Chapter 17
Phytoparasitic Nematodes: Risks and Regulations

Eric L. Davis and David K. Nendick

17.1 Introduction

Nematodes (invertebrate 'roundworms') are the most numerous multi-cellular animals on earth (Poinar 1983), and second to insects (arthropods) in the number of described species. Estimates declare only about 3 % of all nematode species have been identified and studied. Nematodes are often overlooked and relatively unknown when compared with insects because of their small to microscopic size and tendency to live within inconspicuous habitats. Most nematodes average less than 1 millimeter (mm) in length while some animal parasites range up to 8 m long! One cubic foot of soil may contain millions of individual nematodes belonging to many taxonomic groups. Different nematode species feed on algae, bacteria, fungi, and higher plants, as well as invertebrates and vertebrate animals (including humans). Nematodes have broad stress tolerances and they have successfully exploited diverse habitats including every conceivable terrestrial and aquatic environment on earth ranging from temperate to tropical soils, arid deserts, salt and fresh water, hot springs, and polar regions (Nickle 1991; Shurtleff and Averre 2000).

All nematodes belong to the Phylum Nematoda (Decraemer and Hunt 2006) and all nematodes are aquatic (as they must live in association with liquids in an abiotic environment or inside a biotic host substrate). In body form they are triploblastic (derived from three embryonic tissue layers), unsegmented, non-coelomate (pseudocoelomate), bilaterally symmetrical roundworms (Fig. 17.1). All nematodes undergo embryonic development within an egg and become worm-shaped (vermiform). However, a few species achieve swollen (pyroform) and contoured body shapes later in adult life stages (Fig. 17.2). Nematodes have a "tube within a tube"

E.L. Davis
Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA

D.K. Nendick (✉)
Biosecurity and Environment Group, Plants, Food and Environment Directorate, Ministry for Primary Industries, Wellington, New Zealand
e-mail: dave.nendick@mpi.govt.nz

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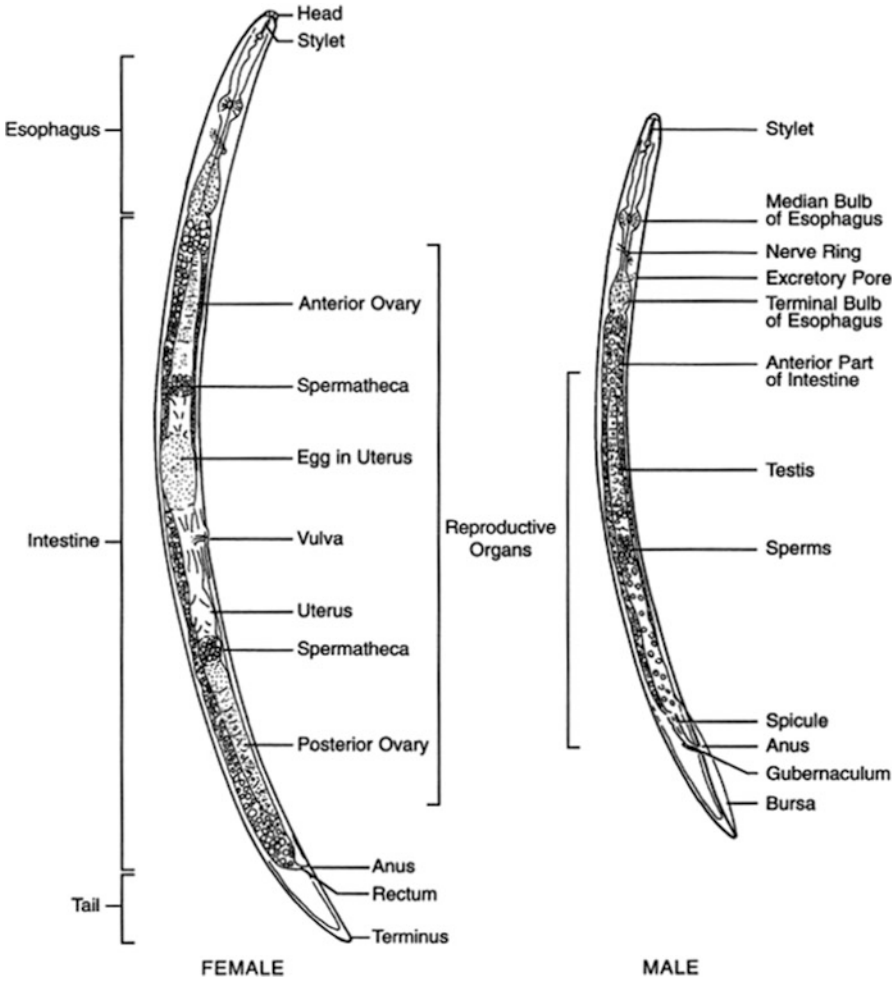

Fig. 17.1 Line drawings of generic phytoparasitic nematodes. Note the stylet used for feeding from plant cells, details of the esophagus including the median bulb (metacorpus), female ovaries and vulva, and male testis and spicules (Reproduced with permission from Shurtleff and Averre 2000)

body plan (Hirschmann 1971) that consists of an outer body wall and an inner digestive system, and the body shape is maintained through pressure of the pseudocoelmic fluid against the flexible body wall (forming a hydrostatic exoskeleton). In all phytoparasitic (plant-parasitic) nematodes (but few other nematode species), the mouth of the digestive system contains a "stylet" – a hypodermic needle-like structure used to pierce plant cell walls for feeding (Agrios 2005; Shurtleff and Averre 2000). All nematodes molt their outer (collagenous) cuticle four times during juvenile growth and maturation to reproductive adult. These four juvenile stages are sometimes termed "larvae". All nematodes have a skeletal,

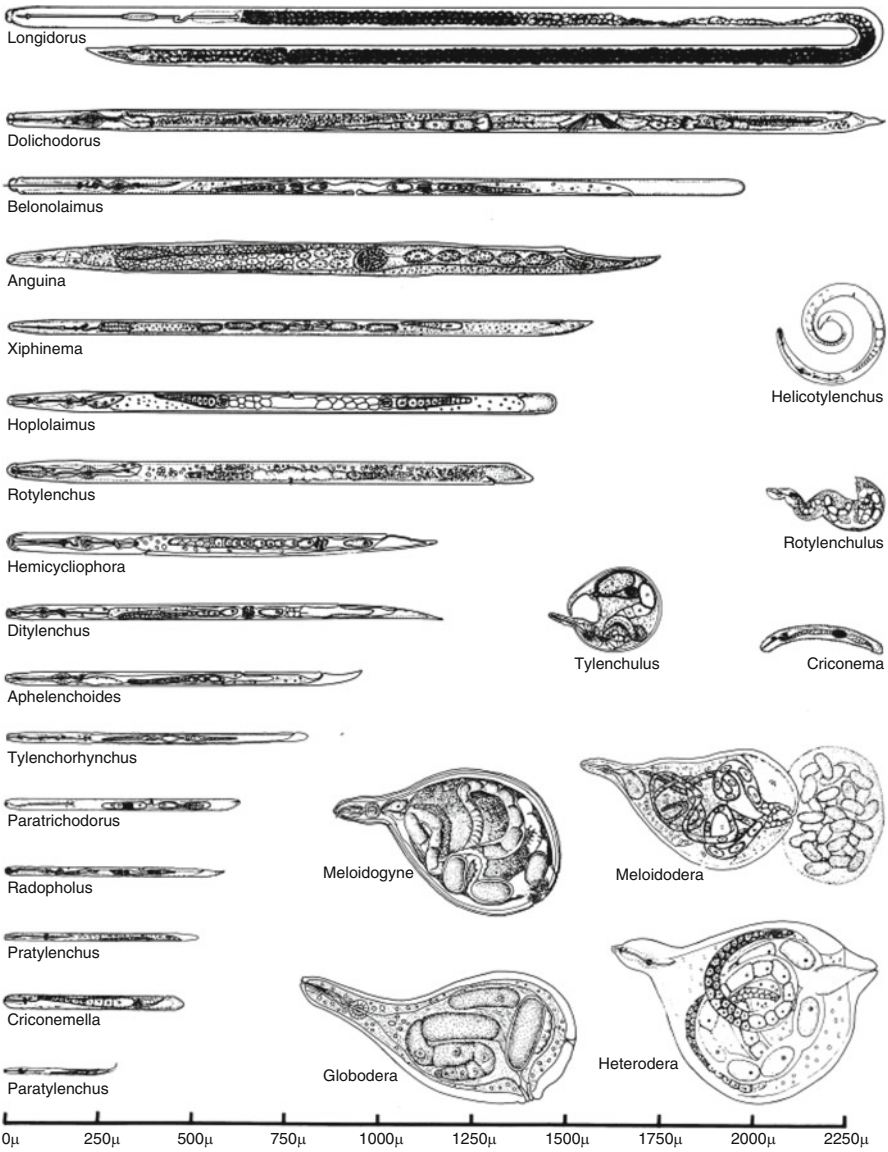

Fig. 17.2 Line drawings of some major Genera of phytoparasitic nematodes that indicate relative size and shape. Genera of nematodes that remain worm-shaped (vermiform) in all life stages are compared on the *left*, and Genera with swollen (pyroform) reproductive adult life stages are shown on the *right* (Reproduced with permission from Shurtleff and Averre 2000)

muscular, excretory, digestive, nervous, and reproductive organ system, but they lack circulatory or respiratory systems. Typically, nematodes are transparent and colourless and usually are dioecious (separate male and female adults that mate).

