NEMATODE QUARANTINE AND CERTIFICATION PROGRAMMES IMPLEMENTED IN FLORIDA

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Contents

Summary. The state of Florida has implemented nematode phytosanitary measures and certification programmes that have protected agricultural interests for more than 40 years. A citrus disease called 'Spreading Decline', found to be caused by the burrowing nematode, *Radopholus similis*, devastated citrus orchards in the 1950s. The damage caused by this disease prompted the adoption of internal phytosanitary measures and the implementation of a citrus nursery certification programme aimed at preventing the spread of major citrus nematode pests. Besides the burrowing nematode, this programme included the citrus nematode (*Tylenchulus semipenetrans*) and the coffee lesion nematode (*Pratylenchus coffeae*). The programme requires that citrus propagative material be produced by following strict sanitation practices. It also requires that non-infested orchards be protected from the introduction of these nematodes from contaminated sources or infested orchards. Another certification programme, similar to that for citrus, was established for ornamentals and other plants for export to domestic and international markets where restrictions against the burrowing nematode are imposed. A similar certification programme for ornamentals is implemented by the Florida ornamental industry for the production of plants free of other Florida nematode pests that are not regulated in Florida, but are regulated in domestic and international markets. These nematodes occurring in Florida and regulated in other states and countries include the reniform nematode, *Rotylenchulus reniformis*, and sting nematodes, *Belonolaimus* spp. Information on other nematode pests prohibited by the state of Florida, such as the potato cyst nematodes (*Globodera rostochiensis* and *G. pallida)* and the red ring nematode [*Bursaphelenchus* (= *Rhadinaphelechus*) *cocophilus*), is also included.

In the United States, plant and animal quarantine functions at the federal level are primarily conducted by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), which enforces protective measures to prevent the entry of foreign plants and noxious organisms into the country. Additionally, individual states can implement quarantine measures within their borders to protect their own agricultural interests. These plant protection actions can be coordinated with federal programmes and vary in proportion to the value of the state's agricultural economy and vulnerability to pest invasion. States with an economically strong agricultural base, such as California, which is the leading state for the production of profitable crops (> $ 25 billion in value, in 2001) (Florida Department of Agriculture and Consumer Services, 2002), have state agencies that implement quarantine functions to protect specific agricultural interests.

In Florida, the Florida Department of Agriculture and Consumer Services (FDACS) is the state organization that deals with all aspects of Florida's agriculture, including quarantine actions. These actions are necessary because of the economic value of Florida crops and because of the geographical location of the Florida peninsula, which makes the agriculture and environment of the state vulnerable to the accidental introduction of exotic pests, such as bacteria, fungi, insects, nematodes and viruses.

In 2001, the Florida farm income was > $ 6.4 billion. Florida has a diversified agriculture with strong citrus and ornamental industries, which had cash receipts of $ 1 billion and $ 765 million in 2001, respectively (Florida Department of Agriculture and Consumer Services, 2002). Foliage and flowering ornamentals produced in Florida are mainly exported to national and international markets and are subject to the impact of quarantine restrictions imposed against plant-parasitic nematodes native to, or that have become established in, Florida. In this paper we discuss i) the quarantine actions implemented by the state of Florida to protect the citrus industry from the major citrus nematode pests and ii) the certification programmes that enable the Florida ornamental industry to export plants to domestic and international markets where restrictions against the Florida-regulated burrowing nematode and other nematode plant pests present in Florida are imposed. A list of the most important exotic plant nematode species that are prohibited in Florida is also provided.

QUARANTINE ACTIONS IMPLEMENTED IN FLORIDA TO PROTECT THE CITRUS INDUSTRY FROM CITRUS NEMATODE PESTS

Historical background

Prior to 1955, phytoparasitic nematode species present in Florida were not formally regulated either inside or outside Florida. However, Florida Department of Agriculture records indicate that nematode pests in plant shipments have been detected since 1917 (Esser, unpublished). In the early 1950s, an outbreak of a citrus disease (Fig. 1) occurred in central Florida. Symptom expression had been known since 1928, but the cause was unknown. Citrus debilitation continued to progress for many years. In 1947, 54 orchards were affected by the disease syndrome; however, the decline caused by the disease reached devastating proportions in the early 1950s. In 1953, it was discovered that the burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949, was the causal agent of the decline. In 1956, 1,053 citrus groves, 130 citrus nurseries, 179 ornamental nurseries and 109 dooryard plantings were found to be infested by this nematode.

