12 Nematodes of Quarantine Importance

Phytonematodes have ever been an unseen and often underestimated menace in horticultural crops across the globe. Besides causing damage due to their own feeding, nematodes also incite or aggravate fungal and bacterial diseases and serve as vectors of many plant viruses. The maxim "prevention is better than cure" is particularly true with nematodes. Once established in a given area, the nematodes are almost impossible to eliminate. Substantial crop losses from phytonematodes could be much greater if species currently causing localized damage became more widespread.

"Quarantine" refers to regulatory actions aimed at preventing or retarding the introduction, establishment, and spread of dangerous pests in crop protection (Maas 1987). In quarantine and certification programs, intensive sampling may be needed to determine if lots or shipments of plants, pots, cuttings, or other units are free of plant-parasitic nematodes and other plant pests. Because it is seldom feasible, or even possible, to examine and test the entire lots for such harmful pests, these determinations must nearly always be made on the basis of samples drawn from the lots.

A "quarantine nematode" refers to a nematode species that can cause a pest of potential economic importance to the area, which can be one specific country or a whole continent, endangered thereby and not yet present there or present but not widely distributed and being officially controlled, as given by the International Plant Protection Convention (IPPC). Opportunities for the introduction of new species of nematodes have been numerous, but its introduction does not necessarily mean its establishment as a pest. Often a complicated set of conditions and events must be present before an organism can survive in a new environment. Frequent introductions, particularly of large numbers of the pest organism, increase that organism's chance of becoming established in a new area. Once a nematode is introduced and established, it may be years before numbers sufficient to cause severe crop injury are produced. Ordinary plant quarantine regulatory action is not well adapted to the detection of plant-parasitic nematodes.

The potential phytosanitary importance of all named phytonematode species is determined by evaluating available information on species characteristics, association with economically important crop hosts, and ability to act as vectors of viruses or form disease complexes with other pathogens. Most named species of phytonematodes are poorly known, recorded from a single location only, not associated with economically important crops, and not known to be associated with other plant disease organisms.

Phytonematodes during quarantine inspections may also act as a bioindicator for consignments that do not meet the phytosanitary requirements of plants being grown in sterile environments and could be carrying other plant pathogens and microorganisms (Hockland and Anderson 2012). Quarantine measures can also prevent spread of nontarget species which are potentially invasive (Schrader and Unger 2003). Quarantine and other phytosanitary measures are particularly important for PPN because other management methods such

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as chemical control or crop resistance can be far more costly and difficult to implement without other adverse effects (Nicol et al. 2011).

First step in implementing quarantine measures is to determine which species should be regulated under international trade rules. The international plant pest convention defines a quarantine pest as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled." Organisms which meet this definition can be regulated. Countries determine their lists of regulated pests according to guidelines set by the International Plant Protection Convention (IPPC) and International Standards for Phytosanitary Measures (ISPM). Countries may also establish lists of regulated non-quarantine pests. The IPPC defines these as follows: "A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party." All lists of regulated pests are dynamic, with species added or removed from lists as phytosanitary risks change or as new species emerge as pests.

Many phytonematodes have low impact in their native range, but much greater impact when introduced to new areas, for example, *Bursaphelenchus xylophilus* (pine wood nematode) in Japan, China, Portugal, and most recently in Spain (Robertson et al. 2011) and the potato cyst nematodes in Europe, the USA, Canada, and Australia (Sun et al. 2007). Quarantine measures against known damaging nematodes are effective in preventing their spread, thus effectively and economically preventing crop losses. The soil habitat that elaborates survival mechanisms of nematodes protects them and enables their quick establishment in a new locality. The low environmental resistance in a new locality also allows their quick growth.

Species other than those on official lists of quarantine pests may also pose a biosecurity risk. Only a small proportion of nematode species have been described (Blaxter 2011; Hodda 2011). New species are being described regularly, and species new to science have been intercepted during phytosanitary inspections (e.g., *Radopholus bridgei* and *Meloidogyne thailandica*, both described from material intercepted in quarantine (Handoo et al. 2005)).

A list of major phytonematodes of quarantine importance in horticultural crops has been furnished in Tables 12.1 and 12.2.

Yield loss caused by phytonematodes is often used to determine economic importance, but

Table 12.1 Nematodes of quarantine importance specific to crop and country

Nematode species	Crop	Country
Globodera rostochiensis	Potato	India
Ditylenchus destructor		
Meloidogyne spp.		
Pratylenchus spp.		
Radopholus similis	Banana	India
Helicotylenchus multicinctus		
Rhadinaphelenchus (*Bursaphelenchus*) *cocophilus*	Palms	Japan, East Asian Nations
Heterodera schachtii	Sugar beet	European countries
Heterodera goettingiana	Vegetables	Europe
H. humuli	Hops	Europe
H. cacti	Cacti	Europe
Aphelenchoides fragariae	Narcissus	Europe
Pratylenchus vulnus	Temperate fruits	Europe and the USA

Table 12.2 A general list of quarantine nematodes

Nematode species
Meloidogyne chitwoodi, Meloidogyne fallax
Globodera rostochiensis, Globodera pallida, Heterodera glycines
Hirschmanniella spp. (except *H. gracilis* and *H. loofi*), *Nacobbus aberrans, Radopholus similis*
Ditylenchus dipsaci, Ditylenchus destructor
Aphelenchoides besseyi, Bursaphelenchus xylophilus
Longidorus diadecturus, Xiphinema americanum, Xiphinema bricolens, Xiphinema californicum, Xiphinema rivesi
Heterodera schachtii, Cactodera cacti, Radopholus citrophilus
Aphelenchoides fragariae, A. ritzemabosi, Heterodera humuli, Heterodera goettingiana
Rhadinaphelenchus cocophilus, Aphelenchoides arachidis

there are limitations in its calculation. Yield-loss calculations from different studies and countries do not necessarily use the same methods, with some reporting damage as percentage yield loss and others reporting as tons per hectare or as percentage yield gained after the application of nematicides or as correlations of yield gains with declining nematode abundance. Indirect losses also generally remain unaccounted for in most yield-loss figures. Yield-loss estimates based on work done long ago and under different nematode management regimes are also likely to require updating. Impacts can also be difficult to estimate or severely underestimated because nematodes may injure plants in many different ways. One way is by direct feeding action, but this may take many different forms including direct damage, root galls, root stunting, or withdrawal of resources from other parts of the plant (Norton and Niblack 1991). Another aspect to consider when assessing phytosanitary status is intraspecific variation. This is often not considered because most phytosanitary regulation is on the taxonomic level of the species. However, species from the economically important genera *Belonolaimus, Ditylenchus, Globodera, Heterodera, Meloidogyne, Nacobbus, Radopholus, Rotylenchulus*, and *Tylenchulus* all have pathotypes or races with distinctive host responses and differences in host range.

