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Influence of the Sting Nematode, *Belonolaimus longicaudatus*, on Young Citrus Trees

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Abstract: The sting nematode, *Belonolaimus longicaudatus*, was associated with poor growth of citrus in a central Florida nursery. Foliage of trees was sparse and chlorotic. Affected rootstocks included Changsha and Cleopatra mandarin orange; Flying Dragon, Rubidoux, and Jacobsen trifoliate orange; Macrophylla and Milam lemon; Palestine sweet lime; sour orange; and the hybrids—Carrizo, Morton, and Rusk citrange and Swingle citrumelo. Root symptoms included apical swelling, development of swollen terminals containing 3–5 apical meristems and hyperplastic tissue, coarse roots, and a reduction in the number of fibrous roots. Population densities as high as 392 sting nematodes per liter soil were detected, with 80% of the population occurring in the top 30 cm of soil; however, nematodes were detected to 107 cm deep. Although an ectoparasite, the nematode was closely associated with citrus root systems and was transported with bare root nursery stock. Disinfestation was accomplished by hot water treatment (49 C for 5 minutes).

Key words: rootstocks, histopathology, population densities, vertical distribution, eradication.

The sting nematode (SN), *Belonolaimus longicaudatus* Rau, was recently associated with severe damage to citrus trees growing in a commercial nursery and in an experiment with 6-year-old citrus rootstocks in central Florida. SN has been associated with citrus plantings (1,4–6,8–10,13,14) and identified as a pathogen of grapefruit seedlings in the greenhouse (12). Growth of young Valencia orange trees was improved when SN-infested soils were fumigated before planting (2,3). Information essential to the evaluation of SN in commercial nurseries as a potential threat to the citrus industry is lacking. It was not known if SN could be dispersed with bare root nursery stock. Furthermore, SN population densities associated with damage to young tree root systems under nursery conditions and the effect of SN on different commercial rootstocks were unknown. No method for eradication of SN from citrus nursery trees had been developed, nor had the vertical distribution of SN been determined in relation to citrus root depth in nurseries. Symptoms and histopathology resulting from natural SN parasitism of citrus roots under field conditions appeared to differ from those generated experimentally under greenhouse conditions (12).

The objective of this research was to increase our understanding of the relationship of SN and citrus and to determine if the occurrence of SN in citrus nurseries posed a threat to the citrus industry.

Materials and Methods

Citrus rootstocks adversely affected by SN were identified by observing poor scion growth and by sampling root systems of young trees (1–8 years old) growing in the field in central Florida. Roots and adjacent soil were collected from two geographically distinct sites from symptomatic and asymptomatic trees growing on the following rootstocks: Changsha and Cleopatra mandarin oranges (*C. reticulata* Blanco); Flying Dragon, and Rubidoux, and Jacobsen trifoliate orange (*Poncirus trifoliata* L. Raf.); Alemow (*C. macrophylla* Wester) and Milam lemon (*C. limon* Burm. f. cv. Milam); Palestine sweet lime (*C. limettioides* Tan.); sour orange (*C. aurantium* L.); and hybrids—Carrizo, Morton, and Rusk citranges (*Citrus sinensis* (L.) Osb. × *P. trifoliata*), Swingle citrumelo (*C. sinensis* × *P. trifoliata*), Rubidoux × Koethen (*P. trifoliata* × *C. sinensis*), and Rangpur (*C. limonia*) × Troyer citrange.

Nematodes were extracted from soil by Cobb's sieving technique using 500-μm-pore sieves to remove debris and 90-μm-pore sieves to collect nematodes. Sediment and nematodes collected on the 90-μm-pore

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sieves were concentrated by backwashing and subsequently extracted by Baermann funnel. Samples were examined after 24 hours for SN. Root systems were photographed, and selected roots were examined histologically.

