as a pathway" http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus/downloads/black_spot/DA-2010-47-FO.pdf

Japan has been less restrictive than the USA with regard to the import of citrus fruit from countries where CBS occurs. Export of citrus fruit from South Africa to Japan requires the production sites to be registered with the South African NPPO, with such registration being conditional on the producer undertaking to implement appropriate pre-harvest control measures (SA DAFF 2011). Furthermore, the export fruit requires official inspection prior to shipping to ensure that the consignments are free of CBS symptomatic fruit.

Prior to the harmonization of phytosanitary regulations across EU member countries in 1990, the import of citrus fruit into Spain, Greece and Italy was not permitted from countries outside Europe, although Italy allowed imports of grapefruit under permit. Exports of citrus from countries where CBS occurs to the rest of Europe were not subjected to phytosanitary restrictions relating to CBS. However, when the EU adopted harmonized phytosanitary regulations, exports of citrus fruit to the entire EU became subject to strict CBS restrictions.

According to EU Council Directive 2000/29/EC (European Union 2000), with regard to CBS, citrus fruit must: Either originate in a country officially recognised as being free from *G. citricarpa*; or the fruits originate in an area officially recognised as being free from *G. citricarpa*; or no symptoms of CBS have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation, and none of the fruits harvested in the field of production has shown, in appropriate official examination, symptoms of this organism; or the fruits originated in a field of production subjected to appropriate treatments against *G. citricarpa* (all strains pathogenic to citrus), and none of the fruits harvested in the field of production have shown, in appropriate official examination, symptoms of this organism.

The technical justification of the EU import regulations pertaining to citrus fruit has been contested by South Africa (EFSA 2008). In 2000, South Africa conducted a PRA on the export of fresh citrus fruit from South Africa to the EU. The PRA concluded that a combination of risk mitigating considerations made the likelihood of commercial fruit trade providing a pathway for the establishment and persistence of CBS in the EU extremely remote. Consequently, it was concluded that the EU regulations represent excessively restrictive barriers to trade and without the technical justification required in terms of IPPC principles (IPPC 2006). The European Commission contested the PRA findings, resulting in extensive subsequent exchanges between South Africa and the EU, including the execution of specific research studies to clarify pertinent aspects of the PRA (EFSA 2008). In the absence of agreement between the EU and South Africa, South Africa called upon the IPPC in 2010 to intervene and facilitate resolution and the initiation of this procedure was declared internationally at the WTO SPS meeting of October 2010 (WTO 2010).

Given that citrus propagation material is the pathway for introduction of CBS into new areas it is appropriate to regulate the movement of such material from areas where CBS occurs to pest-free areas. The cryptic nature of CBS symptoms on vegetative material increases the risk that circumventing quarantine procedures will introduce CBS to an area. Citrus seed is not a pathway for the distribution of CBS, and many countries do permit the import of commercial quantities of seed, but subject to specific risk mitigation measures.

The international movement of heavily infected fruit is unlikely to take place even in the absence of CBS-specific regulatory restrictions because of the unsightly nature of such fruit. The effectiveness of "grading out" symptomatic fruit in packinghouses can be improved by increasing the number of people at inspection points, and reducing the rate at which the fruit moves through the pack line. But these measures cannot consistently eliminate the prospect of missing the occasional symptomatic fruit. Furthermore, the occurrence of latently infected fruit before symptom expression limits the effectiveness of pre-export inspections to eliminate infected fruit from export consignments.

Presently no single treatment or handling procedure can consistently and reliably eliminate all CBS infected fruit when exporting from an area where CBS has reached epidemic levels. However, risk mitigation procedures that can reduce the incidence of CBS infected fruit include effective field treatment, field monitoring, orchard assessment and selection, packinghouse grading, post harvest handling and inspection procedures. Of these, only preventative field treatments and fruit storage at low temperatures (below 8°C) consistently reduced postharvest CBS development (Agostini et al. 2006). Strobilurins are being used for control of CBS (Schutte et al. 1996, 2003; Miles et al. 2004), but the possibility that CBS may develop resistance to the strobilurins, justifies the incorporation of two additional mancozeb sprays before and after the strobilurin applications in October and January (Nel et al. 2003).

Trials have shown that black spot control can be improved by a combination of fungicide applications to ensure coverage of fruit as expansion occurs during the fruit susceptibility period, pruning (Loest 1968) to reduce pycnidiospore inoculum and promote tree vigour and spray penetration, and application of hay mulch over the leaf litter to suppress liberation of ascospores from fallen leaves (Miles et al. 2008). In Brazil, the use of mulching and leaf litter elimination in affected orchards (Spósito et al. 2011) is insufficient to suppress CBS, due to sources of conidia on fruit from multiple and irregular blooms (Baldassari et al. 2009). Various packinghouse treatments are effective in killing pycnidiospores present at the time of treatment, but they do not prevent the subsequent production of spores (Korf et al. 2001).

CBS establishment in a new area requires the presence of susceptible host material, adequate inoculum and favourable environmental conditions, not only for isolated or periodic infection events, but also for the completion of the life cycle in successive generations. Ascocarps of *G. citricarpa* are not found on fruit, so only pycnidiospores are of relevance in terms of the first potential infection event following entry of infected commercial fruit into a CBS-free region. Kiely (1948a) stated "the importance of infected fruit on the ground in providing water-borne inoculum is practically nil". Pycnidiospores lose their viability rapidly and pycnidiospores produced on CBS-infected fruit must be in close proximity to susceptible host material (attached young fruit and new leaf flushes) in the presence of water for a potential infection event to occur (Kiely 1948b; Wager 1952). Detached leaves and leaf litter are not susceptible to infection (Truter et al. 2007). Leaf infections remain predominantly latent until leaf drop and desiccation (Kotzé 1996).

An infection event does not necessarily result in permanent establishment of the organism in a new region. Establishment requires the presence of adequate host material in a region that is exposed to climatic conditions that are conductive to the successful and repeated completion of the life cycle. Winter rainfall regions that experience cool wet winters and dry hot summers are an effective barrier to establishment of CBS (Paul et al. 2005). Magarey et al. (2009) concluded that in Europe, *G. citricarpa* is not expected to have an impact in areas with commercial citrus production, but will be a threat to citrus production in Florida and to a lesser extent Gulf Coast production and is unlikely to be a concern in California. EFSA (2008), by contrast, concluded from their modeling that in some years and at some locations in the EU, climate is favorable for disease development. However, this position was based heavily on arguments focused on infection events as opposed to establishment.

Important citrus producing regions of the world remain free of CBS. Where such areas occur in regions that are suitable for CBS establishment, regulatory controls are required to protect such areas from a CBS incursion. Considering that the movement of infected citrus propagation material has been the means by which CBS has spread, CBS incursion risk management plans should place heavy emphasis on this pathway. The legal international movement of citrus propagation material is tightly regulated and many countries have implemented strict enforcement measures. However, as with many other biosecurity risks, the primary risk pathway is traveler baggage. Public awareness and compliance enforcement at ports of entry are important determinants of the level of risk management.

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Chapter 19
Invasive Pathogens in Plant Biosecurity. Case Study: *Phytophthora ramorum* Werres et al.: Cause of Sudden Oak Death, Ramorum Leaf Blight and Ramorum Dieback

Scott C. Redlin, Sabine Werres, and Thomas Schröder

19.1 Introduction

19.1.1 The Importance of Plant Pathogens in Plant Biosecurity

The term "pest" is defined as any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products (IPPC 2011). Among pests are the pathogenic agents that cause plant diseases. Plant pathogens are regulated in international and domestic trade in agricultural and silvicultural products. The introduction of exotic plant pathogens results in new diseases that can be ecologically costly (loss of plants and habitats), economically costly (loss of revenues from forest and horticultural products) and challenging to manage. Plant health and quarantine regulations focus on the principle of pathogen exclusion and sometimes eradication (Waller et al. 2002). When established into new regions, plant pathogens may cause plant disease epidemics and are often problematic to eradicate. One of the most destructive plant pathogens in human history was, *Phytophthora infestans* (Mont.) de Bary, cause of Late Blight of Potato and inciting the Irish potato famine (Schumann 1991). The genus *Phytophthora*, is one of several plant pathogenic Oomycetes previously classified as fungi but now considered as fungal-like members (pseudofungi) of the Kingdom Chromista (Agrios 2005).

In addition to the plant disease epidemics of herbaceous plants, numerous pathogens cause disease epidemics of woody plants. Examples of tree disease epidemics include: Chestnut Blight of Fagaceae caused by *Cryphonectria parastica*

S.C. Redlin (✉)
USDA-APHIS/Plant Protection and Quarantine, CPHST PERAL, Raleigh, NC 27606, USA
e-mail: Scott.C.Redlin@aphis.usda.gov

S. Werres • T. Schröder
Julius Kühn-Institut – Federal Research Center for Cultivated Plants (JKI), Institute for Plant Protection in Horticulture and Forestry, Messeweg 11/12, 38104 Braunschweig, Germany

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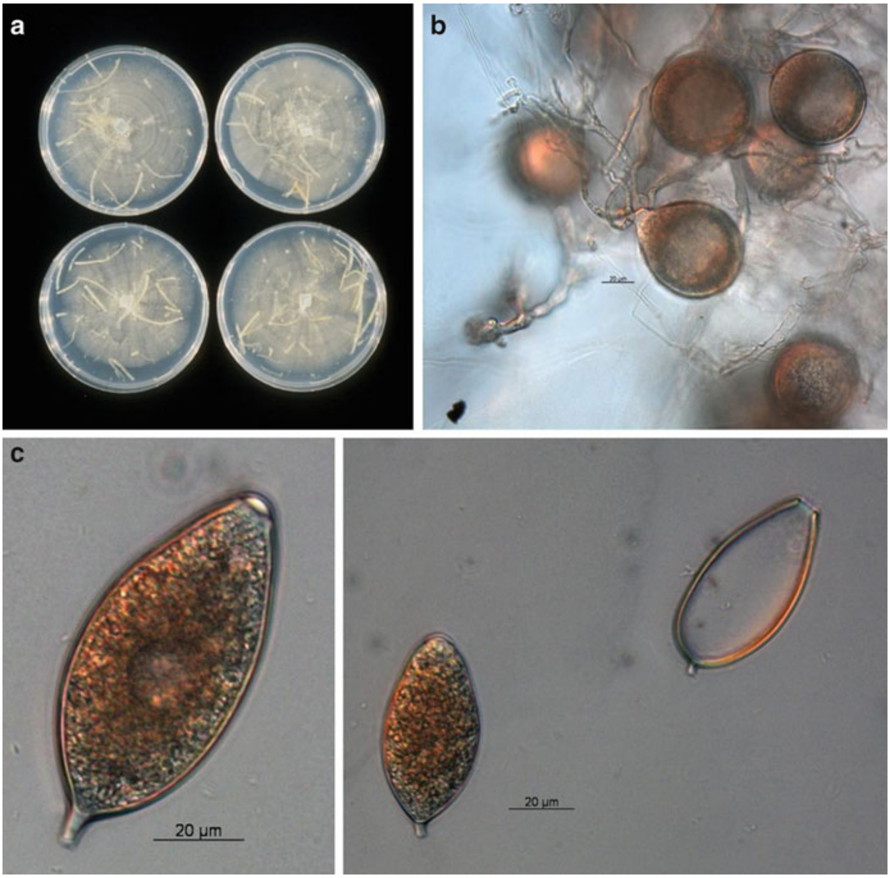

Fig. 19.1 *Phytophthora ramorum* morphology. (**a**) Colonies on carrot piece agar; (**b**) Chlamydospores (*centre*: germinating chlamydospore); (**c**) Sporangia (*right*: empty sporangium after zoospore release)

(Murrill) M. E. Barr; Dutch Elm Disease of Ulmaceae caused by *Ophiostoma ulmi* (Buisman) Nannf.; Scleroderris Canker of Pinaceae caused by *Gremmeniella abietina* (Lagerb.) M. Morelet; White Pine Blister Rust of Pinaceae caused by *Cronartium ribicola* J. C. Fisch; Canker Stain of Platanaceae, Seridum Canker of Cupressaceae, Alder Decline of Betulaceae and Bleeding Canker of Ericaceae and Fagaceae caused by *Phytophthora kernoviae* (Brasier et al. 2004b, 2005; Manion 1990; Wagener 1928).

Disease symptoms on Fagaceae had been noted in California several years before we discovered that Sudden Oak Death was caused by a species of *Phytophthora* new to science, *Phytophthora ramorum* Werres, De Cock and Man In't Veld Werres (Werres et al. 2001). Two other diseases, "Ramorum Leaf Blight" and "Ramorum Dieback" are also caused by *P. ramorum* (COMTF 2012). Figure 19.1 depicts several morphological features of *P. ramorum* (colony morphology, chlamydospores, and sporangia). Symptoms of the diseases caused by the pathogen on several hosts are shown in Figs. 19.2, 19.3, 19.4, 19.5, 19.6, and 19.7.

Fig. 19.2 ***Phytophthora ramorum* disease symptoms** (**a**) on *Pieris, left*: wilting and brown leaves, *right*: discolouration on a twig (→); (**b**) on *Rhododendron, left*: wilting and brown leaves, *right*: discoloured twig and shoot; (**c**) on *Viburnum,* cambium necrosis at stem base further disease symptoms see www.suddenoakdeath.org and http://www.eppo.fr/QUARANTINE/Alert_List/fungi/PHYTRA.htm

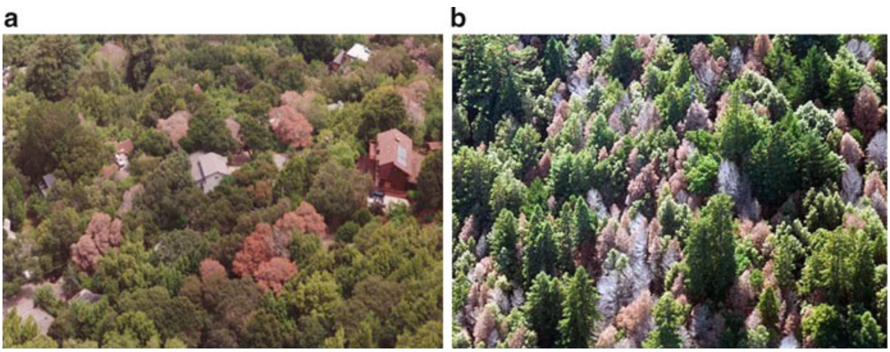

Fig. 19.3 (**a**) ***Phytophthora ramorum* disease symptoms** on infected trees surrounding homes in Marin County, California (Image courtesy Marin County Fire Department). (**b**) ***Phytophthora ramorum* disease symptoms** on *Lithocarpus densiflora,* Marin County, California (Image courtesy Forest Health Protection, USDA Forest Service, Pacific Southwest Region)

Fig. 19.4 ***Phytophthora ramorum*** **disease symptoms** on *Lithocarpus densiflora*, *left*: bleeding canker on lower portion of trunk is not associated with cracks in bark or insect damage (Image courtesy Garbelotto lab, University of California, Berkeley); *centre*: dried exudate from bleeding symptom on outer bark; *right*: outer bark removed to reveal inner bark, canker and canker margin (Image courtesy Bruce Moltzan, USDA Forest Service)

Fig. 19.5 *Phytophthora ramorum* disease symptoms (**a**) on *Umbellularia californica*, natural reservoir for *Phytophthora ramorum;* host is not killed but develops leaf blight (Image courtesy Joseph O'Brien, USDA-Forest Service); (**b**) on *Camellia japonica*; *centre* and *right*, *brown* lesions on leaves are irregular and usually restricted to leaf tips (Image courtesy Oregon Department of Agriculture and Cheryl Blomquist, California Department of Food and Agriculture)

19.1.2 **Phytophthora ramorum** *as a Pathogen of Regulatory Significance*

The high economic impact of *P. ramorum* is due to the disease or mortality it causes to plants, including costs associated with the loss of sale of infected nursery stock, inspection fees, tree removal, and tree deaths in home and business landscapes which reduce real estate value (native trees and shrubs and landscape plantings) (COMTF 2012; Garbelotto et al. 2001). In international and domestic trade, host plant materials including nursery stock and forest products are regulated due to this pathogen. The ecological impact of *P. ramorum* in forested areas results from the

Fig. 19.6 *Phytophthora ramorum* disease symptoms. (**a**) *Rhododendron macrophyllum, left*: shoot dieback, *right*: foliar blight (Image courtesy Everett Hansen, Oregon State University). (**b**) *Kalmia latifolia*, foliar blight (also known as Ramorum Blight) (Image courtesy J. Fallacy, Washington State Department of Agriculture)

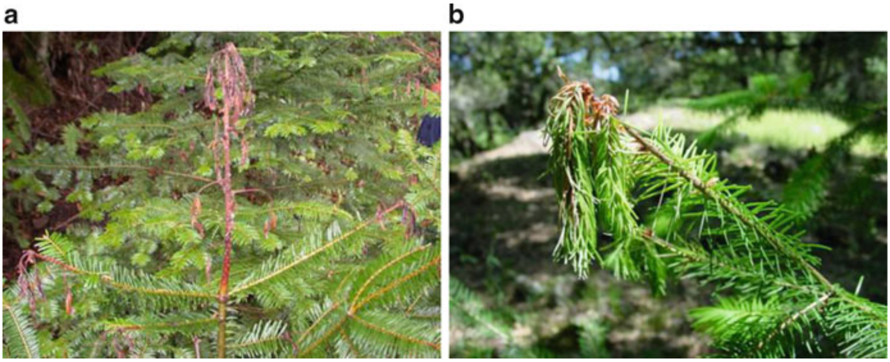

Fig. 19.7 *Phytophthora ramorum* disease symptoms (**a**) on *Abies grandis,* dieback and shoot blight; (**b**) on *Pseudotsuga menziesii,* dieback (Image courtesy Santa Clara County (California) Agriculture Department and David Rizzo, University of California, Davis)

dieback and death of trees, followed by erosion with increased silting of rivers resulting in fish kills, and opportunities for the ingress of invasive plant species (COMTF 2012; Garbelotto et al. 2001). In addition, standing and fallen dead trees of *Lithocarpus densiflorus* (Hook. & Arn.) Rehder and *Quercus* spp. in the western USA increase surface fuels and are a fire hazard in forested areas (Garbelotto et al. 2001). Data in the following text referring to Europe are based on the situation in the 27 European Union (EU) member states and does not include all 47 European countries.

19.2 Geographical Distribution

19.2.1 Europe

The geographical distribution of *P. ramorum* in Europe includes 19 EU countries, where it is under official control: Belgium, Czech Republic (eradicated nursery finding), Denmark, Estonia, Finland, France, Germany, Ireland, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Poland, Portugal, Slovenia, Spain (including Mallorca), Sweden and the UK (England, Wales, Scotland and Northern Ireland, Channel Islands) (Sansford et al. 2009). This pathogen has also been recorded in Norway and Switzerland, and most recently in Greece and Croatia (EPPO 2011; Sansford et al. 2009; Tsopelas et al. 2011).

Phytophthora ramorum was first detected in the Netherlands and subsequently in Germany. The first isolate in the Netherlands originated from a diseased *Rhododendron* plant in a nursery during 1993 (Werres et al. 2001). Subsequently, the pathogen was detected during 1995 in Germany as an unknown new *Phytophthora* species on *Rhododendron*. It was recovered on old *Rhododendron* hedge plants surrounding the farmhouse of a plant nursery and within recirculating irrigation water of the nursery (Werres and Marwitz 1997; Werres et al. 2001).

During 2001, regulatory officials in Poland detected the pathogen on imported *Rhododendron* sp. planted in nurseries (Orlikowski and Szkuta 2002). Subsequently (2002), based on surveys recommended by the European Union (EU) Commission Decision 2002/757/EC, eight EU member states detected *P. ramorum* in their countries for the first time (Belgium, Denmark, France, Ireland, Italy, Spain (Mallorca, Galicia), Sweden, and UK; Table 19.1). The pathogen was also detected in Norway, a non-EU country. During 2003, the Czech Republic and Slovenia reported their first *P. ramorum,* followed by Finland, Poland, and Switzerland during 2004. First detection of *P. ramorum* was reported in France during 2005 and Portugal during 2006. Lithuania reported its first detection during 2007, while Serbia and Estonia followed during 2008. Recent reports are from Greece (2010) and Croatia (2011) (EPPO 2011; Tsopelas et al. 2011).

19.2.2 North America

Canada: Infected ornamental plants in nurseries and landscapes have been detected and destroyed in British Columbia. For survey results in Canada please refer to:

http://www.inspection.gc.ca/english/plaveg/pestrava/phyram/sodmsce.shtml.

USA: During 2011, *P. ramorum* was identified as established in 14 counties of California (Alameda, Contra Costa, Humboldt, Lake, Marin, Mendocino, Monterey, Napa, San Francisco, San Mateo, Santa Clara, Santa Cruz, Solano and Sonoma) and 116 square miles were quarantined for the pathogen in Curry County, Oregon. Infected nursery stock was detected and destroyed in 23 states, including

Table 19.1 First detections of *Phytophthora ramorum* in Europe

Year	Country	Host plant	Location	Reference[a]
1993	The Netherlands	*Rhododendron*	Nursery	Werres et al. (2001
1994/1995	Germany	1. *Rhododendron* 2. recirculating water	1. nursery (imported plants), hedge in a private garden 2.Nursery	Werres and Marwitz (1997) Werres et al. (2001)
2001	Germany	*Rhododendron* *Viburnum*	Nursery	EPPO RS 2003/038
2001	The Netherlands	1. *Rhododendron* + *Viburnum bodnantense* 2. *Rhododendron*	1. nursery 2. public gardens, forest	EPPO RS 2003/014
2001	Poland	*Rhododendron*	Nursery containerized plant	EPPO RS 2002/040
2001	Germany	*Rhododendron*	Nursery	EPPO R S 2002/078
2002	Germany	*Viburnum, Rhododendron*	nursery, private gardens	EPPO RS 2003/038
2002	UK	*Viburnum* *Rhododendron*	Nursery	EPPO RS 2002/077
2002	Spain (Mallorca)	*Viburnum bodnantense*	Nursery	EPPO RS 2002/160 Moralejo and Werres (2002)
2002	Belgium	*Viburnum bodnantense*	Nursery	De Merlier et al. (2003)
2002	Denmark	1. *Rhododendron* 2. *Viburnum*	Nursery	EPPO RS 2003/133
2002	France	*Rhododendron* *Viburnum*	Nursery	EPPO RS 2003/036
2002	Ireland	*Rhododendron* *Viburnum*	Nursery Garden centre	EPPO RS 2003/133
2002	Italy (Piedmont)	*Rhododendron yakushimanum*	Nursery	Gullino et al. (2003) EPPO RS 2004/106
2002	Norway	*Rhododendron*	Nursery	EPPO RS 2003/133
2002	Spain (Galicia)	*Camellia japonica*	Nursery	Pintos Varela et al. (2004)
2002	Sweden	*Rhododendron*	Nursery	EPPO RS 2003/020
2002	UK	*Pieris forrestii*	Private garden	Inman et al. (2003)
2002?	Guernsey (UK)	*Rhododendron, Viburnum, Camellia, Leucothoe, Arbutus*	Gardens	EPPO RS 2003/133
2003	Belgium	*Rhododendron, Viburnum*	Nursery	EPPO RS 2003/019
2003	Czech Republic	*Viburnum bodnantense*	Nursery	EPPO RS 2005/159
2003	Germany	*Rhododendron*	Forest	EPPO RS 2004/139
2003	Slovenia	1 *Rhododendron catawbiense* 'Grandiflorum', *Viburnum farreri* 2 *Viburnum bodnantense*	1. garden centre 2. nursery	Zerjav et al. (2004) EPPO RS 2003/161
2003	Spain (Galicia)	*Viburnum tinus, Rhododendron, Camellia japonica*	Nursery	EPPO RS 2004/172
2003	UK (Scotland)	*Syringa vulgaris*	Nursery	Beales et al. (2004b)
2003	UK	*Camellia japonica*		Beales et al. (2004a)
2003?	UK	*Pieris formosa* var. *forrestii, P. japonica* *Camellia japonica, Kalmia latifolia*	?	EPPO 2003/039
2003?	UK	*Syringa, Arbutus*	?	EPPO RS 2003/133
2003	UK (SW of England)	*Quercus falcata*	Private estate	EPPO RS 2003/145 (Brasier et al. 2004a)
2003	UK (SW of England)	*Fagus sylvatica, Quercus ilex, Aesculus hippocastanum*	Private estates	EPPO RS 2003/162
2003	UK (South Wales)	*Hamamelis virginiana*	Public garden	Giltrap et al. (2004) EPPO RS 2007/210
2003	UK	*Taxus baccata*	Nursery	Lane et al. (2004)
2004	Belgium	*Euonymus, Kalmia, Rhododendron, Viburnum*	Nurseries, Parks, private garden	EPPO RS 2004/171
2004	Finland	*Rhododendron*	Nursery	EPPO RS 2005/159 (Lilja et al. 2007)
2004	Germany	*Pieris japonica*	Forest	EPPO RS 2004/139
2004	The Netherlands	*Quercus rubra*	Park	EPPO RS 2004/024
2004	Norway	*Pieris japonica, Kalmia, Rhododendron*	Nursery, private garden	EPPO RS 2007/095
2004	Poland	*Pieris japonica, Calluna vulgaris, Photinia* sp.	Nursery	EPPO RS 2004/086
2004	Switzerland	*Viburnum*	Nursery	Heininger et al. (2004) EPPO RS 2004/105
2004	UK	*Parrotia persica*	Public garden	(Hughes et al. 2006)
2005	France	*Pieris japonica*	Nusery	EPPO 2007/177
2005	Ireland	*Rhododendron ponticum*	Nursery	O'Connor and Gosling (2008)
2005	Norway	*Viburnum fragans*	Private garden	Herrero et al. (2006) EPPO RS 2007/095
2006	France	*Camellia*	Nursery	EPPO RS 2007/177 Husson et al. (2007)
2006	The Netherlands	*Fagus sylvatica*	?	Anonymous (2006) EPPO RS 1006/102
2006	Portugal	*Viburnum*	Nursery	Gomes and Amaro (2008)
2007	France	*Syringa, Taxus*	Nursery	EPPO 2007/177
2007	Lithuania	*Rhododendron*	Nursery	EPPO 2007/188
2007	UK	*Magnolia stellata, M. loebneri, Griselinia littoralis*		Giltrap et al. (2007)
2008	Serbia	*Rhododendron*	Public garden	Bulajic et al. (2009) EPPO RS 2009/13
2008	Estonia	?	Garden centres	EPPO RS 2010/040
2009	Norway	*Vaccinium myrtilus*	Aboretum	Herrero et al. (2011)
2009	UK	*Larix kaempferi*	Forest	EPPO RS 2010/033
2009/2010?	Serbia	1 – *Rhododendron* 2 – *Pieris*	1. Garden 2. Garden centre	Bulajic et al. (2010)
2010	Ireland	*Quercus phillyraeoides, Kalmia, Michelia, Magnolia, Viburnum, Rhododendron, Pieris*	Historical garden	EPPO 2010/149
2010	Ireland	*Larix kaempferi*	Outdoor	EPPO 2010b
2010	Greece	*Rhododendron*	Nursery	Tsopelas et al. (2011)

Highlighted in *grey* = plants known to be imported; ? = no information

[a]EPPO Reporting Services see http://www.eppo.org/PUBLICATIONS/reporting/reporting_service.htm

Alabama, Arkansas, Arizona, California, Colorado, Connecticut, Florida, Georgia, Louisiana, Maryland, Mississippi, North Carolina, New Jersey, New Mexico, New York, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, and Washington (APHIS 2005). Since January 2005, all nursery stock shipped interstate from California, Oregon and Washington has been regulated to help prevent *P. ramorum* movement (APHIS 2004, 2007).