12.1 Biosecurity Implications

Despite these limitations, making the best systematic predictions of impact and risk possible based on as much data as can be obtained is preferable to the alternative of empirical measurements of actual damage following real introductions of potentially damaging species. Where real introductions of exotic nematodes have occurred and been measured, the impacts have mostly been substantial. Of course, by this stage, eradication is seldom an option. More recently nematode species have been used as bioindicators during quarantine inspections (Hodda et al. 2008). In the UK, *Helicotylenchus dihystera* is often intercepted with planting materials supposedly grown in sterile conditions, indicating that phytosanitary standards were not met (Hockland and Anderson 2012). Other microscopic plant pathogens such as fungi, bacteria, and viruses may also use similar pathways to nematodes (Grousset et al. 2012); hence, targeting the generally larger nematodes during quarantine inspections has assisted in reducing the risks from other microscopic quarantine organisms generally. Phytosanitary risks are specific to countries or regions. Regulated organisms differ among countries depending on species distributions and regulatory or biosecurity policy in different countries and formal pest risk analysis processes.

Most quarantine action utilizes visual inspection supplemented by a hand lens or low-power microscope – to detect parasitic organisms themselves or to check symptoms of infection. Symptoms of nematode injury usually are not diagnostic, because they are largely plant reactions such as poor growth, chlorosis, and other symptoms equally indicative of root injury from many other causes. Specific symptoms also may vary with the reaction of different plants. Large obvious lesions are formed on the roots of certain plants attacked by root lesion nematodes, while such lesions are not found on the roots of many other kinds of plants attacked by the same nematode, although serious injury may be caused on both. Nematodes also may enter and be carried in roots of plants which are not preferred hosts. These nematodes later may prove to be of importance on other crops in the area or to subsequent crops on the same land. Nursery stock is a common means of disseminating plant-parasitic nematodes, either as parasites of nursery plants involved or as contaminants in or on these plants. Regulations established by the Department of Horticulture require that locations, where nursery stock is grown or sold, be inspected for phytonematodes.

Fortunately, the active dispersal of nematodes is rather limited, but this is more than compensated by the ease of passive dispersal with movement of infested soil, plant material, and water. Man has aided in the indiscriminate spread of nematode while carrying the plant materials and other items infested with nematodes for research, trade, charity, or other purposes. With the recent trade liberalization, the bulk movement of commodities has become common, and consequently the risk of entry of pests and diseases has increased manifold. Several devastating phytonematodes have entered into new locations and established successfully on major horticultural crops, leading to losses.

12.2 Nematodes Under Quarantine Act

Nematodes under the following families have been included under quarantine act, viz., Anguinidae, Aphelenchoididae, Atylenchidae, Belonolaimidae, Caloosiidae, Criconematidae, Dolichodoridae, Ecphyadophoridae, Hemicycliophoridae, Heteroderidae, Hoplolaimidae, Longidoridae, Meloidogynidae, Neotylechidae, Parasitaphelenchidae, Paratylenchidae, Pratylenchidae, Psilenchidae, Rotylenchulidae, Sphaeronematidae, Telotylenchidae, Trichodoridae, Tylenchidae, Tylenchulidae, and Tylodoridae. The major phytonematodes include *Ditylenchus angustus, Globodera pallida, Globodera rostochiensis, Heterodera goettingiana, Heterodera schachtii, Heterodera zeae, Meloidogyne chitwoodi, Meloidogyne fallax, Nacobbus aberrans, Radopholus citrophilus, Radopholus similis*, and *Xiphinema index.* However, of all the phytonematodes, in most countries, quarantine measures have been strictly followed in potato cyst nematodes.

12.2.1 Potato Cyst Nematodes

Golden cyst nematode of potato (*Globodera rostochiensis*) and pale cyst nematode (*Globodera pallida*) are the major cyst nematodes infesting potato.

12.2.1.1 Host Range

Potatoes are by far the most important host crop. Tomatoes and aubergines are also attacked. Other *Solanum* spp. and their hybrids can also act as hosts. Both species of *Globodera* have several different pathotypes (Kort 1974). The pathotypes are characterized by their ability to multiply on certain tuberous *Solanum* clones and hybrids used in breeding. Five pathotypes are recognized within *G. rostochiensis* (Ro1-Ro5 international notation) and three in *G. pallida* (Pa1-Pa3) (Kort et al. 1977). Some of these pathotypes are recognized by their almost total inability to multiply on specific cultivars of potato (single-gene resistance); for example, the most commonly grown resistant potato cultivars (based on gene H1 derived from clones of *S. tuberosum* subsp. *andigena*) are resistant to pathotype Ro1 of *G. rostochiensis* only. Other pathotypes show different levels of ability to multiply on different cultivars; the testing of this form of resistance is discussed by Mugniéry et al. (1989). The internationally recognized system of classification of pathotypes refers mainly to the pathotypes present in Europe (and spread from there) and may not have relevance to South America (Kort et al. 1977). It is probable that pathotypes exist there which were never transferred from the Andean region (Canto-Saenz and Mayer de Scurrah 1978).

The golden nematode is one of the world's most damaging potato pests. First detected in the USA in 1941 in Nassau County on Long Island, New York, it was subsequently found in eight other New York counties. For over 60 years, an effective federal and state quarantine program has confined the pest to nine counties in New York. If golden nematode were to become more widely established in US potato, tomato, and eggplant production areas, annual crop losses could reach $4.8 billion. The golden nematode is primarily a pest of potatoes and is also referred to as a species of potato cyst nematode. In the larval stage, it bores into the roots of host plants and feeds on their juices. Because the aboveground damage is not visible during the early stages of infestation, the pest can remain undetected for years. The first sign of a golden nematode infestation is poor plant growth in one or more areas of a potato field. As an infestation builds, the damaged area increases and eventually the entire field displays poor plant growth. Large numbers of the nematodes cause wilting, stunted growth, poor root development, and early plant death. Golden nematodes can also reproduce on the roots of eggplant, tomatoes, and several solanaceous weeds.