The burrowing nematode migrates about 10 metres per year through soil and infected citrus roots, resulting in a rapid spread of the expression of the disease, which was given the appropriate name of 'Spreading Decline'. This disease is known only in Florida as there are no reports of burrowing nematode infections on citrus in other citrus-growing areas of the world. After the discovery that the burrowing nematode was the causal agent of 'Spreading Decline', studies on the biology and ecology of the nematode were initiated and are still ongoing. The serious impact of the disease on the Florida citrus industry, and the fact that the causal agent of the disease was a nematode pest new to citrus, made it necessary to establish strict phytosanitary measures to prevent the spread of the disease in Florida, the United States and worldwide. It is worthwhile mentioning that, when the quarantine actions against the burrowing nematode were enacted in Florida, the US Congress had already promulgated the Golden Nematode Act, which was passed in 1948. This legislation was enacted to protect the potato industry from the movement of the golden nematode *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975, which was introduced into the state of New York some time before 1941.

Phytosanitary measures to contain the spread of injurious organisms are effective if they are based on sound biological data. The results of the studies conducted in Florida on the burrowing nematode have had a profound effect on the implementation and improvement of management and quarantine actions against this nematode. The results of 40 years of research on the citrus burrowing nematode are condensed in the following section.

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 six months.

Burrowing nematode races. Studies have shown that there are two races with different host preferences. 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 and called *Radopholus citrophilus* by Huettel *et al.* (1984), who found differences between the two races in oocyte maturation, sex pheromones, enzymes and proteins. However, recent molecular and mating studies by Kaplan *et al.* (1997) and Kaplan and Opperman (2000) show that *R. similis* and *R. citrophilus* are conspecific and they have been returned to their previous status as races of *R. similis*.

The burrowing nematode severely damages the fibrous roots of citrus trees. The debilitated root system does not adequately support the above ground portion of the tree which exhibits sparse foliage, 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 non-viable.

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%). This soil type makes up much of the subsoil in central Florida known as the 'Ridge' and favours the burrowing nematode. The combination of low soil moisture conditions (5-7%) in the subsoil and nematode damage result 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 expression of the disease (Hughes, 1955; O'Bannon and Tomerlin, 1971).

Surveys and ecological research studies indicate that

'Spreading Decline' occurs only in the central part of the Florida peninsula (Florida 'Ridge') where the soil top layer is 30-60 cm deep, very rich in organic matter, and dark in color. The layer below (subsoil) consists of coarse, deep (2-3 m) of well-drained sand, poor in organic matter (< 1%). The top soil layer is rich in organic matter, which supports biological factors antagonistic to nematodes, such as soil insects, fungi and bacteria. These factors limit the role of the burrowing nematode, a poor competitor with other organisms. The subsoil layer contains fewer antagonistic factors, therefore becoming a more favourable environment for the burrowing nematode. After roots are invaded, the nematode-damaged root tissues are infected by pathogenic organisms that cause root decay. Decayed roots do not support the nematode, which is forced into a continuous migration in search of healthy roots.

Research studies have shown that burrowing nematode populations on citrus seedlings grown in pots containing top soil rich in organic matter declined and remained at very low levels, whereas populations declined slightly but remained at damaging levels in pots containing sandy subsoil poor in organic matter similar to that found in the 'Ridge'.

The findings of these ecological studies had important quarantine implications because they elucidated the relationship between soil factors and nematode damage in the 'Ridge'.

Quarantine actions and management of the burrowing nematode on citrus in Florida

Long term research has shown that the burrowing nematodes were spread in the 'Ridge' with infected citrus propagative material originating from infested nurseries. Extensive nematode surveys conducted in non-cultivated areas of Florida indicated that the nematode was not present on native terrestrial Florida plants. 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. Florida's uncultivated lands are free of the burrowing nematode. These findings allowed the implementation of appropriate phystosanitary actions to prevent the spread of the nematode in uncultivated areas and the adoption of effective management practices in citrus orchards.

Details on the surveys and pest exclusion measures implemented since 1953 are provided in the following sections (see Poucher *et al.*, 1967).

Surveys. More than three million survey samples were taken from 1955 to 1984 to define nematode infested areas.

Fig. 1. A citrus tree infected by burrowing nematodes and showing 'spreading decline' symptoms. Note the tree canopy with sparse leaves, which reveals branches that are not visible in healthy trees. (Courtesy of L. Duncan).