A central Florida nursery planting of 2-year-old Hamlin sweet orange on Carrizo citrange exhibiting symptoms of SN injury was selected for determining soil population densities of SN associated with visible symptoms. Samples were taken at intervals starting in asymptomatic areas and extending through the symptomatic areas. Each sample consisted of a single core (20 cm d × 30 cm deep) of roots and soil taken 20 cm from each nursery tree. Subsequently, nursery trees adjacent to sampled trees were carefully uprooted, and root systems were rinsed free of soil and photographed; nematodes were extracted from soil samples as described earlier. Data were expressed as nematodes per liter soil, and population densities were compared with the amount of damage exhibited by root systems.

The anatomy of parasitized and nonparasitized fibrous roots was compared. Roots were fixed in a solution of 10% formalin in 95% ethanol, dehydrated with a t-butyl alcohol series, and infiltrated with paraffin. Sections (12 μm) were stained with safranin and fast green and examined by light microscopy (LM).

The vertical distribution of SN and roots of Carrizo citrange in the citrus nursery was determined by removing seven successive vertical 15-cm-deep cores within a row of young citrus trees to 105 cm deep with a 7.6-cm-d auger. Samples were taken from two sites in the nursery with four replicates per site. Samples were processed as described earlier, and data were expressed as nematodes per liter soil. Roots from each sample were rinsed free of soil and weighed.

To evaluate methods of disinfesting root systems of SN, 30 nursery trees and surrounding soil (root ball) were carefully removed from the field. Root systems from 10 trees were shaken vigorously and dipped twice in a container of water at 25 C or rinsed with tap water (25 C) at a flow rate of 17 liters per minute until root systems appeared clean. SN removed by these methods from the root systems were recovered on a 90-μm-pore sieve and counted. SN remaining on the root system after treatment were recovered by incubating all fibrous roots from each tree in jars for 3 days at 25 ± 1 C. To determine their thermal inactivation point, SN were subjected to temperatures of 27, 38, 49, and 60 C for 2.5, 5.0, 7.5, 10.0, and 12.5 minutes in a water bath. Nematodes for this experiment were extracted from field soil as described earlier. The nematode suspension (200 SN/ml) was divided equally among 100 conical 15-ml centrifuge tubes, and five tubes were subjected to each temperature-×-time treatment. Surviving nematodes were recovered after 24 hours from Baermann funnels. Controls were handled at 23 C.

To determine if SN transported with bare root nursery trees were capable of establishing new populations, and to determine if SN could be eradicated from bare root trees, the following treatments were applied to 2-year-old Hamlin on Carrizo citrange nursery trees grown in SN-infested soil. 1) Root systems were dipped twice in water at 23 C, and trees were planted into steam-sterilized soil. 2) Root systems were rinsed with running water until they appeared clean, and trees were planted into steam-sterilized soil. 3) Trees were shaken manually until soil particles or debris no longer fell from root systems and were then planted in steam-sterilized soil. 4) Root systems were immersed in water at 49 C for 5 minutes, and trees were planted into steam-sterilized soil. 5) Trees were planted directly into steam-sterilized soil. 6) Trees were planted directly into SN-infested soil. 7) Trees grown in SN-free soil were planted in steam-sterilized soil. Treatments were replicated 10 times. All trees were grown in a greenhouse in 20.4-cm-d clay pots for 3 months. At harvest, trees were removed from pots and nematodes were extracted from all the soil in each pot as described earlier and from all fibrous roots of each tree by jar incubation (15) for 3 days at 26 C. Data were expressed as nematodes per liter soil. Root damage in relation to treatment was also assessed.

Results

Under central Florida conditions, citrus nursery trees growing in SN-infested soil

FIG. 1. Damage to Hamlin orange on Carrizo citrange rootstock caused by *Belonolaimus longicaudatus*. A) Infested area. B) Noninfested area of same field.

were stunted and possessed small stems and chlorotic leaves (Fig. 1). All rootstocks parasitized by SN had coarse root systems with few lateral roots and swollen root terminals with multiple apices (Fig. 2). SN were always associated with these symptoms, and symptoms were similar in all parasitized rootstocks.

