

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

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/http/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 (*Berbera 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 (Guerra 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. erythrae* cannot establish in hot and dry areas. However, adults are highly tolerant of weather extremes (Samways 1990; Aubert 1987b). *Trioza erythrae* 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/plantquarantine00unit>).

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 km², 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.

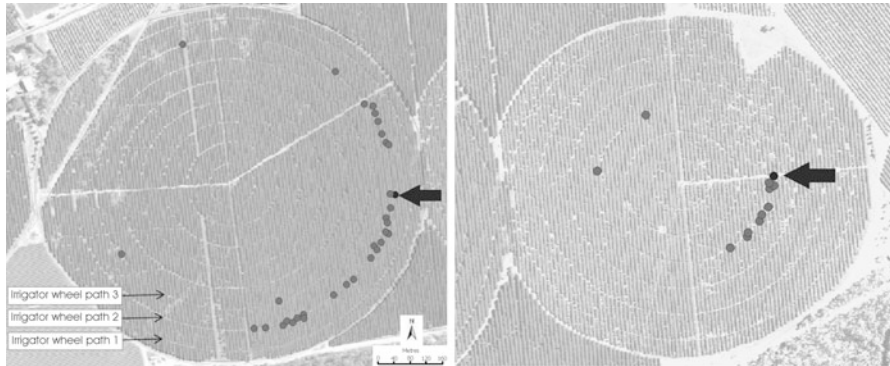


Fig. 18.5 Distribution of canker-infected trees along the pivot irrigator's tracks in blocks 7 and 8 on IP-2 (Taken from Gambley et al. 2009. Courtesy of Queensland Department of Agriculture, Fisheries and Forestry)

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.

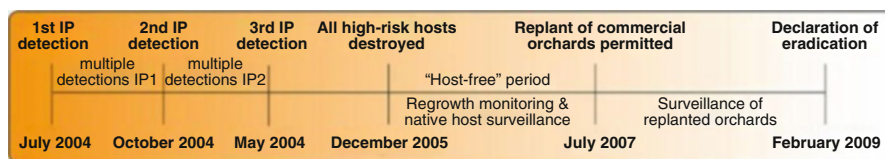
Evidence from other countries has shown that the canker bacterium can only survive in soil at population levels sufficient for infection for a few weeks (Goto 1992; Leite and Mohan 1984; Graham et al. 1989). *Xcc* can survive on several grasses for at least several months while the longevity and level 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 bactericide 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 citriasiatica* 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. citriasiatica* 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 conducive 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.

Acknowledgements The authors thank Rodney Turner (Program Manager, Plant Health Australia, Canberra), Mike Ashton (Plant Biosecurity & Product Integrity, Queensland Department of Agriculture, Fisheries and Forestry) and Fiona Macbeth (formerly of Office of the Chief Plant Protection Officer, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra) for their review and constructive criticism of the section on citrus canker eradication at Emerald. The Australian Government Department of Agriculture, Fisheries and Forestry, the Queensland Department of Agriculture, Fisheries and Forestry, all other Australian state government departments of agriculture, the citrus growers of Australia and the relevant communities should be acknowledged for their support and commitment to the successful eradication of citrus canker from Australia.

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