The pale cyst nematode, *Globodera pallida*, is a major pest of potato crops in cool–temperate areas. It primarily affects plants within the potato family including tomatoes, eggplants, and some weeds. If left uncontrolled, pale cyst nematodes can cause up to 80 % yield loss in potato fields. The pale cyst nematode is widely distributed in many potato-growing regions throughout the world. In North America, besides the current find in Idaho, the nematode is also known to be present on the island of Newfoundland, Canada. Pale cyst nematode infestations may be associated with patches of poor growth in potato fields. At high nematode populations, affected potato plants may exhibit yellowing, wilting, or death of foliage – none of which has been observed in Idaho potato fields.

12.2.1.2 Geographical Distribution

The center of origin of the two species is in the Andes Mountains in South America from where they were introduced to Europe with potatoes, probably in the mid-nineteenth century. From there, they were spread with seed potatoes to other areas. The present distribution covers temperate zones down to sea level and in the tropics at higher altitudes. In these areas, distribution is linked with that of the potato crop.

12.2.2 Golden Cyst Nematode of Potato (*Globodera rostochiensis*)

EPPO Region: Albania, Algeria, Austria, Belarus, Belgium, Bulgaria, the Czech Republic, Cyprus, Denmark, Egypt, Estonia, the Faroe Islands, Finland, France, Germany, Greece (including Crete), Hungary (one locality only), Iceland, Ireland, Latvia, Lebanon, Libya, Lithuania, Luxembourg, Malta, Morocco, the Netherlands, Norway, Poland, Portugal (including Madeira; unconfirmed in Azores), Spain (including Canary Islands), Russia (Central Russia, Eastern Siberia, Far East, Northern Russia, Southern Russia, Western Siberia), Slovakia, Sweden, Switzerland, Tunisia, the UK (England, Channel Islands), Ukraine, and Yugoslavia (unconfirmed). Found in Israel on only two occasions in 1954 and 1965 in a small area in the Sharon region and was successfully eradicated.

Asia: Cyprus, India (Kerala, Tamil Nadu), Japan (Hokkaido), Lebanon, Pakistan, the Philippines, Sri Lanka, Tajikistan, and Russia (Eastern Siberia, Far East, Western Siberia)

Africa: Algeria, Egypt, Libya, Morocco (intercepted only), Sierra Leone, South Africa, and Tunisia

North America: Canada (Newfoundland, British Columbia Vancouver Island only), Mexico, and the USA (New York; eradicated in Delaware)

Central America and Caribbean: Costa Rica and Panama

South America: Throughout the high Andean region: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru, and Venezuela. More southerly in range than *G. pallida*

Oceania: Australia (two outbreaks, one in Western Australia in 1986, the other in Victoria in 1991; both are subject to official eradication programs), New Zealand, and Norfolk Island

EU: Present

12.2.3 Pale/White Cyst Nematode of Potato (*Globodera pallida*)

EPPO region: Algeria, Austria, Belgium, Cyprus, the Faroe Islands, France, Germany, Greece (Crete only), Iceland, Ireland, Italy, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal (mainland), Russia (unconfirmed in European Russia), Slovakia, Spain (including Canary Islands), Sweden, Switzerland, Tunisia, the UK (England, Scotland, Channel Islands), and Yugoslavia

Asia: Cyprus, India (Himachal Pradesh, Kerala, Tamil Nadu), and Pakistan

Africa: Algeria, Tunisia, and South Africa

North America: Canada (Newfoundland)

Central America and Caribbean: Panama

South America: Throughout the high Andean region: Argentina, Bolivia, Chile, Colombia, Ecuador, Peru, and Venezuela. More northerly in range than *G. rostochiensis*

Oceania: New Zealand

EU: Present

Diagnosis: When suspected nematode introductions are found in diagnostic samples, their identity should be confirmed, voucher specimen should be deposited in an appropriate reference collection, and quarantine authorities should be notified. In addition to detecting newly introduced nematodes, diagnostic services may become involved in quarantine issues through their role in assessing samples of produce designated for overseas export market. As international trade increases, there will be an increasing demand from overseas quarantine authorities to ensure that agricultural products (e.g., tubers, rhizomes, rooted plants) are free of specific nematodes. If protocols for collecting and processing samples are set by the importing country, these must be followed by the diagnostic service. In the absence of specific protocols, the level of risk should be assessed and appropriate assessment procedures need to be developed.

Detection and Inspection Methods: Ordinary plant quarantine regulatory action is not well adapted for the detection of phytonematodes. Most quarantine action utilizes visual inspection supplemented by a hand lens or low-power microscope – to detect parasitic organisms themselves or to check symptoms of infection. Symptoms of nematode injury usually are not diagnostic, because they are largely plant reactions such as poor growth, chlorosis, and other symptoms equally indicative of root injury from many other causes. Specific symptoms also may vary with the reaction of different plants. Large obvious lesions are formed on the roots of certain plants attacked by root lesion nematodes, while such lesions are not found on the roots of many other kinds of plants attacked by the same nematode, although serious injury may be caused on both.

The symptoms described for *Globodera* spp. can have many other causes and cannot be taken as proof of presence of nematodes. For positive detection it is necessary to find cysts in soil samples or females or cysts on host roots. Mature females and cysts are just visible to the naked eye and can be seen as minute white or yellow globes on the root surface. Specific identification is just possible by observation of the female color at the appropriate stage of development, either a change from white to yellow in *G. rostochiensis* or prolonged white (slightly cream but no yellow phase) in *G. pallida*. Several methods are available for extracting juveniles or larvae from the soil (Southey 1986; OEPP/EPPO 1991), and thereafter specialist microscopical examination of juveniles, females, or cysts is necessary for precise identification. The species can also be distinguished by the morphological characters and measurements of second-stage juveniles and cysts. The use of biochemical techniques such as DNA probes is being investigated as morphological identification can be difficult.

Means of Movement and Dispersal: These nematodes have no natural means of dispersal and can only move the short distances traveled by juveniles attracted toward roots in the soil. They are spread into new areas as cysts on, in order of importance, seed potatoes, nursery stock, soil, flower bulbs, and potatoes for consumption or processing. The last named are only important if there is a risk of their being planted or if care is not taken with disposal of waste soil.