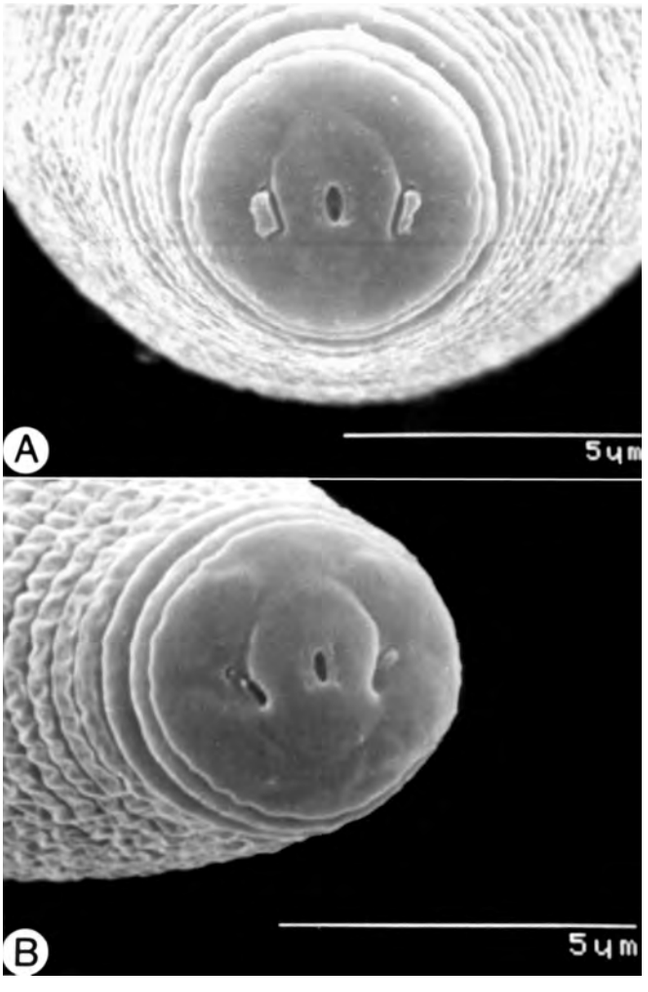

Fig. 2. *En face* electron micrographs of *Pratylenchus coffeae* from citrus in Florida. Note the plain and smooth face due to fusion of the oral disc with lateral and medial lip sectors. This morphological characteristic has important diagnostic value to separate lesion nematode species similar to *P. coffeae* from citrus populations of *P. coffeae* in Florida. (Courtesy of R. N. Inserra and L. W. Duncan).

Eradication. A burrowing nematode eradication programme, called 'Push and Treat', was attempted in Florida by removing infected citrus trees from orchards and applying high doses of fumigant nematicides. This programme was discontinued due to environmental concern and chemical contamination of the water table. However, these eradication attempts on more than 6,000 hectares were effective in containing the spread of the nematode from infested orchards into non-infested ones.

Isolation. Infested orchards can be separated from healthy orchards by areas (called barriers). In the past, these barriers were kept weed and root-free by 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 ploughing and root pruning. Maintenance of the mechanical barriers is costly.

Certification. The damage caused by 'Spreading Decline' to the citrus industry prompted the Florida Legislators to implement the Citrus Nursery Certification Programme 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 that: 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. Details concerning the procedures adopted for the implementation of this programme are listed in the following two sections.

Additional major citrus nematode pests targeted by the quarantine actions

When the Citrus Nursery Certification Programme was passed by the Florida Legislature to prevent the spread of the burrowing nematode in citrus growing areas of the state, two additional citrus nematode pests, the coffee lesion nematode, *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev *et* Schuurmans Stekhoven, 1941, and the citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913, were included in the programme and subjected to the same restrictive measures as those for the burrowing nematode.

The coffee lesion nematode. Pratylenchus coffeae develops and reproduces inside host root tissues in a manner similar to the burrowing nematode. The characteristic plain and smooth face with two lip annuli of this species is shown in figure 2 [see Inserra *et al.* (1997) for more information on the morphological characteristics of citrus populations of *P. coffeae*]. The coffee lesion nematode has a wide distribution in tropical and subtropical citrus growing areas of the world. In Florida, it causes symptoms similar to those of 'Spreading Decline'; however, the coffee lesion nematode is not widespread on citrus in Florida. The nematode occurs on citrus, banana and many ornamentals. The coffee lesion nematode is a regulated pest in citrus growing areas of Florida. It is also regulated in a few counties in California and a few countries in South America.

The Citrus Nematode. Tylenchulus semipenetrans has a sedentary, semi-endoparasitic habit. The vermiform 2nd stage female juvenile (Fig. 3) initiates the infection by inserting its anterior body portion into the root tissues. The juvenile moults to the adult stage, which is sedentary and immobile. In the adult stage, the posterior portion of the female body enlarges with maturation of the reproductive system and protrudes from the root surface. Eggs are deposited in a gelatinous matrix on the root surface. Males are not parasitic and have a very weak stylet. The citrus nematode has a narrow host range that includes citrus, grape (*Vitis* spp.) and olive (*Olea europaea* L.). In the United States, *T. semipenetrans* is a regulated nematode in citrus growing areas of Florida and in citrus nurseries in California. Elsewhere, a few countries in the Middle East and South America impose regulations against this nematode.

The Citrus Nursery Certification Programme

The Citrus Nursery Certification Programme consists of three phases:

i) site approval, ii) pit approval and iii) pre-movement certification of young citrus trees before they are moved from the nurseries and transplanted into orchards.