Microscopy is used to determine nematode body shape, size, and structure of internal organ systems. Microscopy has been the traditional technology used to identify and classify nematodes taxonomically (Mai et al. 1996; Shurtleff and Averre 2000). More recent taxonomy for the Nematoda is based upon DNA sequence data (De Ley and Blaxter 2004). The adoption of molecular technologies for practical identifications of nematodes for diagnostics remains limited (Subbotin and Moens 2006). However, recent technology advances have provided promising new approaches that include direct extraction of total DNA (See Sect. 13.5) from soil samples and subsequent use of species-specific real-time Polymerase Chain Reaction (PCR) primers in multiplex assays to identify different phytoparasitic nematode species within a single sample (Ophel-Keller et al. 2008). Not all nematodes in a soil sample (or any abiotic nematode habitat) are parasitic. Most nematode species are not parasitic on animals or plants. Roughly 50 % of all nematodes live in marine environments as zooplankton and there are relatively few parasitic species among them. About 25 % of all nematodes live in freshwater in lakes, rivers, soils, and streams. Among this group of nematodes most species are microbial feeders that play an intricate role in ecosystem food webs and nutrient turnover (Poinar 1983). An example of bacterial-feeding soil nematode is *Caenorhabditis elegans* Maupas which has become a premier biological model for the study of animal behaviour, development, genetics, genomics and pharmacology (Bird et al. 1999).

The remaining 25 % of nematodes are the parasitic species. About 15 % of these species are parasites of animals (Poinar 1983). Some of the invertebrate parasites (of arthropods) have been exploited to develop commercial control for insect pests in agriculture (Nickle 1991). The remaining 10 % of the parasitic nematodes include about 4,000 species within about 200 genera (Nickle 1991) that are parasitic on plants. Annual global crop yield loss to phytoparasitic nematodes is estimated to be around $USD 100 billion (Chitwood 2003). This figure is probably underestimated because their impact is probably greater since plant symptoms of nematode damage are often non-specific and unrecognised as nematode damage. Economic, environmental and practical considerations dictate the level of management that farmers can use to reduce damage from phytoparasitic nematodes including use of chemical control (nematicides), resistant crop cultivars, or rotations from hosts to non-host crops. Nematode diseases that damage plants that grow in areas of human development, forests, or prairies present an even greater management problem to reduce phytoparasitic nematode populations to non-damaging levels. After a phytoparasitic nematode infestation has occurred, the nematode is rarely eradicated from the infested area. Exclusion and sanitation remain the single best management strategies to prevent phytoparasitic nematodes from becoming established or introduced to new areas (Hockland et al. 2006).

This chapter summarizes some of the more damaging species of phytoparasitic nematodes and those of international regulatory concern. For purposes of this chapter we group phytoparasitic nematodes based upon their parasitic habit (Agrios 2005; Hussey and Grundler 1998; Perry and Moens 2006) as follows: (1) *Ectoparasites* that feed with their stylets from outside of host plant tissues; (2) *Endoparasites* (Fig. 17.3a) that penetrate and feed within host plant tissues; (3) *Migratory*

Fig. 17.3 (**a**) A micrograph (100×) of multiple individuals of the migratory endoparasitic lesion nematode (*Pratylenchus* spp.) stained red with acid fuchsin inside a corn root; (**b**) a healthy, fibrous root system of celery on the *left* and a severely galled celery root system infected with many root-knot nematodes (*Meloidogyne* spp.) on the *right*; (**c**) a micrograph (40×) of several brown, round females of the tobacco cyst nematode (*Globodera tabacum*) on roots of tobacco; (**d**) A micrograph (100×) of females of *G. tabacum* extracted from host roots demonstrates different stages of cyst maturation from *white* (young female) to *yellow* to *brown* (mature cyst); (**e**) leaves of chrysanthemum infected with foliar nematodes (*Aphelenchoides* spp.) display characteristic symptoms of interveinal chlorosis, necrosis and angular lesions; (**f**) extensive damage to a pine forest in Japan from infestation with the pinewood nematode, *Bursaphelenchus xylophilus*

nematodes that feed from a host cell for a relatively short period of time and move to another host cell; and (4) *Sedentary* nematodes that establish prolonged feeding (1 day or longer) from a single site in host plant tissues. Most phytoparasitic nematode species are soil-dwelling pests and feed from the roots of a host plant. Several significant phytoparasitic nematode species, however, do infest and feed in the upper parts of plants in flowers, seeds or shoot tissues (Nickle 1991; Pirone 1978).

17.2 Ectoparasitic Nematodes

*17.2.1 Dagger Nematodes (*Xiphinema *spp.)*

Dagger nematodes primarily are ectoparasites of woody plants (small trees and tree fruits) but can damage annual crops like corn, soybean, some cereals and herbaceous perennials (Decraemer and Geraert 2006). For instance, *Xiphinema* is an important pest in all grape-growing regions of the world because it can also introduce and spread (vector) Grapevine Fanleaf (GVFL) Virus. *Xiphinema* also vectors other NEPO viruses (Comoviridae) such as Prunus Necrotic Ringspot Virus on peach in the northeast USA. Dagger nematodes also cause direct damage by feeding at or near root tips where they often cause slight galling and necrosis. These are relatively large phytoparasitic nematodes (1.5–4.5 mm long) with an odontostylet that has an odontophore with three basal flanges, a distinct 'guiding ring', and a two-part esophagus (no metacorpus). Females have one or two ovaries depending upon vulval position; the female's tail shape can range from conoid to more rounded with a small, distinct peg-like projection (Mucro) at the tip. Males are rare in some species and have a similar body shape with stout spicules and no bursae. Of the approximately 60 species described, the three most agriculturally-important species include: (1) *X. americanum* Cobb – one of the more common nematodes in USA, probably a "species complex"; (2) *X. index* Thorne and Allen – mainly found associated with its natural host – grapevine; and (3) *X. diversicaudatum* Micoletsky – the largest species (4.3 mm long) which is very damaging to strawberry.

Xiphinema species differ in their selection of feeding sites; most feed ectoparasitically at the tips of roots and produce small galls on certain plants. Dagger nematodes have very long stylets that allow them to feed deep into root tissue. They may feed from the same site for relatively long periods of time, and therefore may also be considered as sedentary ectoparasites. Feeding can result in cessation of root growth and swelling (galls) of root tips within 12 h of feeding with transmission of plant viruses often occurring within this period. Necrotic cells are often surrounded by the hypertrophied (over-sized) cells with multiple nuclei at the feeding site that serve as the food source for the nematode's sedentary feeding.

*17.2.2 Sting Nematodes (*Belonolaimus *spp.)*

Sting nematodes can be one of the most devastating nematode pathogens (Smart and Nguyen 1991) due to their severe effects. Hosts include field crops (corn, cotton, peanut, soybean), fruits, grasses and trees. Sting nematodes are primarily found in the southern USA and some Caribbean islands. Sting nematodes also

prefer sandy soils, like the coastal plains of the southeast USA. In Florida, sting nematodes are one of the most important pests of turf, and they have relatively recently been detected in California. The most important agricultural species is *B. longicaudatus* Rau. Sting nematodes are relatively long compared with other phytoparasitic nematodes (>2 mm), with an offset, bulbous head, very long stylet with small knobs, moderate annulation and the esophagus slightly overlaps ventrally. The female has two ovaries and a rounded tail, and males have a pointed tail with long, tapering bursae.