Phytosanitary Risk and Phytosanitary Measures: Both species of potato cyst nematode are A2 quarantine pests for EPPO (*OEPP/EPPO* 1978, 1981). They are also of quarantine significance for APPPC and NAPPO. In addition, *G. rostochiensis* is a quarantine pest for CPPC and IAPSC. The nematodes are already established in most or all areas in the EPPO region that are important for the cultivation of potatoes for consumption or the production of starch; therefore, regular attention to control is needed in such areas. Where domestic legislative measures are in force, import regulations are justified to ensure comparable standards for imported material. It is essential that areas of seed potato production be kept as free as possible from these nematodes. *G. pallida* is generally less common than *G. rostochiensis* in most of the EPPO region (with the exception of the southern part of the UK) and is absent from some countries; it therefore merits greater attention from the phytosanitary standpoint. At some time in the future, it may be worthwhile to consider the individual pathotypes as being the quarantine organisms, rather than the two species themselves. It is obvious that some pathotypes are very widely distributed, and furthermore some are more important economically than others. Unfortunately, information is still limited on the precise distribution of the pathotypes.

Measures to prevent the introduction of the nematodes to areas where they are not already established include soil sampling surveys and regulations concerning movement of seed potatoes, nursery stock, flower bulbs, and soil. These apply nationally as well as internationally (CEC 1969). Consignments of potato tubers, rooted plants, and bulbs from countries where the nematodes occur may be examined to check amounts of adhering soil, if any, or to take samples of soil for laboratory examination. Additional safeguards during transit of consignments could be washing of tubers and flower bulbs to remove soil, although it should be noted that cysts can remain embedded in tubers, especially in the eyes. Alternatively, tubers may be dipped in dilute sodium hypochlorite solution (Wood and Foot 1977). The EPPO specific quarantine requirements (OEPP/EPPO 1990) for these nematodes require that the field in which seed potatoes or rooted plants being imported were grown was inspected by taking soil samples according to an EPPO-recommended method (OEPP/EPPO 1991) and found free from viable cysts of both species. The sampling must have been performed after harvest and after removal of the previous potato crop.

Best Management Schedule: Once established, these nematodes are nearly impossible to eliminate as cysts can lie dormant in the soil without a host crop for decades. A new generation of PCN is produced each time a host crop is grown. However, the following are the best management practices to help prevent infection and reduce the nematode population over time:

(a) *Minimize soil movement:* Avoid sharing farm machinery, equipment, tools, and containers; do not spread tare dirt or debris onto agricultural land or areas where it could be spread to other agricultural land. Tare dirt or debris is the loose soil knocked off either during potato processing operations or during storage filling/emptying operations; never use bags, containers, etc., more than once for potato transport unless they are free of soil. Be sure all commercial transport vehicles are free of soil.
(b) *Keep farm equipment and vehicles clean:* Ensure equipment is available to conduct proper cleaning and disinfection of farm machinery and vehicles; clean and disinfect all used equipment before using on a farm; clean and disinfect all machinery, vehicles, and other equipment before going between fields. This includes those of temporary help, custom applicators, and utility companies.
(c) *Healthy seed:* Plant certified seed potatoes produced on land determined not to be infested with nematode. Avoid continuous planting of potatoes in the same field(s). A long rotation cycle is critical for managing and preventing PCN. During interim years, plant nonhost crops. Contain water and soil during tuber washing to avoid contaminating farmland. Processing facilities should not return soil, water, cull tubers, and debris to agricultural land. This includes managing and limiting waste used as livestock feed. Segregate potatoes in storage – each field should be stored separately.
(d) *Best land practices:* Plant cover crops when fields are not in use to prevent wind and water from moving soil. Keep hedgerows, sod barriers, or sod strips between fields and along highways to provide a physical barrier to soil movement. Do not use common headlands, farm roads, and public roads as turning areas.
(e) *Long-term management:* Successful management or eradication of cyst nematodes

is complex and takes many years. It is important to develop appropriate long-term strategies to deal with the pest. These strategies will help protect the local, provincial, and national potato industries as well as other affected industries while being sensitive to the situation of positive growers. With appropriate management, it will be possible to grow most nonhost crops on affected fields, and it may, over several years, become possible to grow potatoes in an appropriate rotation.

(f) *Regulatory measures:* In the event of cyst nematode detection, immediate regulatory measures are to be taken to contain potential sources of spread. Surveys and investigations to trace the infestation back to an infested field and to trace the infestation forward are also conducted to prevent any further spread. No potatoes could be grown on land known to be infested through soil sampling; movement of farm machinery and construction equipment out of the regulated area was permitted only after steam cleaning and fumigation; no soil nor sod could be moved out of the restricted area; potato grading stations could be operated only under permit, and the tare soil had to be treated; potatoes grown on Long Island could not be used for seed; potatoes shipped for table stock must be washed. Potatoes must be packaged in nonreusable paper bags.

12.2.4 Stem and Bulb Nematode (*Ditylenchus dipsaci*)

D. dipsaci is known to attack over 450 different plant species, including many weeds. However, it occurs in more than ten biological "races," some of which have a limited host range. The races that breed on rye, oats, and onions seem to be polyphagous and can also infest several other crops, whereas those breeding on lucerne, *Trifolium pratense*, and strawberries are virtually specific for their named hosts and seem to have relatively few alternative host plants. The tulip race will also infest *Narcissus*, whereas another race commonly found in *Narcissus* does not breed on tulip. It is known that some of the races can interbreed and that their progeny has different host preferences (Eriksson 1974).

The principal hosts are faba beans, garlic, *Hyacinthus orientalis*, leeks, lucerne, maize, *Narcissus pseudonarcissus*, oats, onions, peas, *Phlox drummondii*, *P. paniculata*, potatoes, rye, strawberries, sugar beet, tobacco, *Trifolium pratense*, *T. repens*, and tulips. It has also been reported on carnations, celery, *Hydrangea*, lentils, rape, parsley, sunflowers, and wheat.

Geographical Distribution: D. dipsaci occurs locally in most temperate areas of the world (Mediterranean region, North and South America, northern and southern Africa, Asia, and Oceania), but it does not seem able to establish itself in tropical regions except at higher altitudes that have a temperate climate. In most countries regulatory measures (e.g., certification schemes) are applied to minimize further spread of *D. dipsaci*.