Site approval. For economic reasons, Florida commercial citrus nurseries are established directly on the ground (Fig. 4). 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 citrus nematode pests.

Tylenchulus spp. populations occur in Florida 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, *T. semi-*

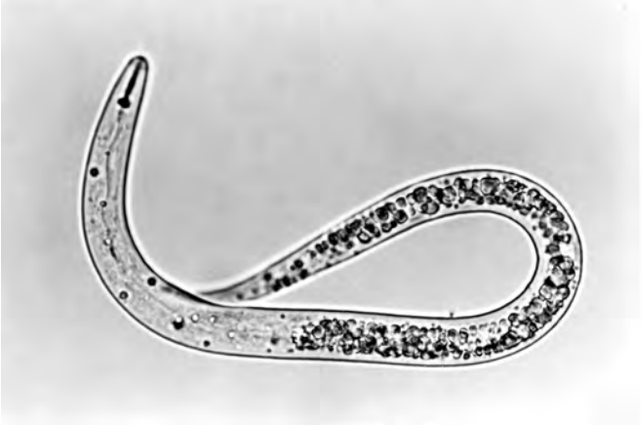

Fig. 3. Photomicrograph of *Tylenchulus semipenetrans* second-stage juvenile female.

penetrans, which attacks citrus. Studies conducted by the senior author from 1985-1988, showed that these populations consisted of two species, now named *Tylenchulus graminis* Inserra, Vovlas, O'Bannon *et* Esser, 1988, which attacks only monocots such as broomsedge and other grasses, and *T. palustris* Inserra, Vovlas, O'Bannon *et* Esser, 1988, which attacks non-rutaceous dicots (Fig. 5). Close examination showed these two species to be morphologically separate from the true citrus nematode and from each other. Neither species parasitizes citrus. The separation of these two species from the true citrus nematode made available potential citrus nursery sites that had previously failed the certification process due to the presence of these wild populations.

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 (Fig. 6). 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.

Pre-movement. Citrus rootstock seedlings are grown in certified media on elevated benches (Fig. 7) that prevent nematode contamination from the ground, and transplanted into nematode-free growing areas. These nematode-free citrus are grown under strict sanitary conditions (Fig. 4) to avoid contamination with nematodes from nematode-contaminated irrigation water, flood water from canals and ponds, contaminated machinery and other sources which may harbour these nematodes. Young trees are sampled before they are moved from the nurseries, and, if nematode-free, can be transplanted into existing orchards or new citrus sites.

The Citrus Nursery Certification Programme requires that any soil or infill unloaded within 30 metres of the borders of a citrus nursery or orchard must be

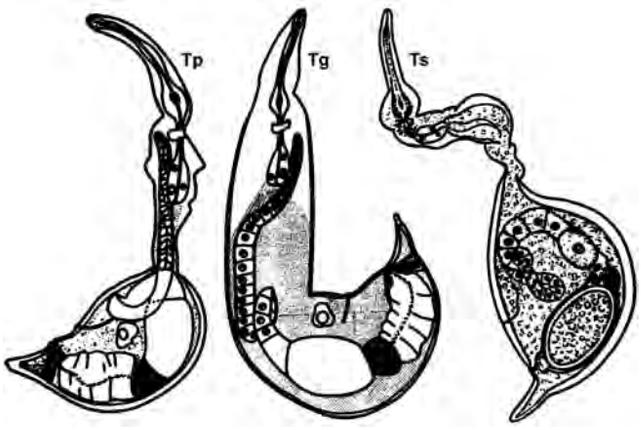

Fig. 5. Drawings of mature females. *Tylenchulus palustris* (Tp), *T. graminis* (Tg) and *T. semipenetrans* (Ts). *Tylenchulus graminis* and *T. palustris* parasitize native plants of Florida, whereas *T. semipenetrans* is a citrus parasitic species. Note the body shapes of *T. palustris* and *T. semipenetrans*, which show < 50% of the body length swollen. In contrast, the shape of *T. graminis* shows > 66% of the body length swollen. The postvulval section of the body is wider in *T. graminis* and *T. palustris* than in *T. semipenetrans.*

Fig. 4. A nematode certified Florida citrus nursery. Three double rows of Swingle citrumelo (X *Citroncirus*) young plants ready to be grafted (on the left) and rows of Hamlin sweet orange (*Citrus sinensis*) grafted on swingle citrumelo rootstock (on the right).

Fig. 6. Top and roots with growing medium attached of nematode certified Swingle citrumelo seedling before being transplanted on a nematode certified site.

Fig. 7. Nematode certified Swingle citrumelo (X *Citroncirus*) seedlings growing in flats placed on elevated benches to prevent nematode contamination from the ground.

Fig. 8. Photomicrograph of a *Rotylenchulus reniformis* vermiform female. Vermiform life-stages of *R. reniformis* are commonly detected in regulatory samples.