Sting nematodes feed as migratory ectoparasites on root tips causing extensive and severe root damage. Their long stylets penetrate deep into cortical and sometimes into vascular tissue. This type of feeding causes cell necrosis in the root apical meristem and can often result in a stunted, "coarse root system". Root tips cease to grow and lesions and necrosis may occur as well. Very few sting nematodes (<10 per 100 cc of soil) can cause severe damage to plants, especially seedlings and grasses. The above-ground symptoms include severe stunting, leaf chlorosis and even seedling death can occur.

*17.2.3 Stubby-Root Nematodes (*Trichodorus *and* Paratrichodorus *spp.)*

The feeding activity of Stubby-root nematodes destroys root tips and results in a shortened, sparse, "stubby root" system (Decraemer 1991). Stubby-root nematodes are distributed globally and prefer sandy, well-drained soils. They have a wide host-range that includes crops like clover, corn, potatoes, turf and many vegetable crops. Stubby-root nematodes cause direct plant damage and can also vector the NETU plant viruses (Tobraviruses). One of the stubby root-vectored viruses causes "corky ringspot" of potato tubers. Both *Trichodorus* and *Paratrichodorus* species have cigar-shaped bodies about 0.5 mm in length, a dorsally-curved onchiostylet without basal knobs or flanges, and they have a two-part esophagus. This type of stylet is very unusual in that it resembles a tooth and it is grooved rather than being hollow! Females of both genera have two amphidelphic ovaries. The genus *Paratrichodorus* includes about 20 species and *Trichodorus* contains about 50 species; the most important agronomic species is *Paratrichodorus minor* (Colbran) Siddiqi.

Stubby-root nematodes feed as migratory ectoparasites over the entire root system, but they mainly feed on epidermal cells at the root tips. Stylet penetration of a plant cell and ingestion can occur in a few minutes but this rapid feeding still allows time for the transfer of plant viruses. Lateral root initials can be produced at feeding sites by the host plant as a response to feeding; other stubby root nematodes, which produce the stunted, branched look of the root system can immediately attack these initials. Above ground symptoms can result in nutrient stress, severe stunting, water stress, and in extreme cases this can lead to plant death.

17.3 Endoparasitic Nematodes

*17.3.1 Root-Knot Nematodes (*Meloidogyne *spp.)*

Root-knot nematodes cause more damage worldwide than any other genus of phytoparasitic nematodes (Eisenback and Triantaphyllou 1991). They are a distributed among all agricultural regions of the world and collectively parasitise over 1,700 different plant species (Agrios 2005). The second-stage juveniles (J2) of root-knot nematodes penetrate plant roots completely and establish elaborate feeding sites. Upon establishment of the feeding site, the J2 begins feeding, becomes sedentary and swells through its molts to the adult stage. Conspicuous galls (knots) form on the roots (Fig. 17.3b) at the site of infection as the result of nematode feeding. The J2 of root-knot nematodes are 0.4–0.6 mm long and have a short, weak stylet, subventral overlapping esophageal gland cells, and a blunt, rounded tail with a hyaline area in the anal region. The rounded adult females may be 1.0 mm in diameter. The pattern of cuticular striations surrounding the female vulval and anal openings (with their distinctive perineal pattern) has traditionally been used for species identification. The four most important species of root-knot nematodes found around the world (Sasser 1980) include *M. arenaria* Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood. These species of *Meloidogyne* collectively cause about $10 billion in annual crop losses (Chitwood 2003) although their existing worldwide distribution mostly reduces them to minimal regulatory concern. However, *Meloidogyne* species of more localized distribution such as *M. chitwoodi* Golden, O'Bannon, Santo & Finley and *M. fallax* Karssen in potato, or *M. exigua* Goeldi in coffee are of regulatory concern and are managed accordingly.

Individual Root-knot Nematode females lay several hundred eggs in a gelatinous mass on the surface of the root-galls that they induce. As with all phytoparasitic nematodes, the first-stage juvenile (J1) molts within the egg and the J2 hatches into the soil matrix. Root-knot nematode J2s migrate in soil and penetrate host plant roots in the zone of elongation just behind the root tip (Hussey and Grundler 1998). The J2 migrates intercellularly (between cells) within the roots using stylet thrusts and they secrete enzymes that loosen the cell wall matrix until it reaches the root vascular tissue. Effector parasitism proteins from secretory cells in the nematode esophagus are then secreted through the stylet into selected root vascular cells to transform them into elaborate feeding cells called "giant-cells" (Davis et al. 2008).

Giant-cells can be 100-times the size of normal plant cells, they also have multiple nuclei, dense cytoplasm, and reinforced cell walls (Hussey and Grundler 1998). Localized root cell division occurs around the feeding site to produce the intercalary root galls (knots) that are the defining visible feature of *Meloidogyne* species infections. The feeding infective juvenile stages undergo three more molts as they become more swollen (pyroform) and rounded to the reproductive adult stage. The pyroform adult female remains embedded within the gall and it is ultimately positioned to lay its eggs in a gelatinous mass on the outside surface of

the root gall. One plant may be host to many hundreds or thousands of root-knot nematodes and consequently the parasitic load on the plant and the damage to the root vascular system results in nutrient and water deficiencies for the host plant, including stunting, wilting, and a greatly reduced crop yield.

*17.3.2 Cyst Nematodes (*Globodera *and* Heterodera *spp.)*

Cyst nematodes are characterized by the hardening and colour change (tanning) of the mature pyroform female body wall after death to form a protective "cyst" (Fig. 17.3c, d) that encloses the eggs (Baldwin and Mundo-Ocampo 1991). Unlike root-knot nematodes, no root galls are formed by cyst nematodes and the swollen adult female is primarily exposed on the outside of the plant's root. Cyst nematodes only have their heads buried within the root to feed. Cyst nematodes are probably the second most economically-important nematode pathogen of major crops after root-knot nematodes. Unlike root-knot species, cyst nematode species have a much more limited plant host-range. However, their effects on successive generations of host plants can be extensive because the eggs contained within the protective cysts can remain viable for a several years in soil. Cyst nematodes are very important nematode pests of (colder) temperate agriculture (for example, potato and winter cereals), but species have also been found on major crops (for example, sugar cane, rice) in warm climates. The J2s look similar to those of root-knot nematodes, but they are slightly larger (0.6–0.8 mm long) with a distinctly more robust stylet (25 μm long) with 'heavier' knobs. While the subfamily Heteroderinae contains seventeen genera, the two most agriculturally-important genera are *Globodera* (having round cysts) (Fig. 17.3c, d) and *Heterodera* (having lemon-shaped cysts).

Important species of cyst nematodes include Beet Cyst Nematode (*H. schachtii* A. Schmidt), Cereal Cyst Nematode (*H. avenae* Wollenweber), Potato Cyst Nematodes (where the two main species include Pale Cyst Nematode (*G. pallida* (Stone) Behrens) and Golden Nematode (*G. rostochiensis* (Wollenweber) Behrens), Soybean Cyst Nematode (*H. glycines* Ichinohe), and Tobacco Cyst Nematode (*G. tabacum* Lownsbery & Lownsbery). Most of these significant cyst nematode species cause considerable economic damage on their respective host crop. However, they are relatively limited in distribution compared with the root-knot nematodes and are subject to strict international regulatory activities and certification requirements.

The cyst nematode life-cycle is similar to that of a root-knot nematode, with some notable differences. Many cyst nematode species require their eggs to be near host-plant roots because egg hatching is stimulated by specific host root exudates. When the J2s penetrate plant roots, they migrate intracellularly (through plant cells) to the root vascular tissue, destroying root cells along their migratory path (Hussey and Grundler 1998). Cyst nematodes also secrete effectors to induce formation of a multinucleate feeding site called a "syncytium" by coordinated cell wall dissolution between neighbouring root vascular cells (Davis et al. 2008). As with root-knot nematodes, after the feeding site is formed the J2s swell, become sedentary and

molt three more times until the adult stage has developed. No root gall is formed, and the swollen female cyst nematode protrudes almost completely from the root and retains most eggs within her body (which eventually falls off the root and becomes the protective cyst). As with root-knot, root damage and parasitic load from hundreds or thousands of nematodes causes extensive disease to the host plant resulting in crop loss.