EPPO Region: Albania, Algeria, Austria, Belarus, Belgium, Bulgaria, Croatia, Cyprus (unconfirmed), the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Latvia, Liechtenstein, Malta, Moldova, Morocco, the Netherlands, Norway, Poland, Portugal, Romania, Russia (European), Slovakia, Spain, Sweden, Switzerland, Syria, Turkey, Tunisia, the UK, Ukraine, and Yugoslavia

Asia: Armenia, Azerbaijan, China (Gansu, Hebei, Henan, Shandong), Cyprus (unconfirmed), India, Iran, Iraq, Israel, Japan (Honshu), Jordan, Kazakhstan, Korea Republic, Kyrgyzstan, Oman, Pakistan, Syria, Turkey, Uzbekistan, and Yemen

Africa: Algeria, Kenya, Morocco, Nigeria, Tunisia, Réunion, and South Africa

North America: Canada (Alberta, British Columbia, Ontario, Prince Edward Island), Mexico, and the USA (Alabama, Arizona, California, Florida, Hawaii, Michigan, New York, North Carolina, Utah, Virginia, Wyoming)

Central America and Caribbean: Costa Rica, the Dominican Republic, and Haiti

South America: Argentina, Bolivia, Brazil (Pernambuco, Parana, Rio Grande do Sul, Santa Catarina, São Paulo), Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay, and Venezuela

Oceania: Australia (New South Wales, South Australia, Tasmania, Victoria, Western Australia), and New Zealand

EU: Present

Detection and Identification: Major symptoms, in general, include swellings and distortion of aerial plant parts and necrosis or rotting of stem bases, bulbs, tubers, and rhizomes.

On Allium spp. (*Onions, Garlic, Leeks, etc.*)*:* The penetration of onion leaves by *D. dipsaci* causes leaf deformation and leaf swellings or blister-like areas on the surface. The leaves grow in a disorderly fashion, often hang as if wilted and become chlorotic. Young plants can be killed by high infestations. The inner scales of the bulb are usually more severely attacked than the outer scales. As the season advances, the bulbs become soft and when cut open show browning of the scales in concentric circles. Conversely, *D. dipsaci* on garlic does not induce deformation or swellings, but causes leaf yellowing and death (Netscher and Sikora 1990).

On Lucerne: The crop declines in patches in the field, and damage is more serious in humid climates. The whole plant becomes desiccated and presents symptoms of stunting and swelling at the base of the stem with conspicuous shortened internodes. With heavy infestation, plants can be killed.

On Tobacco: Invasion by the nematode of the lower part of the stem causes stunting and deformation of the plant followed by "stem break."

On Faba Beans: D. dipsaci causes swelling and deformation of stem tissue or lesions which turn reddish-brown then black, depending on cultivar and environmental factors. Newly formed pods take on a dark brown appearance. The lesions envelop the stem and increase in length, often advancing to the edge of an internode. Leaf and petiole necrosis is also common under heavy infestations, but can be confused with symptoms induced by fungal leaf pathogens. Infected seeds are darker, distorted and smaller in size and may have speckle-like spots on the surface. Heavy infestations often kill the main shoots, stimulating secondary tiller formation. The more severe symptoms are usually induced by the "giant race" on faba beans (Sikora and Greco 1990).

Detection and Inspection Methods: D. dipsaci can be isolated from samples of suspected seed material (according to symptoms) by dissection in water at 20 times magnification. Nematodes leave the dissected tissue and swim actively in the water. Microscopic examination at 800 times magnification is necessary for correct identification of the nematode species.

Means of Movement and Dispersal: In international trade *D. dipsaci* is liable to be carried on dry seeds and planting material of host plants. In the field the fourth-stage, juvenile can withstand desiccation for many years, and although soil densities seem to decrease rapidly, the nematode can survive for years without a host plant. Nematode survival and damage are greater in heavy soils as compared to sandy soils. It can also survive on a number of weeds. Irrigation water and cultivation by contaminated farm tools and machinery are other sources of inoculum dissemination. Nematode-free (certified) seeds and planting material are most essential to prevent crop damage by *D. dipsaci*. Hot water treatments with different temperature–time combinations, depending on type and state of seed material, are major preventive measures.

Phytosanitary Risk and Phytosanitary Measures: At present, the distribution of the different races throughout the region is patchy, and some countries apply official control measures to limit spread. Other countries regard the pest as being a quality pest which can be effectively controlled by production and use of healthy planting material. It is certainly true that, without control, *D. dipsaci* may cause complete failure of host crops within the EPPO region. EPPO lists it as an A2 quarantine pest, and CPPC, IAPSC, and NAPPO also consider it to be of quarantine significance.

The implementation of certification schemes for the production of host plants of *D. dipsaci* can provide planting material free from the pest. Imports of soil and plants for planting and seeds of host plants from countries where this nematode occurs should be restricted.

12.2.5 Potato Rot Nematode (*Ditylenchus destructor*)

Host Range: Potatoes are the main host of *D. destructor*, but the nematode can also occasionally be found on bulbous *Iris*, carrots, *Trifolium* spp., groundnuts, and garlic. Overall, some 70 crops and weeds and a similar number of fungus species have been recorded as hosts.

Geographical Distribution

EPPO Region: Albania, Austria, Belarus, Belgium, Bulgaria, the Czech Republic, Estonia, Finland (intercepted only), France, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Romania, Russia (European), Slovakia, Spain, Sweden, Switzerland, Turkey, and the UK

Asia: Azerbaijan, Bangladesh (unconfirmed), China (Hainan, Hebei, Jiangsu, Liaoning, Shandong), India (unconfirmed), India, Iran, Japan, Kazakhstan, Malaysia (unconfirmed), Saudi Arabia, Tajikistan, Turkey, and Uzbekistan

Africa: South Africa

North America: Canada, Mexico, and the USA (Arkansas, California, Hawaii, Idaho, Indiana, New Jersey, North Carolina, Oregon, South Carolina, Virginia, Washington, West Virginia, Wisconsin)

South America: Ecuador

Oceania: Australia (New South Wales, Victoria, South Australia, Western Australia, restricted distribution in Tasmania), and New Zealand (on hops only; Foot and Wood 1982)

EU: Present

Detection and Identification

Symptoms

On Potatoes: There are, in general, no obvious symptoms in the aerial parts of the plant, although heavily infested tubers give rise to weak plants which usually die. Early infections can be detected by peeling the tuber which can reveal small, off-white spots in the otherwise healthy flesh. These later enlarge, darken, are wooly in texture, and may be slightly hollow at the center.