Population densities of SN in a planting of 2-year-old Hamlin on Carrizo citrange rootstock ranged from no nematodes in areas where trees were asymptomatic to 415 SN per liter soil in areas where severe symptoms occurred. However, trees showed damage in areas with nematode densities as low as 40 SN per liter soil.

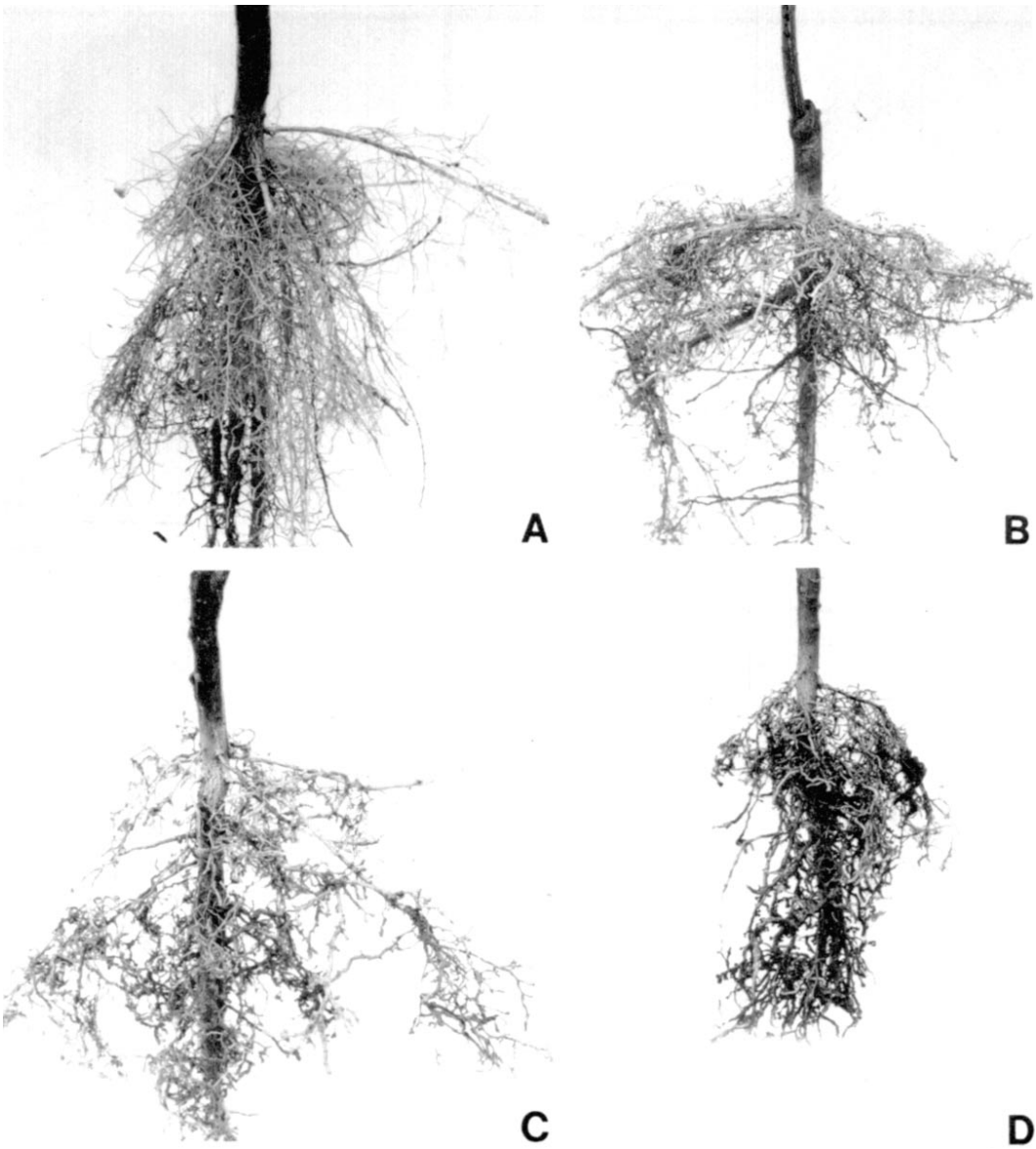

FIG. 2. Population density of *Belonolaimus longicaudatus* (SN) in relation to damage of naturally infected Carrizo citrange root systems. A) No SN/liter soil. B) 58.5 SN/liter soil. C) 100.0 SN/liter soil. D) 364.5 SN/liter soil.