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. Presently, no Florida citrus nurseries are known to be infested by the burrowing nematode.

In conclusion, 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 hectares in 1984. Present distribution of burrowing nematode in citrus orchards is < 1% of the total area.

Since 1985, the Florida citrus industry has expanded to the flatwood areas of the Florida peninsula where soils are higher in organic matter than in the 'Ridge'. The water table is also much higher than in the 'Ridge'. These areas are not conducive to the expression of 'Spreading Decline' and, due to the certification programme, they have been planted with nematode-free citrus rootstocks and should remain free of the problem.

Cultural practices in nematode-infested citrus orchards

As mentioned before, the burrowing nematode has not been eradicated from all the citrus growing areas of Central Florida. Appropriate cultural practices are implemented in these infested areas. These cultural practices reflect the findings of long term field studies.

The results of field studies on the root distribution of citrus rootstocks indicate that young trees (5 years old) have 66% of their root system in the top soil layer (30-60 cm deep). Old trees (25-30 years old) have < 40% in the top soil layer. Since the burrowing nematode is less active in the top soil of the 'Ridge', the young trees are less damaged by the nematode because the majority of their roots are in the top soil and are only lightly infected by the nematode. In contrast, the old trees suffer devastating damage because the majority of their roots (60-90%) are in the deep sandy subsoil where the nematode is more active and attains high population levels. Only 10-40% of the roots of older trees are in the top soil.

In non-irrigated citrus orchards, burrowing nematode soil populations decline during the winter months because the trees are stressed by the nematode root damage, which is exacerbated by drought and prevents the production of root flushes. During these months, the debilitated root system is not able to support the tree canopy, resulting in decline symptoms and fruit drop. Nematode populations begin to increase in the late spring and early summer at the beginning of the rainy season. Due to the rains, tree stress is reduced, which suppresses 'Spreading Decline' symptoms. However, yield losses are great because of the fruit drop in the spring. Nematode populations peak in the fall because the available moisture stimulates root flushing, which provides fresh root tissues in which the nematodes reproduce freely.

A condensed list of the cultural practices adopted in

nematode-infested citrus orchards is given below.

– Supplemental irrigation during the low rainfall months alleviates the stress caused by the low moisture in the root zone infected by the nematode. Since roots in the top soil are relatively healthier, surface irrigation allows trees to tolerate nematode damage.
– Non-tillage and the use of herbicides rather than disking also avoid damage to the roots in the topsoil. These roots play a major role in supporting the canopy.
– Organic amendments improve water holding capacity and ion exchange and facilitate the activity of biological control agents.
– These cultural practices can be supplemented with the application of non-fumigant nematicides in order to suppress the nematode population (O'Bannon and Tarjan, 1978).
– Resistant citrus rootstocks have been developed. One of the most effective is Milam lemon. However, a strain of the nematode, which has been found in a limited area, is able to break the resistance of this rootstock.

While 'Spreading Decline'-infected orchards are not "cured", management practices result in economic production provided that trees are never allowed to become stressed.

CERTIFICATION PROGRAMMES ENABLING EXPORT OF FLORIDA ORNAMENTALS AND OTHER PLANTS TO NATIONAL AND INTERNATIONAL MARKETS

These certification programmes concern i) two Florida regulated nematode citrus pests, the burrowing nematode and the coffee lesion nematode, because these two nematodes infect many ornamentals, and ii) other Florida nematode plant pests, which are not regulated in Florida, but are regulated in other states and countries.

Certification programmes for the burrowing and coffee lesion nematodes on plants for exportation

Because of the regulatory and management practices in Florida, the burrowing nematode is no longer a threat to the citrus industry in this state. However, the damage caused to citrus by the burrowing nematode prompted quarantine actions in other citrus producing states and countries in order to protect their citrus industries from both races of the burrowing nematode. Unfortunately, this ban has a serious economic impact on the ornamental industry of Florida and also on other states and countries where the banana race is present. *Radopholus similis* is regulated by four states besides Florida (Arizona, California, Louisiana and Texas) and > 50 countries including Bermuda, Chile, the European Union, Japan, Mexico and Switzerland.

Both races of the burrowing nematode infect many plants including:

– aroids (anthuriums, pothos, epipremnums etc.), bamboo, ginger, *Musa* spp. and palms (parlor palms).

The coffee lesion nematode, *P. coffeae*, is regulated by only a few countries (mainly in South America) and by many counties in California. The host range of this nematode among ornamentals includes aroids such as *Aglaonema* sp., *Ficus* and Musaceae species.