Symptoms of dieback, general decline and death of *Lithocarpus densiflorius* (tan oak) and *Quercus* spp. (oak) were noted from the early 1990s in Marin County California, but the cause remained unknown. By the late 1990s, environmental factors or damage caused by arthropods was suspected as a cause of sudden oak death (Garbelotto et al. 2001).

Isolations made from cankers of symptomatic trees resulted in cultures of an undescribed species of *Phytophthora*. Clive Brasier, plant pathologist from the UK, Sabine Werres from Germany and Robert Baayen from the Netherlands visited Dave Rizzo in the USA during 2001 to compare living cultures from the Netherlands and Germany with cultures from the USA. The pathologists noted that the organism isolated into pure culture from *Lithocarpus* and *Quercus*, was morphologically similar to species of *Phytophthora* consistently isolated from *Rhododendron* spp. in Europe. A description of the causal organism and its name in the genus *Phytophthora* was published by Werres et al. (2001). Researchers in California and Europe exchanged pathogen ITS sequences, and found a match of the sequences from isolates collected in Germany, the Netherlands and California (Garbelotto and Rizzo 2005). This verified that the organism in North America was conspecific with the taxon detected and isolated in Europe since 1993 (Werres and Marwitz 1997).

Isolating the pathogen from symptomatic plant material during initial surveys was very difficult. Samples from symptomatic plant materials frequently yielded a negative result, depending on the freshness of the sample, the type of host plant material from which the sample was derived and the handling procedures that were used after specimen collection. To address that challenge, researchers at the University of California, Berkeley developed a Polymerase Chain Reaction (PCR) technique to determine whether the organism was present and not dependent on whether the organism was viable. Further refinements allowed the DNA of the organism to be sequenced (Kliejunas 2010).

19.3 Location of First Detection

19.3.1 Europe

Nearly all first-detections in three countries were on *Rhododendron* and *Viburnum* species produced in nurseries. Some pathogens originated from garden centers (Table 19.1), with two exceptions. In 1995 the pathogen was detected on

Rhododendron sp. in recirculating irrigation water of a nursery in Germany, and in Serbia during 2008 on a plant in a public garden. Some of the infected plants were imported but in most cases detailed information was not available.

The first positive report from forest areas came in 2002 on *Rhododendron* in the Netherlands and later (2004) in Germany. In Netherlands a few trees (*Fagus sylvatica* L.) were found infested during subsequent years. In Germany, *P. ramorum* could only be isolated from salvaged *Rhododendron* and *Pieris* in the understory, but not from other tree species within the forest stand. Most detections outside nurseries have been reported from the UK, which may be caused by different climatic conditions compared with the European continent.

19.3.2 North America

Symptoms of a disease that became known as "Sudden Oak Death" (SOD) were first detected in forests on *Quercus* spp. and *L. densiflorus* during 1995 at Mill Valley (Marin County), California. The causal organism was not formally described until 2001. In addition to forested areas, SOD was detected on new hosts and nurseries outside quarantined and regulated areas during 2004 (Frankel 2008). Detailed information and full timelines for the distribution, hosts and diagnostics of *P. ramorum* in forests and nurseries in the USA and Canada are available (Kliejunas 2010) and the California Oak Mortality Task Force (COMTF 2012).

19.4 Host Range

19.4.1 Europe

The main host plants (*Rhododendron* and *Viburnum* species) for the pathogen were identified first in European countries. Species in these two genera are susceptible taxa as confirmed by the yearly monitoring on *P. ramorum* carried out in the EU-member states. *Camellia japonica* Linnaeus was confirmed as a nursery host during 2003 on Mallorca (Spain). Since 2003 the host range of *P. ramorum* has been increasing. Tree species as hosts were reported first during 2003 (*Quercus ilex* L. in UK) followed by the Netherlands during 2004 for *Q. rubra* L. and during 2006 for *Fagus sylvatica* L. Although additional trees of different species are routinely found infested in the UK, no further infestation was detected after the initial detection in the Netherlands. The first gymnosperm (*Taxus baccata* L.) infected by *P. ramorum* was detected in the UK during 2003. Since 2003 the range of naturally infected tree species has been increasing. Recently, the pathogen was detected on *Larix kaempferi* (Lamb.) Carr. in the UK (Webber et al. 2010) and in Ireland where *Q. ilex, Q. phillyraeoides* A. Gray and *Picea sitchensis* (Bong.) Carrière (one tree) have been found as natural hosts (EPPO 2010a, b).

19.4.2 North America

Canada: Infected ornamentals in nurseries and landscape plantings have been detected and destroyed in British Columbia. The nursery repeatedly was positive for several years. The nursery had placed many plants in close proximity in a greenhouse and provided overhead irrigation. The plant species added to the list of plants associated with *P. ramorum* by Canadian Food Inspection Agency (CFIA) include *Acer davidii* Franch., *Ardisia japonica* Blume, *Berberis diversifolia* (Sweet) Steud., (= *Mahonia aquifolium*), *Cercis chinensis* Bunge, *Cornus kousa* Buerger ex Miq., *Corylopsis spicata* Siebold & Zucc., *Daphniphyllum glaucescens* Blume, *Distylium myricoides* Hemsley, *Hamamelis* × *intermedia* (*H. japonica* × *H. mollis*), *Ilex purpurea* Hasskarl, *Leucothoe axillaris* (Lam.) D. Don, *Loropetalum chinense*, *Magnolia cavalieri*, *Magnolia, M. ernestii* (= *Michelia wilsonii*), *M. foveolata* (Merr. ex Dandy) Figlar, *M. grandiflora* L., *M. kobus* DC., *M. maudiae* (= *Michelia maudiae*), *Manglietia insignis*, *Osmanthus decorus*, *Parakmeria lotungensis*, *Physocarpus opulifolius*, *Prunus lusitanica*, *Pyracantha koidzumii*, *Rosa* spp. (hybrid roses), and *R. rugosa*. For survey results in Canada please refer to:

http://www.inspection.gc.ca/english/plaveg/pestrava/phyram/sodmsce.shtml.

USA: The host range for *P. ramorum* is broad and continues to expand. During February 2010, 45 plant species in 20 families were considered "proven hosts" and regulated for *P. ramorum* (APHIS 2010). Naturally infected associated plants are considered host plants regulated for *P. ramorum* upon completion, documentation, review, and acceptance of traditional Koch's postulates (APHIS 2010). For a complete list of this of this regulated host list visit the following website:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf

A second group of 82 hosts (including representatives of 32 plant families) are considered plants associated with *P. ramorum*. These host plants are naturally infected by *P. ramorum* and detected using PCR. Traditional Koch's postulates have not been completed or documented and reviewed for each of these associated plants. For a complete list of the regulated hosts please refer to:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/usdaprlist.pdf

19.5 Population Diversities and Epidemiology

19.5.1 Population Diversity

When *P. ramorum* isolates from Europe and USA were first compared morphologically during 2001, the populations seemed to be identical. Subsequently, detailed studies with more isolates revealed consistent morphological differences between

Table 19.2 Clonal lineages of *P. ramorum* (Grünwald et al. 2009)

Clonal lineage	Mating type	Geographical distribution	Habitat
NA1	A2	North America	Forest, nurseries
NA2	A2	North America	Nurseries
EU1	Predominantly A1; rarely A2	Europe and North America	Nurseries, public green, gardens

both populations and that they belong to different mating types (Brasier 2003; Werres and Kaminski 2005; Brasier et al. 2006).

The first isolates detected in Europe (designated "EU1") were determined as isolates of mating type "A1" (Werres et al. 2001). The first North American isolates (designated NA1) belonged to mating type "A2" regardless of whether they originated from nurseries or woodlands. Mating type A2 was detected during 2002 and 2003 in Europe (Werres and De Merlier 2003; Vercauteren et al. 2010) and during 2003 in the USA (Hansen et al. 2003). Both detections involved nursery plants. In Europe A2 was recovered from the hybrid *Viburnum* × *bodnantense* (*V. farreri* × *V. grandiflorum*) imported to Belgium with unknown origin, and in the USA on *Viburnum* and *Pieris* plants in northern Oregon. Since 2003, isolates of mating type A2 have not been detected in Europe. In North America, more isolates of mating type A1 have been subsequently identified. All of the A1 mating types found to date in the USA are EU1.

Initial molecular studies with European and USA *P. ramorum* isolates detected two different lineages: One recovered in North America and the other in Europe (Ivors et al. 2004). These lineages were considered as correlated with the mating type. The North American lineage was of mating type A2; the European lineage was of mating type A1. Further studies showed the presence of three distinct lineages (Grünwald et al. 2008; Elliott et al. 2009c). Grünwald et al. (2009) standardised the nomenclature of these lineages as U1, NA1 and NA2 (Table 19.2). According to the current nomenclature, EU1 isolates belong to mating type A1 with one exception: The single European isolate of mating type A2 detected in Belgium during 2002 belongs to lineage EU1 (Ivors et al. 2006). Both NA lineages belong to mating type A2.

Within a lineage the isolates show genotypic diversity (Britt and Hansen 2009a, b; Dart et al. 2009; Goss et al. 2009c). Dominance of single genotypes within a local lineage distribution indicates differences in aggressiveness of the genotypes. Nevertheless, infection studies with isolates of the three lineages or with different genotypes showed few differences (Britt and Hansen 2009a; Elliott et al. 2009a, b) or low variability between lineages and high variability within lineages (Elliott et al. 2009a). Other studies revealed EU1-A2 and EU1-A1 isolates were similarly aggressive and more aggressive than the NA1 isolates tested (Vercauteren et al. 2010).

Following introduction into new areas, development of new genotypes and interpopulation genetic exchanges can occur (Goss et al. 2009b; Prospero et al. 2009). Currently, we see no evidence for sexual reproduction under natural conditions (Prospero et al. 2009) although simultaneous occurrence of A1 and A2 isolates has been reported in USA nurseries (Hansen et al. 2003; Grünwald et al. 2008). Based on genetic drift, we postulate that these clonal lineages have probably been separated for several centuries and may have lost the ability to sexually reproduce. In vitro mating studies showed that sexual reproduction is possible (Boutet et al. 2010). Fitness and aggressiveness of progenies depend on the parents: EU1-A1 × EU1-A2 pairings result in a high number of unfit progenies with lower aggressiveness on *Rhododendron* leaves differing from EU1 × NA1 progenies which exhibit higher aggressiveness (Boutet et al. 2010). To lower the risk of new aggressive *P. ramorum* genotypes developing after adaption to new hosts and environments, the introduction of the two NA lineages into Europe and of further EU1-A1 isolates to North America should be avoided through plant quarantine regulations (refer to the work of Grunwald for up-to-date information on lineages and sexual reproduction of *P. ramorum*).

19.5.2 Origin, Distribution and Pathways of **P. ramorum**

Determining the origin, distribution and pathways of pathogens is critical as for invasive vertebrates, invertebrates and weeds. The microscopic size of pathogens severely limits anatomical criteria for identification. Regulatory officers must rely upon genetic data for accurate identification (See Ch 13). Concerning *P. ramorum,* detailed genetic studies suggest that the three clonal lineages originated from separated geographical locations and that they were introduced to Europe and North America independently (Goss et al. 2009a). Furthermore, the European lineage is hypothesized to be older than the two North American lineages (Goss et al. 2009a).

The EU1-A1 lineage appears to be most widespread: It has been detected in European nurseries, public gardens and forests as well as North America nurseries, but not in forests. The EU1-A2 lineage seems comprised of single detections in Belgium and the population appears to have been eradicated (Vercauteren et al. 2010). Current evidence suggests that both NA lineages only occur in North America. NA1 occurs in nurseries and forest; NA2 occurs only in nurseries.

Several genetic studies on the distribution of *P. ramorum* populations indicates that different migration pathways exist, including human mediated (via plant trade) and natural spread (Prospero et al. 2007, 2009; Mascheretti et al. 2008, 2009; Goss et al. 2009b). In California, the epidemic is hypothesized as based on historical human-mediated spread, with new infestations by infrequent medium-range to long-range natural movement of the pathogen and by local generation of new genotypes (Mascheretti et al. 2008). Later studies on the *P. ramorum* population of USA nurseries showed that isolates of the dominant NA1 lineage could be clustered into two groups: One containing isolates from Connecticut, Oregon and Washington state, and the other group containing isolates from California and other USA states (Goss et al. 2009b). These results correlated with the trace-forward analysis of the USDA-APHIS Plant Protection and Quarantine (APHIS PPQ). Trace-forward locations are any locations that received potentially infected plants from a confirmed infested source nursery, including residential or commercial landscapes (APHIS 2010).

Studies of isolates from Washington nurseries showed high levels of genotypic diversity within the *P. ramorum* population. This high diversity and the lack of sexual recombination suggests multiple introductions of the pathogen into Washington nurseries (Dart et al. 2009). The situation is different in Oregon because the local *P. ramorum* population is characterized by low genetic diversity (Prospero et al. 2007). Significant differentiation and low gene flow were detected between nursery and forest populations. Two nursery genotypes were also found in the forest, but at a low frequency. The authors concluded that nursery infestation in Oregon resulted through introduction of novel genotypes from nurseries outside of Oregon. In Canada, where all three lineages have been detected, the most common clonal lineage was NA2, not NA1 (the lineage predominantly distributed in the USA) (Goss et al. 2011). Analysis of EU1 isolates, detected in Canada, showed similar genotypes with USA and European isolates, but estimation of migration rates indicate that migration from Europe to North America was higher than North America to Europe (Goss et al. 2011).

Detailed microsatellite-based genotypic analysis of the *P. ramorum* population in Europe is in progress (studies within COST FP0801 Action, Heungens et al., personal communication).

19.6 Emergency Measures and Regulations

19.6.1 European Union

Immediately following the description of *P. ramorum* by Werres et al. 2001, the European and Mediterranean Plant Protection Organisation (EPPO) listed *P. ramorum* on their alert list. This list contains harmful organisms of phytosanitary interest for all 50 EPPO countries but does not include data on their geographic distribution and potential threat. Following a Pest Risk Analysis (PRA), the UK informed the EU-member states about the occurrence of *P. ramorum* on their territory and the implementation of additional measures to prevent introduction and spread within the European-Community. Concomitantly, Netherlands and Germany notified EU members about the occurrence of *P. ramorum*. At that time *P. ramorum* was not listed in EU quarantine legislation (EU-Directive 2000/29/EC). The EU Commission took provisional emergency phytosanitary measures against the introduction and spread within the Community. These measures were enacted on November 1, 2002 (Commission Decision 2002/757/EC (EU 2002)) and were revised in 2004 and 2007. The decision regulates the import and movement of susceptible plants, susceptible wood and susceptible bark and recommends phytosanitary measures if *P. ramorum* is found in production sites. Nurseries producing host plants must be registered by the National Plant Protection Organisation (NPPO) and must be inspected at least twice a year. For details of the Commission Decision 2002/757/EC including its amendments please refer to:

http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002D0757:20070330:EN:PDF.

In addition to recommendations for import or movement of host plants, each EU-member state must conduct a yearly survey for *P. ramorum* in its territory. Results must be reported to other member states and the EU Commission.

Monitoring must be carried out in nurseries, parks and woodlands. The EU-Commission issued a guideline for management and survey of host plants in forests, woodlands, heathlands, parks and gardens.

During 2004–2007 a European research project (RAPRA) was carried out with the aim to develop a comprehensive PRA. A more recent review supported the findings in the RAPRA PRA (Sansford et al. 2009).

19.6.2 North America. Regulations in Canada

The CFIA has promulgated the Plant Protection Act and subsequently several Plant Protection Policy Directives (D-08-04, D-02-12, D-01-12, D-01-01, D-96-20 D-98-08, D-95-26) that include nursery stock, soil, wood packing material and firewood (CFIA 2010).

Regulations in USA: Code of Federal Regulations (7 CFR §301.92) Domestic regulations *Phytophthora ramorum* has been under regulation in the USA since February 2003. Regulated articles as listed in 7 CFR§301.92 include the following: Nursery stock, logs, lumber, wood, chips, bark, collected natural materials, and green waste/biomass. PRAs for *P. ramorum* by PPQ's CPHST Plant Epidemiology and Risk Analysis Laboratory (PERAL) was the scientific basis of APHIS regulations (Cave et al. 2007).

Nurseries in the quarantined areas must be inspected, sampled, and tested annually for symptoms of disease caused by *P. ramorum.* Pre-shipment inspections are required before interstate movement. The Emergency Federal Order Restricting Movement of Nursery Stock from California, Oregon and Washington Nurseries (December 21, 2004) and 7 CFR §301.92 also requires nurseries in regulated areas of California, Oregon and Washington to have annual and pre-shipment inspections of host material before interstate shipment. Eradication efforts are initiated if the pathogen is detected during any inspection process.

Eradication or regulatory measures in forests (natural stands) or in ornamental landscape settings (horticultural plantings) include the removal and destruction of infected hosts. This was attempted in locations where geographical distribution was limited. The procedure included cutting down and destroying hosts followed by revisiting the site and treating any new sprouts with herbicides. Eradication was verified by sampling soil for the presence of spores (chlamydospores, sporangia, zoospores). Determining the source of origin of potentially-infested plants and their final shipping destination or possibly the location of outplanting is accomplished through a process referred to as "trace back" and "trace forward" by regulatory agency pest management programs of APHIS-PPQ Western Region and State Cooperators (APHIS 2010).

19.7 Conclusion and Summary

Several challenges are associated with *P. ramorum* and the diseases it causes. Several positive outcomes resulting from the threat of *P. ramorum* include: (1) Recognition that as many as 50 undescribed taxa of *Phytophthora* (Brasier 2009) exist, including two taxa of regulatory interest (*P. kernoviae* Brasier and *P. alni* Brasier & S. A. Kirk) (Brasier et al. 2004b, Brasier et al. 2005); (2) development and refinement of diagnostic tools including PCR (nested, Multiplex), Realtime (ITS, elicitin) (Kliejunas 2010) and lab-on-a-chip techniques (Julich et al. 2011); (3) advancement of risk modeling and mapping, which has been instrumental in tracking *P. ramorum* and other exotic pests (Magarey et al. 2007); (4) implementation of quality control measures for nursery stock; (5) establishment of the National Ornamentals Research Site to study pests and diseases of nursery stock, particularly *P. ramorum,* at the Dominican University of California (NORS-DUC) through a cooperative agreement with APHIS PPQ's Center for Plant Health and Science Technology, and (6) development of nursery best management practises for *P. ramorum* (CANG 2008), and gives additional visibility for further evaluation of 7 CFR §319.37 related to movement of plant pests including plant pathogens with propagative plants. The role and prognosis for *P. ramorum* and the diseases it causes in global ecosystems is unknown and depends on numerous factors including virulence, survival and spread in the environment via aerial dispersal, climate change, and human assisted movement of host material and live plants.

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Chapter 20
The Importance of Weeds in Plant Biosecurity

Andy W. Sheppard, Anthony L. Koop, and Richard Hill

20.1 Introduction

Biosecurity represents the array of research and regulatory strategies used to assess and manage the risk of incursion and impact of harmful organisms (Meyerson and Reaser 2002b), including weeds and harmful invasive pest plants (IPPC 2009). Weeds have a broad definition ranging from plant species with very harmful impacts (Esler 1988; IPPC 2009) to species with simple undesirable qualities such as growing where they are not wanted. The term "weed" is generally well understood, but usage in relation to specific impacts may be subjective. The concept of an invasive plant species is more recent and more scientific in definition, but definitions vary. From an ecological perspective, an invasive plant is an alien species that naturalises, spreads and persists (colonizes). In an applied and regulatory context, this definition also implies impacts, through attaining densities that suppress resident species or biodiversity, and/or disrupt ecosystem function and services. The applied terms of weed and invasive plant represent slightly different concepts (Rejmánek 1994). In this chapter we use the terms interchangeably as they both relate to the IPPC concept of a pest plant (IPPC 2009). Weeds are categorised by National Plant Protection Organisations (NAPPO) and departments under legislation and regulations based on perceived potential to cause harm, current distribution, abundance and impacts. Specific categories invoke different regulatory actions. The term "noxious weed" is often used for weeds that require a regulatory action, but the precise definition varies among regulatory agencies.

A.W. Sheppard (✉)
CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, ACT 2601, Australia
e-mail: Andy.Sheppard@csiro.au

A.L. Koop
USDA-APHIS-PPQ-CPHST, 1730 Varsity Drive, Suite 300, Raleigh, NC 27606-5202, USA

R. Hill
Richard Hill & Associates, Private Bag 4704, Christchurch, New Zealand

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Weeds are often the most costly biosecurity threats for realised social, economic, and environmental impacts (Pimentel et al. 2000; Hulme, Chap. 1). Estimates suggest that weeds cause direct and indirect losses of about $20–34 billion per year in the USA (Gunn and Ritchie 1988; Pimentel et al. 2000; Westbrooks 1998). In Australia, weeds result in agroforestry losses of $3.5–4.5 billion per year, and possibly more (Sinden et al. 2004). In Canada, weeds in 58 crops result in a loss of about $1 billion annually (Swanton et al. 1993). These costs are likely to be underestimated because they usually ignore environmental and social impacts. Because of the long-term impacts and costs associated with pests and invasive species, incursion prevention is often the first priority in a biosecurity strategy (Meyerson and Reaser 2002a; Pimentel et al. 2000; White and Schwarz 1998).

The biosecurity threat posed by all types of pests (including weeds and invasive plants) is increasing as human society globalizes (Hulme 2009; Meyerson and Reaser 2002a). More people are moving about the globe with a sharply increasing volume and diversity of goods that are traded among nations (Hulme 2009). Increased trade is due to population increase, income growth, and transport modernization (Hulme 2009) as well as the removal of unjustified trade barriers and the adoption of free trade agreements among nations (Perrings et al. 2005). Some authors believe that globalisation and the associated increase in movement of species is inducing a third phase of (anthropogenic) global biological invasion (Hulme 2009). Globalisation of biodiversity is also seen as evidence the world is entering the Anthropocene (Ellis et al. 2012).

We begin by discussing the principal driving force for plant invasions, namely the intentional introduction of plant species. Then, we examine ways plants are introduced to new areas. Later, we summarize the types of impacts weeds and invasive species may have in agricultural and natural ecosystems. Because threats from particular plant species can be recognised before and after introduction, we review a range of biosecurity strategies for weeds. Finally, to support concepts discussed in the chapter, we devote a section to mini-case histories. These histories highlight the diversity of ways plants may be problematic and how regulators respond to them.

20.2 The Driving Force for Plant Invasions

Plants pose different biosecurity challenges compared with pathogens, arthropods and vertebrates. People intentionally introduce, promote, breed, and cultivate plants outside their native range at levels far exceeding those of any other taxonomic group. This shows the importance and diversity of plants and plant parts in human societies. Plants are used as food for human consumption, livestock feed, household fuel, building materials and fibre, and chemicals for manufacturing (production of paper and packaging, textiles and pharmaceutical products). Plants are also important as icons of human culture and ornamentals (Simpson and Conner-Ogorzaly 1986). Plant exploration, introduction, and the trade of plants, plant parts and plant products (e.g. wood or wooden items) (Dosmann and Del Tredici 2003; Fairchild 1938; Whyte 1959) have been important drivers of plant invasions.

Early civilizations moved valuable plant species with the development and spread of agriculture. Most invasion biologists consider the era of European exploration as marking the beginning of global trade in plants. European colonisation of other continents led to the discovery, cultivation and export of many new valuable crop (grasses and root vegetables) and forestry (pine, maple, eucalyptus) species (Mack and Lonsdale 2001). As "new" species were introduced to Europe, European species were transported to the colonies, to support local sufficiency and initiate local agrarian economies. Since the end of the Middle Ages, global trade and transportation have increased steadily (Hulme 2009; Pyšek et al. 2011). Horticultural and ornamental demand for new and exotic plants has also contributed to the massive unrestricted global movement of plants (Mack 1991; Mack and Lonsdale 2001). During the past few hundred years, tens of thousands of plant species have been introduced to new areas. For example, 300,000–350,000 species of vascular plants exist globally. About 20,000 species are endemic in Australia (Australian Plant Name Index: http://www.cpbr.gov.au/apni/index.html). During the last 200 years, 26,000 species have been introduced to Australia and about 25,000 of these are currently in cultivation (Randall 2007), representing 7.4–8.7 % of the world's flora. The situation is similar in New Zealand and probably similar in other developed countries. However, reliable data are not available because many cultivated exotic plants are in private collections and centralized databases of introduced and cultivated plants do not exist in other countries. During the last 30–40 years the movement of plant species between countries has come under regulation, but only in a few countries.

Floristic studies show that large portions of regional floras are composed of exotic naturalized and invasive species. For example, Australia, New Zealand, Canada and the USA have about 3,000, 2,400, 1,200 and 5,000 naturalised, alien plant species respectively (Howell and Sawyer 2006; Randall 2007; CFIA 2008; NRCS 2012). Often, the percentage of exotic species in regional floras reach 25–50 % (FNA Editorial Committee 2003; Wagner et al. 1999). Without restrictions, new plant species continue being introduced and naturalised. Where data are recorded (Australia and New Zealand) this equates to more than ten species of newly naturalised plant species per year (Bourdôt et al. 2007; CFIA 2008; Groves and Hosking 1997; Williams and Newfield 2002). About 1 % of all introduced species escape and naturalize in the surrounding landscape and 0.1 % become problem weeds (Williamson and Fitter 1996). Due to long time-lags in plant invasions, new invasive plants introduced today may not be detected or recognized as invaders for many decades (Crooks 2005; Kowarik 1995). Often, plant species likely to make up the invasive harmful plants of the future are already introduced and naturalised. An important challenge facing biosecurity agencies involves identifying potentially harmful weeds among alien species already introduced or naturalised in countries (Pheloung et al. 1999; Cunningham et al. 2003; Virtue et al. 2006).

20.3 Pathways of Introduction

A pathway is anything that allows the entry or spread of a pest (IPPC 2009). Understanding the ways that species can be introduced to new areas is critical for developing effective biosecurity strategies, policies, regulations, and laws. Hulme et al. (2008) proposed a simplified framework for invasion pathways for all types of pests in terrestrial and aquatic ecosystems. They identified six pathways for introduction (release, escape, contaminant, stowaway, corridor, and unaided) and potential regulatory approaches to minimize the risk for each pathway type. Table 20.1 defines each of these pathways and provides plant examples for each type. Here we focus on the four most important pathways for plant introductions.

In contrast to other types of noxious organisms, most naturalized plant species have been deliberately introduced for agriculture, forestry, horticulture or as ornamentals (Cook and Dias 2006; Groves and Hosking 1997; Hulme et al. 2008; Mack 1991). Studies from Canada (CFIA 2008), Australia (Groves and Hosking 1997), New Zealand (Howell 2008), and the USA (Reichard 1997) report that 58 %, 90 %, 66 %, and 82 %, respectively, of naturalized plants were intentionally introduced. Intentionally introduced species are either directly released into the environment with the intention that they establish and spread (Lonsdale 1994), or they escape from botanical gardens, private collections, or homeowner's backyards (Groves et al. 2005).