*17.3.3 Reniform Nematode (*Rotylenchulus reniformis *Linford & Oliveira)*

Reniform Nematode is widely distributed in tropical and subtropical areas (Jatala 1991). This pathogen has a fairly wide host range of at least 140 plant species that span 30 plant families. Primary hosts in the tropics include banana, citrus, coffee, ginger, pineapple, and tea; primary hosts in the subtropics include cotton, cowpea, sweet potato, and soybean. This nematode has become increasingly important during the past two decades (Robinson 2007), and appears to be expanding its distribution in warmer climates. Reniform Nematode is distributed in many areas in the USA, especially the southeast where concerns for root-knot and cyst nematodes have left the Reniform Nematode relatively unmanaged. Pathogenicity of different reniform populations varies, and sometimes high population densities can cause relatively little damage. In Hawaii, reniform populations are extremely aggressive and survival of the pineapple industry is dependent on maintaining adequate Reniform Nematode control. The J2 of Reniform Nematodes are relatively small (about 0.5 mm long) with a rounded sclerotized lip region and pointed tail similar to other members of the Hoplolaimidae. Reniform Nematode juveniles undergo superimposed molts through J3/J4 stage in soil without feeding; the emerging immature female nematode penetrates the root and forms a syncytium along the endodermis of the root vascular system. The developing female feeds from the syncytium as a sedentary endoparasite and swells to become a kidney-shaped (renale) female protruding from the infected root that is characteristic of this species. Although *R. reniformis* is not presently subject to specific regulatory action (other than generic phytosanitary controls), it's rapidly increasing geographic distribution, relatively high soil population densities, and plant damage potential combined with a wide plant host-range should make it a phytoparasitic nematode species of concern.

*17.3.4 False Root-Knot Nematode (*Naccobus aberrans *(Thorne) Thorne & Allen)*

False root-knot nematode, *Naccobus aberrans*, is a sedentary endoparasitic nematode that also forms a feeding site in infected roots (Jatala 1991). This

nematode is generally limited in distribution and global damage in agriculture, but it has been a problem in potatoes grown in Central and South America. The nematodes can infect potato tubers and be very destructive in these crops. However, its current limited distribution has made this species subject to strict regulatory practises. *Naccobus aberrans* was originally isolated in Utah, and is primarily found in the Americas. It has a wide host range including chenopods, crucifers, cucurbits, leguminous and solanaceous plants. The nematode forms similar galls as *Meloidegyne* spp. on roots of host plants giving it the name "false root-knot nematode". *Naccobus aberrans* is a member of the Family Pratylenchidae. Juveniles are relatively small (0.4–0.5 mm long) with a flat sclerotized lip region, strong stylet, and rounded tail. The J2, J3, and J4 stages penetrate plant roots and feed as migratory endoparasites of roots until the immature female forms a syncytium (not giant-cells) and feeds as a sedentary endoparasite. The female swells to become rounded and a gall forms around the developing female, but the gall forms laterally from the root (on one side only), as compared with the root-knot nematode gall that extends from all sides of the root.

*17.3.5 Lesion Nematodes (*Pratylenchus *spp.)*

This group is considered the third most economically important Genus of phytoparasitic nematodes (behind root-knot and cyst nematodes) because of its global distribution, wide host-range, and very destructive parasitic habits (Duncan and Moens 2006). For example, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans, is considered as the most economically important nematode in the northeastern USA. About 70 species are identified in *Pratylenchus*; they vary and overlap geographically and in plant host preference (Loof 1991). All life stages are vermiform, 0.3–0.9 mm long, and display a flat sclerotized lip region, rounded tail, and esophageal glands that overlap ventrally. All species and life stages are migratory endoparasites of subterranean plant parts (roots, pods, tubers etc.). They cause extensive plant tissue necrosis (lesions) by their feeding activity and intracellular migration. More important species include: (1) *P. penetrans* – very wide host-range (>400 plant host species from row crops to fruit trees); (2) *P. vulnus* Allen & Jensen – important crop hosts include fruit trees and ornamentals; (3) *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven – important crop hosts include peanut and soybean; (4) *P. zeae* Graham – the most important crop host is corn; (5) *P. coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven – important crop hosts include banana, citrus, coffee and strawberry; and (6) *P. scribneri* Steiner – may be the most widely distributed lesion nematode in the USA. The wide global geographic distribution of lesion nematodes makes them subject to relatively limited regulatory activity. However, lesion nematodes are extremely destructive species in warmer climates. Species such as *P. coffeae* are of considerable concern for further geographic spread.

*17.3.6 Burrowing Nematode (*Radopholus similis *(Cobb) Thorne)*

Burrowing nematodes are taxonomically related to and very similar to lesion nematodes in morphology and parasitic habit as migratory endoparasites of underground plant parts (Duncan and Moens 2006). Dorsal overlap of esophageal gland cells and strong sexual dimorphism of males are key features that distinguish *Radopholus* from *Pratylenchus* (Loof 1991). Burrowing nematodes are an important pest causing "spreading decline" of citrus plantations in Florida, "toppling disease" of banana in Central and South America, and "pepper yellows" disease in Indonesia. *Radopholus similis* is present in tropical Africa, parts of Central and South America, Australia, within some ornamental plant industries in Europe, in the Indonesian archipelago and it is found in Florida and Hawaii in the USA. Strict quarantine regulation has helped limit the spread of burrowing nematodes within countries where it is found and around the world. However, the wide range of host plant species (ca 250) for *R. similis* and its immensely destructive capabilities make it an important pest in its range and a constant threat for spread into new areas (Duncan and Moens 2006; Hockland et al. 2006).

17.4 Shoot Parasites

This group of phytoparasitic nematodes feeds on above-ground plant tissues including buds, floral primordia, leaves, seeds and stems (Agrios 2005; Horst and Nelson 1997; Pirone 1978). Some species may also feed on below-ground plant tissue; notably, most shoot parasites can also use their stylets to alternatively feed on fungi. This group of nematodes are mainly migratory endoparasitic but some feed ectoparasitically during parts of their life cycles. The number of shoot parasitic nematode species is relatively limited and may be due to the relatively fluctuating and potentially adverse environmental conditions above ground. Most shoot parasites have evolved extreme adaptations for surviving unfavourable environmental conditions, most notably the ability to "dry-down" (in a process called anhydrobiosis) into a long-term (which can extend for several years to decades!) survival stage (sometimes called "dauer") that can be revived with the addition of water.

*17.4.1 Stem and Bulb Nematodes (*Ditylenchus *spp.)*

Ditylenchus species exist in many ecological niches (Sturhan and Brzeski 1991). *Ditylenchus* is among the genera of phytoparasitic nematodes with the greatest variety of feeding habits. Species feed upon fungi, roots, bulbs, and stems. These nematodes have a global distribution but are most damaging in temperate regions. Male and female bodies are fairly long (1–2 mm) and attenuated, the flat lip region

is not sclerotized or offset, includes a fine, short stylet with small knobs, esophageal glands form a non-overlapping bulb, and females have one ovary. Species feed mainly as migratory endoparasites within shoots; they also infect below-ground plant parts and can migrate to feed in the shoots. The most important economic species include: (1) *D. dipsaci* (Kühn) Filipjev – a name that comprises about 80 species that form a "species complex." They are among the nematodes of greatest economic impact globally. Taxa under this name parasitise over 500 plant host species and feed on flowers, leaves and stems. This species complex has excessive intra-specific variation (with about 20 host races including differential chromosome numbers) that in heavy infestations can cause crop losses of 60–80 %; (2) *D. destructor* Thorne – causes Potato Dry Rot. This nematode is a migratory endoparasite on underground plant parts and can feed on fungal mycelia. Fortunately, *D. destructor* is limited in distribution within the USA and other temperate regions; and (3) *D. myceliophagus* Goodey – a fungivoral pest of the mushroom industry that feeds upon mycelia in commercial mushroom beds.