On badly affected tubers, there are typically slightly sunken areas with cracked and wrinkled skin which is detached in places from the underlying flesh. The flesh has a dry and mealy appearance, varying in color from grayish to dark brown or black. This discoloration is largely due to secondary invasion of fungi, bacteria, and free-living nematodes (the latter are easily confused with *D. destructor*).

In contrast, the skin of potatoes infested with *D. dipsaci* is not usually cracked, and the rot darkens toward the inside of the tuber. The symptoms are more obvious in the foliage, which is shortened and malformed.

On Iris and Tulips: Infestations usually begin at the base and extend up to the fleshy scales, causing gray to black lesions; roots may be blackened and leaves poorly developed, with yellow tips.

On Groundnuts: Hulls of groundnuts show black discoloration which appears first along the longitudinal veins. The kernels are shrunken. The infected testae are brown to black and the embryo shows a brown discoloration (Jones and De Waele 1988).

Detection and Inspection Methods: Prior to planting, soil can be sampled using a standard extraction procedure for nematodes of this size (Hooper 1986). It is difficult to detect the presence of *D. destructor* from external tuber appearance alone. Sample tubers should be cut or peeled to look for the characteristic whitish pockets in which most of the nematodes are found. Microscopic examination of the nematode is necessary for correct identification of the species.

Means of Movement and Dispersal: The nematodes can move only short distances in the soil and have no natural means of long-range movement. The main means of dispersal is with infested potato tubers or other subterranean organs of host plants, e.g., bulbs and rhizomes (especially of *Iris*). Transport in infested soil is another important means of spread. Irrigation water can also carry the nematodes.

Phytosanitary Risk: D. destructor was considered to be an EPPO A2 quarantine pest (OEPP/EPPO 1978) but was deleted from the quarantine list in 1984 because of its minor importance and very wide distribution throughout the EPPO region, in particular in those areas where it would be likely to cause crop damage. *D. destructor* is of quarantine significance for the APPPC and COSAVE. The requirement of the nematode for high relative humidity means it would be unlikely to become a problem in areas with warm, dry soils; it may therefore be of concern to potato production only in the northern parts of the EPPO region. However, its establishment as a groundnut pathogen in South Africa has shown its potential to adjust to different (and normally unfavorable) climatic conditions (De Waele and Wilke 1990).

Phytosanitary Measures: Fumigation under vacuum (650 mmHg) with hydrogen cyanide (initial dose, 4 g/m^3) for 1 h at above 10 °C gives good control of the nematode in bulbs, rhizomes, and tubers and especially asparagus roots and strawberry plants. Infestation in *Iris* bulbs can be controlled by immersion in water containing 0.5 % formaldehyde at 43.5 °C for 2–3 h, but some varieties may be injured during this treatment. In garlic bulbs, nematodes were controlled by drying at 34–36 °C for 12–17 days (Fujimura et al. 1989).

12.2.6 The Burrowing Nematode (*Radopholus similis*/*R. citrophilus*) on Citrus

12.2.6.1 Parasitism and Ecology of the Burrowing Nematode on Citrus

The burrowing nematode is a dimorphic species. Females have a well-developed stylet and are endoparasitic. Males have a poorly developed stylet and are not considered to be parasitic. Development and reproduction take place inside root tissues of plant hosts. Nematode feeding and migration in roots cause large cavities and necrosis in the cortical and vascular tissues. Nematode life stages require healthy root tissue for development and reproduction. They migrate out of roots that have been colonized and damaged as a result of nematode feeding activity and the subsequent decay caused by the invasion of bacteria, fungi, mites, and other organisms. The migratory habit of the nematodes in soil provides the opportunity for burrowing nematodes to search for healthy roots. Burrowing nematodes can survive in the absence of a host for about 6 months.

Burrowing Nematode Races: Studies have shown that there are two races with different host preferences (Inserra et al. 2005). One race, called the banana race, parasitizes banana, but not citrus. The other race, called the citrus race, parasitizes both citrus and banana. Both races have extended, overlapping host ranges that include ornamentals, agronomic crops, and weeds. There are no obvious and specific morphological differences between the two races. The citrus race was eventually elevated to the status of species, *Radopholus citrophilus* and had differences between the two races in oocyte maturation, sex pheromones, enzymes, and proteins. However, recent molecular and mating studies by Kaplan and Opperman (2000) show that *R. similis* and *R. citrophilus* are nonspecific and they have been returned to their previous status as races of *R. similis*.

Several biological factors need to be considered for regulatory management of different phytonematodes, including burrowing nematode (Table 12.3) (O'Bannon and Esser 1987).

Damage and Symptoms: The burrowing nematode severely damages the fibrous roots of citrus trees. The debilitated root system does not adequately support the aboveground portion of the tree which exhibits sparse foliage and weakened and dead branches. This condition results in severely reduced yields of as much as 80 % fruit loss compared to healthy trees, rendering infested orchards economically nonviable.

Soil factors play a major role in the expression of "spreading decline" symptoms, which occur mainly in deep well-drained sands (90–95 % sand) poor in organic matter (<1 %).

Table 12.3 Comparative biological factors to be considered for regulatory management of major phytonematodes

Factor	Burrowing nematode	Golden cyst nematode	Soybean cyst nematode
Soil depth occurrence	15 cm–5 m	7–30 cm	7–30 cm
Longevity, without food	6 months	8–10 years	4 years
Host range (plant species)	290	3	500
Parasitic habit	Endoparasitic	Semi-endoparasitic	Semi-endoparasitic
Natural protection	None	Cyst	Cyst
Climate	Tropical	Temperate	Temperate

This soil type makes up much of the subsoil in some locations known as the "ridge" and favors the burrowing nematode. The combination of low soil moisture conditions (5–7 %) in the subsoil and nematode damage results in "spreading decline" symptoms. Soils rich in organic matter, silt and clay components, and high moisture levels inhibit the increase of burrowing nematode populations and are not conducive to the expression of the disease (O'Bannon and Tomerlin 1971).