Contamination of traded commodities is another important pathway for weeds and invasive plants (Mack et al. 2000; CFIA 2008; Hulme et al. 2008). Weed-seed contaminants in crop seed are a high-risk pathway because weed seeds will receive the same favourable growing conditions as crop seed. In addition to other agricultural pathways such as animal, wool and hay contamination, weeds can also enter as contaminants through other commercial pathways such as attached to imported ornamental plants, floral display, in bird seed or attached to uncleaned timber and imported equipment. For example *Acaena novae-zealandiae* Kirk was imported into the UK on fleeces from New Zealand; this weed established in the nineteenth Century and is now spreading in the UK. Contamination as a pathway is much more important for agricultural weeds than environmental weeds. They move through trade between agricultural communities, using similar farming systems. In New Zealand, the arrival of agricultural weeds, mostly as contaminants, peaked before 1900 and most naturalized before 1950 when there were nearly twice as many agricultural weeds as conservation weeds (Williams et al. 2010).

The unintentional introduction of organisms attached to or within a transport vector (i.e., stowaways) is a less important pathway for plant introduction (Hulme et al. 2008). However, this is the most important pathway for marine invasions because many organisms colonize ship hulls and contaminate ballast water (Carlton 1989; Davidson and Simkanin 2012). Many non-trade related means of transportation or conveyance (e.g. military movements or as ships ballast) can also act as a pathway for plant introduction and spread (Hulme et al. 2008; Ruiz and Carlton 2003). Seeds of some species can readily stick to equipment in transport (Whinam

Table 20.1 Categories of pathways for pest introduction with weed and invasive plant examples (Categories and definitions from Hulme et al. 2008)

Pathway	Definition	Examples
Release	Intentional introduction for release/propagation in environment	*Pueraria montana* was intentionally planted in USA during early 1900s to reduce soil erosion. Over 85 million seedlings were provided to private landowners by government agencies (Forseth and Innis 2004) For more than 70 years, (until 1980s), Government sponsored introductions into Australia comprised 145,000 accessions of 8,200+ species. Accessions include 2,200 grass (Poaceae) and 2,200 legume species, representing about twice the indigenous flora in those families and about 22 % and 18 % of global flora of grasses and legumes. Introductions targeted at improving fodder for grazing industry (Cook and Dias 2006). Gamba Grass is a species that has escaped deliberate plantings and is transforming native Australian savannahs (Rossiter-Rachor et al. 2008)
Escape	Intentional introduction as a commodity but escapes unintentionally	*Mimosa pigra* was introduced into Darwin Botanical Garden as a curiosity, escaped the garden and spread to replace 80,000 ha of native vegetation on wetlands in northern Australia (Parsons and Cuthbertson 2001) Many other exotic plant species have been brought into countries for commercial, horticultural, or ornamental purposes and this has been an important pathway of invasive plants. Examples include gorse (*Ulex europaeus*), brooms (*Cytisus* and *Genista* spp.) and olives (*Olea europaea*)
Contaminant	Unintentional introduction with specific commodity	*Avena fatua* was probably introduced into Canada as an impurity in seed and feed, over 300 years ago (Sharma and Born 1978) Most weeds of grazing systems in southern Australia and New Zealand entered country and spread as contaminants of wool, pasture seed mixes, or in emergency fodder shipments. Contaminants include most thistles (*Cirsium, Carduus, Onopordum, Carthamus*), knapweeds (*Centaurea* spp.) and fireweeds (*Senecio* spp.) (Parsons and Cuthbertson 2001)
Stowaway	Unintentional introduction attached to or within a transport vector	Marine macroalgae *Undaria pinnatifida* native to Japan, Korea, and parts of China, most likely established in New Zealand as a fouling organism on hulls of ships or in ballast water (Hay and Luckens 1987) Foreign propagules from various plant species found as stowaways on cargo equipment and containers going to Antarctica. Similarly, propagules also found on equipment and clothing (particularly

(continued)

Table 20.1 (continued)

Pathway	Definition	Examples
		Velcro) of expeditioners (e.g., *Acaena novae-zelandiae*, *Bidens pilosa*, and *Plantago coronopus*. (Whinam et al. 2005))
Corridor	Unintentional introduction via human infrastructures linking previously unconnected regions	Roadways, waterways, and railways have facilitated the spread of *Polygonum cuspidatum* and *Artemisia vulgaris* in the USA (Barney 2006 and references therein) and Scotch broom in New Zealand (Syrett et al. 1999)
Unaided[a]	Unintentional introduction via natural dispersal of alien species across political borders	*Oeceoclades maculata* is a terrestrial orchid native to Africa, and possibly South America. Except for this species, the entire genus is native to Africa. Since this plant was first discovered in Brazil 200 years ago, it rapidly migrated northward into Florida in the USA (Stern 1988)

[a]Plants transported aerially long distances between land masses as species or known to spread routinely by natural agents seaborne across long ocean distances (e.g. *Ipomoea* spp.) include no species that have become detrimental (i.e., weedy) (Mack and Lonsdale 2001)

et al. 2005), clothing (Waterkeyn et al. 2010), vehicles (Lonsdale and Lane 1994; Von Der Lippe and Kowarik 2007) and containers (Hulme 2008). Mud, which contains many plant seeds, is probably the best mechanism by which seeds stick to some of these pathways (c.f. Waterkeyn et al. 2010), but not the only mechanism. For example, propagules can also be sucked into the intake grills of refrigerated cargo containers (Fowler 2010).

All pathways for weed introduction described above can be managed with appropriate pre-border and border biosecurity measures (Chaps. 5 and 6 and discussions below). Species proposed for introductions can be screened for invasive potential with risk assessments. The risk of contaminating and stowaway taxa can be mitigated through various risk management strategies (e.g. disinfestation). Such measures are effective, particularly in Australia and New Zealand (Williams and West 2000).

20.4 Impacts of Weeds and Invasive Plants: A Review

Understanding the impacts and potential impacts of weeds and invasive plants is critical for protecting plant resources. Such understanding helps: (1) Regulators decide which species should be excluded and managed at ports and borders; (2) Land managers evaluate which species should be prioritized for eradication or management; and (3) Scientists identify and design weed management programs. Like other types of pests, pest plants have a wide range of direct and indirect impacts in natural and manmade landscapes. The concepts of direct and indirect impacts (and how they are categorized) varies (IPPC 2009; Pimentel et al. 2000;

Vilà et al. 2011), but include the costs of managing pests, mitigating pest impacts, and the costs associated with the impact of management itself.

In production systems, weeds reduce crop yield by competing for water, light, and nutrients. A study of 58 commodities in Canada estimated that about 10 % of the potential yield is lost due to weeds, resulting in a loss of about $1 billion annually for just these crops (Swanton et al. 1993). The USA and Australia report similar losses in the billions of dollars with up to 20 % of supermarket prices being due to these weed and pest management costs (Pimentel et al. 2000; Sinden et al. 2004). Other direct impacts include harvest interference and grain contamination (Smith et al. 2000). In grazing systems, weeds reduce pasture productivity by competing with desirable plants, restricting livestock access, and reducing livestock health through unpalatable and toxic weeds (Bourdôt et al. 2007; Smith et al. 2000). The economic losses to agriculture can be staggering, as well as the indirect costs associated with controlling weeds. These include the herbicide and labour costs associated with weed management, and changes in market value or access due to weed contaminants in commodities (Bridges 1994; Sinden et al. 2004).

The environmental impacts of weeds are much harder to assess, because they are difficult to express in monetary terms, and much harder to quantify and express because natural systems are very complex. In natural systems, weeds are generally referred to as invasive species because they naturalize and spread throughout the landscape. Invasive species cause diverse impacts. The most obvious impacts are the displacement of native plant species, and alteration of plant community composition (Coutts-Smith and Downey 2006). Through trophic interactions, plant invaders indirectly affect populations of all other species, including pollinators, herbivores, frugivores, and predators. Called "transformers" by some researchers, the worst invasive species readily form dense populations that reduce biodiversity, change the physical structure of the habitat, and alter ecosystem processes (Richardson et al. 2000; Vilà et al. 2011; Vitousek et al. 1987). In the process of changing the biotic and physical environment, invasive species may also hybridize with natives (Vila et al. 2000) and alter the evolutionary pathways of the species they affect (Mooney and Cleland 2001). In addition to habitat loss and climate change, biological invasions contribute to global environmental change (Mooney and Hobbs 2000; Pyšek and Richardson 2010; Vitousek et al. 1996).

Weeds also have diverse impacts in cities and other public areas where people interact with plants and the environment. The social impacts of weeds have a higher profile when there is significant overlap with dense human populations. For example, the common dandelion (*Taraxacum officinale* Wigg) is easily recognized as a weed by most people. Homeowners who maintain a lawn or garden will affirm that weeds blemish their lawns and compete with their garden plants. As a result, homeowners spend large sums of money on herbicides and other weed control products. Weeds also pose a health and safety hazard because they contribute to seasonal allergies (Wopfner et al. 2005), increase the frequency of brush fires (D'Antonio and Vitousek 1992), and are toxic to people and pets (Burrows and Tyrl 2001; Derraik 2007). Other species (particularly aquatic weeds) interfere with swimming, fishing, and hunting activities (Pieterse and Murphy 1990), or are a nuisance (CFIA 2008).

Weeds cause other less obvious issues. Weeds block transportation and utility right-of-ways making it difficult for affected companies to provide service. During storms, some species that grow along storm water drainage systems (e.g. Alligator weed) break and clog culverts or city drainage systems (Bossard et al. 2000). In developing countries and rural settings, weeds sometimes threaten the livelihoods of entire communities (Pieterse and Murphy 1990; Wilson et al. 2007). Differences in attitudes and management of weeds among neighbours can also lead to potential conflicts and stress (e.g., invasive bamboo in the USA; Chilean Needle Grass in New Zealand).

The impacts of weeds are not always confined to one sector. Many weeds impact two or three of these systems (Table 20.2). For example, a stand of *Ulex europaeus* Linn. (gorse) prevents livestock access to grazing land. It also suppresses native plant communities and access to tracks and waterways in natural ecosystems (Richardson and Hill 1998; Parsons and Cuthbertson 2001). Many other weed species have similar types of across-sector impacts (e.g. *Lygodium* and *Rubus* species) (Ferriter 2001; Parsons and Cuthbertson 2001). Such species usually rank highest in weed risk assessment and scoring systems and are most frequently targeted for control and management. Table 20.2 provides several examples of species with impacts across multiple sectors.

Table 20.2 Types of impacts of weeds and invasive plants in natural, production, and anthropogenic systems

Production systems (croplands, rangelands, pastures, nurseries, plantations, etc.)
1. Reduce grazing value of pastures and rangelands, resulting in economic losses *Euphorbia esula* (Leistritz et al. 2004), thistles (Parsons and Cuthbertson 2001), *Prosopis* spp. (Scanlan et al. 1991), *Opuntia* sp. (Grice 2006), *Nassella trichotoma* (Campbell and Vere 1995)
2. Reduce crop yield *Cyperus rotundus* (William and Warren 1975), *Imperata cylindrica* (Chikoye et al. 2007), *Lolium rigidum* (Paynter and Hills 2009), *Raphanus raphanistrum* (Eslami et al. 2006)
3. Toxic to cattle and other farm animals *Senecio* spp. (Johnson et al. 1989), *Echium* spp. (Piggin and Sheppard 1995), *Solanum* spp. (Buck et al. 1960) and other shrubs like *Lantana* spp.
4. Reduce forest productivity and increase control costs *Pueraria montana* var. *lobata* (Forseth and Innis 2004), *U. europaeus* (Richardson and Hill 1998)
5. Interfere with irrigation, reduce water oxygen and light levels, and increase evapotranspiration rates *Eichhornia crassipes, Salvinia molesta, Pistia stratiotes* and other aquatic weeds (Abdeen 2008; Mathur and Mathur 2006)
6. Allelopathic forbs disrupt nitrogen fixation and suppress recruitment of native vegetation in pastures *Carduus nutans* (Wardle et al. 1998)
7. Act as reservoirs (alternate or secondary hosts) for pest and pathogens of crops *Echinochloa crus-galli* Beauv (Tindall et al. 2004)

(continued)

Table 20.2 (continued)

Natural systems (national parks, conservation lands, wildlife preserves etc.)

1. Increase fire frequency and intensity
   Bromus tectorum (Knapp 1996), *Imperata cylindrica* (Lippincott 2000), *Andropogon gayanus* (Rossiter-Rachor et al. 2008), gorse (Richardson and Hill 1998)
2. Alter nitrogen and phosphate cycling, and ecosystem development
   Myrica faya (Vitousek et al. 1987), *Asparagus asparagoides* (Turner et al. 2011) *Cytisus scoparius* (Fogarty and Facelli 1999), *Tradescantia fluminensis* (Standish et al. 2001)
3. Change ecosystem water usage and evapotranspiration
   Acacia mearnsii (Dye and Jarmain 2004)
4. Convert plant habitats from one type to another (e.g., prairies to forests, mud flats to grass swards)
   Melaleuca quinquenervia: (Rayamajhi et al. 2009; Schmitz et al. 1997), *Spartina anglica* (Nehring and Hesse 2008), *Agrostis stolonifera* (Gremmen et al. 1998), *Pinus* spp. (Richardson 1998)
5. Reduce reproductive success and survival of native plants and animals species
   Lythrum salicaria (da Silva and Sargent 2011; Grabas and Laverty 1999), *Chromolaena odorata* (Leslie and Spotila 2001), *Cryptostegia grandiflora* (Tomley 1995)
6. Intercept light availability and prevent native plant recruitment underneath
   Nymphoides peltata (Kelly and Maguire 2009), *Syzygium jambos* (Avalos et al. 2006), *Tradescantia fluminensis* (Standish et al. 2001), *Miconia calvescens* (Meyer 1996)
7. Reduce plant diversity
   Pennisetum clandestinum (Heyligers and Adams 2004), *Miconia calvescens* (Meyer and Florence 1996), *Ageratina riparia* (Fröhlich et al. 1999)
8. Change abundance and distribution of native plants and animals through trophic interactions
   Mimosa pigra (Braithwaite et al. 1989), *Hymenachne amplexicaulis* (Houston and Duivenvoorden 2002)

Anthropogenic systems and cultural impacts (cities, parks, right-of-ways, etc.)

1. Reduce water yield from mountain catchment areas
   Hakea species (Le Maitre 1996; van Wyk 1987)
2. Disrupt power to communities and endanger train lines
   Pueraria montana var. *lobata* (Forseth and Innis 2004; McNeely 2001)
3. Alter the appearance of natural landscapes, affecting aesthetics and ecotourism
   Hakea gibbosa (Le Maitre 1996), *Spartina anglica* (Kriwoken and Hedge 2000), *Pinus* spp. (Richardson 1998)
4. Induce severe dermatitis in humans
   Heracleum mantegazzianum (Derraik 2007)
5. Limit access to recreational areas for boating, fishing, hiking, hunting, etc.
   Nymphoides peltata (Countryman 1970), *Eichhornia crassipes* (Wolverton and McDonald 1979)
6. Compete with and displace desirable garden and landscape plants
   Lonicera japonica (Daves Garden 2011)
7. Aesthetically displeasing in lawns and turf grasses
   Taraxacum officinale (Holm et al. 1997)
8. Damage homes and other property
   Hedera helix (Daves Garden 2011)

Allergen sources

Ambrosia artemisifolia and *Lolium spp*. (Léonard et al. 2010; Freidhoff et al. 1986)

Spiritual and cultural values of indigenous peoples

Cenchrus pennisetiformis (Marshall et al. 2011)

Some weeds are beneficial in one type of system and detrimental in another. For example, of 463 tropical pasture grasses introduced into Australia between 1947 and 1985, 17 were considered valuable fodder species in pastures and weedy in other ecosystems (Lonsdale 1994). *Caulerpa* is an economically important genus because it is used as an ornamental in salt-water aquaria (Frisch and Murray 2002; Walters et al. 2006). However, it is highly invasive in marine ecosystems, causing negative environmental and economic impacts (Levi and Francour 2004; Muller 2000; Relini et al. 1998). When introduced and established in a country, plants that are both beneficial and harmful cause conflicts and challenge regulatory agencies pressured by opposing interest groups to take different kinds of action (i.e., regulate or don't regulate). We will return to the issue of these conflict species under management challenges below.

20.5 Risk Assessment and Management Across the Biosecurity Continuum

Weed management encompasses diverse approaches. Like other types of pests, the approach taken depends on whether the species is present or absent in the area at risk. The overwhelming human desire to intentionally introduce and cultivate plants poses particular challenges for weed managers and complicates approaches to weed management. Here we explore some of these challenges, and the general approaches used in weed risk assessment and management.

20.5.1 Conventions, Legislation, Policy and Regulations

Strategies to manage weed risks are determined by international conventions/treaties, national legislation, local laws, and agency policy/responsibility. International conventions are a basis for global principles and guidelines for managing threats to humanity. Certain conventions relating to pests aim to minimise the risks of their introduction (accidental or deliberate) and impacts. They achieve this through three mechanisms:

1. *International Treaties and Agreements* (e.g. around preventing introductions, undertaking eradications where appropriate, and reducing environmental impacts – i.e. the Convention on Biological Diversity, Article 8(h), Decision XI/23, Decision X/31, and Aichi target 9 within the Strategic Plan 2011–2020) www.cbd.int/decision/cop/?id=7197.
2. *Regulatory Approaches* (e.g. International Marine Organisation ballast water convention and regional biosecurity systems) globallast.imo.org/index.asp?page = mepc.htm.

3. *Frameworks and Guidelines* for Risk Assessment (e.g., Import Risk Assessment (IRA) under the International Plant Protection Convention, www.wto.org/english/tratop_e/sps_e/spsagr_e.htm, and risks associated with trade).

The impacts of invasive species generally have not achieved headline recognition under global sustainable development through the United Nations (e.g. the Rio and Rio + 20 Declarations).

National weeds-based legislation and regulatory responsibility originated in national and state departments of agriculture, because of direct economic impacts of weeds to agriculture. More recent recognition of the environmental impacts of weeds has led to a greater role for weed management in government departments with responsibility for the natural environment and water (e.g., the Working for Water program in South Africa). Legislation generally addresses the pre-border importation and post-border control and management of noxious plants. Such legislation invariably adopts a blacklist or prohibited-list approach which lists species that cannot be imported or moved post-border. All other species have unrestricted entry assuming they meet all other regulatory criteria including freedom from plant pests. For this regulatory approach to be effective, all plant taxa likely to be imported must be evaluated before listing. The USA and Canada use prohibited lists and national plant protection organisations take responsibility for such lists. Under legislation relating to environmental protection some sea-locked countries (Australia and New Zealand), have adopted a white-list or permitted-list approach in which only plant species on the white-list may be imported (Sheppard et al. 2003; Roberts et al. 2011). This approach is more precautionary and allows evaluation of species, as requests for importation are submitted. The white-list approach also defines knowledge on which species are present so detections can quickly be identified as new arrivals.

These two approaches for weed regulation are fundamentally different. Ultimately, the permitted-list approach represents a more secure biosecurity approach but encounters more resistance from plant importers.

When a weed establishes within a country, regulatory processes are relatively consistent across countries and jurisdictions. Weeds are usually categorised based on their distribution, abundance and impacts. Specific regulatory requirements are applied to each category around eradication, control, management or containment. When noxious weeds become too widespread to effectively regulate, they are generally categorised as “widespread weeds” or some similar status, and are deregulated. Subsequently, they may still be subject to local legislation and laws, particularly in areas where they are not yet present or common.

International conventions, national legislation, policies and regulatory processes to assist prevention and management of the impacts of invasive species are always changing to reflect improved processes and understanding about weed risks and impacts, and to reflect changes in societal values.

20.5.2 Pre-border: "An Ounce of Prevention Is Worth a Pound of Cure"

Managers and economists accept that preventing the introduction of invasive species is a far more effective strategy than ongoing species management (Leung et al. 2002; Pimentel et al. 2005; Thomas 2001; Westbrooks 1991). The primary aim of pre-border biosecurity strategies is to prevent the entry and establishment of pests. Prevention strategies require a mechanism for identifying foreign weeds posing a potential risk and for listing them to prevent their entry.

Weed Risk Assessment (WRA) is a process used to evaluate the likelihood of the introduction and spread of a plant species, and of its potential consequences should the species become established in the area at risk. Like other types of Pest Risk Assessments, WRAs are tools that allow regulators and managers to make informed decisions that will reduce future economic and ecological harm caused by weedy and invasive plants. The concept of risk assessment has been applied for a long time in the fields of business, finance, and human health (Chap. 9). During the last 20 years it has been applied to plant pests and weeds (Esler 1988; Forcella and Wood 1984; Hazard 1988). Weed Risk Assessments used to identify or screen plants for invasiveness are known as pre-border WRAs. The first WRA tool was developed in the late 1980s for screening plant importations for invasiveness (Hazard 1988). Since then, additional WRA systems have been developed: Some for small geographic areas (Jefferson et al. 2004; Widrlechner et al. 2004), others for specific taxonomic or ecological groups of plants (Reichard and Hamilton 1997; Rejmánek and Richardson 1996; Tucker and Richardson 1995), and others with a much wider scope (Champion and Clayton 2000; Lehtonen 2001; Panetta and Mitchell 1991; Pheloung et al. 1999).

A popular pre-border screening tool is the Australian WRA, which consists of 49 predominantly "yes/no" questions about a species' traits and its status elsewhere in the world (Pheloung et al. 1999). The questions evaluate whether a plant possesses traits typically associated with weedy and invasive species; the higher the risk score, the more likely a given plant will become invasive in the WRA area. The Australian WRA has been modified and tested globally for use in nearly a dozen areas. In each case, the questions relating to climatic compatibility are modified to identify species capable of establishing in the local climate and environment. Overall, the Australian WRA has demonstrated its ability to consistently identify invaders (e.g., Daehler et al. 2004; Gordon and Gantz 2008; Nishida et al. 2009). However, it is better at identifying major-invaders than non-invaders (e.g., mean ~90 % vs. 70 % accuracy; Gordon et al. 2008).

Scientists with the USDA, Plant Protection and Quarantine (PPQ) recently developed and validated a WRA model for the USA. This new model (based on the Australian WRA) increased non-invader accuracy to 97 % and maintained major-invader accuracy at 94 % (Koop et al. 2012). The PPQ screening tool uses a logistic regression model to evaluate the likelihood a plant species will be invasive. Model results are expressed in terms of low risk, evaluate further, and high risk. A unique feature of the PPQ WRA is its evaluation of the consequences of uncertainty on the final risk score. That is, it determines which other risk scores are

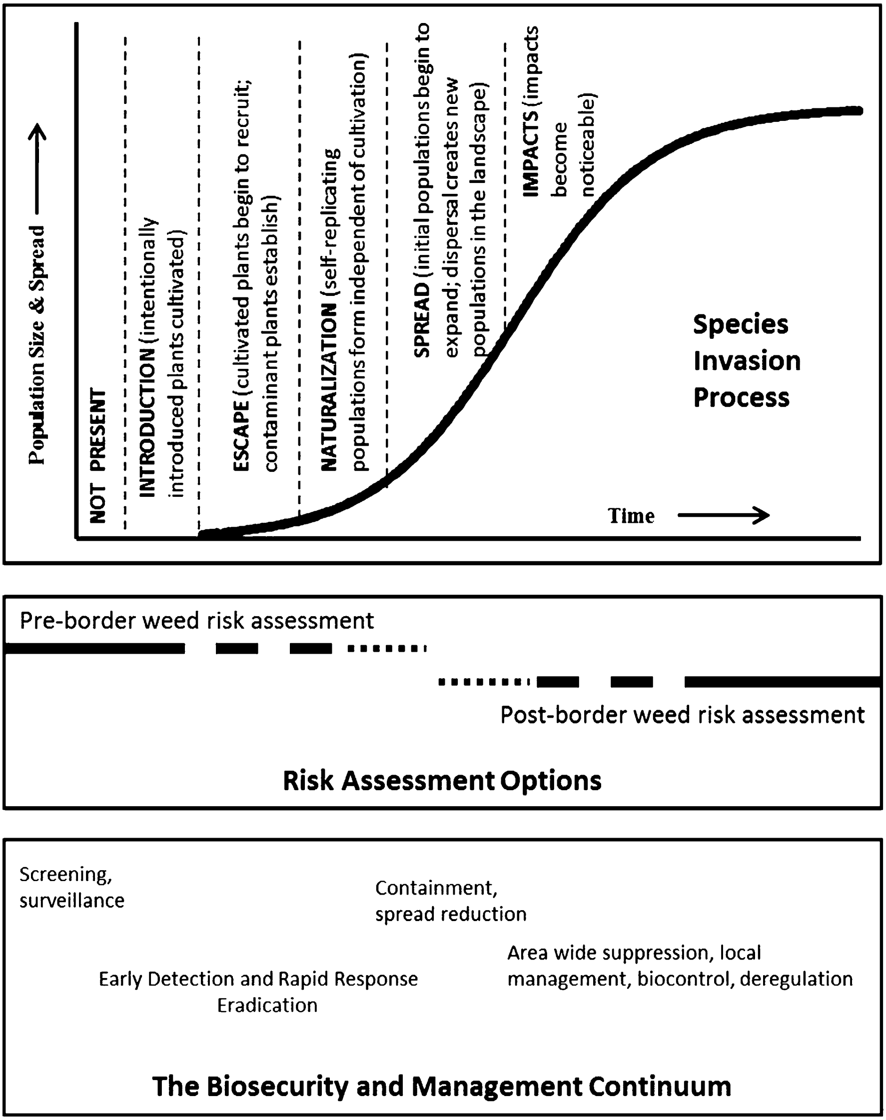


Fig. 20.1 Relationship of the species invasion process to risk assessment, biosecurity, and management options

possible if the questions were answered slightly differently based on the level of uncertainty associated with each question. Uncertainty is a fundamental aspect of risk that must be considered because our knowledge about the factors that contribute to risk is never perfect (National Research Council 2009).

The use of screening tools to evaluate weed risk potential has become very popular and is generally believed to be an important part of a country's biosecurity strategy (Fig. 20.1). Assuming all necessary information is available, WRAs can be easily conducted and incorporated into a regulatory framework. However, several authors argue that most WRAs are rather simplistic and cannot truly predict invasiveness because they focus primarily on species traits (Noble 1989). They add that the outcome of species introductions is determined by complex interactions among several types of factors: Species traits, environmental properties, propagule pressure, chance events, biotic interactions, and anthropogenic factors (Daehler and Strong 1993; Kolar and Lodge 2001; Lodge 1993; Mack 2005). Further, because the probability of an introduced species becoming invasive or weedy is inherently very low (i.e., the base rate of the probability), the reliability of predicting a rare event will be low (Caley and Kuhnert 2006; Hulme 2011). This has led to arguments that WRA lacks scientific rigour and that a paradigm shift in weed biosecurity is necessary (Hulme 2011), but this has not halted its widespread adoption by regulatory agencies.