The fourth-stage juvenile (J4) of *Ditylenchus* is the survival and infective stage (Sturhan and Brzeski 1991). *Ditylenchus dipsaci* migrates and feeds within parenchymous tissues of the stem and can move below ground to invade host roots and storage organs such as bulbs, rendering all infected organs malformed, necrotic and twisted. After several generations of reproduction, the J4 form a dried mass of thousands of juveniles called "nema wool" on infected plant parts (mainly in or on storage organs like bulbs). The extensive lesions of dry rot caused on potato by *D. destructor* also contain dried aggregations of J4 nematodes. A sanitation program to reduce *D. destructor* infestation in USA growing regions had been relatively successful until recently… *D. destructor* appears to be on the increase again in some USA growing regions (Hafez et al. 2010). The regional/world-wide distribution and the destructive and survival ability of *D. dipsaci* and *D. destructor* keep them permanently under international regulatory control.

*17.4.2 Wheat (Cereal) Gall Nematodes (*Anguina *spp.)*

Anguina species have similar above-ground (but not below-ground) parasitic habits as *Ditylenchus*, but mainly feed on or in developing buds and floral primordia, and colonize cereal seed heads where they reproduce in large numbers within infected seeds (Krall 1991). *Anguina tritici* (Steinbuch) Filipjev (Wheat Gall Nematode) forms darkened "cockles" in place of wheat seeds that may contain thousands of anhydrobiotic nematodes that may remain viable for 30 years! A sanitation program to remove infected cockles within sown wheat seed has been largely effective to virtually eliminate *A. tritici* as a threat in major wheat growing regions; it remains under regulatory phytosanitary control in wheat growing countries around the world. *Anguina funesta* Price, Fisher & Kerr and other *Anguina* species (e.g., *A. agrostis* (Steinbuch) Filipjev in earlier reports) attack seedheads of rye and other cereal crops. In Australia, these *Anguina* species vector a bacterium, *Rathayibacter toxicus* Sasaki,

Chijimatsu & Suzuki (Riley & Ophel) (syn. *Clavibacter toxicus*, *Corynebaterium toxicus*) that can be infected with a bacteriophage. This entire disease complex produces a powerful neurotoxin (corynetoxin) in developing seedheads that causes a major disease of grazing livestock called "annual ryegrass toxicity" that often produces fatal poisoning. This disease complex is also subject to international phytosanitary regulation.

*17.4.3 Foliar Nematodes (*Aphelenchoides *spp.)*

Members of this Genus are primarily endoparasites of foliar tissue and buds; some minor species are fungivores (Duncan and Moens 2006; Nickle and Hooper 1991). They are limited in their distribution, and primarily prefer temperate climates (USA and Europe). They can be a serious economic threat when infesting ornamental plant production nurseries. The most important species are (1) *A. fragariae* (Ritzema Bos) Christie which causes "Spring crimp" of strawberry and has at least 250 host plant species including ornamentals like *Begonia* spp., ferns, and *Hosta* spp.; (2) *A. ritzemabosi* (Schwartz) Steiner & Buhrer which is a primary pest of plants in the Compositae such as *Chrysanthemum* sp., *Fragaria* sp. (strawberry) and many other host species; and (3) *A. besseyi* Christie which prefers warmer climates and causes "summer crimp" of strawberry and "white tip" disease of rice. All 180+ species of *Aphelenchoides* have a prominent rectangular metacorpus; they are long (1 mm), slender nematodes with a short, fine stylet with small knobs, raised lip region, and dorsal overlapping esophageal glands.

Foliar nematodes often overwinter within decomposing foliage on the ground and unusually do not infest soil (Agrios 2005; Duncan and Moens 2006). They can infect emerging shoots of germinating plants and can feed ectoparasitically on leaf primordia. They climb the shoots of plants in a film of water and usually enter leaves through the stomata. Foliar nematodes feed on parenchymous mesophyll tissue, destroying cells and resulting in leaf blotches (Fig. 17.3e). The infected leaves turn brown, then black, and the discolourations are often delineated by the primary leaf veins (angular appearance). Interveinal necrosis is common and severe infections can lead to extensive defoliation and poor plant growth and appearance. The foliar nematode life cycle is relatively short (2 weeks), promoting multiple generations and as many as 15,000 nematodes can occur in a single leaf! Since plant symptoms often lag behind substantial nematode infestation, foliar nematode spread has been on the increase, especially within the ornamental plant industry (McCuiston et al. 2007).

*17.4.4 Pinewood Nematode (*Bursaphelenchus xylophilus *(Steiner & Buhrer) Nickle)*

Bursaphelenchus xylophilus is the primary economically important member of the Genus (Duncan and Moens 2006). It is one of two important phytoparasitic

nematode species that are unique in that they are vectored by insects and feed on both plant cells and associated fungi in host trees. They were originally reported to be the causal agent of "pine wilt" disease, but several associated organisms and plant toxins are believed to act in association with *B. xylophilus* to induce "pinewilt disease complex" (PWDC). The nematode may have been imported into the USA from Japan where it is a devastating pathogen of endemic genotypes of pine trees (Fig. 17.3f). The predominant species of pines and other tree hosts in the USA, however, are not as susceptible to PWDC as the Japanese pines. (For example, it does not produce epidemics, but some localized problems do occur in the USA). Pinewood nematodes also have a prominent metacorpus and they are long (0.4–1.5 mm), slender nematodes with a high, offset lip region, short, fine stylet with small knobs, excretory pore posterior to metacorpus, and the esophageal glands are weakly developed and slender (Nickle and Hooper 1991).

The pinewood nematodes are vectored by cerambycid beetles (*Monochamus* sp.) and thus have a life cycle closely associated with the life cycle of the beetle. The nematodes feed on plant cells and perhaps mainly on fungi. A blue-stain fungus (*Ceratocystis* spp.) is almost always associated with this nematode infection in trees, and other fungi such as *Botrytis cinerea* (De Bary) Whetzel are occasionally found and serve as a food source for the nematode. Essentially, two life cycles of *B. xylophilus* are observed: (1) During active feeding of "newly infected" trees the nematodes feed on epithelial cells of the xylem and cortex and the life cycle can be as short as 4.5 days (life span 15 days, produces 80 eggs) and produce many generations in the tree. The infected tree foliage becomes discoloured (grayish) and often the needles are retained (where they normally would drop). The pattern of discolouration and wilting is irregular (patchy) among the tree at first, and at this point growth the symptoms induced by the blue-stain fungus are obvious. Also the tree produces phytotoxins (as a defense response) that may become systemic and actually harm the tree itself. Eventually the entire tree becomes discoloured, wilted, and eventually dies. (2) The dispersal cycle occurs in dead or dying wood, perhaps after overwintering, where the nematode feeds primarily on associated fungi. The nematodes molt to become resistant third-stage dauer-larvae with a thickened cuticle and stored food (lipids and glycogen) reserves. The third-stage dauer-larvae migrate to the pupal chambers of beetles that have invaded the same tree. The nematodes molt to become fourth-stage dauer-larvae, and then enter the respiratory system (spiracles) of developed, young beetle adults.

An average of 15,000–20,000 dauer-larvae can be vectored (this does not hurt the insect) by one emerging adult beetle and transferred to healthy trees by the feeding of the beetle. The presence of the nematodes in both the trees and beetles compounds potential spread and regulatory concerns. The regulatory concern extends to all wood products derived from infected trees, including packing materials for shipping. The development of the International Standards for Phytosanitary Measure No. 15 (ISPM 15) by the IPPC occurred due to concerns about the introduction of forestry pests via international trade in wooden articles (https://www.ippc.int/). This international regulatory standard (developed in 2006 and modified in 2010) has led to a significant increase in import protection via the

use of heat–treatment and pre-shipment fumigation to disinfest logs, lumber and wooden products to eliminate pest such as the Pinewood Nematode and wood boring insects.

A related species, *Bursaphelenchus cocophilus* (Cobb) Baujard (syn. *Radinaphelenchus cocophilus*) has a similar disease cycle (Duncan and Moens 2006) in palm trees. This nematode is vectored by a Palm Weevil (*Rhynchophorus*) and is an important pathogen of coconut and ornamental palms in Central America, South America and the Caribbean. The nematode infects the foliage and eventually the stem (trunk) of palms and forms the characteristic "red ring" disease that can be seen in the cross-sections of infected tree trunks. Again, problems associated with eventual disruption of vascular transport will eventually kill the tree and the nematode is vectored to new trees by the weevils.