Quarantine Actions and Management of the Burrowing Nematode on Citrus: Long-term research has shown that the burrowing nematodes were spread in the "ridge" with infected citrus propagative material originating from infested nurseries. The nematode occurs mainly in areas where burrowing nematode-infected plants, such as citrus, banana, and many ornamentals, were introduced by human intervention. Nematodes may also occur on introduced nematode-infected aquatic plants, such as aquatic aroids. It is essential to implement appropriate phytosanitary actions to prevent the spread of the nematode in uncultivated areas and the adoption of effective management practices in citrus orchards.

(a) *Eradication:* A burrowing nematode eradication program, called "Push and Treat," was attempted in Florida by removing infected citrus trees from orchards and applying high doses of fumigant nematicides. This program was discontinued due to environmental concern and chemical contamination of the water table. However, these eradication attempts on more than 6,000 ha were effective in containing the spread of the nematode from infested orchards into noninfested ones.

(b) *Isolation:* Infested orchards can be separated from healthy orchards by areas, known as barriers. In the past, these barriers were kept weed and root-free by the application of large amounts of fumigant nematicides to chemically devitalize the citrus roots in the barrier and prevent nematode migration. Today they are maintained by mechanical plowing and root pruning. Maintenance of the mechanical barriers is costly.

(c) *Certification:* In several locations like Florida, the Citrus Nursery Certification Programme has been implemented to prevent the spread of citrus nematode pests in Florida citrus orchards (Rule Chapter 5B-44.003, Florida Administrative Code). The Citrus Nursery Certification Programme requires the following: Commercial citrus nurseries produce propagative material free of citrus nematode pests. Soil, peat, and rock material for use in citrus orchards and nurseries must be certified free of these nematodes by the Florida Department of Agriculture and Consumer Services.

The Citrus Nursery Certification Programme: The Citrus Nursery Certification Programme consists of three phases, viz., site approval, pit approval, and premovement certification of young citrus trees before they are moved from the nurseries and transplanted into orchards.

(a) *Site approval:* For economic reasons, commercial citrus nurseries are normally established directly on the ground, as in Florida. Pasture lands are often used for commercial citrus nursery sites. These potential nursery sites are sampled to check for the presence of nematode pests and certified if found free from regulated

Table 12.4 Enzymes/toxins released from nematode-infected plant tissues

Crop	Nematode	Enzymes/toxins released
Potato	*D. destructor*	Polygalacturonase (PG) pectinase
Onion	*D. dipsaci*	Endo-polygalacturonase (E-PG) alkaline phosphatase
Potato	*G. rostochiensis*	Amylase ß-galactosidase, ß-glucosidase
Tubers/cuttings	*Meloidogyne* spp.	Acid phosphatase, alkaline phosphatase, amylase, proteolytic cellulase, esterase, proteases, pectinase
Banana	*R. similis*	Cellulase, invertase
Sugar beet	*H. schactii*	Amylase, cellulase
Citrus	*T. semipenetrans*	Alkaline phosphatase, amylase

citrus nematode pests. Populations of *Tylenchulus* spp. occur in several pastures and uncultivated lands. At one time these populations were called "wild" strains of the citrus nematode and, because of their morphological similarity, were confused with the regulated citrus nematode.

(b) *Pit approval:* Land destined for citrus nurseries and certified nematode-free must be planted with nematode-free citrus rootstock seedlings, which are grown from seeds or tissue culture in soil mixes containing peat and sand free from regulated nematode pests that parasitize citrus. In Florida, peat and sand for use in citrus nurseries are mined from deposits located in natural fields free from the citrus nematode pests. Construction material, such as gravel, clay, and shell, to be used in citrus orchards for construction of roads or other purposes, must be extracted from sites free from nematode pests of citrus. These deposits of construction material are sampled as for pit and nursery sites.

(c) *Premovement:* Citrus rootstock seedlings are grown in certified media on elevated benches that prevent nematode contamination from the ground and transplanted into nematode-free growing areas. The Citrus Nursery Certification Programme requires that any soil or infill unloaded within 30 m of the borders of a citrus nursery or orchard must be certified free of any citrus nematode pest. This successful Citrus Nursery Certification Programme resulted in a rapid reduction in the number of burrowing nematode-infested citrus nurseries. Hence, the implementation of the eradication, isolation, or barrier and Citrus Nursery Certification Programmes initiated in the 1950s reduced the area of orchards infested with the burrowing nematode to less than 4,000 ha in 1984. Present distribution of burrowing nematode in citrus orchards is <1 % of the total area.

Nature of Damage: Since nematodes affect the storage/planting materials which are dormant, their tissues are physiologically different from actively growing plants. Nematodes employ mechanical energy and biochemical components, viz., enzymes and toxins (Table 12.4) to cause diseases. Nematodes degrade either the planting material (rotting or drying) or the growth and development of plant. Nematodes come in the way of respiration and photosynthesis. "True seeds" may be shriveled/distorted and become chaffy resulting in crop loss.

12.2.7 Procedure for Quarantine Inspection for Nematodes

The following is the practical procedure for quarantine inspection of plant material for nematode infestation/contamination:

- Maintaining of records relating to the type of plant material, country of origin, destination, date of receipt and date of disposal, phytosanitary certificate, etc.
- Visual examination of underground and aerial parts of plant material for symptoms of nematode injury, if any, for example, surface or deep necrosis of roots, root rot, root galls, lesions, excessive root branching, stubby root,

coarse root, curly root tips, dead or devitalized buds, crinkled or distorted stems and foliage, seed galls, leaf lesions or spots, leaf galls, and distorted or discolored seed. Each of these symptoms could give indications of infestation by nematodes which cause these specific symptoms.

- Use of appropriate technique for the recovery of nematodes from plant tissue and separation of plant debris, soil clods, free soil, and other extraneous inert matter mixed with true seed or adhering to the seed or plants. Examination of such soil and debris for incidence of nematodes, using appropriate techniques.
- Detailed examination and identification of the nematodes intercepted, under microscope, followed by risk analysis of the species intercepted.
- Decision on the disposal of the plant sample (clearance or rejection) in view of the regulations, risk involved, and the possibility of salvaging the material.
- Salvaging of infested material by fumigation; hot water treatment; nematicidal dip; manual cleaning, washing, and drying; etc., whenever appropriate methods of denematization are available. It should be mentioned here that the salvaging methods used in quarantine must ensure a hundred percent kill of the nematode without injury to the planting material. Since tested and standardized methods (for quarantine operations) are available only for a limited number of plants and planting material (and these too pertain to limited number of species of endoparasitic nematodes), the most ideal procedure for vegetative propagation is to grow the material in nematode-free soil, especially when the plant consignments are large and the suspected nematodes have not been recorded from the country.