Because the burrowing and coffee lesion nematodes have so many hosts, especially foliage ornamentals such as the aroids and Musaceae species, a certification programme has been established in Florida nurseries to prevent the movement of burrowing and coffee lesion nematodes to states and countries that prohibit the introduction of these pests. This certification programme for ornamentals and other plants to be exported is similar to those described for citrus nurseries. The implementation of strict sanitation practices is the most important aspect of this certification programme. Educational programmes promoting the adoption of good sanitation practices in nurseries were initiated in Florida in 1961 (Esser, 1996). These sanitation guidelines require that ornamental nurseries must be established on land free from the burrowing and coffee lesion nematodes. Clean plants should be grown in artificial soil mixes free from the nematodes and in clean containers placed on disinfected and elevated benches or slabs to avoid the direct contact of the containers with the ground or nematode-contaminated run-off water. Plants should be kept under strict sanitation practices, i.e. using clean tools, clean watering devices and clean hands. Plants are sampled periodically for nematodes by FDACS inspectors and are only certified for export if no burrowing nematodes are found. Certification programmes provided by the state of Florida enable Florida growers to ship plants to national and international markets.

Certification programmes for the reniform nematode and other plant nematodes indigenous or established in Florida

These certification programmes concern Florida plant nematodes that are not regulated in Florida.

The reniform nematode: *Rotylenchulus reniformis* Linford *et* Oliveira, 1940 (Fig. 8) is a quarantine pest having serious economic impact primarily on the ornamental industry of Florida. This species has a sedentary, semi-endoparasitic habit. Vermiform mobile adult females initiate root infection by penetrating the host root with the anterior portion of the body. The nematode establishes a permanent feeding site in the root stele and becomes sedentary and immobile. The posterior portion of the body enlarges with maturation. Eggs are deposited in a gelatinous matrix. Males and juveniles are not parasitic.

The life cycle of *R. reniformis* is completed in 17-23 days at 25 °C. The period from root penetration to egg deposition is 41, 13, 7 or 7 days at 15 (59), 20 (68), 25 (77) or 34 (93) °C (°F). Vermiform stages retain the cuticles of earlier stages, which enable the nematode to tolerate adverse soil conditions. Life stages with re-

tained cuticles persist in the soil in the absence of a host for 16-27 months.

The reniform nematode is distributed in tropical countries, including those of North, Central and South America and the Caribbean. It is widely distributed in the southeastern part of the United States, where it is an economic pest of cotton. The nematode has not been found in Arizona, California or New Mexico.

Reniform nematode infection and reproduction is favored by loamy soils and it occurs throughout southern and northern portions of Florida.

Within the United States, Arizona, California and New Mexico impose quarantine restrictions on plant shipments contaminated or infected by *R. reniformis* to protect their cotton industries from this pest. Outside the United States, this nematode is prohibited by a few countries which include Argentina, Chile, Korea and South Africa (see the website http://excerpt.ceris.purdue.edu/ for more information).

Rotylenchulus reniformis is known to reproduce on 39 ornamentals including aloe, dracaena, schefflera, sansevieria and zigocactus species. The nematode is a serious problem in palm nurseries of south Florida, because the ornamental palm trade from Florida to Arizona and California is disrupted by the frequent rejection of palm shipments found infected or contaminated with this pest.

Host tests conducted by the Nematologists at the Division of Plant Industry in the1990s showed that the majority of ornamental palms are not hosts of the reniform nematode. Nematode infection and reproduction was observed only on *Acoelorrhaphe wrightii* and *Washingtonia robusta* [see Robinson *et al.* (1997) for more information on the biology and host range of the reniform nematode].

The reniform nematode infects and reproduces on many weeds, some of which are: artillery plant, malanga, morning glory, purselane, spiny amaranth and wild taro. These are a source of contamination for non-host palms and ornamentals growing in the presence of these weeds. Reniform nematode management in nurseries is difficult because of the long nematode survival time and a lack of nematicides registered for ornamental nurseries. The management is complicated by the ability of *R. reniformis* to reproduce on many weeds and ornamentals, which leads to contamination problems in nurseries with a record of *R. reniformis* infestations.

The nursery certification programme established for burrowing nematode certification is very effective for the production of reniform nematode-free plants. Thus, the basic principles of nursery sanitation for the exclusion of the burrowing nematodes are valid for the exclusion of the reniform nematode and other nematodes. Since weeds are a major source of contamination, effective weed control must be implemented. This is especially true in nurseries with a record of this nematode.