Climate matching software and ecological niche modelling represent another class of tools that can be used to evaluate one component of the risk potential of a species: Habitat suitability in the areas at risk (Lindgren 2011). These approaches identify environmental and climatic variables associated with a species' distribution, usually in its native range, and then project those variables onto a different region where the species may invade or is invading (Peterson 2003). They produce risk maps that identify which regions are ecologically suitable for invasion. Differing in the specific methodologies used and assumptions made, numerous ecological niche models have been developed (e.g., BIOCLIM, GARP, MAXENT, etc.). All of them rely on occurrence or presence/absence data that are readily available today on Internet databases (e.g., The Global Biodiversity Information Facility, http://data.gbif.org) (Elith et al. 2006; Peterson 2003). The outcomes from such models must be treated with extreme caution however, as a matched climate is a very simplistic way of assuming a species' potential distribution (Kriticos and Randall 2001). More sophisticated models (e.g. CLIMEX/DYMEX) include habitat and reproductive requirements of particular weeds using GIS layers of soil type, aspect, vegetation, and other variables (Kriticos and Randall 2001). Through climate matching, it may prove possible to estimate future potential biogeographic impacts on agriculture or biodiversity and estimate bio-economic impacts in space and time (Venette et al. 2010). At a broader scale, climate matching can also be used to match regions with similar climates and thus identify potential sources of invasive species (Thuiller et al. 2005; Kriticos 2012). This can be particularly valuable for port authorities charged with border biosecurity and who must prioritize their limited resources on the riskiest pathways.

20.5.3 Post-border Strategies

Despite best efforts to prevent the entry of weeds and invasive plants, some will always cross national borders. A comprehensive biosecurity framework also needs post-border strategies to address new detections and ongoing invasions within countries. Post-border strategies for plants are particularly important because many species (thought benign) are intentionally introduced and cultivated in environments suitable for their establishment. Sometimes, plants remain "well behaved" for many decades before showing any evidence of naturalization, spread, or impact (Crooks 2005; Groves 2006). This is often called the "lag phase of invasion" (Crooks 2005). Unfortunately, by the time an invading species is noticed, it may be too widely distributed and naturalized to eradicate or contain (Forcella 1985).

High costs associated with ongoing management of weeds have prompted some workers to promote the concept of Early Detection and Rapid Response (EDRR) as a framework for invasive species management (FICMNEW 2003; Westbrooks 2004). The basic premise of this strategy is that weedy species caught early in the naturalization phase of invasion are more readily eradicated (if that is the goal) than species that are more widely distributed across the landscape (Harris and Timmins 2009; Simberloff 2003). EDRR is a cost-effective and environmentally-sound approach because it does not restrict trade. EDRR only targets naturalized species, and has minimal impact of control measures if applied early (Westbrooks 2004). Furthermore, eradicating small naturalized populations, even if their future risk is uncertain, is much more cost-effective than waiting until they are more firmly established and distributed (Harris and Timmins 2009). EDRR management strategies incorporate the following elements: (1) Early detection and reporting; (2) identification and vouchering; (3) risk assessment; (4) planning; (5) rapid response, if warranted; and (6) monitoring and review (Westbrooks 2004; Wotton and Hewitt 2004). For an EDRR response system to be effective, a plan must be in place and explain how to respond to new incursions. The explanation must include tools with which to respond, and the capacity and resources to carry out the response (Wotton and Hewitt 2004).

Perhaps the most difficult aspect of EDRR involves establishing a centralized communication and reporting network, and finding groups with some basic plant identification skills that can routinely survey regions at risk. EDRR communication and database networks should allow users to: (1) Record new observations of pests, (2) upload descriptive information, photographs, and georeferenced data, (3) automatically report new records to relevant authorities and identifiers, and (4) plot presence/absence data on a map (Bowen 2007; Simpson et al. 2009). A critical aspect of these networks is the ongoing participation of relevant agencies and groups (e.g., state agricultural departments, local park managers, native plant societies, gardening clubs, science museums, taxonomists, extension personnel), each contributing specific components to the EDRR framework (Lambert 2004). Invasive plant detection networks can incorporate trained citizen volunteers to regularly monitor areas (e.g., The Invasive Plant Atlas of New England, http://nbii-nin.ciesin.columbia.edu/ipane/). Smart phone applications ("apps") that record location, photographs, and other information have been developed to assist workers and volunteers submitting information to EDRR networks (Box 20.1). In the USA, several networks have been created but most have a regional focus (Simpson et al. 2009). The Cooperative Agricultural Pest Survey (CAPS), sponsored by the USDA APHIS, has a national focus but only involves federal and state agricultural agencies (http://pest.ceris.purdue.edu/). In Australia such networks are operated by state or local agencies with weed surveillance responsibilities, based on agreed lists of national and regional alert weeds (i.e. new weeds that are considered high likelihood of detection and risk for a nation or region but have not yet been detected http://www.weeds.gov.au/weeds/lists/alert.html).

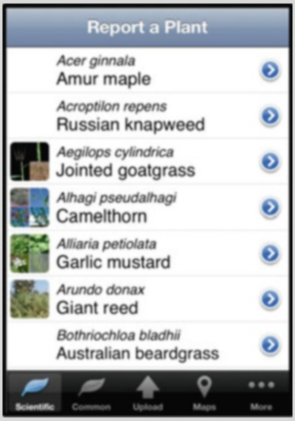
When detected, weed species should be evaluated to determine their risk potential. Post-border WRAs are as diverse as pre-border assessments (e.g., DPI 2008; Hiebert and Stubbendieck 1993; Morse et al. 2004; Virtue et al. 2006; Crossman and Bass 2008). While post-border WRAs examine many of the same kinds of plant traits and risk factors as pre-border WRAs, they tend to focus less on behaviour elsewhere, entry potential, and establishment potential, and more on impacts, ratio of current to potential distribution, and feasibility of control. When complete, managers can decide which actions or management options are appropriate in each case. Management options include eradication, containment, suppression, and no action. In Australia, the detection of a national alert weed for the first time requires a state or national response that usually starts with an eradication program (Panetta et al. 2011).

Field survey data (Dewey and Andersen 2004) on species distribution are valuable for decision makers because they influence management decisions. However, information on the extent of cultivation within the area can also be very useful because these will act as sources for reinvasion into the landscape. When the risk potential of a species is understood, risk managers can then prioritize species, or certain populations of the species, for management as there are never enough resources to manage everything.

Species eradication offers an attractive alternative to perpetual species management (Simberloff 2003). However, eradication is not simple and represents the complete elimination of all reproductive individuals and potentially viable propagules from an area (Regan et al. 2006). To be successful, eradication campaigns usually require substantial funding, long-term commitment, and support from all parties involved, including private landowners (Gardener et al. 2010; Myers et al. 2000; Regan et al. 2006). For example, out of 30 eradication pilot projects on the Galapagos Islands, only four succeeded. The four eradicated species all occurred in less than 1 ha, occurred on only one landowner's property, and did not possess any long-term seed dormancy (Gardener et al. 2010). Managers understand that species with relatively restricted distributions (i.e., <1000 ha) stand the best chance of being eradicated (Rejmánek and Pitcairn 2002). However, large infestations can also be eradicated under some circumstances. Australia has had only a few successful weed eradication programs. Nevertheless, eradication programs remain a common EDRR strategy for new discoveries of alert weeds in Australia.

If eradication is not feasible, then managers may pursue other broad tactics including species containment, spread reduction, area wide suppression (Myers et al. 2000) or asset protection (Grice 2009). Again, the best strategy will depend upon various factors including: The species' biology; its potential for spread; current distribution with respect to natural and political boundaries; effectiveness of management strategies; financial, logistical, and political support; and resources at risk. Species caught early in the invasion process may be contained or at the very least their spread slowed (Fig. 20.1). In these cases, identifying and eliminating the most invasive populations (i.e., source populations) may be the most effective approach (Maxwell et al. 2009). For more widely distributed species, perpetual population suppression/control is often the only option left, but may prove uneconomic. This strategy is often carried out by local resource managers and agricultural groups (farmers, nurseries). In these cases, plant populations may be managed through a combination of physical, chemical and biological control. Plant populations and species posing the greatest threats can be identified and prioritized for management (Grice 2009; Skurka Darin et al. 2011). In Australia, when a weed is considered beyond the capacity for area-wide suppression, managers resort to applying weed management only to those areas under threat that have high asset value e.g. due to the presence of regionally or nationally relevant rare or threatened species or communities (Turner et al. 2010; Grice et al. 2011).

Box 20.1 The Missouri River Watershed Coalition Mobile Phone Application



(continued)

Box 20.1 (continued)

Sophisticated mobile phones allow third party applications that enhance Early Detection and Rapid Response (EDRR). These applications enable mobile phones to:

- Display information normally contained in field guides
- Access information stored in centralized databases
- Provide diverse tools for plant identification
- Take photographs of plants and their surrounding habitat
- Obtain and record plant location (e.g., lat/long) with the built-in GPS unit
- Provide a template for recording field information
- Submit occurrences wirelessly to centralized information networks
- Provide contact information for relevant authorities and experts

These types of mobile phone applications are an important resource for weed managers working in the field and greatly facilitate their jobs. However, these apps also allow private citizens who don't work in weed management to contribute to EDRR networks. This is critically important because many more private citizens are potentially interested in conservation and invasive species, than there are weed managers. With mobile phone applications citizen scientists and conservationists can form a vital component of invasive species detection networks.

Several mobile apps have been developed to help detect, identify and report species in the field. Here we showcase one that was developed for the Missouri River Watershed Coalition (MRWC). The MRWC is comprised of multiple agencies in six states responsible for managing the watershed. The app was developed by the Center for Invasive Species and Ecosystem Health

(continued)

Box 20.1 (continued)
(University of Georgia), and the Center for Invasive Plant Management (Montana State University-Bozeman). The MRWC app includes a database of weed factsheets searchable by either their scientific or common names (top image). This app allows users to record and upload weed observations to the Early Detection & Distribution Mapping System (EDDMapS) database (mid image). Location information can be either automatically generated by the phone or it can be manually entered with the aid of Google Maps. Photographs can be appended to the record as well. Finally, the MRWC also allows users to retrieve distribution information in the form of a map from the EDDMapS database (bottom image).

20.5.4 Biocontrol and Integrated Weed Management

Biocontrol in weed management has a broad definition around the use of biological (rather than chemical) methods to manage weeds. For example, such approaches include the use of mycoherbicides; the formulations of plant pathogens that can be applied like chemical herbicides for "once off" treatment of weed infestations. Classical biological control of weeds has the longest history and overall success, and is a management strategy where natural enemies of weeds are imported from the plant's native range, and released to control a weed in its exotic range. Classical biological control of weeds is built on accepted ecological theory: Alien weeds may have become invasive because they have escaped their native natural enemies in their native range (Maron and Vilà 2001; Keane and Crawley 2002). Where successful, biocontrol is a self-sustaining, cost-effective and low-risk tool for weed management, with released biocontrol agents suppressing the invasive weeds to lower or non-impactful densities.

Classical biological control has been practiced for over 100 years. A review of completed programs in Australia, South Africa, New Zealand, Hawaii and Mauritius (Myers and Bazely 2003) shows that more than 75 % (range 50–85 %) of target weeds have been significantly or permanently controlled. Chance of control success is not particularly constrained by target life-history strategy or genetic variability (Chaboudez and Sheppard 1995) and climate or continent (Julien et al. 1984).

Biological control is more effective than other kinds of weed control because the efficacy is widespread and permanent. Controlled targets cease to be a concern to society. The released biological control agents (usually highly specific insects or plant pathogens) provide widespread control through natural spread or explicit distribution programs. Only biological control can provide effective and long-term control of a target invasive weed with very low follow-up costs. A limitation of biological control as a weed management strategy is the high initial research and development costs. Most biological control programs take 10–20 years before the first signs of success. The method cannot provide a quick solution but can provide a permanent one. Unlike herbicides, it is completely "target specific" and so weed replacement (sometimes by something worse) is also a frequent perverse outcome. The ecological risks of introducing one exotic organism to control another also attracts criticism (Louda et al. 2003), but more than 100 years of publicly available data, shows the benefits far outweigh the risks. All direct non-target impacts are, using current internationally accepted risk assessment protocols, highly predictable. Where biological controls have occurred, they have been accepted as part of the independent decision process for permitting release (Sheppard et al. 2003).

For all these reasons, biological control remains the only option available for widespread permanent reductions in the impacts of alien invasive plants. Further, it has also been used as an effective strategy to slow weed spread through limiting seed production (Paynter et al. 1996). Biological control has been used successfully against all growth stages and life history types of weeds and for weeds affecting agro-forestry or the natural environment. Aquatic weeds have been more successfully controlled than terrestrial weeds, but some studies suggest that the main factor limiting the long-term effectiveness of a biological control program is the duration and level of investment (Fowler 2000). That is, achieving effectiveness simplifies to a simple decision around cost over benefit.

Many studies have favourably evaluated the effectiveness of weed biological control (Myers and Bazely 2003). Where negative impacts have been headlined, they have been far outweighed by the economic benefits to agriculture and the eased capacity to manage plant invasions in natural ecosystems (Meyer et al. 2012). Biological control will be less effective when the target is only one of several alien plants impacting one type of agriculture or one location in the environment. Successful classical biological control programs against weeds have occurred on every continent where practiced. Successful controls include prickly pear cactus throughout the world, water hyacinth control in most situations worldwide, various thistles and knapweeds in pasture, paperbark trees in Florida and more than 20 other examples (Myers and Bazely 2003). With increasing incidence of herbicide resistance and de-registration, biological control remains the critical weed management tool into the foreseeable future for long-term weed control of widespread weeds, particularly in natural ecosystems.

Biological control also can be integrated with other weed management programs when it does not independently generate the desired level of control. Biological control may suppress the invasiveness of particular weeds, through reduced competitiveness or seed production, to the point where conventional weed control options (such as chemical, mechanical or strategic- grazing based strategies) provide the required level of control. By designing strategies that do not interfere with biological control agent effectiveness, the desired level of weed control may be achieved, particularly for high-impact weeds or in habitats sensitive to other types of management strategies (Huwer et al. 2005).

20.5.5 Conflict Species

Opinions vary concerning the harmful impacts and benefits of a particular introduced plant species. The ambiguity is an inherent consequence of intentional introductions that have led to intended positive and unintended negative impacts. When harmful alien plants also generate economic benefits then a conflict-of-interest around a permanent effective control solution (e.g. biological control) has arisen, and will continue to arise (Friedel et al. 2010). This presents a real challenge, from a regulatory and weed management perspective. Most invasive plants were deliberately introduced for a perceived agricultural, forestry, horticultural or ornamental benefit (Groves and Hosking 1997). Often the benefit is not realised; occasionally such species have negative impacts on the environment. The conflict arises when they have both a benefit in the intended sector (e.g. agriculture), and a harmful impact in another sector, (i.e. on natural ecosystems). Many examples occur – most notably in certain pine, forage shrub and grass species. A high-profile example is Buffle Grass (*Cenchrus ciliaris* Linn.) from Africa and introduced into Australia as a forage species. Buffle Grass is invading enormous undisturbed tracks of central Australia and outcompeting native aboriginal food plants. Also, it is changing landscape appearance through standing biomass and generating fire risk. In its intended pastoral agricultural setting, Buffle Grass remains highly valued as a fast-growing nutritive species if well managed and is tolerant of high-grazing pressure (Marshall et al. 2011). The impacts of invasive Australian acacias in South Africa or pines in New Zealand, where these plants are valued for their wood/timber production, are other examples. We must find effective regulatory processes to manage the negative impacts of such species, while exploiting their benefits.

Unfortunately, ideal nursery species also make good invaders. Crop breeding is increasingly undertaken to enhance plant traits that would improve the species capacity to persist and multiply in more stressful environments (Blum 1988). The global energy crisis and the associated developing green economy (particularly around biofuels and biofactory plants) is also in the process of pushing a suite of "new" cultivated plants onto our landscapes. We must avoid the mistakes made around historic tropical grass and forestry/horticultural tree introductions. We must introduce and plant new genotypes in a manner where whole new suites of future negative environmental impacts are avoided (Sheppard et al. 2011). We need smart regulatory processes to manage future potential risks, or society will quickly lose its grip on the global environmental sustainability challenge. Internationally, new crop breeding and new crop importing and planting accepted codes of conduct may be required.

20.6 Summary and Conclusions

We have described what constitutes a weed and the major drivers and pathways of plant invasions. We briefly characterised the impacts of weeds with multiple examples (Table 20.2). Finally, we described a range of biosecurity frameworks for weeds present or not present in the country and at differing stages of invasion. Options are also presented for regulating/managing plant invaders and why weeds present unique challenges compared with other noxious organisms. We conclude with several weed case histories, that include examples of different weed life forms, weeds at different stages in the invasion process, weeds that have a range of different impacts and weeds that require a range of different control strategies (from eradication to area-wide management). We also include examples of weed scenarios that are controversial because of the economic or environmental benefits they provide.

Weeds are challenging biosecurity targets because they are more likely to be introduced deliberately, and offer some benefits to society, compared with other kinds of invasive organisms. For introduced plants, regulatory agencies must weigh potential benefits with their potential impacts as weeds and invasive species. Some managers muse that they are not doing their job when not being sued by both opposing groups. Risk assessors and managers are challenged by plants without history of introduction elsewhere; such plants are difficult to evaluate due to invasion lag phases. A species may be well behaved in its native range, but when introduced to a location away from regulating organisms, it becomes problematic. Thus, an early detection and rapid response program is critical. Currently, however, many regulatory systems do not apply the same EDRR imperative for new plant detections as they do to other pests, probably because potential new weed impacts will be slow to realise. The long-term economic, social and environmental impacts of weeds often far outweigh the impact of pests (Pimentel et al. 2000); delaying strategic response because weeds are slow at spreading may prove counterproductive. The false perception is that there is more time for a response; in reality the time window for eradication for some weeds (e.g. *Striga* and *Orobanche* spp., Parsons and Cuthbertson 2001) may be as short as for other pests. Weeds therefore offer unique biosecurity challenges around societal perceptions of impacts and ease of control.

Some groups advocate draconian restrictions on movement of plants or plant parts, but this is not in the general interest of society. Importation, domestication, and cultivation of plants are important to society for many reasons. Still, we should not blindly import new plants without considering their potential consequences. Regulatory mechanisms are available to reduce the risks of importing potential weeds and invasive species, either accidentally or deliberately. We have discussed the most widely applied of these in this chapter. Many opportunities exist for improving the efficiency of such mechanisms and the decision-making process through the better application of science (Hulme 2009). Opportunities include improving weed prediction tools through ecological, biogeographic and climate modelling (Kriticos 2012). Broader use could be made of “white lists” developed from evidence-based scientific approaches. Easier mechanisms for species detection and disinfestation at borders and optimal post-border surveillance strategies designed around knowledge of ecology of target weeds could also be developed.

For naturalised potentially invasive plants, the science-based mechanisms of post-border risk assessment are becoming widely adopted through national and regional post-border biosecurity protocols. This trend must continue to ensure optimum use of scarce resources available for response to new weed threats. Also, science and science-based processes should be used when considering the optimal risk-management strategy for a given plant. Weed management options are continually changing as herbicides are now being de-registered in many countries faster than they are being developed. Compounded by increasing levels of herbicide resistance in weeds, new management systems may be required that are not herbicide dependent, but built more on other options such as robotic mechanical control or novel biological control strategies.

The scientific understanding of plant invasions is expanding rapidly. New science-based approaches to biosecurity that address current and future weeds are continually being developed and improved. Science must inform policy and provide policy-relevant and regulation-ready tools, and not just academic knowledge or impractical ideas. Insufficient invasive plant science currently is focused on practical solutions to recognised biosecurity needs. If regulators don't see the benefits of this science they will continue to apply best-bet or hunch-based policy instruments.

Acknowledgements We thank Cory Lindgren (Plant Health & Biosecurity Directorate CANADA) and Richard Hansen (USDA-APHIS-PPQ-CPHST) for contributions to the development of this chapter. Craig Ramsey (USDA-APHIS-PPQ-CPHST) developed the case study for Cogongrass.

Appendix. Case Studies

Case studies provide examples of the diverse range of weeds, highlight some challenges they pose and discuss approaches for weed management. Species described below do not represent the worst or most significant weeds or invasive plants in the world. Plants were selected as examples of the wide range of life forms, pathways of entry, impacts, invasion status, and management strategies for weeds.

A.1 Andropogon gayanus *Kunth. (Poaceae): Gamba Grass*

This grass is a good example of a species intentionally introduced to support economic development, but later became a significant invader. Gamba Grass was deliberately introduced into Australia as a tropical pasture grass to increase productivity of the tropical grazing industry. If well managed in pastoral systems, it can augment livestock production because it is a fast-growing, high-biomass grass. But its negative impacts far outweigh potential benefits. Gamba Grass has become one of the worst ecosystem-transforming weeds of Australia, particularly in tropical latitudes of Northern Australia (Rossiter-Rachor et al. 2008). This species recently was declared an Australian Weed of National Significance and requires a national management strategy.

Gamba Grass transforms native communities by altering the natural grass-fire cycle of northern tropical savannahs. Australian tropical savannahs are tree-dominated. Frequent, low-intensity fires during the dry season spread quickly through the short grass herb layer leaving the trees intact. Gamba Grass grows through the wet season, attaining several metres in height and generating many times the biomass of native grasses. During the dry season, the tall, dried grass layer increases the fire intensity by a factor of 8 and wicks the high flames into the tree canopy. This kills the tree canopy and changes the ecosystem into a tree-free, high biomass grass-dominated savannah to which none of the native species are adapted. Hotter fires also alter the nutrient, water and carbon cycles of these ecosystems. Less carbon is stored and more CO_2 is released into the atmosphere (Rossiter et al. 2003). These effects are extremely difficult to manage on the scales they occur and the affected land management agencies spend most of their resources preventing the spread of Gamba Grass into new areas. Gamba Grass generally spreads into areas along water courses with little hope for the recovery of affected ecosystems without a specific control option for this grass. Biocontrol is now being considered but may face strong opposition from the large livestock enterprises of northern Australia that see this grass as being beneficial to their industry.

A.2 Asphodelus fistulosus *L. (Liliaceae): Onion Weed*

Pest status is never static and changes as species spread and interact with local environments, as additional information becomes available, and as human values and perspectives change. Onion Weed is an herbaceous annual to perennial plant that forms dense clumps of onion-like leaves up to 0.75 m tall (Parsons 1973). It is native to dry regions of the Mediterranean region eastward into Central Asia, and has been introduced to other countries including the USA and Australia (NGRP 2012). It is particularly well suited to dry, open conditions. Onion Weed reproduces by seeds that are readily dispersed by wind, water, animals, and machinery (Parsons 1973).

Normally, Onion Weed has difficulty establishing in areas dominated by plants with shallow roots due to strong competition for water at the soil surface. However, under disturbed conditions, plants readily establish (Parsons 1973). When Onion Weed establishes, it can dominate an area, particularly in grazed rangelands because it is unpalatable to livestock. The carrying capacity of many of the drier rangelands in southern Australia have been depleted by as much as 75 % due to this plant. This species may also threaten biological diversity (Coutts-Smith and Downey 2006).

Onion Weed was considered a significant weed in Australia (Parsons 1973), but recent data suggests it may not be as important (Pitt et al. 2006). This change in status highlights the importance of following initial observations of invasive plant behaviour and impact with rigorous scientific studies. In the USA, Onion Weed is viewed as an emerging and potentially significant weed (Holm et al. 1991). Data from monitoring surveys indicates it is spreading in the USA (Craig Ramsey, unpublished). Onion Weed is a U.S. Federal Noxious Weed (7 CFR § 360 2012). Typical of most widely naturalized weeds, there is no national effort to eradicate or control this species. Rather, control is left to local management agencies. Fortunately, Onion Weed can be easily managed under proper conditions (Parsons and Cuthbertson 2001).

A.3 Caulerpa taxifolia *(M. Vahl) C. Agardh 1817 (Caulerpaceae): Caulerpa*

The pet and aquarium trade is an important source of many invasive aquatic weed species, including marine algae. Algae fall outside the regulatory scope of some National Plant Protection Organizations (NPPOs), but they can have significant impacts on national resources. *Caulerpa* is a genus of macrophytic green algae that is distributed throughout tropical and subtropical marine habitats worldwide (Guiry and Rindi 2005). These seaweeds are important in the marine aquarium industry and are sold in retail stores as live plants for home and commercial aquaria (Frisch 2003). *Caulerpa taxifolia* has a nearly global distribution, including Florida, the Caribbean, Hawaii, and other pacific islands (Guiry and Rindi 2005). The taxonomic status of *C. taxifolia* is in constant flux (Chisholm et al. 1995; Meusnier et al. 2002; Olsen et al. 1998). It includes a complex of sibling species and subspecies that must be teased apart with genetic, morphological, and common garden studies (Benzie et al. 2000; Meusnier et al. 2002) due to large variation in plant morphology.

Unlike the native population the Mediterranean strain is asexual and is the most widely known invasive form of *C. taxifolia*. This strain forms dense monospecific meadows that reduce biodiversity and alter habitat structure and fish communities (Levi and Francour 2004). Molecular studies show the Mediterranean strain most likely originated from coastal waters of Australia (Meusnier et al. 2001, 2002). It was first detected at Monaco, in 1984, in front of the Oceanographic Museum, covering only a few square meters, where it was cultivated (Meinesz and Boudouresque 1996). In hindsight, eradicating it then would have saved countless dollars in control efforts and prevented the degradation of entire communities. Unfortunately, action was delayed (Simberloff 2003). By 2001, *C. taxifolia* had spread to several other Mediterranean countries and covered over 32,000 acres of seafloor in Spain, France, Italy, Croatia and Tunisia (Williams and Grosholz 2002).

In 1998, about 100 scientists alerted the Secretary of the U.S. Department of Interior concerning the potential threat of *C. taxifolia*. In response, the U.S. Fish and Wildlife Service drafted a risk assessment. In March 1999, the invasive Mediterranean strain of *C. taxifolia* was listed as a U.S. federal noxious weed, which prohibited its entry into and movement throughout the USA (7 CFR § 360 January 1 2006). U.S. Federal Noxious Weeds cannot be imported or moved interstate without a permit e.g. for research purposes (U.S. Plant Protection Act of 2000). In the summer of 2000, two populations of *C. taxifolia* were detected in southern California (SCCAT 2005). The incursion was rapidly treated and since 2002, no new patches of *C. taxifolia* have been observed in California (Anderson 2005). Law forbids the import and interstate movement of the Mediterranean strain of *C. taxifolia* into the USA, Nevertheless it is quite probably still present in USA trade, misidentified or mislabelled as some other taxon (Walters et al. 2006).

A.4 Eichhornia crassipes *(Mart.) Solms (Pontederiaceae): Water Hyacinth*

Globally, freshwater aquatic weeds are well known for their impacts in natural and anthropogenic systems. They are good examples of conflict species because they are widely sold in the aquarium trade as tank and water garden ornamentals. Some species have been intentionally released into public waters for propagation and harvest by aquarists. *Eichhornia crassipes* (Water Hyacinth) is a floating aquatic weed that is problematic in all tropical/subtropical regions of the world including Australasia and the southern USA. Despite prolific flowering, it primarily reproduces by forming vegetative clones. This leads to the development of extensive floating mats that readily move with wind and water flow. Water Hyacinth reduces water quality through increased turbidity, decreases light penetration into water bodies, and lowers dissolved oxygen levels. This reduces the establishment and persistence of native submerged and emergent aquatic plants. Decaying plants can decrease oxygen levels further, leading to fish mortality and the collapse of the entire aquatic ecosystem (Julien et al. 2012).

Water Hyacinth impacts human society by reducing water quality for drinking, access to water for fishing or washing, and blocking irrigation pumps and lines. These weeds can increase siltation rates and decrease water flow, causing stagnation and thus increasing the occurrence of disease-carrying mosquitoes and snails. In dams, especially in dry areas, the evapotranspiration rates of water hyacinth are considerably higher than natural evaporation rates, depleting water supplies in dams and reducing availability of water for stock or irrigation (Julien et al. 2012). In waterways with high nutrification, these problems are magnified as plant populations are more vigorous than normal.