17.5 Detection and Management of Phytoparasitic Nematodes

Efficient sampling is a critical component of any pest detection scheme, and phytoparasitic nematodes are no different (Barker and Davis 1996; Been and Schomaker 2006). Regulatory management of nematodes also will be discussed in the section below. A brief discussion on sampling, detection, and identification of phytoparasitic nematodes for grower use is presented here. Sampling for diagnostic purposes should include soil and/or plant (roots or shoots, where appropriate) samples taken at the margins of the diseased area since the nematodes will migrate from dead tissues to infect living plant tissues. Fields with a history of nematode infestation and sampled for advisory purposes should collect samples in a systematic pattern to obtain an average representation of the nematode population density across the entire growing area. Samples should be submitted to a certified nematode assay lab in a timely manner for extraction of nematodes from samples and subsequent nematode identification and quantification.

The type (public or private) and availability of nematode assay labs differs across geographic regions, and even among different states within the USA (Barker and Davis 1996). Various flotation methods are used to separate nematodes from soil samples (Barker and Davis 1996; Shurtleff and Averre 2000), with the floating nematodes subsequently captured on fine-mesh sieves (25–50 μm openings). Extraction of nematodes from plant samples (and sometimes soil samples) can include methods to stimulate nematodes to emigrate from plant tissues or simply to macerate the host tissues to release the nematodes and capture the nematodes on sieves. Nematode assay labs then depend upon highly-trained personnel to identify (usually only to Genus-level) and quantify the nematodes present in a sample using microscopy and make nematode management recommendations as appropriate (Shurtleff and Averre 2000). As indicated earlier, molecular tools may soon be more widely adapted to assist in accurate and practical identification of phytoparasitic nematodes (to Species-level) for diagnostic and advisory purposes (Ophel-Keller et al. 2008; Subbotin and Moens 2006).

Available management options to reduce potential phytoparasitic nematode damage depend upon several factors, most importantly the type and population densities of nematodes present, crop history and future cropping systems, and predicted cost-efficiency of an appropriate management tactic. Nematicides have been a staple for nematode management in high-value crops for decades, but the high cost, inherent toxicity, and potential environmental damage of many nematicides have limited their use (Haydock et al. 2006). Soil fumigants such as Methyl Bromide, 1, 3 dichloropropene (Telone II), methylisothiocyanate (MITC) emitters (Vapam), dazomet (Basamid), and chloropicrin (tear gas) greatly reduce pre-plant levels of nematodes to provide crop protection. The relatively recent listing of methyl bromide as an ozone-depleting agent, however, has virtually resulted in its elimination from commercial use globally except for official pre-shipment or quarantine use and where exemptions apply. (http://ozone.unep.org/Publications/MP_Handbook/Section_1.1_The_Montreal_Protocol/Article_2H.shtml; Schneider et al. 2003).

Granular and liquid non-fumigant nematicides are generally nerve toxins (acetylcholinesterase inhibitors), such as the organophosphates fenamiphos (Nemacur), terbufos (Counter), and ethoprop (Mocap) or carbamates such as aldicarb (Temik), oxamyl (Vydate), and carbofuran (Furadan). These non-fumigant compounds can be applied post-plant and usually provide some systemic activity in plants. However their high costs, groundwater concerns, and mammalian toxicity have greatly restricted their authorized use.

A viable alternative to nematicides is the use of crop cultivars that are genetically resistant to phytoparasitic nematodes. Cultivars of several major crop species such as cotton, soybean, tomato, and others have been bred for resistance to specific species of phytoparasitic nematodes and provide excellent crop protection and yields (Starr et al. 2002). The risk of selection of resistance-breaking races within a nematode species and the lack of identified sources of resistance to other nematode species and for other major crop species, however, suggests the urgent need for the development of new nematode-resistant crop cultivars.

Rotation to a non-host crop species presents an effective cultural practise to reduce phytoparasitic nematode population densities to levels that are below economic damage thresholds (Noe 1998). This technique works well for infestations of nematodes with a limited crop host range and lack of long-term survival strategies with the availability of practical and economically-viable rotation crops. Incorporation of nematode-resistant cultivars into a crop rotation scheme can provide the potential for long-term sustainability to manage some nematode infestations.

17.6 International Biosecurity Regulations of Phytoparasitic Nematodes

17.6.1 Introduction

We provide a basic outline of biosecurity (quarantine/plant health) related to the management or regulation of phytoparasitic nematodes in international trade. This

introduction to biosecurity gives examples of historical events, international agreements, legislation, phytosanitary requirements and techniques that prevent or limit the spread of nematodes via trade in plants, plant products and commerce. Effective regulation is essential because nematodes are often difficult to detect and easily overlooked. Indeed, many people are unaware of their existence.

The importance of nematodes as pests in agriculture and horticulture is relatively poorly known to the general public, compared with other pests such as insects. Nematodes often go undetected because they are typically microscopic and tend to be located in plant roots or in soil where they are virtually impossible to see without knowledge of nematode symptoms, detection techniques and/or training. In addition to being found in soil, roots, and underground plant organs, nematodes can also spread in the upper parts of plants such as stems, floral organs, seeds, and wood (Agrios 2005; Duncan and Moens 2006; Hockland et al. 2006; Shurtleff and Averre 2000; Taylor 1971).

17.6.2 Historical Spread of Nematodes via Trade

Without effective biosecurity regulation, phytoparasitic nematodes can easily spread to new areas/regions/countries via trade in plants and plant products. Nematodes also are spread in association with farm/garden tools, machinery or shipping containers that are contaminated by plant material or soil (Agrios 2005; MAF Biosecurity New Zealand (MAFBNZ) 2009a; Norton and Niblack 1991; Shurtleff and Averre 2000). Introduction of nematodes in this manner has occurred frequently with serious agricultural consequences. Spread is only stopped or restricted by effective biosecurity regulation that may include specific phytosanitary activities being implemented before importation or after detection in new areas (Cotten and Van Riel 1993; Hockland et al. 2006).

Distribution and establishment of phytoparasitic nematodes can also be influenced strongly by climatic conditions. For example, tropical nematode species will usually not do well in temperate regions and vice-versa. Nematodes with a narrow host range may also struggle to establish in the global distribution of their hosts species (Noe and Sikora 1990). Early examples of phytoparasitic nematodes reported in the literature include: Needham's initial observations (1743) of the Wheat Weed Gall Nematode; Schmidt's identification of *Heterodera schachtii* (1850) from "exhausted" sugar beet fields in Europe; reports by Cobb (1891) of nematodes associated with plants from Fiji; Treub's identification of *Meloidogyne javanica* from Indonesia in 1885, and Prayer's description (1901) of a nematode disease of banana in Egypt (El-Sharif 1997; Luc et al. 1990; Thorne 1961).

Discovery of the Golden Nematode (*Globodera rostochiensis*) in 1941 in New York State was reportedly associated with military equipment and vehicles returning to the USA after World War 1. This infestation led to millions of dollars being spent on the Golden Nematode Control Project by the USDA to prevent further spread. It also resulted in a tightening of import regulation for plant material

entering the USA (Brodie and Mai 1989; Cotten and Van Riel 1993; Hockland et al. 2006). Despite tight regulations and surveillance, Golden Nematodes have been detected in other areas of North America.

The enforcement of strict Potato Cyst Nematode quarantine regulation delayed detection of *G. pallida* (Pale Potato Cyst Nematode) until 2006 when eight fields in Idaho were found infested (Skantar et al. 2007). Similar finds of Potato Cyst Nematodes in New Zealand (1983) and Australia (1986) emphasizes that very stringent international biosecurity regulations may only slow spread before other regulatory measures must be implemented (Cotten and Van Riel 1993; Marshall 1998; Stanton 1986; Wood et al. 1983).

17.6.3 The International Basis for Phytosanitary Agreements and Regulation of Nematodes

Exclusion via effective biosecurity regulation is the most important tool or strategy to prevent phytoparasitic nematodes from becoming established or introduced to new areas. When introduced into a new area or country, phytoparasitic nematodes are rarely eradicated because initial infestations may often go unnoticed or undiagnosed for considerable periods of time. This offers time and opportunity for nematodes to spread further from the first point of introduction via movement of infested machinery, plant material, soil, tools, vehicle tire treads or by water and wind. Incidentally, exclusion via biosecurity (quarantine) regulation of plant products in international trade is certainly the most effective way of limiting the movement of phytoparasitic nematodes (Barker 1997; Hockland et al. 2006).