12.2.8 Molecular Aids to Nematode Diagnosis

The traditional morphological methods of identification have formed the basis of nematode taxonomy for more than 100 years, and for the foreseeable future, they will continue to be important. However, rapid advances in biotechnology have provided taxonomists with a wide range of new tools, which may eventually revolutionize nematode identification. These new technologies are already widely used in areas such as medical and veterinary diagnosis, where they have been adopted because of their discriminatory power and their ease of use and interpretation. Protein electrophoresis and DNA-based technologies are major methods that may eventually be used for the routine identification of nematodes.

Morphological characters have long been used for nematode taxonomy and will continue to be the mainstay. It is an essential component of any higher level classification of nematodes, and in many cases morphology provides a quick and unambiguous diagnosis to species (Ravichandra 2008). Nevertheless, morphological taxonomy suffers from some limitations. Morphological taxonomy cannot be used for intrasubspecific groups such as races, pathotypes, and strains. Identification by other methods including host range testing is very time consuming and not full proof. Since the genotype of the nematode is examined directly in case of molecular markers, problems associated with phenotypic variation in taxonomic characters are avoided.

Furthermore, greater discrimination is possible because the entire genome is available for study, including 75–80 % of the genome, which is noncoding and contains many highly variable sequences. This DNA sequence variability provides a large pool of information that can provide useful diagnostic characters for the separation of taxa and intrasubspecific categories for the unraveling of evolutionary relationships between nematode groups. Diagnostic kits can be developed for the identification, which can be used for advisory services and intercepting the consignments for domestic as well as international quarantine purposes. Another advantage of molecular markers is that it is independent of the stage of the nematode to be identified and one or few nematodes are enough. The results are interchangeable among laboratories worldwide and reproducible. DNA analysis also helps to understand the genetic pool similarity of any nematode species in a given geographical area,

Table 12.5 Hot water treatment for denematizing planting material

Sl. No.	Nematode	Planting stocks	Time (min.)	Temp. (°C)
1.	*A. ritzemabosi*	Chrysanthemum stools	15	47.8
2.	*A. fragariae*	Easter lily bulbs	60	44.0
		Strawberry runners	10	46.0
3.	*Ditylenchus dipsaci*	Onion	120	43.5
		Narcissus bulbs	240	43.0
4.	*D. destructor*	Irish bulbs	180	43.0
		Potato	60	48.0
5.	*Meloidogyne* spp.	Potato tubers	120	46–47.54
		Sweet potato	65	46.7
		Grape rooted cuttings	10	50.0
		Strawberry cuttings	30	47.8
		Rose cuttings	5	52.8
		Ginger rhizomes	60	45.5
		Peach root stocks	10	55.0
		Tuberose tubers	5–10	50–51.0
		Begonia tubers	60	49.0
		Yam tubers	30	48.0
		Caladium tubers	60	45.0
		Cherry root stocks	5–10	51.0
6.	*Pratylenchus penetrans*	Strawberry	7.5	51.0
7.	*Radopholus similis*	Banana suckers	30	50.0
		Citrus rooted cuttings	20	55.0
8.	*Tylenchulus semipenetrans*	Citrus rooted cuttings	10	46.7
			25	45.0

and this information is highly useful in resistance breeding. There are various molecular markers available at present, which can be used in nematode identification depending on the availability of time, facilities, purpose, and quantity of nematodes.

12.2.9 Avoiding Infiltration of Nematodes/Restricting the Spread

- Set up a nematode diagnostic unit with basic apparatus for extraction, observation, and decontamination and a trained nematologist at each port of entry. Measures like hot water treatment of the planting stock can detect and kill nematodes (Table 12.5).
- Set up regional laboratories with equipments and two nematologists (one taxonomist and one applied nematologist).
- Establish a centralized database of indigenous and exotic nematode pests.
- Carry out pest risk analyses (PRA) for exotic and localized indigenous nematode pests.
- Introduce mandatory phytosanitary inspection of bulk imports of plant materials at the time of entry.
- Introduce post-entry periodic monitoring of sites where imported materials are sown/planted.
- Introduce mandatory destruction at entry-time and post-entry decontamination and, if necessary, destruction of infested material.

The use of treated planting stock is one of the best measures to avoid nematodes, which may be carried out in the following manners:

1. *Heat: steam* (potato cyst nematode, stem, and bulb nematode) and autoclave small quantities of soil.
2. *Hot water:* More common. Time and temperature factors are specific (Table 12.5).

3. *Irradiation: G. rostochiensis* and *D. myceliophagus*.
4. *Washing processes:* Proper and careful washing of material to remove adhering nematodes.
5. *Seed cleaning:* Mechanical seed cleaning to remove dormant structures in the seed lot.

12.2.10 Some Requirements and Solutions

These include basic information on occurrence and distribution of nematode species already present in the country. Categorization of nematodes depending on the information collected into groups based on their damage potential is important. Prohibited species, restricted species, unrestricted species, and import policy-bulk assignments are difficult to examine. Importing small quantities and multiplying of seed/planting material are more suitable. Post-entry quarantine facilities and post-entry isolation growing facilities are essential requirements at all quarantine agencies. Sometimes, mere lab examinations may not be sufficient to detect the nematodes. Operational requirements like socioeconomic factors of the country, lack of public awareness on the nematodes, and programs of educating the public through films, lectures, and pamphlets require immediate attention of the authorities. Training and communication on various aspects of nematodes is also an essential requirement.

12.2.11 Future Thrusts

Major future thrusts include detailed and precise information on the occurrence, distribution, pathogenicity, and host range of phytonematodes of quarantine significance, classification of nematode species into categories according to their damage potential to be incorporated into quarantine regulations under DIP Act, regulation of quantity of hazardous material by the authorities, post-entry quarantine facilities to be improved, attempts to create more public awareness, qualified nematologists to be appointed at quarantine centers, minimum usage of chemicals, safer and eco-friendly approaches like bio-agents, and the use of genetically modified planting material.

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