Water Hyacinth is difficult and costly to manage using conventional techniques. Herbicides are usually banned on water bodies; mechanical removal is costly and short-term. Fortunately Water Hyacinth and many other aquatic weeds have been successfully managed using biological control. The weevils *Neochetina eichhorniae* Warner and *N. bruchi* (Hustache) have been introduced to control water hyacinth around the world. Both species establish quickly and achieve effective control within a few years (Cilliers 1991). They are less effective at lower temperatures and in more polluted environments. Other agents also are being used, but these have not been as effective as the weevils. Water hyacinth is a declared noxious and high priority weed in most tropical and subtropical regions of the world.

A.5 Imperata cylindrica *(L.) Beauv. (Poaceae): Cogongrass*

NPPOs typically focus on pests that are not present in their country or have a limited distribution and can be contained or eradicated. Sometimes NPPOs must develop and maintain regulatory and management programs for more widespread species that are exceptionally problematic. Cogongrass is considered one of the worst 100 invasive species globally. It is a tropical/subtropical grass estimated to have invaded over 100,000 ha of land throughout the southeastern USA and over 500 million ha across 73 countries globally (Bryson and Carter 1993; Ramsey et al. 2003). Cogongrass is considered one of the most difficult invasive species to control throughout the world (Ramsey et al. 2003). It easily displaces crops and native herbaceous shrub and tree species. Cogongrass is extremely tolerant of infertile soils, and is well-adapted to a wide variety of soil conditions of upland forests. Cogongrass readily invades forests, pastures and fields, utility rights-of-way, roadsides, and natural areas. It typically invades an area after disturbances, such as grazing, weed control, timber harvesting, fire, and hurricanes (Lippincott 1997).

A primary adaptive strategy of Cogongrass is to allocate more of its photosynthate into below-ground biomass production. Below-ground production can reach 300–1,100 g/m^2. Cogongrass has a low shoot/root ratio, with up to 75 % of its total biomass in the root system. Investment in belowground biomass has several competitive advantages, including an ability to quickly respond to above-ground disturbances by re-vegetating from rhizomes. These adaptations facilitate both the aggressive growth of Cogongrass into new areas as well as a high resistance to new disturbances.

Spatial analysis of 26 patches of Cogongrass in Florida and Alabama during 2008 demonstrated the aggressive behavior of this species (Craig Ramsey, unpublished data). In this study, the perimeter of each patch was mapped several times during the study period using a GPS unit capable of recording a point every second. The resulting distributions were then used to estimate changes in the population size over time. Patches growing underneath mature forest plantations had an average growth rate of 15 ft^2/day during the study period representing a 19 % increase in area. Patches growing in clear-cuts grew at about 25 ft^2/day representing a 143 % increase in area (Craig Ramsey, unpublished data). In addition to aggressive growth rates, Cogongrass continues to out-compete native plants due to its allelopathic properties (Bryson and Carter 1993). Cogongrass is widely distributed across several states in the southeastern USA. Unfortunately, its distribution is too broad for eradication (http://plants.usda.gov/java/). The USDA lists this species as a

U.S. Federal Noxious Weed with the intent of containment. Meanwhile, several of the states containing Cogongrass are regulating this plant as a state noxious weed. Other agencies are trying to eradicate and control it locally depending on the resources at risk (Bryson and Carter 1993; Ramsey et al. 2003).

A.6 Miconia calvescens *DC (Melastomataceae): Miconia*

Miconia (or Velvet Tree, Bush Currant) is considered another of the "world's worst invasive species" and has been intentionally spread for ornamental appeal. This species is a large-leaved fast-growing tree species endemic to the tropical rain forests of South and Central America. The bicoloured purple and green leaves have led to a keen ornamental attraction to this plant and its horticultural-driven spread around the Pacific. In addition to a fast growth rate, the plant produces many small bird-dispersed seeds that can persist as a seedbank under closed canopy forests. A large tree can produce up to 50 million seeds annually. Miconia has been introduced into Australia, and all of the large islands and many small islands in the Pacific. Introduced into Tahiti during 1937, this species has invaded the island forests of Tahiti and Moorea, replacing nearly 70 % of the indigenous forests with dense, monospecific stands (Meyer and Florence 1996). The impacts have been phenomenal in terms of suppression of native biodiversity on these islands and these impacts have led to pan-pacific concerns about this weed in areas where it has been introduced. Miconia is a significant transformer because it over-tops the indigenous forest and prevents native forest recruitment by forming a closed canopy of very large shade leaves, effectively altering forest succession. This behaviour is dramatic and puzzling because in its native range, it does not persist in closed canopy forests.

Concern about *M. calvescens* has led to two Miconia-specific international conferences (www.hear.org/miconia/1997conference/; www.hear.org/conferences/miconia2009/proceedings/) in Hawaii, where more than 1 million dollars is spent annually to eradicate the weed from some islands. On other islands, as in Australia, the weed is now too widespread to be eradicated, Containment is an ongoing strategy with high ongoing costs. Management of Miconia requires great care as the seeds are widely dispersed by frugivores and detection of recruiting individuals is difficult in dense forest structure. Few weeds can invade and disrupt undisturbed native forests in this way.

Community awareness of Miconia is high in affected areas. Where eradication is not possible, biological control programs have been initiated through collaborations between the governments of Tahiti and Hawaii, and researchers in South and Central America. These efforts have led to the introduction into Tahiti of a Miconia-specific fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *miconiae* Killgore and L. Sugiy (Melanconiales, Coelomycetes, Deuteromycetinae) found in Brazil during 1997. The fungus causes lesions on the leaves that allow light through the closed canopy and increases natural regeneration of the native forest plants (Meyer and Fourdrigniez 2011; Meyer et al. 2012). This agent is starting to break the total shade caused by Miconia and reduce the impacts to native species. Native trees are expected to eventually overtop the diseased Miconia canopy and bring this weed under natural control. The US Forest Service in Hawaii is also looking at other biological control agents to bring about greater control of Miconia.

A.7 Striga asiatica *(L.) Kuntze (Scrophulariaceae): (Witchweed)*

Parasitic weeds comprise a small subset of all weeds, but are readily recognized for their direct impacts on hosts. Here we highlight a parasitic plant that has been the target of one of the USDA's longest eradication programs. Witchweed is an herbaceous, obligate, hemiparasitic plant that grows to about 30–40 cm in height (CABI 2012). It primarily attacks grasses (mostly C4 species), but hosts in other families have been documented (Cochrane and Press 1997). Major crop hosts include sorghum, maize, sugar cane, pearl millet, finger millet, and rice (CABI 2012). Seeds of *S. asiatica* require a chemical signal from the roots of their hosts to germinate. The seedlings, which are underground, then produce a primary haustorium that penetrates the host's roots and enters the xylem. As the parasite grows, it produces more haustoria, and eventually produces herbaceous shoots that are green with bright orange-red flowers. Plants are self-fertile, and produce thousands of seeds that are about 0.3 mm long. Seeds can survive in soil for 14 years (CABI 2012).

Striga asiatica causes wilting, stunting, and scorching of the foliage of its hosts (CABI 2012). These symptoms appear even before the plant has emerged from the soil. Crop damage is worse under marginal rainfall and low soil fertility. Estimates suggest that one *S. asiatica* plant per square meter reduces plant yield by about 1 %. Under heavy infestations, crop losses of 21–28 % have been reported (CABI 2012). Given the difficulty in detecting this parasite, and its impact on major grains, Witchweed is considered a major pest threat, even in more developed countries (Patterson et al. 1982).

This parasitic plant was first detected in the USA during 1956, when a specimen was brought to a plant disease clinic at North Carolina State University for identification (Eplee 1992). Because of the pest's threat to U.S. grain production, the USDA and North and South Carolina Departments of Agriculture imposed quarantines around the areas affected. Detection and delimiting surveys identified about 175,000 ha of infested fields in a small region near the coast of both states (Iverson et al. 2011). The USDA responded promptly by establishing a research lab to develop methods for eradication, resulting in several innovative methods and a creative management plan (Eplee 1992). The Witchweed program of the USA is one of the oldest and longest eradication programs in the country and is nearing completion. In 2011, officials announced that *S. asiatica* has been eliminated from 99 % of all infested sites (Iverson et al. 2011). Work continues to fully eradicate the species. This weed has just been detected for the first time in Australia.

A.8 Vitex rotundifolia *L. f. (Verbenaceae): Beach Vitex*

Common names include Beach Vitex, Chasteberry, Roundleaf Chastetree, Monk's Pepper. Most pest regulatory programs managed by NPPOs are initiated by government or industry representatives responsible for plant protection. However, regulatory programs also may be initiated due to widespread interest and concern by citizens. Beach Vitex is a low growing, deciduous shrub with ascending and decumbent stems. Plants typically grow to 1 m in height, but can reach 2 m if protected from the wind. Prostrate, vine-like stems, which root along the nodes, can reach more than 10 m in length (Cousins et al. 2010). Beach Vitex is native to coastal habitats in the western Pacific, from Korea and Japan southward throughout tropical southeastern Asia, as well as northern Australia, Fiji, and New Caledonia (USDA-ARS, 2005). It is also native to Hawaii. It was promoted in the southeastern USA to help stabilize sand dunes after Hurricane Hugo damaged coastal communities in the Carolinas in 1989 (Cousins et al. 2010). However, after about a decade, the plant was recognized to be highly invasive (USDA-NRCS 2005).

Because of the density of its growth and the production of allelopathic chemicals, Beach Vitex outcompetes native vegetation, including the iconic sea oats (Cousins et al. 2010; Gresham and Neal 2005). Beach Vitex leaves and fruit contain cuticular alkanes that leach onto the sand substrate (Cousins et al. 2009). This results in intense sand hydrophobicity (i.e., water repelling) that persists in the soil for many years and may limit the growth and recruitment of native species (Cousins et al. 2009; Gresham and Neal 2005). The density of its stems and roots lead some people to fear that it impedes successful nesting by endangered sea turtles that lay their eggs in sand dunes along the southeastern USA (Dorell 2009). Coastal communities, already threatened and extremely fragmented by overdevelopment, are threatened by this species which has been called the "kudzu of the coast". In addition to the ecological impacts, Beach Vitex changes the overall appearance of coastal dunes that are highly valued by beach goers.

Unlike most other weeds or invasive species, the pressure to manage Beach Vitex has been mostly a "bottom-up process". Homeowners in South Carolina formed the Beach Vitex Task Force to organize action and management (http://www.beachvitex.org/). As a result of these grass roots efforts, many local communities have passed ordinances prohibiting the species. In 2009, North Carolina listed Beach Vitex as a State Noxious Weed (Cousins et al. 2010). Virginia subsequently erected quarantines around the few locations where it wass known to occur. Some weed experts have asked USDA- APHIS to list the species as a Federal Noxious Weed. APHIS has prepared a weed risk assessment of the species, but is trying to resolve how to regulate a plant species that is native to a part of the USA.

A.9 Ulex europaeus *L. and* Cytisus scoparius *(l.) Link (Fabaceae): Gorse and Broom*

Widely distributed weeds are beyond hope of eradication and must be suppressed indefinitely to minimize their negative impact. These weeds are ideal candidates for biological control programs. Invasive in North and South America, Australasia, and on some oceanic islands, these European leguminous shrubs are the worst of several global temperate to Mediterranean-climate weeds in the Tribe Genisteae. They have showy yellow flowers and reproduce entirely by seed produced in copious quantities (ca 30,000/m^2). These species were introduced for several reasons including horticultural use as a hedge plant (gorse), and sand-dune stabilisation (broom) in the USA (Richardson and Hill 1998; Syrett et al. 1999).

The spread from their intended plantings have led to widespread production losses in livestock and forestry systems. Beekeepers in New Zealand value them as nectar and pollen sources. This perceived benefit has led to some local conflict, but an analysis showed that the bee industry would be unaffected if the density of these plants could be radically reduced. As grazing-tolerant nitrogen fixers, they are frequently altering the nutrient status of the invaded soils to the detriment of native plants. Their entry into and spread in natural ecosystems may have been facilitated by livestock, mining operations and natural "dispersal down river" systems. These species have become weeds of disturbed and grazed grasslands, dunes, and native forests, as well as important weeds of commercial forestry, particularly pine plantations. Unchecked, they form dense, monospecific stands, which prevent livestock access and smother the native herb layer. Gorse and Broom can also slow or prevent natural regeneration in woodlands. In addition to altering soil nitrogen levels, they can also affect the intensity and frequency of natural bush-fire regimes.

Gorse and Broom have been the focus of weed management approaches for many years. Integrated management strategies have been developed based on slashing, mulching, ploughing, goats, fire and herbicides, but stands rapidly regenerate from the seed bank without a thick layer of mulch or a strong growth of over sown perennial grass cover. The long-lived seed bank requires long-term follow-up treatments. Unfortunately, these treatments can't be economically justified for the often poor productivity systems gorse and broom dominate. Both species have been the focus of biological control programs for many years in North America and Australasia. Several insect biocontrol agents have been tried in each case, but beyond some direct reductions to seed production, the effects have been minor. A gall mite released into Australasia may now be showing some promise against broom. Established stands tend to senesce once they are beyond 20 years old and this leads to a build-up of soil pathogens limiting subsequent recruitment of seedlings. However, such effects are negated by a major disturbance such as ploughing or fire. Gorse and brooms are two of the 32 "Weeds of National Significance" in Australia, requiring development and implementation of national management strategies.

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Chapter 21
Climate Change and Plant Biosecurity: Implications for Policy

Jo Luck, Ian D. Campbell, Roger Magarey, Scott Isard, Jean-Philippe Aurambout, and Kyla Finlay

21.1 Introduction

Climate change is a major threat to global agriculture and forestry production but it may also be considered a risk for the effective management of weeds, insects and pathogens (pests). Plant-based industries are vulnerable to climate change but limited research has addressed changes in the risks associated with pests. Understanding these threats will enable industry and quarantine agencies to better prepare and adapt to any increased risks. For example, including the projected effects of climate change in Pest Risk Analyses we highlight new and emerging biosecurity risks and changes that may be needed to pre-define quarantine zones, containment

J. Luck (✉) • K. Finlay
Biosciences Research Division, Department of Environment and Primary Industries, Victoria AgriBio, 5 Ring Rd, La Trobe University, Bundoora, 3083 Victoria, Australia

Plant Biosecurity Cooperative Research Centre, Innovation Centre, University of Canberra, Bruce, ACT 2617, Australia
e-mail: jo.luck@pbcrc.com.au

I.D. Campbell
Integrated Natural Resources Division, Agriculture and Agri-Food Canada, 1341 Baseline Rd., Ottawa, ON K1A 0C5, Canada

R. Magarey
North Carolina State University and Co-operator with USDA-APHIS-PPQ-CPHST PERAL, 1730 Varsity Drive, Suite 300, Raleigh, NC 27606, USA

S. Isard
Departments of Plant Pathology and Meteorology, Pennsylvania State University, 205 Buckhout Lab, University Park, PA 16802, USA

J.-P. Aurambout
Plant Biosecurity Cooperative Research Centre, Innovation Centre, University of Canberra, Bruce, ACT 2617, Australia

Future Farming Systems Research, Department of Primary Industries Victoria, PO Box 4166, Parkville, VIC 3052, Australia

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strategies, threat lists, industry biosecurity plans, pest management strategies and contingency plans, surveillance activities and post-entry quarantine guidelines.

Climate change and increased climate variability will alter the performance and distribution of crops and the pests that may affect them. Pest outbreaks occur when changes in climatic conditions (such as temperature and moisture) are most favourable for growth, development, survival and dissemination (Aurambout et al. 2006). This may cause pests to expand their normal range into a new environment, potentially extending agricultural losses.

We anticipate new pest interactions will occur and cause existing innocuous organisms to emerge as major issues, or provide conditions for introduced pests to become established. Close interactions between insects and pathogens, host plants and the environment necessitate examining the effects of climate change on the pests's own biology and the system in which a pest interacts (Luck et al. 2011). The complexity of these interactions presents a significant challenge in providing accurate information for decision-making. Analysis of the pest's response to climate in isolation ignores critical changes that may occur in the host plants and habitats and in the host-insect/pathogen relationship as a result of climate change. Understanding the effect of climate change on these interactions is essential to understanding the threat climate change poses to plant biosecurity (Aurambout et al. 2006).

The nature, extent and intensity of climatic changes will vary strongly both spatially and temporally (Aurambout et al. 2006). The responses of pests to these changes will be species- and region-specific and made more complex by their interactions and interdependencies. Climate change will also alter global agricultural production and trade patterns (IPCC 2007), and may result in policies (such as those intended to reduce atmospheric greenhouse gases) that may further affect production and trade as well as more directly affect the tools used to combat plant health risks. Due to the inherent complexity of biological interactions, no general rules can be developed to define the impact of climate change on plant biosecurity. However, new computer models will improve our understanding of these processes and potentially estimate outcomes of specific cases or extract general trends for commodities, regions, or categories of pests.

As defined in a discussion of risk assessment (Chap. 9), the analysis of biosecurity threats involves four steps as determined by the International Standards for Phytosanitary Measures (ISPM) No. 11, (FAO 2006): (1) Pest categorisation, (2) Assessment of probability of entry, (3) Establishment and spread and (4) Assessment of consequences and combining the probabilities to estimate risk. Assessing the likely economic, environmental and social impact of a potential pest threat helps define management options. Climate change represents a new layer of complexity because the extent to which pests will respond is unknown (Aurambout et al. 2006). In the future, climate change will influence pest biology and distribution resulting in some pests becoming more severe while others may become less of a threat. Understanding these potential new responses may determine new directions in biosecurity management, such as the re-prioritization of threats and changes to contingency planning (Fig. 21.1).

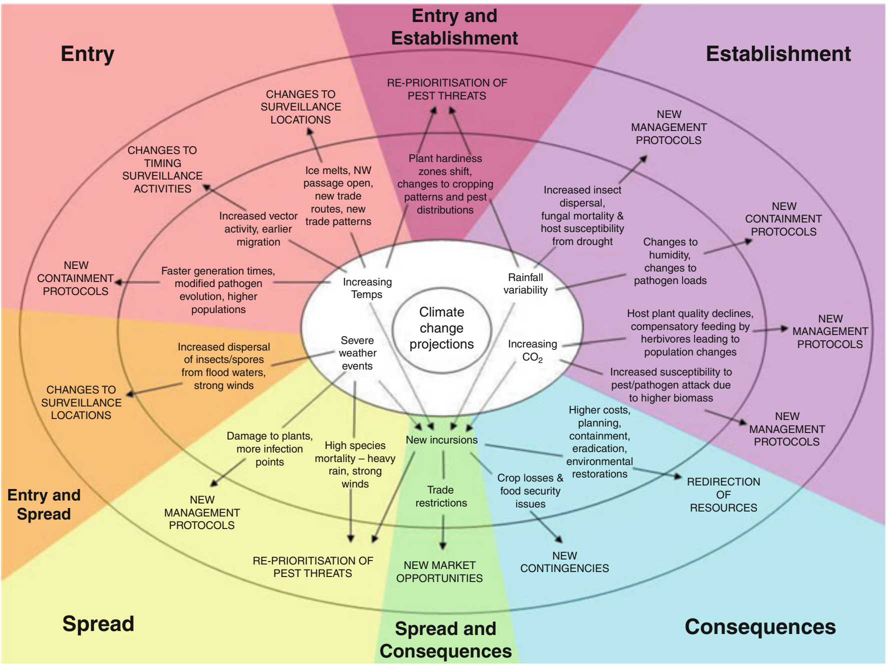

Fig. 21.1 Summary of possible effects of climate change on the entry, establishment, spread and consequences of a pest, pathogen or weed with potential implications for plant biosecurity policy

This chapter outlines the likely effects of climate change on invasive species and illustrates these effects using two case studies. We extrapolate these effects to describe the potential implications to plant biosecurity policy with recommendations to address any research or policy gaps.

21.2 IPCC Global Projections

Global climate projections as detailed in the Intergovernmental Panel on Climate Change Fourth Assessment Report (IPCC 4AR; IPCC 2007a) indicate indisputable climate warming as evidenced by increase in global average ambient and ocean temperatures, widespread ice melting and rising global average sea level (Meehl et al. 2007). These observed changes in global average temperatures are very likely due to the observed increase in greenhouse gas emissions, particularly carbon dioxide (CO_2) concentrations (currently 397 ppm http://co2now.org, July 2013). Atmospheric CO_2 concentration is expected to increase to 500–900 ppm by 2,100 depending on emission scenarios (see Special Report on Emission Scenarios, Nakićenović and Swart 2000).

Coupled Atmosphere-Ocean General Circulation Model outputs indicate that global average warming will continue at an average rate of about 0.2 °C per decade for the next two decades, though the warming may not be linear. Depending on the emission scenario, global average surface air temperatures for 2,090–2,099 will be 1.1–6.4 °C warmer than 1980–1999, with best estimates ranging from +1.8 °C for a low range (B1) emission scenario to +4.0 °C for the high level (A1FI) emission scenario. Warming will be heterogeneous globally: greatest over land and at high northern latitudes and least over the Southern Ocean and northern North Atlantic Ocean (Meehl et al. 2007).

Thermal expansion due to warmer seas is the most significant factor contributing to sea level rise, which is projected to be 0.18–0.59 m (1.5–9.7 mm $year^{-1}$) between 1980–1999 and 2090–2099 over the full range of IPCC emission scenarios. Geographical variability is likely to be high with smaller than average rises in the Southern Ocean and larger than average rises expected in the Arctic and across the southern Atlantic and Indian Oceans. Sea-ice in the Arctic and Antarctic is expected to be reduced in future climates; indications are that Arctic late summer sea-ice will disappear during the latter part of the twenty-first century (Bo et al. 2009).

Following an increase in mean temperature, we anticipate a greater risk of high temperature extremes, accompanied by a reduced risk of low temperature extremes. In concert, we anticipate an increased risk of longer-lasting and more intense heat waves, particularly in Western Europe, the Mediterranean and the south-eastern and western USA.

Mean annual precipitation will increase in tropical regions, particularly in the tropical Pacific, and at high latitudes with decreases expected in the subtropics. Overall mean water vapour, evaporation and precipitation is projected to increase. An increase in the intensity of precipitation events is expected for all areas but particularly in the tropics and at high latitudes where increases in mean precipitation are apparent. Such increases, along with a higher likelihood of very wet winters, bring a greater risk of flooding. In addition, tropical cyclones (typhoons and hurricanes) will increase in intensity with larger peak wind speeds and greater frequency of heavy precipitation. These projections of increased intensity have a higher degree of certainty over the north Atlantic Basin (IPCC 2007a). Models also predict a pole-ward shift of these storm tracks for both Hemispheres, particularly evident in the Southern Hemisphere, with greater storm activity at higher latitudes (IPCC 2007a).

Longer periods of drying between heavy precipitation events are also projected and therefore, in mid-continental areas where summer drying will be greater, a greater risk of drought is indicated, despite possible increases in precipitation. This may be intensified in many regions by increased evapo-transpiration resulting from warmer growing season temperatures. In cold regions, winter snowpack may either increase or decrease depending on the balance of increased winter precipitation and warmer winter temperatures; changes in snowpack will affect spring flooding and ground and surface water recharge.

21.3 Direct Climate Change Impacts on Weeds, Insects, Pathogens and Host Plants Affecting Plant Biosecurity

21.3.1 Increasing Temperature

Increasing temperatures are projected to cause changes in pest distribution (Cannon 1998; Root et al. 2003). Temperate species are likely to extend their distributions to higher latitudes and altitudes while cold-adapted species may experience restricted distribution (Bale 2002). For example, northward shifts of the Codling Moth (*Cydia pomonella* (Linnaeus)) and the Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) are projected for Norway (Rafoss and Sæthre 2003). In the southern Hemisphere, models have predicted a southward range expansion for the Queensland Fruit Fly (*Bactrocera tryoni* (Froggatt)) as the temperature increases (Sutherst et al. 2000). In Australia, the population genetics southern populations of the Vinegar Fly (*Drosophilia melanogaster* Meigen) have changed corresponding to a shift of 4 °C latitude which probably represents a genetic adaptation to the warmer and drier climate (Umina et al. 2005).

Temperature directly influences insect life cycle parameters such as growth and development, survival, fecundity, feeding behaviour, range and abundance (Bale 2002). Under increasing temperatures, insects may respond with shorter development times and shorter life cycles (Harrington et al. 2001; Bale 2002), although this can vary with different insect groups (Masters et al. 1998) indicating the effect is likely to be species specific. For annual temperate species, higher temperatures during the active period of growth could speed development and reduce predation as less time is spent in the vulnerable nymphal and larval stages. With faster development times this could lead to an increase in the number of generations per season as recorded for some insect taxa such as Lepidoptera, Coleoptera and Hemiptera (Kiritani 2006; Gomi et al. 2007; Jönsson et al. 2007).

Higher winter temperatures may impact species with no diapause requirements, such as the Green Peach Aphid (*Myzus persicae* (Sulzer)), which shows increased winter survival and higher population densities in warmer temperatures (Bale et al. 1988). For some multivoltine species, diapause occurs in response to other environmental cues such as longer photoperiods so rising temperatures may not trigger a diapause response as seen for the Green Stink Bug (*Nezara viridula* Linnaeus) in Japan (Musolin 2007).

Increasing temperatures may favour broad categories of plants in some environments. For example, plants using the C_4 photosynthetic pathway are better adapted to warm dry conditions than are C_3 plants; thus C_4 grasses may become more aggressive invaders in some temperate ecosystems (Ehleringer et al. 1997; White et al. 2001). Extreme cold events in winter, expected to decrease in both frequency and severity with climate change, may control some pests. In Switzerland, an increase in the frost-free growing season over recent decades has already resulted in significantly increased success of invasive alien broad-leaved species in some areas (Walther 2002).

Increasing temperatures may disrupt synchronized activity between host plant phenology and herbivorous insects affecting successful life cycle completion and leading to unexpected responses such as decreased pollination or insect infestations (Root et al. 2003). The relative asynchrony may disadvantage some species that have very close associations between budburst of the host plant and emergence (Straw 1995) and benefit species which can take advantage of longer periods of growth (Zhou et al. 1995). Predictive modelling in the UK suggests that with a 2 °C increase in mean annual temperatures, life cycles of certain insects could be completed 2–3 weeks earlier (Dewar and Watt 1992). Further, meta-analysis of the breeding cycles of temperate insect species have shown cycles starting 5.1 ± 0.1 days earlier over the period of a decade in the UK (Cannon 1998).

Migration and movement of species may be affected by increasing temperatures by disrupting thermal thresholds for development and flight. For example, a warmer climate would result in flight thresholds being reached earlier and result in early and possibly prolonged immigration such as seen in aphids and moths (Zhou et al. 1995; Woiwod 1997). This may be counteracted by limitations to flight due to upper thresholds being reached more frequently.