Regulation is chiefly conducted cooperatively under the auspices of the IPPC (See Sect. 2.2). As stated the IPPC was formed in 1951 to promote and manage international cooperation in managing plant pests (Cotten and Van Riel 1993; https://www.ippc.int/). The IPPC is managed by the Commission on Phytosanitary Measures (CPM), which promotes cooperation between member nations in order to protect the world's crop plants and natural plant environments from the introduction and spread of plant pests, while aiming to minimise interference with usual international trade and the movement of people (Chap. 2). A major revision of the IPPC occurred in 1997; currently 177 signatory nations agree to the timely exchange of phytosanitary information and use standardised phytosanitary terms. We recognise 34 International Standards for Phytosanitary Measures (ISPMs) that include export and import phytosanitary guidelines. For example, ISPM No. 1 is the guidance document for "*Phytosanitary Principles for the Protection of Plants and the Application of Phytosanitary Measures in International Trade*" (http://www.ippc.int/) (Chap. 2). Additionally, Regional Plant Protection Organisations (RPPOs) operate cooperatively in specific regions of the globe. For example, the Pacific Plant Protection Organisation was formed in 1994, includes 27 Pacific member countries//territories, and is recognised by the IPPC (https://www.ippc.int/index.php?id=pppo).

17.6.4 Country or Region Specific Regulation of Nematodes

Typically, regulated pathogens or pests do not occur in the country imposing the regulatory importation requirements. Biosecurity regulation of phytoparasitic nematodes is conducted similar to ways used for plant pathogens (bacteria, fungi, viruses etc.). Regulated (biosecurity status) nematodes are designated by the IPPC as being "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled" (https://www.ippc.int/). Biosecurity-status nematodes (like other pests and pathogens) of concern are identified or determined by a National Plant Protection Organisation (NPPO) or equivalent regulatory agency after conducting risk analysis or risk assessment (Chap. 5). For example, this work is conducted by the NPPO of a country such as MAFBNZ in NZ, DAFF-AQIS in Australia or USDA-APHIS in the USA. In addition, RPPOs may also develop pest lists and biosecurity strategies for wider areas, such as the European Plant Protection Organisation (EPPO) for the European and Mediterranean region. Using IPPC guidelines, country or regional organisations determine and impose biosecurity requirements on the importation pathways to prevent introduction of pathogens and pests of concern (Cotten and Van Riel 1993; Hockland et al. 2006; Mathys and Smith 1984; https://ippc.int/). For our purposes here, the regulatory efforts of MAFBNZ will serve as an example of similar efforts among other participating countries.

17.6.5 Development of Phytosanitary Requirements for Import Regulation of Nematodes

In NZ, MAFBNZ conducts risk analyses and develops Import Health Standards (IHSs) for importing plants and plant products that hold specific pest lists, and also hold generic and targeted import requirements (as per IPPC guidelines). IHSs usually undergo regular revision and are periodically modified to reflect new pest risks and the discovery of new pests that become established (in addition to reporting new pests as is required under IPPC guidelines). The IHS that applies most particularly for regulating most nematodes is the one for propagable plants and plant parts – IHS for Nursery Stock: 155.02.06 Importation of Nursery Stock (MAF Biosecurity New Zealand 2010a; http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf). MAFBNZ also publishes an Unwanted Organisms Register on its website (MAF Biosecurity New Zealand 2010b; http://www.biosecurity.govt.nz/pests/registers/uor).

This register holds a list of 368 nematodes that NZ considers to be unwanted biosecurity organisms and having likelihood to cause serious economic or environmental harm. Examples of some of the world's most serious phytoparasitic nematodes are listed and include: *Aphelenchoides besseyi* (Rice White-tip Nematode), *Aphelenchoides bicaudatus* ((Imamura) Filipjev & Schuurmans Stekhoven) (Foliar Nematode), *Belonolaimus longicaudatus* (Sting Nematode and one other species), *Bursaphelenchus xylophilus* (Pine Wilt Nematode), *Ditylenchus dipsaci*

[stem and bulb nematode strains not occurring in NZ], *Heterodera cruciferae* Franklin (Cabbage Cyst Nematode), *H. glycines* (Soybean Cyst Nematode), *Hoplolaimus columbus* Sher (Columbia Lance Nematode) *H. galeatus* (Cobb) (Crown-headed Lance Nematode), *Longidorus* spp. (Needle Nematodes and 25 other species), *Meloidogyne chitwoodi*, (Columbia Root-knot Nematode and another 14 RKN species), *Naccobus aberrans* (False Root Knot Nematode), *Paralongidorus* spp., (virus-vectoring and stunt nematodes, three species in total), *Paratrichodorus* spp. (Stubby Root Nematodes, four species in total), *Pratylenchus brachyurus* (Root Lesion Nematode), *P. indicus* Siddiqi (Root Lesion Nematode) *P. scribneri* Steiner (Scribner's Root Lesion Nematode and another 12 species), *Radopholus similis* (Burrowing Nematode and another three species), *Rotylenchulus reniformis* (Reniform Nematode and another three species), *Trichodorus* spp. (Stubby Root Nematodes, eight species in total), *Tylenchorhynchus* spp. (Stunt Nematodes, 16 species in total) and *Xiphinema index* (Dagger Nematode and another 34 species).

Like most NPPOs, MAFBNZ requires that imported plants and plant parts are accompanied by a Phytosanitary Certificate (PC) that officially certifies that the plants and plant material has been inspected and/or treated in the exporting country in accordance with appropriate official procedures and found to be free of any visually detectable regulated pests, and conforms with NZ's import requirements. For whole plants (with roots) the following additional declaration must also to be written on the PC: (1) "The plants were raised from seed/cuttings in soil-less rooting media in containers maintained out of contact with the soil"; or (2) "The roots of the plants have been dipped in fenamiphos (Note this is an organophosphate nematicide) at 1.6 g active ingredient per litre of water for 30 min)".

In addition to the requirement for official certification after inspection, MAFBNZ also specifies particular pesticide treatment requirements for regulating nematodes on plants and plant parts. These requirements are applied generically across multiple imported host plant species. For dormant bulbs both fumigation or hot water treatment and dipping in fenamiphos are required for nematode control (the methods of treatment must be written on the PC in the Treatment Section) as follows:

(1) Methyl Bromide fumigation: Fumigation for 2 h at atmospheric pressure using one of the following combinations of rate (g/m^3) and temperature (°C):

Rate (g/m^3)	Temperature (°C)
48	10–15
40	16–20
32	21–27
28	28–32

OR Hot water treatment: Immersion in hot water at a constant temperature of 24 °C for 2 h, followed by immersion in hot water at a constant temperature of 45 °C for 4 h (period required at the stated temperatures excluding warm-up times), AND (2) Chemical treatment: Immersion in fenamiphos (1 g active ingredient per litre of dip) for 1 h.

By comparison, the USDA maintains a Regulated Pest List associated with imported plants and plant products. This list was developed by using pest data obtained from the US Code of Federal Regulations (7 CFR 300–399), biosecurity

data on pests found on imported goods at the USA border, data on biosecurity pests determined by USDA – Animal and Plant Health Inspection Service (APHIS) and by stakeholders in the USA as having potential to cause serious economic or environmental damage (http://www.aphis.usda.gov/import_export/plants/plant_imports/regulated_pest_list.shtml; United States National Archives & Records Administration 2008).

This list includes the Potato Cysts Nematodes, *Globodera pallida* and *G. rostochiensis* which are strictly regulated by the USDA from entering the USA and from spreading internally in the USA from areas where they have established. Information on US Federal Domestic Quarantine requirements are located at the following address: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&rgn=div5&view=text&node=7:5.1.1.1.2&idno=7. In particular, information relating to the US domestic quarantine requirements for *G. pallida* and *G. rostochiensis* are specified here also. These Potato Cyst Nematode species are very good examples of serious economic pests that have spread widely from their origin in the Andes of South America to the potato growing regions of the world. Due to the importance of potatoes in world agricultural trade potatoes, these pathogens are currently managed extensively and strictly regulated. Presently *G. pallida* and *G. rostochiensis* are specifically biosecurity regulated by 55 and 106 countries respectively (Hockland et al. 2006; Jones and Kempston 1982; Skantar et al. 2007).