This nursery certification programme is effective also for excluding other Florida nematode plant pests targeted by external phytosanitary measures and listed below. These nematodes include the awl nematode (*Dolichodorus heterocephalus* Cobb, 1914), dagger nematode (*Xiphinema vulgare* Tarjan, 1964), rice root nematode [*Hirschmanniella oryzae* (van Breda de Haan, 1902) Luc *et* Goodey, 1964], rice white-tip nematode (*Aphelenchoides besseyi* Christie, 1942) and sting nematode (*Belonolaimus* spp.), which are regulated by California. It is worthwhile to mention that, in addition to phytosanitary practices, Florida nurseries must comply with other requirements in order to ship plants to California, such as a compliance agreement with the state. The compliance agreement includes a master permit which lists all the procedures and requirements the grower must follow under the supervision of FDACS inspectors in order to be eligible to export plants to the California market. Arizona regulates the burrowing nematode and reniform nematode. However, it also may reject nematodes prohibited by California to protect common agricultural interests with California. Texas and Louisiana regulate only the burrowing nematode.

Common nematode pests occurring in Florida and prohibited by major trade partner countries are listed below. Bermuda prohibits the burrowing nematode and cyst forming nematodes. Canada regulates the soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952). The European Union, Japan and Mexico regulate the burrowing nematode. The European Union also prohibits the pinewood nematode [*Bursaphelenchus xylophilus* (Steiner *et* Buhrer, 1934) Nickle, 1970], rice white-tip nematode and the dagger nematodes of the *Xiphinema americanum* group. The pinewood nematode does not infect ornamentals, but it can be present on pinewood by-products used as a component of soil mixes. Mexico also prohibits foliar nematodes (*Aphelenchoides* spp.), lesion nematodes (*Pratylenchus* spp.) and root-knot nematodes (*Meloidogyne* spp.). Switzerland prohibits the burrowing nematode, reniform nematode and root-knot nematodes (*Meloidogyne* spp.). Detailed quarantine requirements are usually specified in the import permits of plant shipments that are issued by other countries not mentioned above.

COST-BENEFITS OF QUARANTINE AND CERTIFICATION PROGRAMMES AND REGULATORY RESEARCH IN FLORIDA

Cost-benefits for Florida citrus industry of quarantine and certification programmes for citrus nematode pests

Conservative estimates made in 1958 indicated that, without the implementation of the phytosanitary programmes, burrowing nematodes would have spread to an additional 18,000 hectares in Central Florida citrus growing areas in a period of 10 years. The cumulative benefits for Florida citrus growers during a 35-year-period, by avoiding the spread of the disease in these 18,000 hectares, are estimated to be $1.4 billion. The

actual cost of the implementation of the regulatory programmes for this period was $ 100 million. Thus the cost of the regulatory programmes was about 7% of the benefits (Dwinell and Lehman, 2004).

An additional benefit of the citrus nursery certification programme was the reduction of the percentage of the citrus area which would have become infested with citrus nematode pests other than *R. similis.* Studies conducted by Lehman (2004) indicated that the annual benefit, in 2000, to Florida citrus growers from excluding *T. semipenetrans* was $ 33 million. This value is based on an estimated average crop loss induced by the nematode of 5% in the Florida citrus growing areas where susceptible citrus rootstocks were planted. In 2000, the total annual benefit to Florida citrus growers by excluding both *T. semipenetrans* and *R. similis* was estimated to be $ 50 million. These estimates do not include the additional benefit received by the exclusion of *P. coffeae* (Lehman, 2004).

Benefits to Florida ornamental industry and California plant industry of nematode certification programmes for nematode plant pests indigenous or established in Florida

Florida ornamental growers face many operative restrictions due to the sanitation practices that they must implement to meet the quality standards required by the nematode certification programmes. However, the cost and burden associated with the implementation of these programmes are compensated by the increased availability of national and international markets for their certified plants. The benefits realized by these certification programmes are remarkable considering that the value of the Florida ornamental industry expressed as cash receipts was about $ 1 billion in 2000.

California growers receive considerable benefit by preventing the introduction of many Florida nematode plant pests that are not present in California. More information concerning these benefits to California growers was provided by Lehman (2004). Estimates made by Dwinell and Lehman (2004) indicated that, in 1995, the benefit to California cotton growers of excluding the reniform nematodes alone was $8.5 million.

Benefits of regulatory research

As mentioned above, pasturelands are often converted to commercial citrus nursery and pit sites. These potential sites are often inhabited by *Tylenchulus* spp. populations formerly thought to be 'wild' strains of the citrus nematode. These 'wild' strains were confused with the regulated citrus parasitic species, *T. semipenetrans,* because of their morphological similarity. A regulatory research project funded by the citrus growers, conducted in 1985-1988, resulted in the discovery of morphological differences between the 'wild' strains of the citrus nematode and *T. semipenetrans*. Two new species of *Tylenchulus* (*T. graminis* and *T. palustris*) that do not infect citrus were described (Inserra *et al.*, 1988). The result of this research was very beneficial to the citrus and soil pit industries and made available potential citrus nursery and pit sites that had previously failed the certification process due to the presence of these nematodes. During 1977-1986, before the separation of the two new *Tylenchulus* species from *T. semipenetrans*, one of every 13 potential citrus nursery and pit sites failed to meet the certification requirement because of the presence of these new *Tylenchulus* species. After their description, no certification failures occurred in areas where they were present. This taxonomic research was particularly useful to the citrus industry because the resulting regulatory changes were implemented during a period of great demand for citrus seedlings by the industry due to the freezes that occurred at the end of the 1980s and beginning of the1990s. These freezes affected many citrus growing areas in the northern portion of the 'Ridge' (Lehman, 2004; Lehman *et al.* 1996) and induced the expansion of the citrus industry in new lands located in the flatwood areas of the Florida peninsula south of the 'Ridge'.