Plant pathogens may be particularly responsive to climate change given their dispersal capabilities and relatively short generation times (Brown and Hovmøller 2002; Scherm and Coakley 2003); also see reviews (Manning and Tiedemann 1995; Chakraborty et al. 1998; Chakraborty 2005). Milder winters favour pathogens such as Powdery Mildew (*Erysiphe grammis* (DC.) Speer), Brown Leaf Rust (*Puccinia hordei* Otth) and Stripe Rust (*Puccinia striiformis* Westend.), Milder winters associated with very hot weather could promote the development of Cercosporea Leaf Spot Disease (*Cercosporea beticola* Saccardo), Powdery Mildew (*Erysiphe betae* (Vaňha) Weltzien) and Rizomania Disease (*Rizomania*) (Chakraborty et al. 1998; Patterson et al. 1999). Further, warm and humid conditions have led to stronger, earlier outbreaks of Potato Blight (*Phytophthora infestans* (Montagne de Bary)); hot dry summers have reduced infestations of many fungal diseases including Rhynchosporium Leaf Blotch (*Rhynchosporium secalis* (Oudem.) Davis) and Septoria Leaf Spot Diseases (*Septoria tritici* Roberge and *S. nodorum* (Berk.) Berk.) (Patterson et al. 1999). Rising temperatures are also likely to impact host-pathogen interactions. Chakraborty et al. (1998) warn that even a small temperature increase could cause increases in heat sums above a threshold suitable to maintain disease resistance. Warmer winters and higher overall temperatures will favour the winter survival of pathogens and accelerate pathogen and vector life cycles (Harvell et al. 2002).

Finally, higher temperatures may also be associated with structural changes in the plant. Increased lignification can confer host resistance and protect against pathogen infection (Chakraborty et al. 2000a; Fuhrer 2003). Alternatively, infection at higher temperature changes may be expressed as reduced defence responses in host plants. For example, increased infection severity was found for bacterial spot-resistant pepper carrying a temperature-sensitive resistance gene (Padgett et al. 1997; de Jong et al. 2002; Romero et al. 2002) and a reduced resistance in oat-resistance cultivars to Oat Stem Rust was caused by *Puccina graminisavenae* (Chakraborty et al. 1998).

21.3.2 Increasing Atmospheric CO_2

Information on the impact of elevated CO_2 on plant-herbivore interactions is still relatively scarce (Coviella and Trumble 1999). Herbivorous insects have been shown to respond to plants grown under elevated CO_2 by "compensatory feeding" or increasing food consumption rates to compensate for higher carbon to nitrogen ratios (Hunter 2001). This effect has been observed for several insect taxa: Lepidoptera (Roth and Lindroth 1995; Lindroth et al. 1997; Goverde et al. 1999; Agrell et al. 2000), chrysomelid beetles (Veteli et al. 2002), sawfly (Williams et al. 1997) and grasshoppers (Johnson and Lincoln 1991). Low nutritional quality of plant material can decrease herbivore growth rates and extend development times (Lawler et al. 1996; Smith and Jones 1998; Johns and Hughes 2002; Goverde and Erhardt 2003; Coll and Hughes 2008).

Species with the capacity to adapt to low-quality food may adjust more readily to climate change (Bale 2002). If elevated CO_2 results in slowed development, then herbivores may be impacted by extending the exposure time to natural enemies (or higher proportions of pathogenic bacteria and viruses) through feeding which could ultimately lead to reduced herbivore abundance (Stiling et al. 1999; Kopper and Lindroth 2003). However, the effects are highly specific to each insect-plant system and vary between insect groups (Coviella and Trumble 1999).

Increased host resistance at elevated CO_2 has been attributed to several plant physiological and morphological changes such as: an increase in net photosynthesis leading to more photosynthates available to partition into resistance activity, reduced stomatal conductance accumulation of carbohydrates in leaves, more leaf waxes, extra layers of epidermal cells, increased fibre content, more mesophyll cells and increased biosynthesis of phenolic compounds (Bowes 1993; Hibberd et al. 1996; Hartley et al. 2000; Fuhrer 2003).

Changes to the architecture of a crop due to increasing CO_2 may lead to increased humidity within the canopy and more favourable conditions for pathogen survival (Chakraborty and Datta 2003). Increased photosynthetic rate under elevated CO_2 levels (Fuhrer 2003) may lead to plant growth earlier in the season enabling insects and pathogens to colonise earlier and a subsequent increase in plant biomass will result in a larger reservoir for pathogen colonisation and replication. Increased fecundity has been shown at elevated CO_2 concentrations for the pathogen *Blumeria graminis* (DC). Speer (= *Erysiphe graminis*) (Hibberd et al. 1996) and the anthracnose pathogen *Colletotrichum gloeoporioides* of the pasture legume *Stylosanthes scabra* Vogel (Chakraborty and Datta 2003) and may counteract host resistance. Enlarged canopies of *S. scrabra* associated with increased plant growth under elevated CO_2 have been shown to increase the humidity of the canopy microclimate, which in turn has led to a higher spore count and increased rates of anthracnose infection (Coakley et al. 1999; Chakraborty et al. 2000b; Pangga et al. 2004). Increased fecundity in a favourable microclimate could accelerate pathogen evolution (Chakraborty and Datta 2003).

Pathogen aggressiveness (the ability to invade, damage and reproduce within a host, Shaner et al. 1992) has been shown to increase under elevated CO_2 for

C. gloeoporioides on resistant cultivars of *S. scabra* over 25 successive infection cycles (Chakraborty and Datta 2003). If the number of infection cycles increases due to increased fecundity or faster life cycles, then we see obvious implications for the robustness of resistant crops (Coakley et al. 1999).

21.3.3 Altered Precipitation Patterns

Changes in precipitation and evapotranspiration are likely to have greater impact on areas where non-irrigated cropping is already subject to frequent failures due to inadequate rainfall. Under changing climate conditions in southern Australia, cropping is likely to become non-viable in the dry margins particularly if rainfall is substantially reduced. This trend may be partially offset by the CO_2 fertilisation effect. In Northern Australia, forecasts predict that cropping will persist with reducing frost risk and potentially increasing yields (Howden 2002).

Plants have specific physiological temperature and moisture/precipitation thresholds, and climate change will influence plant distributions. Large trees would be slow to adapt to changing bioclimes leaving them vulnerable to fast-adapting pests. For example, nearly 25 % of Australian eucalypts could not tolerate a 1 °C shift in temperature or more than a 20 % variation in rainfall (Hughes et al. 1996). These shifts in non-crop plants will have consequences for crops via the effect on availability of alternate hosts for crop pests, pollinating insects and natural enemies of crop pests.

The slow response of trees and other long-lived species may result in the widespread occurrence of what may be termed "biomes in transition" – climatically disturbed, disequilibrium vegetation associations. Some biomes are adapted to frequent large-area disturbances (like the forest-fire-prone eucalyptus forests in Australia or the conifer forests of north-western North America). Other biomes experience large-area disturbances less frequently and are less well-adapted to them. Widespread disturbance can occur on broad regional or subcontinental scales in response to human impacts such as extensive land use conversion or an introduced pest. Rare natural events can also cause widespread disequilibrium vegetation assemblages. For instance, the rapid devastating spread of a forest pathogen in eastern North America *ca*. 3000 BC eliminated 90 % of the forest-dominant eastern hemlock (*Tsuga canadensis* (Linnaeus) Calliere) (Allison et al. 1986). Also, the current climate-change abetted rapid and devastating spread of the Mountain Pine Beetle (*Dendroctonus ponderosae* Hopkins) in western Canada (Carroll et al. 2003). Although less well-recognized, rapid climate fluctuations can also effect such broad disequilibrium, as occurred with the Little Ice Age in eastern Canada (Campbell and McAndrews 1993). The coming climate change is expected to be more rapid and larger in amplitude than the Little Ice Age, and could therefore have a correspondingly more widespread impact on global biome equilibria. In such an environment, invasive alien species (IAS) may find opportunities for establishment and spread that would not occur in less disturbed landscapes.

21.3.4 Extreme Events (Hurricane, Drought, Floods, Extreme Heat)

Increases in the frequency of climatic extremes are now considered potentially more damaging to agriculture than changes in the mean. Long-term yields will be affected by heavy damage and/or severe disruption to farming practices (Porter and Semenov 2005). An increase in the frequency of droughts may reduce a tree's ability to recover between drought years leading to decline and death. Trees are also likely to be affected by the increased storms and cyclones, through direct damage to tree and forest stand structure. Irrigated trees with shallow root zones may be at more risk of failure with the predicted increase in severe storms. Trees with deeper root zones will therefore be better adapted to survive reduced rainfall and increased storm events. Increased forest stand blow-downs will also increase disturbed habitat.

Drought and accompanying tree stress affect plant physiology, causing some plant hosts to be more susceptible to pest attack especially when combined with higher temperatures (Rosenzweig et al. 2001; Fuhrer 2003). The increase in insect vector populations (e.g. aphids) caused by dry conditions and warm temperatures can aid the dissemination of plant viruses, for example (Rosenzweig et al. 2001). Conversely, drought may reduce the incidence of some pathogens that require water or humidity for development (Chakraborty 2005).

Increased winds and driving rains will enable widespread dispersal of pests, possibly resulting in new incursions in previously uninfected sites and facilitating infection/establishment and survival rates (Rosenzweig et al. 2001; Anderson et al. 2004). For example, Citrus Canker bacteria ooze from lesions in the presence of free moisture, associated with dew, rain or irrigation (Pruvost et al. 2002; Mavrodieva et al. 2004). In Florida the winds associated with Hurricane Wilma spread Citrus Canker bacteria widely leading to the destruction of 170,000 acres (one-third of the industry) of fruit trees in commercial groves (Gottwald and Irey 2007) (see Chap. 18). Desert locusts have historically been prevented from reaching the Americas by the cold temperatures and variable winds encountered at the atmospheric elevations required for the long-distance trans-Atlantic flight. Winds and warm temperatures associated with Hurricane Joan and Tropical Disturbance Eighteen, however, allowed locusts to reach South America and the Caribbean in 1988 (Richardson and Nemeth 1991; Rosenberg and Burt 1999; Lorenz 2009).

Other natural disturbances related to extreme weather events may also be affected by climate change in complex ways. For example, the response of forest fire regimes will vary depending on the direct impacts climate change will have on fuel type (perhaps converting dry coniferous forest to open parkland or grassland) as well as the likely increase in extreme fire weather (Campbell and Flannigan 2000). Invasive alien species have complex interactions with fire; fire may kill the invaders, whose seeds/eggs/spores and propagules/larval stages may not be able to withstand the fire, but the disturbance itself may provide an opening for invasion (Keeley et al. 2003). Insects and pathogens may also colonize fire-damaged stands; they may themselves promote fire by weakening a stand and producing standing deadwood fuel (Fleming et al. 2002). The net effect will be complex and will vary from region to region and species to species.

21.4 Biosecurity Implications for Pest Risk Analysis

21.4.1 Pest Risk Assessment Process

The Australian quarantine system is underpinned by international standards and agreements set out by the World Trade Organisation (WTO) for the application of sanitary and phytosanitary measures (the SPS agreement) and, for plant pests, various guidelines and standards developed by the International Plant Protection Convention (IPPC) (See Sect. 2.2). The ISPM No. 11 (FAO 2006) defines the risk assessment phase of a Pest Risk Analysis which involves the initial assessment of the pest for likely economic, environmental and social impacts and categorisation according to whether it poses a significant threat to industry and therefore whether to continue with the Pest Risk Analysis process (See Chap. 9). If the organism is considered a high-priority pest, then an assessment is made of the probability of entry through an analysis of the documented and potential pathways from the source country to destination. Also, the likelihood of the pest being associated with those pathways at the source should be considered. The probability of establishment and spread involves examining factors associated with the likelihood that a pest will survive, successfully propagate on a host then disperse to other suitable hosts. Finally, an assessment of direct and indirect consequences is made through analysis of the likelihood and extent of harm that might be caused to economic, environmental and social factors. Direct-consequence criteria include harm to plant life and health as well as other environmental variables. Indirect criteria refer to the economic, environmental or social costs associated with the pest threat. Assessment and quantification of these variables will determine management options for the likely incursion of an exotic pest. However, an oft overlooked and confounding factor is the unknown extent to which a pest will respond to a changing climate (Aurambout et al. 2006). The process as described above, and as usually practiced, shows a high degree of reliance on historical data, including historical pest range, history of past introductions and detections in shipping, and history of past impacts. All these histories are to some degree climate-dependent, and are therefore unreliable guides to future potential under a changed climate.

Here we discuss the potential impacts of climate change on three common types of PRAs: Commodity Risk Assessment, Weed Risk Assessment and Species-level (or Pathway) Risk Assessment. We will also make some general recommendations for incorporating climate change scenarios into the risk assessment process.

In a “Commodity” Pest Risk Assessment, we typically see many pests that are identified in a preliminary pest list. From this list, pests that are likely to follow the pathway, and are also of quarantine significance, are selected for analysis. In the current USDA-APHIS system, plant hardiness zones are used to analyse a pest’s likelihood of establishing in the USA. The plant hardiness zone approach represents a quick and practical way to analyse the pests, especially for those that have limited distribution or developmental data. A risk analyst will compare the plant hardiness zones occupied by a pest in its current distribution with plant hardiness zones that

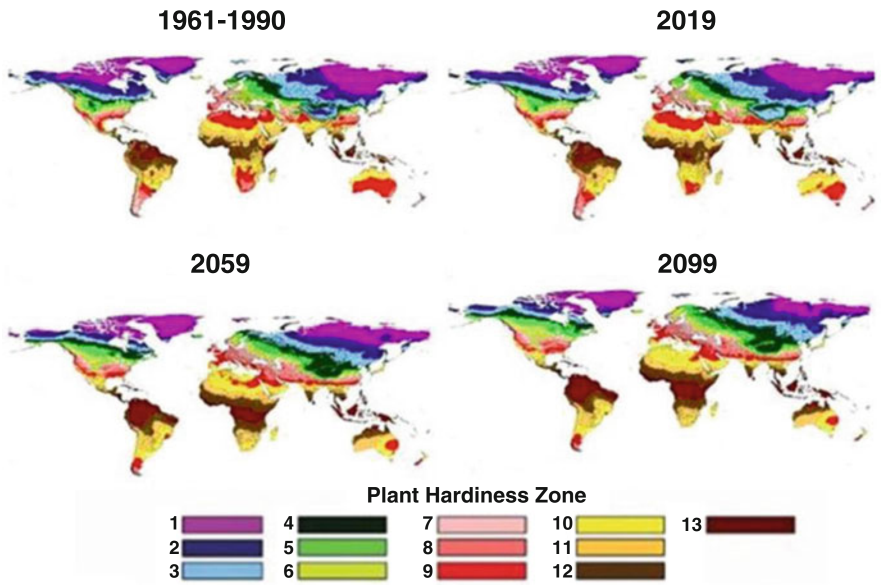

Fig. 21.2 Global plant hardiness zones based on climate change scenario B1 (IPCC) for 1961–1990, 2019, 2059 and 2099. Plant Hardiness Zone definitions described in Magarey et al. (2008)

are present in the USA. To support this analysis, global plant hardiness zones have been created from historical weather station records that represent 10 and 30 year-climatologies (Magarey et al. 2008). In addition, recent maps have been created showing how these plant hardiness zones are expected to change over the next 90 years (http://www.nappfst.org) based on the IPPC B1 scenario. These scenarios assume low to moderate increases in atmospheric greenhouse gasses and thus project low to moderate rates of climate change. The maps were generated in decadal increments and show a poleward migration of the hardiness zones (Fig. 21.2).

Weed Risk Assessment processes are being developed to assess the weediness of plants imported for propagation (Chap. 21; Pheloung et al. 1999). The process uses several subjective questions to classify plants as potential invaders, as non-invaders or requiring additional analysis. A commonly included question relates to geographic potential or climate suitability. Most plant species (unlike other pests), usually have sufficient numbers of geo-referenced observations from herbaria and museums worldwide to support species distribution modelling including climate matching or environmental niche modelling. These modelling techniques infer the potential distribution of a plant species from a mathematical relationship between its current distribution and environmental (e.g. climate) variables (Venette et al. 2010). This approach is amenable to incorporating climate change scenarios and many studies have been published showing the potential of the approach (Pheloung et al. 1996; Kriticos et al. 2003; Thuiller et al. 2005). Several potential implications of climate change will likely increase the number of weed introductions. We anticipate increased demand for the introduction of tropical plant species for which the climate would have previously been too cold. A second underestimated impact is that as the climate warms, biofuel production will likely increase and this may also result in introduction of new crop species, including genetically modified strains, that have potential for escape and invasion (Sheppard et al. 2011).

A Species Risk Assessment provides more scope for a detailed analysis of the PRA areas climatologically suitable for a pest. For arthropods and pathogens, insufficient geo-referenced observations often preclude construction of species distribution models. An alternative, if experimental data is available, is to create a model based on available data such as infection days, degree-days, mortality thresholds or growth indices. These types of models are also amenable to climate change studies and many examples exist in the literature for pests including Karnal Bunt, Colorado Potato Beetle, Mediterranean Fruit Fly and Prickly Acacia (Baker et al. 2000; Scherm et al. 2000; Rafoss and Sæthre 2003).

Methods are available for investigating the potential economic impact of climate change on a pest incursion (Ackerman and Finlayson 2006; Ha et al. 2010), including the useful incursion management cost-benefit modelling framework (Beare et al. 2005). However, the climate change implications are usually not included. Researchers may want to identify potential climate change impacts at each stage of a Pest Risk Assessment to determine whether they can be reliably quantified.

21.4.2 Surveillance Response

Exotic species invade through human mediated pathways (such as trade and travel) or through natural, often wind-borne dispersal. Understanding where an invasive species is likely to cross a country's national or state borders through natural transport is critical in the deployment of insect traps, sentinel plants and detection protocols used in biosecurity surveillance. The role of natural dispersal in the spread of plant pests across borders has been largely under explored, despite considerable evidence that atmospheric pathways are critical factors for determining the introduction and spread of pests (Aurambout et al. 2006) and the invasive potential of organisms (Isard et al. 2005). A good example is *Phakospora pachyrhizi*, Asian Soybean Rust (ASR) (illustrated below). With increased wind speeds and higher intensity cyclones and hurricanes projected to increase in future climates, surveillance locations and entry points will need to be more closely monitored and regularly revised.

Knowledge of any increased risks with climate change would improve the targeting of exotic plant pests and assist in prioritising 'high-risk' locations for surveillance. If surveillance can be better focused on the times and places where there is an elevated possibility of detection, then resources can be allocated more effectively to meet the threats.

For the purpose of surveillance networks, pest arrival can be forecast after host or habitat availability and climate variables are integrated with knowledge of the biology of the organism. Atmospheric transport models for aeroecological applications, such as the National Oceanic and Atmospheric Administration's HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) model can accurately track region-to-region movement (Isard et al. 2006; Kuparinen 2006; Cardwell and Hoffman 2009; Shamoun-Baranes et al. 2010). The HYSPLIT model requires interpretation to understand how the pest's biology will influence transport along the forecasted trajectory. This includes an understanding of aerobiological processes including spore production, canopy escape, turbulent transport, survival and deposition (Isard et al. 2005).

21.4.3 Incursion Management

When an invasive species breaches a border, it is critical from an incursion management perspective that adequate boundaries are established around the central incursion point in order to contain the pest. This "quarantine zone" may need to be pre-emptively increased or decreased based on the behaviour of the pest in future climates. Area freedom or containment is costly in terms of the development and implementation of contingency plans detailing the requirements for managing the pest. These plans may include: monitoring; diagnostic capability; delimiting surveys; destruction of infected host plants; regulation of movement of pest-free material; chemical spraying; management of insecticide use to avoid resistance; biological control research; awareness and detection education and development of awareness and communication strategies. Further, in the event of an incursion, decisions often must be made quickly. Although costs are involved in having an expert permanently available, the benefits of a knowledge source in an incursion are invaluable.

An example of changes to biosecurity contingencies and future policy as a result of severe weather occurred in Florida during 2005. Winds associated with Hurricane Wilma rapidly spread Citrus Canker (*Xanthomonas citri citri*), which destroyed 170,000 acres (one-third of the industry) of fruit trees in commercial groves. This movement of the bacterium breached the pre-defined 579 m (1,900 ft) quarantine zone already in place for Citrus Canker management (see Chap. 18). Before Wilma, an eradication program was in place that was discontinued as a result of the hurricane spreading the disease to a point where containment or eradication was no longer economically feasible. Severe weather events had not previously been accounted for in containment strategies (Irey et al. 2006; Gottwald and Irey 2007). Because the intensity of these events is predicted to increase with climate change (particularly for the northern hemisphere), specialists must adjust the current containment zones for key wind-borne invasive species.

21.4.4 Trade and Market Access

Pest incursions, when not due to natural migrations, are primarily due to trade. With the products of agriculture and forestry (both highly climate-dependent) accounting for a large fraction of global trade, we can expect that trade patterns will be influenced by climate change in several ways. These will include changes in global food production and consumption patterns, changes in trade routes, and changes in trade due to more direct geopolitical effects climate change and less direct effects of greenhouse gas reduction policies that may be developed to mitigate climate change.

Changes in global production and consumption patterns Global food production and consumption patterns are dependent on political geography, climate, and economic geography. As climate changes, some areas of the world will become less viable for the production of their traditional crops due to changes in temperature and precipitation. Other areas will find a change in the crops planted due to changes in global competitive advantage: An area's ability to produce the crop may not be negatively affected by climate change, but if another country has increased productivity of that same crop, then their own production may become less economic in global markets.

The IPCC (2007b) has determined (albeit with only "medium to low confidence") that the net effect of climate change will be increased food-import dependence of most developing countries. Lobell et al. (2008) identify southern Asia and southern Africa as regions likely to see the most significant declines in food production unless significant adaptation measures are taken. The IPCC also concludes, with the same level of confidence, that global trade in food and forest products is likely to increase. An increase in trade generally means increased opportunities for the spread of pests; an increase in food trade with the developing world means increased opportunities for pest transfers between developing and developed countries.

Specific commodities expected to experience significant changes in global production patterns include maize and wheat (which are likely to experience significantly reduced production in southern Africa) and millet (likely to experience reduced production in central Africa) (Lobell et al. 2008). In other regions of the world, changes in crop production are less dramatic, but may nevertheless impact trade patterns. For example, Canada is expected to increase wheat production (Lobell et al. 2010), consequently increasing export to countries where production is expected to decline.

Changes in trade routes Climate change is broadly expected to increase storminess (IPCC 2007a). This will impact seagoing trade by making some trade routes slightly more dangerous or expensive to operate in some seasons, with impacts on pest entry and establishment potential. For example, the island of Newfoundland in Canada is dependent on ferry links to the mainland for much of its animal feed supply. A stormier North Atlantic winter could reduce the reliability of the ferry link, thereby driving a need for larger shipments and increased storage capacity on the island to withstand potentially longer and more frequent periods of

Fig. 21.3 A typical cargo ship travelling between Europe and Asia via the Panama Canal (*red line*) takes 22–23 days to complete the more than 24,000 km trip; travel through the Northwest Passage (*blue line*) would require 14–15 days to travel a distance of less than 15,000 km, thereby saving considerable time and fuel. The pest risks would be significantly different, due to different landfalls in transit, different weather in transit, and a shorter travel time

transportation interruption (AAFC 2011). Possible impacts on pest introductions would be: reduced opportunities for introductions due to the reduction in the number of shipments per year; or the remaining shipments would be larger and stored for longer periods of time.

A possibly more significant change in trade routes may be occasioned by the rapid loss of perennial sea ice in the high Arctic. The Northwest Passage (Fig. 21.3) is a potential trade route that reduces the travel distance between Western Europe and Eastern Asia by several thousand kilometres. The Passage has the added advantage of not limiting vessel size to one that can navigate the Panama Canal (known as the "Panamax"). Combined, these advantages have the potential to dramatically increase trade between the North Atlantic region and the North Pacific region. This is particularly true of trade in perishable goods, which often carry pests. Although the Northwest Passage has not yet been open to significant commercial shipping, recent years have seen sea ice disappearing at a faster-than-expected rate, and the first tentative uses of the Passage for commerce. The Passage could be reasonably reliably seasonally ice-free by 2030 (Stroeve et al. 2008). Although this has not previously occurred in the historic record, it has occurred during pre-historical warm-climate intervals (Fisher et al. 2006).

The implications that an open Northwest Passage could have on global spread of pests is not clear, because much depends on trade that is displaced by the opening of the Passage. Nevertheless, Canada probably will be more exposed to exotic pests because shipping that did not approach Canada when passing through the Panama Canal will instead pass through Canadian waters and possibly stop for refuelling or other reasons in Canadian ports, even if not destined for Canada. Alternatively, much of the increased exposure will occur in the high Arctic, where climate conditions are likely to remain unfavourable for the establishment of most pests of concern. The Arctic environment is very sensitive to disturbances of any type, and is among the lowest naturally occurring biodiversity regions on Earth. Any pest that becomes established in the Arctic may therefore be of particular concern to the ecology of the region.

A positive effect of opening of the Passage would be to reduce transit through the Panama Canal. This may reduce the likelihood of pests being acquired while travelling between the North Atlantic domain and North Pacific domain. The Canadian Arctic is a very low biodiversity region and is not a likely source of many pests of concern elsewhere. Also, cold weather likely to be encountered in an Arctic passage (even in summer) could reduce the viability of many pests that are not cold-adapted, and thus reduce the likelihood of establishment at destination. Conversely, the reduced transit time between the North Atlantic and North Pacific may increase viability. The overall impact on global spread of pests, though likely mildly negative for Canada, is not easy to assess.

Other changes in trade Climate change has often been described as having potentially devastating effects in some regions of the world, through sea-level rise and desertification which may lead to large movements of refugees and major economic displacements (IPCC 2007b). These scenarios notwithstanding, we can anticipate that climate change will induce some geopolitical change, which in turn will likely affect trade patterns.

The policies that may be developed to reduce atmospheric greenhouse gases could have a very significant impact on trade in agricultural and forest products. If "carbon foot-printing" becomes widespread and starts to affect trade (e.g. through a local-food movement such as the "100-mile diet"), then we could see an increased emphasis on locally-produced foods. Diversifying local production could increase the number of crops in any given location that are vulnerable to exotic pests and possibly reduce the movement of exotic tropical produce into developed (temperate) countries.

Some crop production techniques may be altered, with reductions in tillage and other changes in practice being favoured for their carbon sequestration potential; these changed practices may have unknown impacts on plant pests. No-till cropping, for example, is being promoted in part for the soil carbon storage benefits it provides. However, unless changes in rotation or other practices also are made, no-till cropping may increase the abundance of some pests while decreasing others (Anderson 2008). Furthermore, some pest control measures such as methyl bromide (which is already under pressure as a greenhouse gas) could become increasingly disfavoured. This could have an impact on trade in products between countries where methyl bromide is required as a preventative measure unless equally low-cost and effective but more atmosphere-friendly alternatives are developed.

21.5 Case Studies

To illustrate the projected effects of climate change on quarantine policy and the potential consequences to biosecurity operations, we present an analysis of the effects on the biology and potential distribution of the exotic insect pest, Asiatic Citrus Psyllid in Australia (if introduced) and the exotic fungal pathogen, Asian Soybean Rust in the USA.

21.5.1 Asiatic Citrus Psyllid, Diaphorina citri *Kuwayama (Hemiptera: Psyllidae)*

Diaphorina citri Kuwayama (the Asiatic citrus psyllid or ACP) is one of two known vectors of the debilitating bacterial citrus disease known as Huanglongbing (HLB) or Citrus Greening (Aubert 1987; da Graça 1991; Halbert and Manjunath 2004). HLB is caused by numerous strains of the phloem-limited bacterium '*Candidatus* Liberibacter spp.' (α-Proteobacteria) (Jagouiex et al. 1994) and infection leads to chlorosis, tip dieback, reduced foliage and eventually tree failure combined with the production of small, lopsided, bitter tasting, discoloured fruit which are unmarketable (Barkley and Miles 2006; Bové 2006; Stokstad 2006) (see Chap. 18 for more detail).