The importation requirements for *Solanum tuberosum* L. (potato) nursery stock into NZ are quite restrictive in that only plants in tissue culture are permitted to be imported by people or organisations that are granted import permits. The tissue culture can be imported in two ways: (1) Imported from facilities accredited by MAFBNZ as meeting certain production and pest free/sanitary standards. The cultures must be accompanied by official certification attesting to freedom from designated pathogens/pests, visually detectable pathogens/pests. Following inspection (on entry to NZ) the tissue cultures are not required to be held in post-entry quarantine; and (2) imported from facilities that are not accredited by MAFBNZ. As above, they must be accompanied by official certification attesting to freedom from designated pathogens and pests, visually detectable pathogens pests and once inspected (on entry to NZ) the tissue cultures are required to be held in post-entry quarantine for a minimum of 3 months (MAF Biosecurity New Zealand 2010a; http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf). Although nematodes are not specifically mentioned in the pest list for *S. tuberosum* (as the potato cyst nematodes and *Ditylenchus destructor* occur in NZ and are not subject to complete biosecurity movement control) it is unlikely that other nematodes that could be associated would escape the testing and inspection scrutiny associated with the tissue culture pathway.

17.6.6 Phytosanitary Management of Exported Plants and Plant Products

As a major agricultural and horticultural exporting nation, NZ is also required to meet an importing country's phytosanitary requirements for plants and plant

products. MAFBNZ is tasked with preventing the entry of new pathogens and pests into NZ and also with preventing the spread of pests found in NZ to other countries on exported products. One example of an export certification program (ECP) relating to potato exports is aimed at phytoparasitic nematodes. The program is run by MAFBNZ. MAFBNZ provides a rigorous ECP for the export of table potato exports (for human consumption) to Taiwan (these requirements may also cover exports other countries). Details of this program may be found at http://www.biosecurity.govt.nz/files/regs/exports/plants/potatoes/potato-pcn-wart.pdf. The program was developed in cooperation with the NZ Potato Export Access Committee and is aimed at meeting Taiwan's phytosanitary requirements for entry of potatoes where freedom from *G. pallida* and *G. rostochiensis* (and Potato Wart Fungus – *Synchytrium endobioticum* (Schilbersky) Percival) is required. The specification listed in the ECP defines MAFBNZ's operational requirements for exporters, growers, Independent Verification Agencies (IVAs as approved by MAFBNZ to carry out export certification activities), packing facility operators and storage facility operators. Requirements for this program are extensive so they are summarized as follows:

1. Production Requirements: Producers of intended export crops must:
    - Agree to the terms and conditions of the ECP by signing a compliance agreement;
    - Only use production sites that have a history of freedom from the Potato Cyst Nematodes (and Potato Wart Fungus);
    - Have official approval and registration for the site (by MAFBNZ via an IVA);
    - Use certified seed potatoes (proof required);
    - Maintain production records;
    - Manage movement and cleanliness of equipment and machinery into production sites to prevent introduction of Potato Cyst Nematodes; and
    - Apply a sprout inhibitor at harvest or ensure this is done during post-harvest processing.
2. Packing Facility and Storage Facility Requirements: These facilities must:
    - Be registered by MAFBNZ via IVA;
    - Have specialists staff who are aware of the ECP requirements;
    - Ensure potatoes are identified with specific MAFBNZ code numbers;
    - Ensure potatoes from Taiwan are segregated and sorted/packed separately from other lines; and
    - Notify MAFBNZ immediately if specific export pests are identified.
3. Exporter Requirements: Exporters must:
    - Be registered with Horticulture NZ;
    - Be registered with MAFBNZ, and provide traceability and post-inspection security; and

- Ensure where the potatoes are subject to MAFBNZ endpoint inspection, an authorized IVA or MAFBNZ approved organisation provides traceability and post-inspection security.

All MAFBNZ approved organisations involved in the ECP must be registered and meet the requirements of applicable MAFBNZ standards. The ECP and other standards form part of the MAFBNZ export phytosanitary certification system. These standards provide delegation of authority by MAFBNZ to authorised IVAs and approved organisations to carry out certification services and activities that contribute to the issuance of MAF phytosanitary certificates. For example, Potato Cyst Nematode sampling must be conducted by MAFBNZ-approved persons using approved sampling methods and techniques. The soil/plant samples may only be processed for identification by MAFBNZ approved labs that must meet the requirements of MAFBNZ Standard: Plants Export Operations Pest Identification Requirements (MAF Biosecurity New Zealand 2009b; http://www.biosecurity.govt.nz/exports/plants/certification/peo-pir.htm). These labs must follow a written (MAFBNZ approved) lab management system for the identification of specific nematodes. Finally, MAFBNZ periodically assesses the lab management systems for nematode identification using MAFBNZ auditors and a nematologist as a technical audit expert. MAFBNZ is extremely careful to ensure the ECP requirements are met in full in order to meet Taiwan's (and other countries) import requirements and prevent further spread of the potato cyst nematodes.

17.6.7 Phytosanitary Methods for Detecting, Excluding, and Treating Nematodes

As nematodes are most commonly found in/on plant tissues or in soil associated with plants, the main emphasis of phytosanitary regulatory management is logically focused on plants (plant nursery stock, propagative parts, some seeds) and soil. Importation requirements for nursery stock plants and plant propagative parts may involve a range of possible phytosanitary activities. These activities could be conducted in the country of origin (such as specific sampling or operating an ECP) or upon importation in the destination country. Detection of nematodes for regulatory purposes (to meet import export or phytosanitary requirements) most often relies on sampling. Usually this includes looking for symptoms of nematode damage in the field or place of production and soil and/or plant samples (roots or shoots depending on the type of nematodes) are taken for analysis.

Knowledge of nematode life cycles and habits in plant parts and soil is very important in determining the best time to conduct sampling. Soil samples should be taken when soil is moist, not dry or wet, and sampling is usually conducted systematically across fields. Where possible, sampling should be focused near host plants or where they were grown as nematodes are usually highly clustered around those areas in fields. However, the success of sampling may also depend on a number of different factors including the host plant, type of nematodes, sampling

depth and soil type (Barker and Davis 1996; Been and Schomaker 2006; Fortuner 1991; Shurtleff and Averre 2000).

A significant problem can occur when nematodes may have been recently introduced to an area and/or occur at levels below the usual level of detection for some particular reason. Here there is the possibility of falsely attributing freedom from target species in these areas or crops even where plant and soil samples have been taken systematically and appropriate analysis and identification processes have been conducted. Timeliness of sending samples to appropriate nematology labs is also important as samples can degrade by drying out and reducing the survival of nematodes. There is a great variation in facilities that deal with extraction and identification of nematodes around the world and those that have official government certification and/or meet international standards are preferable (Barker and Davis 1996; Luc et al. 1990; MAF Biosecurity New Zealand 2009).

Several standardised lab methods are used for the extraction of nematodes from soil samples (Barker and Davis 1996; Fortuner 1991; Shurtleff and Averre 2000). Extraction of nematodes from plant samples (mostly roots) also varies widely. Methods have been developed to stimulate nematodes to leave plant tissue samples. For example, extracted plant material can be placed on funnels above beakers in mist chambers to stimulate the gradual movement of the nematodes outwards. Another approach is to coarsely grind the plant material to release the nematodes and capture them on fine mesh sieves. Nematode analysis and identification labs then rely on specialist nematology professionals (scientists and technicians) to identify to genus level and quantify the nematodes extracted in a sample using microscopy.

Reports on nematodes that are identified are then used for biosecurity or regulatory decisions as required (Cotten and Van Riel 1993; Fortuner 1991; Shurtleff and Averre 2000). In addition, the ongoing development of molecular tools may become further refined to help with the accurate and rapid identification of such nematodes (to species level) for regulatory and phytosanitary purposes (Ophel-Keller et al. 2008; Subbotin and Moens 2006).

When phytoparasitic nematodes are identified as being problematic in certain areas or in certain plants or plant materials, other techniques can be used for eradication. These techniques may include the use of designated biosecurity treatments on bulbs, rootstocks, other plant parts and seeds including dipping in nematicides, the use of fumigation at specific rates and temperatures, and hot water treatment (Bridge et al. 1990; Cotten and Van Riel 1993; Hockland et al. 2006; MAF Biosecurity New Zealand 2010). Other commonly used strategies for excluding nematodes by managing imported materials include post-harvest or pre-export inspection, banning the importation of plant host material or specific types of host material, banning the importation of plant bedding material and soil, and requiring that imported plants are certified as being grown in artificial planting media (Barker 1997; Cotten and Van Riel 1993; Hockland et al. 2006). These strategies often involve the additional use of phytosanitary treatments and may also require post-entry quarantine for important or high value planting material.

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