Another regulatory study that benefited the citrus industry was conducted in 1994-1999, resulting in the separation of a lesion nematode occurring in Florida pastures and citrus nursery and pit sites from the regulated coffee lesion nematode, *P. coffeae*. The results of this research indicated that this lesion nematode is a new unnamed species morphologically similar to *P. coffeae* and *P. loosi* Loof, 1960 (Inserra *et al.*, 1996). This new lesion nematode species is not a citrus parasite and infects mainly grasses (*Panicum hemitomon* and *Paspalum notatum*) that commonly occur in pastures potentially useful for citrus nursery sites. The findings of this regulatory and taxonomic research resulted in the availability of more potential citrus nursery and pit sites that had previously failed the certification process due to the presence of this lesion nematode.

SELECTED NEMATODE PLANT PESTS PROHIBITED BY FLORIDA

Many exotic nematodes pose a potential menace to Florida agriculture. They include potato cyst nematodes: *Globodera pallida* (Stone, 1973) Behrens, 1975 and *G. rostochiensis,* red ring nematode [*Bursaphelenchus* (= *Rhadinaphelenchus*) *cocophilus* (Cobb, 1919) Baujard, 1989], stem nematode [*Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936] potato rot nematode (*D. destructor* Thorne, 1945) and root-knot nematodes infecting citrus in the Far East. Section 5B-3.0038 of Chapter 5B-3 of Florida Administrative Code contains a complete list of plant parasitic nematodes that are prohibited by the state of Florida. This list can be accessed from the Division of Plant Industry (DPI) website http://agweb.doacs.state.fl.us under Regulations-Rules.

The state of Florida does not have quarantine inspectors at Florida ports of entry and relies on the inspection services provided by USDA-APHIS officials for the

protection of Florida agriculture from the introduction of exotic nematode pests. However, Florida quarantine inspectors may intervene and enforce quarantine actions if plant shipments released by APHIS officials contain plant nematode pests regulated in Florida. Periodic surveys of field and ornamental crops, conducted by Florida nematologists and inspectors, for nematodes continue to be an effective method to detect exotic nematode pests. Unfortunately, introduced nematodes that are detected during the field surveys are already established. Typically, it takes almost five years from the time a nematode plant pest is first accidentally introduced into a new area until it is actually detected on its hosts there.

In recent years, a Cooperative Agricultural Pest Survey programme (CAPS) has been conducted by the FDACS at the state level. This programme is assisting the Homeland Security initiative in protecting the food production and natural resources of the United States from exotic pests and bioterrorism. For example, the CAPS programme has been very useful in defining the geographical distribution of the root-knot nematode, *Meloidogyne mayaguensis* Rammah *et* Hirschmann, 1988, which is a pest new to Florida (Brito *et al*., 2004). This programme will be very useful in detecting other plant nematode species new to Florida.

CONCLUDING REMARKS

For almost 40 years, quarantine and certifications programmes have protected the Florida citrus industry from the spread of nematode pests. These programmes have been an effective means of nematode pest management on citrus. They are safe for the environment because their implementation does not require the use of chemicals. They are also financially remunerative for the growers. The implementation of the Citrus Nursery Certification Programme probably will prevent the spread of new exotic nematode pests of citrus if they are accidentally introduced into Florida.

A nematode certification programme for ornamentals, based on the adoption of strict sanitation practices, remains the best option for the Florida ornamental industry for the production of certified plants for export to national and international markets. The high cost of this programme is a limiting factor that can affect the competitiveness of nurseries producing low-value plants. It is difficult to make long term predictions about the number of nurseries able to implement this nematode certification programme in the future. Many nurseries located in south Florida are very competitive in the national and international markets because the climatic conditions of south Florida are favorable for the fast growth of ornamental plants, especially palms. These nurseries can afford the cost of such a programme. However, the future of these nurseries in south Florida is uncertain because of the pressure that they receive from residential developers who are looking for new land for housing. The protection of Florida's agriculture and environment from exotic nematode pests will remain a difficult challenge in the future because of the intensification of international trade and tourism. The inspections at the ports of entry and quarantine measures implemented by USDA-APHIS in excluding exotic nematode pests from Florida have been effective, but should be complemented by a more intensive cooperation between federal and state nematologists and inspectors.

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