ACP and HLB are widely distributed throughout the citrus growing regions of the world (Chap. 18, Aurambout et al. 2009; Beattie and Barkley 2009). ACP was reported from northern Australia in 1915 but was eradicated by chance during the 1916–1922 campaign to rid the Northern Territory of an outbreak of Citrus Canker (Bellis et al. 2005). Re-introduction of ACP (and HLB) would have serious repercussions for the AUD$446 million per annum Australian citrus industry (Johns 2004; Australian Citrus Growers 2007). Indeed, HLB is considered one of the highest priority exotic pathogen threats to Australian citrus (Dempsey et al. 2002).

The Australian climate varies greatly over its large land mass and has numerous climate classification zones including equatorial, tropical, subtropical and, temperate. The impact of climate change is also likely to be spatially heterogeneous directly affecting ACP growth, development and behaviour. ACP can also be affected by climate change indirectly via changes to the hostplant. This case study outlines the potential impact of climate change on the future biosecurity risk status of ACP.

Probability for entry A high-risk pathway for the entry of ACP (and HLB) into Australia is through the introduction of infected citrus material or psyllids from neighbouring countries particularly New Guinea, Indonesia and Timor Leste where the disease and vector are present (Weinert et al. 2004). The Torres Strait separates Australia from Papua New Guinea by only 150 km at its narrowest point and includes over 200 islands. Greater intensity tropical cyclones and projected increases in wind speeds over northern Australia may increase the likelihood of wind-borne assisted incursions. The entry of the Leucaena Psyllid (*Heteropsylla cubana* Crawford) into northern Australia is considered due to air currents from the Western Pacific probably associated with tropical Cyclone Winifred (Bray and Sands 1986). Further, for ACP long distance movement (90–145 km) associated with hurricanes has been hypothesized to account for rapid disease dissemination in Florida (Gottwald et al. 2007).

In the north, extreme precipitation events are projected to be more widespread in summer and autumn (CSIRO and BoM 2007) that will be detrimental to ACP population growth both in terms of direct mortality and availability of host.

Probability for establishment and spread Cultivated citrus is produced commercially in every state of Australia except Tasmania. Two major citrus growing regions are southeast Australia (encompassing parts of Victoria, New South Wales and South Australia) and southeast Queensland. Minor growing areas also exist in Queensland, New South Wales, the Northern Territory and Western Australia (CAL 2009). Preliminary bioclimatic CLIMEX™ matching (Sutherst and Maywald 1985) has indicated that ACP would survive in most of Australia's citrus growing regions except the parts of northwest Victoria and southern New South Wales (Beattie 2002).

ACP has been observed to rest, feed and/or complete its development on numerous species and varieties of Citrus, including six native Australian citrus species. As a consequence, we see a high likelihood of ACP finding a suitable host after it has entered Australia. Several species of native Australian citrus occupy the north and northeastern coastline of Australia filling the gap where cultivated Citrus is absent (Sykes 1997; Mabberley 1998; AVH 2009). Further, the native form of the rutaceous orange jessamine (*Murraya paniculata* (Linn.) Jack), has an extensive distribution along the northern coastline of Australia (Mabberley 1998; AVH 2009) and the cultivated form (*M. paniculata*var *exotica*) is a common ornamental plant in Australian parks, gardens and backyards (Mabberley 1998).

To test the potential impact of climate change on the behaviour, distribution and fecundity of ACP in Australia, a model was developed incorporating its biology in relation to its *Citrus* host (Aurambout et al. 2009). The model was applied to three timeframes; a baseline (1990) and projected temperatures for 2030 and 2070 using the 'mid-range' (A2) emission scenario (Nakićenović and Swart 2000). Increasing temperatures affected the emergence date and duration of new vegetative growth critical to ACP feeding and egg-laying (Husain and Nath 1927; Lin et al. 1973). Flushing cycles for Citrus in Australia are not well known and vary spatially. The model was based on flushing-cycle dates from a study of water use efficiency for Valencia orange trees grown in the major citrus growing region of

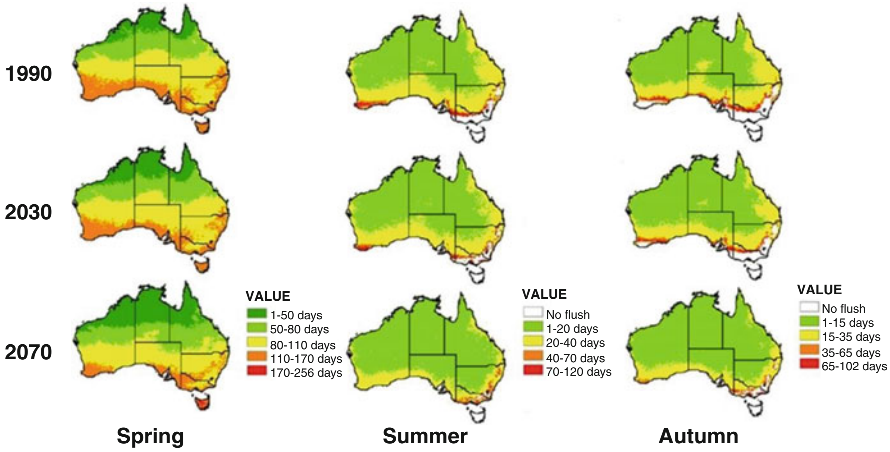

Fig. 21.4 Number of days when the first (Spring), second (Summer) and third (Autumn) flush is present for 1990, 2030 and 2070 (Aurambout et al. 2009)

southeast Australia. Here, three flushes per year generally occur: The main one in Spring followed by smaller flushes in summer and autumn (Hutton 2004). With increasing temperatures all three flushes will occur earlier in the year and be of shorter duration (Fig. 21.4). All three flushes will show a gradual southward expansion of shorter durations, and a geographic expansion in terms of the area over which the summer and autumn flushes can occur (Aurambout et al. 2009). In short, southeast Australia will become progressively more climatically suitable, and experience longer periods of summer and autumn flushes, as the temperature increases. One of the implications of these findings are that surveillance activities to detect potential ACP incursions should start progressively earlier in the year as the temperature increases.

The model indicated that increasing temperatures led to a shortening of the psyllid developmental period, thereby partially compensating for the shortening of the time period when young flushes are available for ACP reproduction.

Overall, the model showed ACP's response to be spatially heterogeneous with a general decrease in the number of successful generations across all of Australia, associated with increasing temperature. Warmer climate change scenarios (Figs. 21.5 and 21.6) also led to very significant decreases in ACP successful reproduction rate, particularly during Easter and in the northern half of Australia. This decrease in the number of generations of ACP successfully reaching the adult stage can be explained by the shortening period when flush was available for feeding, thus leading to an overall reduced population of psyllids (Figs. 21.5 and 21.6). Different climate change scenarios showed that a modest decrease in the number of successful generations of ACP could be expected by 2030 across all scenarios in the northern part of Western Australia (Fig. 21.5).

Climate change impact should become more prominent by 2070 where all scenarios showed a strong decrease in ACP reproductive success (particularly

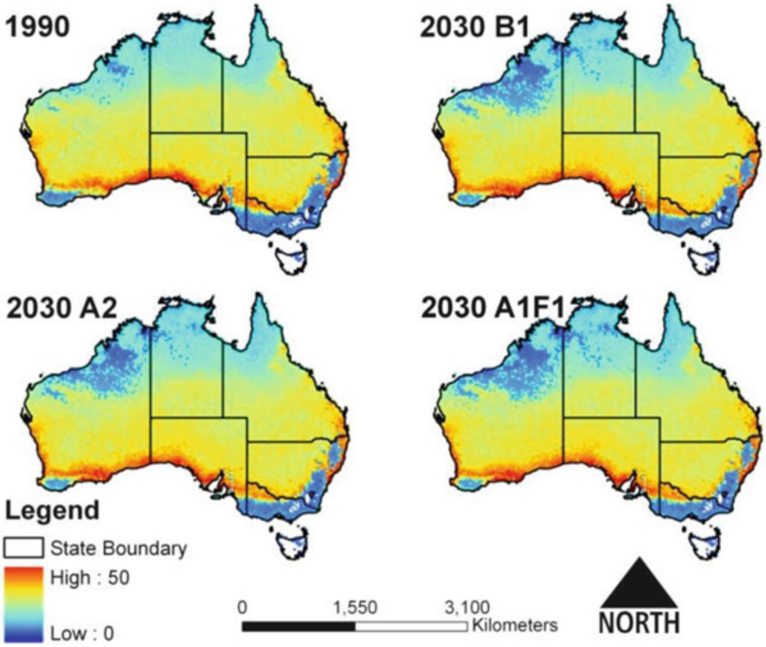

Fig. 21.5 Number of Asiatic Citrus Psyllid generations successfully reaching adult stage (after a 3-year simulation), for 2030 under three climate-change scenarios

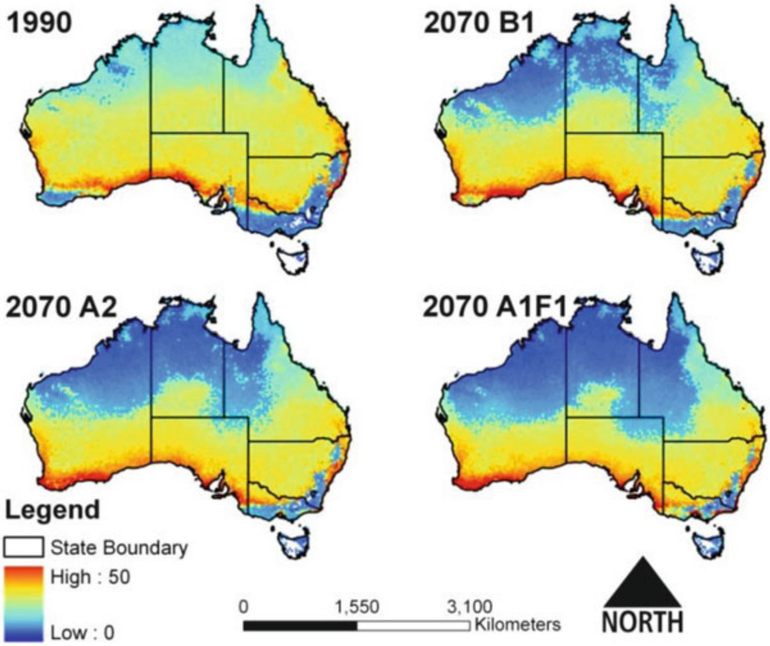

Fig. 21.6 Number of Asiatic Citrus Psyllid generations successfully reaching adult stage (after a 3-year simulation), for 2070 under three climate-change scenarios

pronounced under the A1FI scenario) in the northern part of Australia (Fig. 21.6). The model showed progressively larger decreases in numbers of successful generations along the northern coastline as the temperature increases and making the southwest and southeast Australia more suitable for the establishment of the psyllid.

Climate change impact also indicated a progressive trend towards earlier emergence and higher populations in the southern parts of Australia as the temperature increases consistent with the advancement in spring flush initiation. The benefits of a spatially enabled model are the ability for multiple locations to be examined simultaneously aiding large-scale surveillance planning by identifying high-risk locations and time periods.

Temperature is an important environmental variable controlling ACP development (Liu and Tsai 2000) and its distribution (Yang et al. 2006). The model also confirmed projected changes in psyllid behaviour and geographic range as temperature increased (Aurambout et al. 2009). However, given that the citrus industry is present in a few discreet locations, determining the risk to the industry as a whole must consider potential changes to citrus phenology and distribution in response to other climatic variables. In the major citrus growing regions of south-eastern Australia, annual precipitation is expected to decrease 2–5 % by 2030 particularly during winter and spring and up to 10 % by 2070. As a consequence, drought is also expected to increase with 20 % more drought months predicted by 2030 over most of Australia and up to 40 % by 2070 (CSIRO and BoM 2007). Irrigation for crops (competing against water for human consumption and environmental needs) in the Murray Darling basin, for example, may significantly reduce citrus plantings in the area and the potential for high-risk threat. However, as regions further south become warmer, they may also become more climatically suitable for citrus growth and see the development of a local citrus industry.

Consequences of establishment The presence of the disease elsewhere in the world has led to a drastically reduced life expectancy of citrus with significant production losses (Halbert and Manjunath 2004). Australia is at substantial risk from the establishment of ACP in Australia with severe downgrading or loss of the entire citrus industry through reduced or static production, unmarketable fruit and cost associated with control measures. The impact will depend on the location of the outbreak and whether it can be satisfactorily contained. Indications are that the likelihood of ACP entering Australia through the northern borders will be greater in future climates but that establishment in citrus orchards is much less likely than in the southeast of Australia.

Locally, the farmer will be subject to immediate quarantine restrictions and may bear the cost of orchard destruction. Also, a long lead time exists for citrus trees between replanting and bearing fruit, which equates to lost income for the grower (Beare et al. 2005). Restriction of interstate trade from the affected area will equate to similar losses on the ground and significant losses to export revenue. Nearly 25 % of Australian citrus is exported overseas which represents about half the total value of production (PHA 2009). Although trade is unlikely to be stopped due to the high prevalence of HLB worldwide, Australia's reputation as a "clean and green" supplier may suffer.

Australia's valuable genetic resource in native citrus is also at risk (Finlay et al. 2009). Australia is one of the richest biodiversity centres for citrus (Mabberley 2004). Recent revisions of citrus systematics (Guerra et al. 2000; Samuel et al. 2001; Mabberley 2004; Zhang et al. 2008; Bayer et al. 2009) suggest that the six Australian indigenous species may comprise up to 50 % of all extant citrus species. The conservation and maintenance of genetic diversity is a high priority for *Citrus* because it is recognised as one of the 35 most important food crops globally (FAO 2009). Australian wild *Citrus* spp. are ecologically adapted to diverse conditions, from tropical rainforest to desert, and therefore have a significant genetic variability useful as rootstock for breeding new varieties (Sykes 1997). Loss of genetic diversity could be an unforeseen cost to the natural environment and could have wide ranging effects on the global community through lost resource for genetic manipulations.

Australia also has an extensive native Rutaceae flora of about 1,200 species (ABRS 2005). Two species are listed as critically endangered, 28 species are endangered and 38 species are vulnerable under the *Environment Protection and Biodiversity Conservation Act* (Anon 2009). In the wet tropics of northeast Queensland, nine species of Rutaceae (including *Citrus inodora* F. M. Bailey) are listed as being of conservation significance (Anon 2005). If these plants are susceptible to HLB, and ACP can transmit HLB to them, then HLB's adverse effects on Australian floral biodiversity could be substantial. Both citrus and ornamental Rutaceae are commonly grown as garden plants in Australia. Mandatory destruction of backyard trees will be costly, logistically difficult and we may see social and political backlash from owner-growers over tree and fruit loss.

21.5.2 Asian Soybean Rust (ASR)

Before its entry into the USA, Asian Soybean Rust (caused by *Phakopsora pachyrhizi* Sydow & P. Sydow) was considered the most serious foliar disease of soybean (*Glycene max* (Linnaeus) Merrill) that could potentially impact in the USA (Sinclair and Hartman 1996). For example, during 2003, ASR was detected in 90 % of the soybean fields in Brazil, with yield losses equivalent to US$759 million, and an additional US$544 million was spent on fungicide applications to control the disease (Yorinori et al. 2005). ASR is an obligate parasite that requires green tissue to survive and reproduce (Bromfield 1984). Since its arrival in the USA, this pathogen has overwintered on kudzu (*Pureria Montana* (Loureiro) Merrill var. lobata (Willdenow) Maesen & S. Almeida) in a narrow geographic zone along the Gulf Coast region and has spread to the North Central soybean production region each year by the aerial transport of ASR urediniospores (USDA 2011). To date, this spread has occurred late in the growing season, and thus the impact of ASR on the North American soybean industry has been less than anticipated. ASR is a pest whose geographic distribution in North America will likely be impacted by climate change due to the frequency and pathways of atmospheric motion systems.

Further, the geographic distribution of the overwintering population has a pronounced effect on the timing and extent of aerial spread of this pathogen during the growing season.

Probability of entry On 6 November 2004, ASR was discovered on the North American continent (Schneider 2005). ASR had been identified during the early twentieth century in Japan and by mid-century, it had spread throughout the soybean production regions of south-eastern Asia, Australia, and India (Bromfield 1984). During the mid-1990s, ASR was reported in central Africa and over the next 5 years spread throughout soybean growing areas of the continent. By 2001, the disease had crossed the Atlantic Ocean, infecting soybean fields in the Rio Parana valley of South America (Miles et al. 2003). The disease remained in the Southern Hemisphere for the next 3 years but in June 2004, soybean foliage from north of the equator in Colombia tested positive for the pathogen (Isard et al. 2005).

To investigate the aerial transport of ASR and assess the risk of spread to North America, researchers developed the Integrated Aerobiology Modelling System (IAMS; Isard et al. 2005). The IAMS includes sub-models for spore release and escape from the plant canopy, atmospheric transport, mortality due to exposure to solar radiation, wet and dry deposition of spores, host development at sources and destinations, and disease progress on these hosts. Aerobiological model simulations suggested that ASR spores were blown from north-western South America to the south-eastern USA in September 2004 on winds associated with Hurricane Ivan (Isard et al. 2005). A map showing the IAMS prediction of where viable spores were deposited in rainfall was provided to the USDA Soybean Rust Rapid Response Team in early November 2004 as a guide to field scouting sorties for the disease. Within 3 weeks of the initial discovery, ASR was confirmed in diseased plant tissue from "volunteer" soybean plants and kudzu at multiple locations within or immediately adjacent to the region of heavy spore deposition predicted by the IAMS (Fig. 21.7). In each of the following years, this pathogen has spread from kudzu into commercial soybean fields, first in the southern USA and then in the continental interior of North America, causing infection in soybean as far north as Ontario in 2007 (USDA 2011).

ASR provides an example of an airborne pathogen that has demonstrated intercontinental movement via tropical and mid-latitude cyclones. ASR was likely blown by atmospheric disturbances associated with the equatorial trough from Africa to South America and then later by Hurricane Ivan to North America. Given the projections for more intense tropical cyclones with larger peak wind speeds and heavier precipitation, the likelihood of entry into the country is increased (CCSP 2008). This conclusion has important implications for other important plant pathogens or new pathotypes that may follow the atmospheric pathway used by *Puccinia melanocephala* Sydow & P. Sydow (the causal agent of Sugarcane Rust, Purdy et al. 1985) and Soybean Rust, first crossing the Atlantic Ocean from Africa to the South American continent and/or the islands in the Caribbean basin, and then moving northward into the USA.

One approach to assessing the likelihood of a pest following the atmospheric pathway is to break the aerobiology process into four component indices: Source

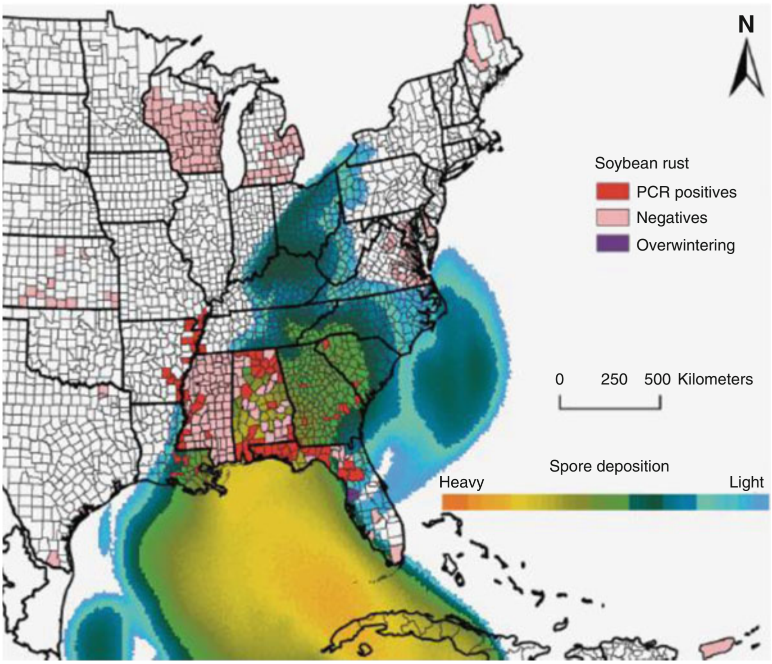

Fig. 21.7 Predicted deposition pattern for ASR from the IAMS model and observations from PCR analysis (Isard et al. 2005)

strength, transport, host distribution, and epidemic potential. This approach was used before incursion of the pathogen to predict where and when ASR was most likely to enter the USA (Fig. 21.8) (Isard and Magarey, unpublished data). In this application, the source strength index was based on the area of susceptible host in potential source regions. It also incorporated the seasonality of inoculum production by dividing the year into six, 2-month periods. This first criterion quickly led researchers to discount Africa as a potential source region for direct transport of ASR into North America. While Nigeria (Africa's largest producer) grew 6,000 ha of soybean, South America had over 30 million ha of soybean (FAO 2002), a significant proportion of which was infected with soybean rust in 2003 (Miles et al. 2003).

IAMS output was used to create the transport index. This assessment was made by recording the number of daily transport events over the previous 10-year period that had the potential to carry viable spores from each potential source area to each of ten USDA agricultural regions. The susceptible-host index was compiled for each region and a 2-month window by considering agricultural census data including harvested acres as well as planting and harvesting dates. The epidemic potential was assessed for each region and 2-month window by using an infection model in the NAPPFAST pest forecasting system (Magarey et al. 2007). Epidemic potential declined as the season progressed because there was less time for epidemics to develop before the onset of winter. To make the final risk assessment, each index

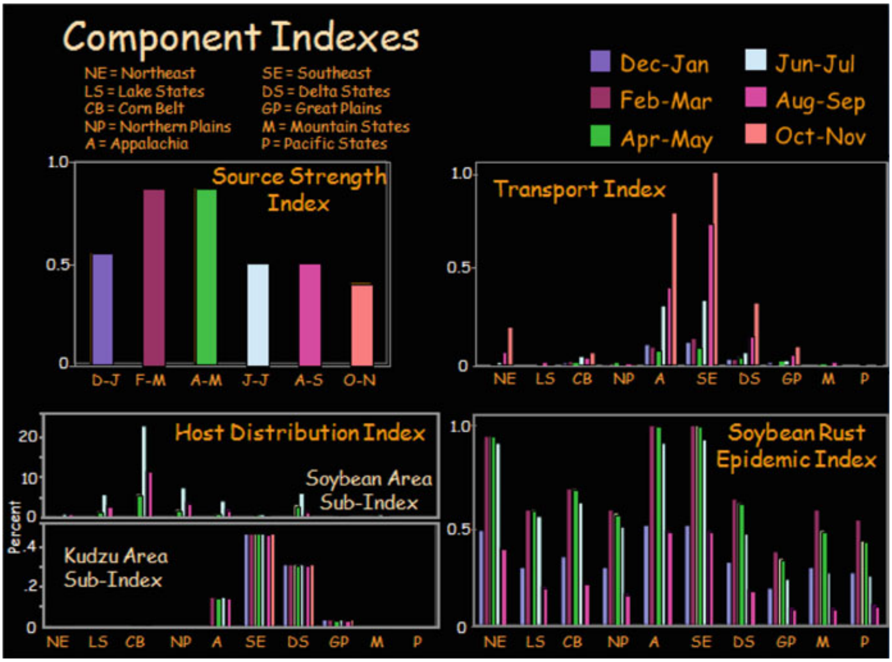

Fig. 21.8 Component risk indexes for predicting when and where soybean rust might enter the USA (Isard and Magarey, unpublished data)

was scaled between 0 and 1 and multiplied together. The final risk index (not shown in Fig. 21.8) indicated the highest risk areas (Appalachia and Southeast) and seasons (June and July) suggesting where and when to scout. As indicated above, soybean rust entered the USA during late September, arriving in the Southeast agricultural region as the index predicted for that time period but too late during the growing season to initiate an epidemic in 2004.

Probability of establishment Although the pathogen is known to have many alternative hosts, ASR has been predominantly found on soybean and kudzu in the USA (USDA 2011). Consequently, during the winter, ASR is restricted to coastal zones of states bordering the Gulf of Mexico (Gulf Coast) and to areas of the Caribbean basin, Mexico, and Central America where either kudzu retains its foliage or soybeans or jicama (*Pachyrhizuserosus* (Linn.) Urban, an alternative host that is commonly grown in Mexico) are grown year-round (Isard et al. 2007; USDA 2011). To cause significant yield losses in North America, *P. pachyrhizi* uridiniospores must move from these areas into the major soybean production region in the continental interior after early May and before September, when the crop is susceptible to the disease (Livingston et al. 2004).

An assessment of the overwintering area for ASR in the USA (Pivonia and Yang 2004) using the CLIMEX software (Sutherst and Maywald 1985) and a long-term data set suggested that the pathogen was only likely to overwinter in central and southern Florida and Texas (USDA Plant Hardiness Zone 10, see Fig. 21.2), where soybean production is minimal. So far, this assessment has been correct. Over the past six winters, soybean rust has overwintered on kudzu in these regions and occasionally along the Louisiana coast and a few urban heat islands outside Zone 10 but within the Gulf Coast states (USDA 2011). Specialists speculate that changes in temperature and precipitation will lead to an increase in ASR in USA soybean production regions (Li et al. 2010). By the end of the century, according to the IPCC A1B scenario, large areas will be suitable for overwintering ASR in southern Texas and Florida as well as areas in Louisiana, Mississippi Alabama, and Georgia. These latter states currently plant almost 1.6 million hectares of soybean; future large acreages of commercial soybean may be planted in close proximity to infected kudzu. Where the early growing season is wet and warm in these states, rapid ASR build-up in commercial fields could provide a large source of inoculum for long-distance aerial transport northward into the central USA at a time when the crop in this important soybean production region is susceptible to yield loss from the disease.

Consequences of establishment The interior of North America was the last major soybean production region in the world to experience Soybean Rust. After the pathogen crossed the Atlantic Ocean and gained a foothold in South America, an unprecedented level of cooperation arose among USDA agencies, state Departments of Agriculture, universities, industry, and grower organizations to prepare for ASR entry into the USA. During the 2004/2005 winter, the USDA Soybean Rust Information System was constructed to integrate soybean rust monitoring, data basing activities, IAMS output, and communications to stakeholders into a state-of-the-art cyber infrastructure (Isard et al. 2006). As part of this effort a monitoring network of >700 Soybean Rust sentinel plots was established across 35 USA states and five Canadian provinces. The majority of the plots were planted in soybean, with 10 % established in kudzu and another 5 % in other leguminous crops (Giesler 2006). In later years, the number of plots diminished as Extension specialists learned more about the distribution and spread of the disease (USDA 2011). By 2007, the network had expanded to include sites in Mexico. Cultivars, planting date, and scouting frequency varied throughout the network in accordance to the USDA sentinel plot protocol (USDA 2011). The first positive find of Soybean Rust in each state, as well as many of the additional positive identifications, were confirmed by the USDA National Plant Diagnostic Network labs (Harmon et al. 2005). The sentinel plot network was augmented by mobile scouting in areas threatened by the disease. In 2006 at the height of monitoring activity, about 18,000 observations were submitted to the national soybean rust database from 2000 different geographic locations throughout the USA and southern Canada (USDA 2011).

The impact of the coordinated response to the challenges of managing the ASR pathogen was enormous. After harvest in 2005, the USDA Economic Research Service reported that many millions of USA soybean hectares that would have received unnecessary fungicide sprays remained untreated for Soybean Rust due to this application of aerobiology and advanced IT for Integrated Pest Management

(IPM). The information disseminated through the USDA Soybean Rust Information System increased the profits of USA soybean producers by between US$11 and US $299 million at a cost of between $2.6 and $5 million (Roberts et al. 2006). In 2006, the USDA Soybean Rust Information System was renamed the IPM Pest Information System for Extension and Education (ipmPIPE) and gradually expanded over the next few years to include monitoring programs focused on soybean aphid, diseases of common beans, Downy Mildew of cucurbits, Pecan Nut Caseborer, and Southern Corn Rust. Use of the Internet platform by producers, crop consultants, Extension specialists, and administrators throughout this period was high, especially with the introduction of a bilingual (Spanish-English) format in 2009. An analysis by Extension specialists indicate that growers' use of the SBR component of the platform alone saved $207 million in fungicide application costs in 2007 (Giesler and Hershman 2007) and roughly $200 million/year in 2008 and 2009 (Hershman 2010). More than 90 % of 361 Certified Crop Advisors who responded to a survey in 2008 indicated that they valued the Soybean Rust ipmPIPE website and sentinel plot network, and that they felt "somewhat" to "very" confident in the information obtained from them (Bradley et al. 2010).

Soybean Rust has not had the level of impact in the USA that was initially expected by stakeholders. Since establishment, ASR has remained in the southeast USA during most of the growing season, spreading into the major production region after the soybean crop was no longer susceptible to yield loss from the disease (Isard et al. 2007; USDA 2011). Regional spread of ASR may be limited by the slow disease progress on kudzu during the first half of the year combined with the short period available for disease establishment on soybean during the vulnerable phase of host reproductive development (Christiano and Scherm 2007). Efficacious management of the pest in the South seems to have helped by retarding the build-up of inoculum in commercial soybean fields in that region as well. We speculate that the impact of the disease may increase over time if ASR develops greater virulence on kudzu. Climate change appears likely to increase the frequency of tropical storms in the Caribbean basin and extend the overwinter geographic zone for *P. pachyrhizi* northward into areas that currently produce larger acreages of soybean. Together these factors may result in earlier build-up of inoculum in commercial soybean fields in the southern USA and earlier and perhaps more frequent transport of the pathogen to the major soybean production region in the continental interior of North America.

21.6 Conclusions

Any model projections or field based records that suggest a weed, insect or pathogen may shift its geographic host range through the effects of climate change will undoubtedly provide valuable information on the future distribution of these species. Modelling predicted impacts on species ecology and changes in distribution of species is a more objective and less history-dependent approach to assist future decision-making. Incorporation of any climate change projections in a model should reflect the most recent and comprehensive science as determined by the IPCC. In most cases we recommend that the high emission scenario be used for these projections (A1FI) as this is the most representative of current trends.

Surveillance operations can be re-focussed on key species with new knowledge of climate change impacts, e.g., increasing temperature or severe weather events. This will inform pre-border and border surveillance activities as they relate to "probability of entry". The Asiatic Citrus Psyllid case study provides evidence that increasing global temperature will result in the earlier arrival of the psyllid coupled to the earlier timing of citrus growth flush which will have implications for the timing of deployment of surveillance for this species.

Knowledge of changes in the biology and "invasiveness" of species and the effects on host and alternate host distribution and biology as a result of climate change will underpin decisions on "probability of establishment" of a particular pest.

The use of atmospheric transport models such as IAMS and HYSPLIT will be integral in determining the movement of species under future climates as it relates to "probability of spread" as demonstrated with the Asian Soybean Rust incursion in the USA.

Agricultural landscapes are likely to alter in response to climate change. PRAs should also determine changes to commodity-based industries and continually assess production prospects in marginal areas. New regions may be identified as becoming more suitable for production to a particular crop especially where mitigation measures are no longer viable. For example, continuing decreases in water availability in the drier cropping zones of southeast Australia may result in a retreat of these cropping areas and establishment in the wetter north. Alternatively, land management and land use transformational changes may occur for some industries in some areas that are unable to adapt to the changing climate. In each case, new pest and host plant interactions, or lack thereof, must be assessed to determine changes to pest risk and will help determine re-prioritisation of the risks.

The effects of climate change on a pest should be considered in PRAs where there is evidence to support its inclusion. The implications of climate change are greater with decreasing size of the considered PRA area. For example, relatively small countries such as New Zealand or United Kingdom or provincial or state jurisdictions have relatively few climate zones. Consequently, climate change can result in additional climate zones being added to the jurisdiction and opportunities for pests to establish in previously inhospitable areas. A regular revision of the PRA will be required relative to new global assessment reports released by the IPCC. In each case, revision of the PRA in light of emerging or recent climatic events may be required. For example, a severe storm event may warrant the revision of an existing PRA for climate-sensitive pests.

Global climate projections should be applied to these decision-making processes for a reasonable time period (e.g. 2020–2030) as opposed to longer-range forecasts. We recommend the use of a 30-year scenario for the following reasons: (1) This might represent a potential timeframe over which measurable numbers of pests might enter, establish and spread; (2) longer time frames are subject to greater uncertainties both in terms of actual emissions and climate change; and (3) changes in political, trade and phytosanitary conditions and practices may be even greater than climate change factors over periods longer than 30 years. Minimising this uncertainty will enable industry and quarantine agencies to better prepare and adapt to any increased quarantine risks posed by future climates. Although the impact of climate change may be smaller than other changes such as trade, many of the impacts of climate change are much more uncertain and unpredictable. Given this uncertainty, we make the following recommendations for future research:

1. Improve atmospheric transport models and the associated pest and crop distribution data sets to enable timely prediction of pest incursions following major tropical storms and hurricanes.
2. Identification and prioritization of new and existing pest threats in jurisdictions most impacted by climate change.
3. Identify climate change impacts that may result in abnormal trade or cropping patterns and consequently introductions of pests.
4. Develop guidelines to incorporate climate change into PRAs and other quarantine policy guidelines such as emergency response.
5. Assess climate change impacts on pest status by incorporating climate change scenarios in currently deployed operational pest models.

The entry, establishment and spread of weeds, insects and pathogens will undoubtedly be influenced by our changing climate. As new knowledge comes to hand it is vital that regulatory agencies incorporate the likely consequence of these changes into their existing policy framework and consider the implications this has for global trade, quarantine and food security.

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Chapter 22
The Future of Regulatory Plant Science

Robert Griffin

The struggle between man and insects began long before the dawn of civilization, has continued without cessation to the present time, and will continue, no doubt, as long as the human race endures. It is due to the fact that both men and certain insect species constantly want the same things at the same time… wherever their interests and ours are diametrically opposed, the war still goes on and neither side can claim a final victory. If they want our crops they still help themselves to them… Not only is it true that we have not really won the fight with the world of insects, but we may go farther and say that by our agricultural methods, by the extension of our commerce, and by other means connected with the development of our civilization, we often actually aid them most effectively in their competition with ourselves.

(Forbes 1915)

22.1 Introduction

The history of mankind is marked by thousands of years cultivating and trading plants and plant products. Ancient consumers coveted an exotic variety of plants, spices, and other agricultural products from faraway lands, but modern urbanites take it for granted that a vast array of fresh agricultural products from anywhere in the world will be available in the local supermarket. Indeed, the availability of food has been a key factor in the rise and fall of civilizations throughout history and trade in plants and plant products has been the cornerstone of economic stability and the engine of innumerable industries (Anonymous 1958; Cox and Large 1960; Ullstrup 1972).

R. Griffin (✉)
Director, Plant Epidemiology and Risk Analysis Laboratory, Plant Protection and Quarantine, APHIS, USDA, Raleigh, NC 27606, USA
e-mail: Robert.L.Griffin@aphis.usda.gov

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Access to sufficient, safe, and nutritious food is central to the concept of food security. At the same time, the ability to trade in plants and products is fundamental to commerce and essential to many national economies. The United Nations estimates the world's population will increase from seven billion in 2011 to ten billion by the end of the century (UN 2011a, b). Despite recent increases in production, world food supplies are barely keeping up with demand (FAO 2011a). In order to feed the burgeoning global population, food production must increase at least 70 % in the next few decades (FAO 2011b). Based on current trends, the anticipated rise in living standards is also expected to cause a comparable increase in the demand for greater diversity and higher quality agricultural products in the marketplace (Hall 1995).

The fundamental reality of the past is the same as the future: food is a basic necessity and trade happens. Agricultural production and agricultural trade are inextricably linked in history and for the foreseeable future. The difference going forward is the rapid rate of evolution and the magnitude of the challenges for future generations to provide the quantity, quality, and diversity of agricultural products that consumers expect.

Strategies for increasing production are limited to expanding the areas of production, increasing the intensity of production, and decreasing losses to pests and waste. Past and present advances in science and technology have made it possible to cope with the challenges of increasing productivity as well as reducing losses. The twentieth century saw enormous progress in genetics, engineering, and chemistry with huge positive impacts on agricultural productivity. Advances in transportation, handling, and storage greatly improved distribution possibilities and reduced waste. Pest control strategies and tools, both in the field and post-harvest, also benefitted from leaps in science and technology that had similar effects on reducing losses, including where the movement of people and goods becomes a pathway for the introduction of pests which can be harmful in new environments. This is where science and technology crosses paths with the regulatory aspects of trade under the traditional rubric of plant quarantine which, depending on the source, comes under the aegis of plant protection or plant health.

A key challenge for the twenty-first century is adapting agricultural policy frameworks to promote productivity while also cultivating a trade environment that accommodates the demand for increasing quality and expanding the availability of agricultural products. Efforts to boost agricultural productivity are already complicated by unprecedented global challenges including the loss of arable land, lack of water, reduced effectiveness and acceptability of chemicals, and global warming (Brown 2004). Fewer producers and the growing influence of corporate farming also signal changes in the political voice of agriculture while globalization accelerates and magnifies agricultural trade to levels unimagined by past generations.

The relationship of regulatory responses to pest risks and the scientific underpinnings of such responses define the future role of regulatory science for plant quarantine. The dizzying pace of evolution combined with complex global challenges and intimidating uncertainty promise to test regulatory plant science as never before and demand the highest possible level of effort, collaboration, and thoughtfulness from the phytosanitary community in the new century.

22.2 Evolution of the National Plant Protection Organization

The National Plant Protection Organization (NPPO) is situated squarely at the intersection of production and commerce, and on the edge of a new age in regulatory plant science. These relatively small regulatory agencies exist in nearly every country where they carry the seemingly contradictory charge of protecting the plant resources of the country from the introduction and spread of pests, including pests which may be introduced via trade, while also authorizing imports and promoting exports with trading partners.

Today's NPPO is the result of more than three centuries of evolution beginning in 1660 with the earliest efforts to apply regulatory measures in the management of plant health by authorities in France legislating for the destruction of barberry (*Berberis vulgaris*) to control black stem rust of wheat (Large 1940). In an significant leap from national legislation to cross-border collaboration, the French and other European countries fashioned the first multilateral effort for plant protection in response to the introduction of the American vine louse (*Viteus vitifolia*; formerly known as *Phylloxera vasatrix*), a pest from North America which was having a devastating effect on the vineyards of Europe in 1878. The step toward multilateral cooperation was significant because it required national governments to establish offices, officials, authorities and policies for cross-border collaboration toward shared objectives – the precursors of today's NPPOs.

National legislation for other pests followed in Great Britain and other European countries. By 1912, the United States which had been aggressively importing new plant species for over a century, established the Plant Quarantine Act which aimed to provide authority for quarantine actions on imports as well as for domestic pest control and eradication programs (Weber 1930). These early regulatory efforts gave birth to the first national organizations devoted to plant protection. This too was significant because the same organizations that were working outwardly on cross-border issues were also responsible for managing domestic challenges. It may seem a normal and logical approach which is taken for granted today, but this arrangement stands in contrast to the traditional and more common approach of governments to separate the authorities and agencies working on domestic and trade or foreign issues beginning at the highest political levels. The point is significant because this logic carried forward to the International Plant Protection Convention (IPPC) and continues today in most countries.

The early part of the twentieth century saw slower progress in regulatory plant protection owing to a depressed global economy and World War I. By 1929, interest in multilateral cooperation for plant protection had been revived and the International Convention for the Protection of Plants was established following a series of technical conferences hosted by the International Institute of Agriculture in Rome (Rogers 1914). This effort was stifled as the global economy dipped again and attention shifted to World War II. The further evolution of plant quarantine was largely stagnant until the end of the war brought new prosperity with large investments in nation-building, including agriculture. The United Nations also took root with strong global support for peaceful cooperation on large-scale agricultural initiatives as the world-view moved from isolationism to globalization. The formation of the General Agreement on Tariffs and Trade (GATT) in 1947 was another significant indicator of change as the path to lasting peace was seen as shifting from protectionist economic policies to liberalized trade on the road to globalization. Decades later, these world-changing philosophies would converge with the evolution of plant quarantine in a significant way to reshape the role of NPPOs (WTO 1999).

By 1945, the Food and Agriculture Organization of the United Nations (FAO) had replaced the International Institute for Agriculture and a flourish of "plurilateral" activities were initiated including a series of efforts to draft an international plant protection agreement. These efforts culminated in September 1951 with agreement on a final draft that became the IPPC (Ebbels 2003). The original Convention was stable for many years and enjoyed wide acceptance but spotty implementation without an active infrastructure. In 1977, the Convention underwent minor changes associated with phytosanitary certification. More substantial changes were agreed in 1997 following the Uruguay Round negotiations of the GATT that established the World Trade Organization and Agreements on Agriculture, including the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement).

From its beginnings in 1952, the IPPC established the roles and responsibilities of the NPPO and most governments followed the general principles and design outlined by the Convention, notably a dual focus on plant pests established in the country and also those which may become established via commerce or natural spread (IPPC 1997). The linkage created by the Convention to bridge the management of domestic plant pests and preventing the introduction and spread of pests from trade has become a defining feature for NPPOs, and continues to be the paradigm for many modern NPPOs, but at least two other design elements from the IPPC are frequently overlooked. One of concepts that has always been a central feature of the Convention is multilateral cooperation in response to regional pest outbreaks (e.g., Desert locust). In practice, such programs have not been directly addressed by the IPPC or through the collaboration of governments under the aegis of the IPPC, but rather by FAO and other international organizations or national and international aid agencies.

Another key point in the IPPC that faded in importance is the scope of its application. The IPPC was never limited to agriculture but extends to all plants, including the protection of both cultivated and uncultivated conditions, forests, urban environments, and fresh-water aquatic systems. The problem is that governments have historically given priority to the application of the IPPC in agriculture and agricultural trade, leaving the perception that it was limited in this respect. In recent decades, as the environmental movement grew, the IPPC was overlooked as a key player in environmental questions of plant health and protection. In fact, it was more often cast as the opposite; a promoter of environmental degradation and an antagonist to biodiversity due to its close relationship with agriculture and trade. The lack of a strong infrastructure for the IPPC in FAO, and the timid nature of NPPOs generally, led to a situation where ambiguous new concepts such as invasive species and the precautionary principle emerged from the environmental community in contrast to the well-established and practiced concepts of pests and risk analysis in the IPPC. As a result, governments find themselves with officials from ministries representing environment and agriculture representing vastly different positions in different international forums – often without realizing the conflict exists.

Although no change in the scope of the IPPC is planned for the present and near future, it appears that the die is cast for a growing focus on harmonization with increasing attention to technical capacity building associated with trade and the implementation of international standards for phytosanitary measures (ISPMs). The current emphasis on the application of the IPPC to trade and the enormous effort that has been devoted to harmonization as a result of the role assigned to the IPPC in the SPS Agreement could not have been anticipated in 1951, but the foundation for these efforts was laid with the establishment of the phytosanitary certificate. In fact, it was the existence of the phytosanitary certificate and its global recognition as a point of harmonization for the phytosanitary community that made the IPPC attractive as a standard setting organization to be attached to the SPS Agreement.

As the GATT marched through eight rounds of negotiations between 1947 and 1995, global efforts toward trade liberalization evolved through stages that first emphasized free trade (removing tariffs), then fair trade (removing non-tariff and technical barriers), and finally safe trade (the SPS Agreement). There was not the same magnitude of evolution occurring in plant quarantine over the same period, but a collision course was set when Article XX:b, the provisions in GATT that allowed countries to put measures in place to protect “human, animal or plant life or health”, was identified in the Uruguay Round as a key area for discipline among the negotiations related to agriculture. The carefully crafted and delicately balanced SPS Agreement was the result. Two key outcomes for NPPOs from the establishment of the SPS Agreement were: first, the focus on international harmonization through standard setting and specifically identifying organizations committed to providing this service; and second, the establishment of a binding dispute settlement mechanism (WTO 1998, 1999). Both have had profound effects on the phytosanitary community and quickly brought new importance to the role of NPPOs in both political and commercial circles.

Aside from the phytosanitary certificate established when the original convention came into force in 1952, the IPPC had no standard-setting history and no mechanism for international collaboration toward the adoption of standards that might be recognized by the WTO. This was in stark contrast to the other two organizations named in the SPS Agreement for standard setting; the Codex Alimentarius for food safety, and the International Organization for Animal Health (OIE). OIE has been creating standards since 1924 and Codex Alimentarius since 1963. After realizing the role that the IPPC was likely to inherit from the SPS Agreement and the enormous challenge to establish some parity with sister standard-setting organizations, the phytosanitary community was driven by the efforts of the Technical Consultation of Regional Plant Protection Organizations to persuade FAO to create a Secretariat. Standard setting began in earnest in 1992, and by 1995 a process to revise the Convention was underway. This sudden burst of activity resulted in a substantially revised Convention by 1997 with a Secretariat in FAO and a Commission for members committed to meeting annually for the adoption of standards coming from numerous expert committees and working groups focused on the harmonization expectations of the SPS Agreement.

In many ways, the SPS Agreement shocked NPPOs to action from a low-key role as accessories in agriculture ministries to central players in the fast-paced, high-stakes and strongly political world of globalized commerce. The change in profile corresponded to a substantial shift in emphasis and effort as NPPOs moved to be part of the international standard-creation process as well as understanding and implementing the finished products. But the leap into the global trade arena has not been without some discomfort. A community whose regulatory judgments historically began from a position of distrust and worst-case assumptions found itself grappling with harmonization and trying to accept the need for evidence and analyses to justify restrictive actions. A long history of decisions and policies must be revisited while also trying to fit current and future policies into the new global framework. Concepts and principles that had been variously understood and diversely interpreted in the past suddenly became fixed as the new rules for doing business and need to be taken more seriously by both NPPOs and politicians because of the potential for challenge by a disputing trading partner. Where the balance between protection and trade had historically tilted more toward protection ("when in doubt, keep it out"), the SPS Agreement swung the pendulum in the other direction, toward globalization and greater emphasis on defendable technical justifications for restrictive actions.

As the analytical capacities of NPPOs improve and as precedents accumulate from challenges to unjustified phytosanitary measures, a better sense of equilibrium would be expected to develop. In the meantime, the motivation for seeking this equilibrium will continue to be the potential for disputes with binding consequences. A counterbalancing motivation comes from commercial interests who prefer to enjoy the advantages of protectionism wherever they can. The challenge for NPPOs is navigating the politics and overcoming historical momentum to stay within the global regulatory framework and actively contribute to its improvement without disputes.

The provisions for binding dispute settlement in the WTO created an unprecedented motivation for governments to take their WTO obligations seriously. This was no less the case with the SPS Agreement and the recent history of jurisprudence on SPS disputes contains many important lessons for NPPOs interested in understanding how harmonization and the framework of principles which surround international trade policy affect real-world decision-making. Likewise, there are lessons for the private sector which can be quick to promote a challenge when they feel wronged but often temper their enthusiasm for a fight when their government is faced with the expense and effort required to mount a formal dispute which has the possibility of an undesirable outcome. Indeed, even a clear "win" in a WTO dispute is unlikely to benefit the damaged sector as one might expect. In the instance where the losing party changes the offending measures, there is no provision for compensation of past damages. In the instance where the losing party does not change its measures, compensation is paid to the government, not the damaged industry. The primary value of winning a dispute for the damaged sector is "setting the record straight" by establishing precedents from a solid interpretation of the application of the Agreement in practice.

Because of the extensive structure that the IPPC and WTO-SPS have constructed around the role of the phytosanitary community, and the importance of stable and predictable trade to all countries, it is likely that the traditional design and function of NPPOs will continue into the foreseeable future, especially as regards their relationship to the application of phytosanitary measures in trade. The main difference going forward is the enhanced role of the RPPO in harmonization and plurilateral collaboration, and the unprecedented global challenges to policy-making generally. Other adjustments, such as increasing analytical capacity, dispute avoidance, and updating policies are questions of time and resources that each country must work through based on the political will and priority given to them. It is clear for instance that consumer issues have played an increasingly more important role in policy-making in recent years and that this also affects the phytosanitary community with issues such as labeling, traceback, organic certification, quality standards, and residue and contamination concerns. Industrialized countries have led responses to these issues in response to affluent and politically vocal consumers where the importance of such issues in developing countries is more likely to be with exports than with domestic production or imports.

If the evolution of the last two decades is any indication, the challenges for NPPOs in the future can be expected to be both large and fast-moving. This argues for a nimble organization that embraces change and responds quickly and effectively to its charge and to unanticipated challenges. One way to prepare is by taking account of current trends.

22.3 Selected Trends

22.3.1 Continuum Approach

The conceptualization of an NPPO based on the Convention is a collection of elements that are adapted by each country for their particular needs and situation. Over time, NPPOs have found there are elements which are common and fundamental and others which can vary considerably from country to country. This Handbook is designed around the premise that biosecurity is a continuum, i.e., a holistic or systemic approach to plant quarantine rather than a collection of disparate pieces. It takes the increasingly more popular and contemporary view that an integrated approach to plant quarantine is most effective. To some, it may seem reasonable to expect every phytosanitary program to be comprehensive and continuous so that each element supported the other and the whole was more than the sum of the parts. In fact this is not yet the prevailing philosophy across the world, but one that has emerged in recent years as NPPOs search for better ways to demonstrate performance. For instance, it is not unusual for border inspection to be characterized as "the last line of defense", and domestic surveillance to be seen as a response to border "failures". In a continuum, the system is seen as continuous functions which are deliberately connected and "tuned" for maximum effectiveness; moving from an offshore function to a border function and finally a domestic function, each with its supporting elements. In this scenario, a certain realistic level of efficacy for inspection is expected which then requires a corresponding level of domestic surveillance which has its own level of efficacy. The whole program will have some level of failure which must be accepted but also used as feedback to improve the system within the limits of resources to further increase effectiveness and efficiency.

The key to a continuum approach is not simply stringing programs together, but rather the analytical background for the design. For example, if fruit flies are a concern, then offshore treatments might be required which are supported by some level of verification at the border and trapping domestically. The available infrastructure for treatment validation, insect detection and identification, and trapping processes must be critically examined to understand how well each is expected to support the overall objective and the combination and strength of elements needed to be most effective.

22.3.2 E-quipment and Big, Fast Data

Every country will have different needs for analytical capacity but it will be essential for all countries to "be connected". As the digital revolution rapidly advances, both the private sector and the government attempt to adopt processes, procedures and policies that depend on electronic communication, data collection – storage – analysis – distribution, and the Internet. The Internet may be one of the greatest equalizers in leveling the field for countries with severely limited resources. PRA for example, can be done nearly as well with a laptop connected to the Internet as in a national agriculture library. Phytosanitary certification is another example where electronic processes not only facilitate certification, but also simplify document security and greatly enhance the possibilities for data collection, reporting, notifications and myriad other information, analysis and communication needs.

While many in the current generation of phytosanitary officials struggle to transition, the coming generation is expected to be fully literate and entirely comfortable with digital technology and managing large amounts of data. The extent of changes that this transition may bring is difficult to imagine, but based on recent trends one can speculate that the phytosanitary world will be dramatically transformed.

22.3.3 Pest Identification: Old and New

Pest identification is a central and crucial function in practically every area of plant quarantine; offshore surveillance, border interceptions, and domestic detections all require a degree of taxonomic expertise to support their operation. Historically, pest identification has relied on taxonomists who were specialists in their area of expertise and mainly used morphological characteristics for identification. Two trends have significantly changed this paradigm. The first is the dwindling number of traditional taxonomists. The profession is not attractive to the younger generation and experienced identifiers are quickly fading into history. At the same time, the area of molecular diagnostics has attracted a large following and has expanded exponentially in recent years.

While the present situation still relies primarily on traditional identification methods, molecular techniques are growing in importance and occupying an increasingly larger role in regulatory programs. The ability to exact precise identifications from organisms or life stages that were previously unidentifiable or only partially identified, and the potential to distinguish species and discover new species, has both positive and negative implications in the phytosanitary world. On the positive side, molecular techniques have provided considerably more precision and allowed for distinctions to be made which would have been otherwise impossible. On the negative side, the technology can be expensive and requires specialized equipment and expertise that is not always feasible in operational situations. In addition, genotypic distinctions sometimes result in "new" organisms that are not morphologically or phenotypically distinct and, from a practical standpoint, not really different for phytosanitary purposes but could technically be considered to be a "new" pest.

A more serious problem may be that there is no internationally agreed method for determining how much of a genotypic difference constitutes a new species and how phenotypic characteristics factor into this determination. Until there is a useful level of harmonization around this aspect of the technology, there will always be the potential for abuse and misunderstandings whenever the results are used in regulatory decision-making.

22.3.4 Benefit-Cost

The SPS Agreement anticipates a marriage of biology and economics which represents a highly unorthodox relationship that has proven difficult to achieve in practice. The level of economic analysis typically practiced by the phytosanitary community is relatively simple accounting and has been adequate for the needs of the past. Note for instance that the definition of a quarantine pest in the IPPC only refers to "potential economic impact". Any organism, by virtue of being a pest, can be qualified as having "potential" impact. Further, the IPPC is somewhat backward in considering all consequences to be economic while the SPS would seem more progressive in recognizing both biological and economic consequences (WTO 1994).

At the working level, NPPOs have typically justified their existence by pointing out that huge negative impacts can be avoided by preventing the introduction of one devastating pest; invoking the adage that an ounce of prevention is worth a pound of cure. Surely a small investment in plant quarantine inspection is justified by the disasters that are avoided! Surely the cost of eradication to save an industry justifies the investment in a program! The examples are extensive, and intuitively these comparisons seem logical and defendable. Problems arise however when trying to determine how much a 5 % reduction in budget will reduce the level of protection, or whether the eradication of a pest is necessary if growers are already treating for similar pests and there is no impact on trade. In sum, the future demands much more sophisticated economic analyses, especially benefit-cost type analyses to help NPPOs prioritize and measure in a truly meaningful and defendable way.

22.4 Final Thoughts

The future evolution of plant quarantine begins with a trajectory from its current path and extends as far as our best view of the horizon. The past two decades have seen transformative change unlike any previous period in the history of regulatory plant science. There is no reason to believe that the rate of change and number of challenges will decrease in the future. Indeed the prognosis is daunting, but the mega-trends in trade, technology, and agriculture tell us that the role filled by this small and unique community will continue to be critical.

Harmonization and breaking-down the walls of distrust with hammers of science will be essential for evolving into future relationships that support safe trade rather than constantly oscillating between trade and protection depending on the direction of political winds. Analytical capacity must gradually replace political weight as the driver of policy, and countries must learn to devote greater effort to developing a common understanding than challenging opposing positions. Science will occupy an increasingly more prominent role and greater attention will be devoted to linking organizational design to performance measures based on more, better, and faster data. Economics will take its rightful role in the analytical toolbox as NPPOs strive to break from the assumptions of the past to reinvent plant protection for the future in the biosecurity continuum.

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