Cortical cells within coarse SN-parasitized roots appeared enlarged (Fig. 3). Root terminals typically contained 3–5 damaged apical meristems. Disorganized hyperplastic tissue occurred between these meristems. Small cavities contiguous with the root surface and cavities within the anterior end of apical meristems were lined by cells which were devoid of cytoplasm and had thick, differentially stained walls. Some cavities contained remnants of cells but did not appear to be specialized feeding sites. Xylem elements extended well into the root tip.

The vertical soil distribution of SN paralleled the nursery tree root system. The upper 45 cm of soil contained 93% of the SN population and 100% of nursery tree root systems (Table 1). Nematodes were detected to 105 cm deep.

Attempts to disinfest root systems of SN by shaking, dipping, or rinsing roots were

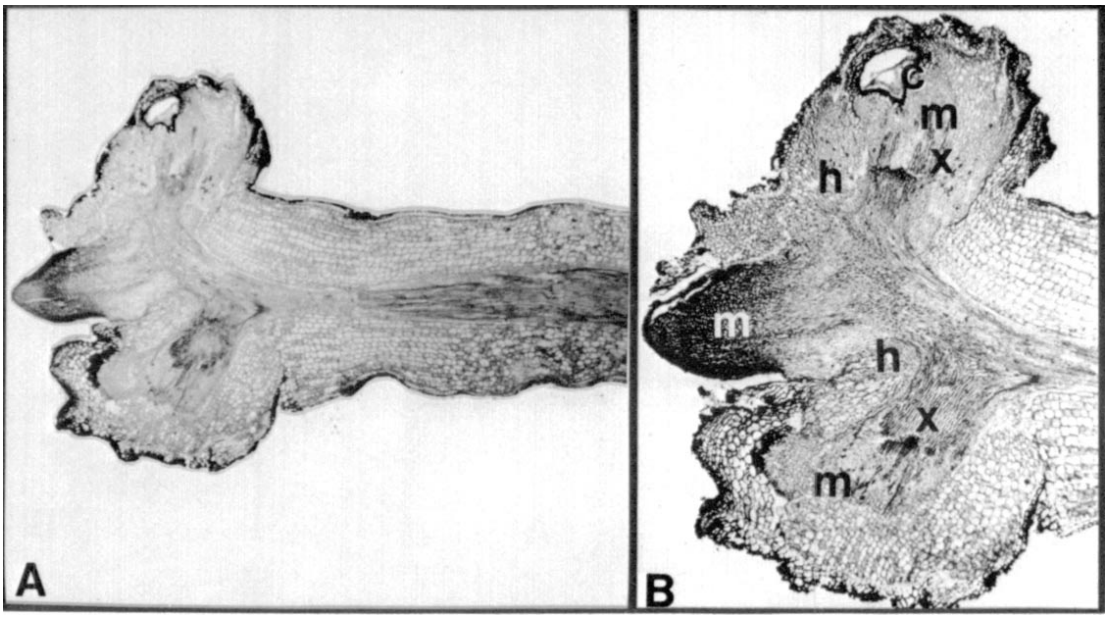

FIG. 3. Histopathology of sour orange roots parasitized by *Belonolaimus longicaudatus*. A) Swollen terminal on coarse feeder root. B) Longisection of root with three meristematic zones (m), cavity believed to result from feeding (c), hyperplastic tissues (h), and xylem elements in root tip zone (x).

unsuccessful (Table 2). However, exposure in vitro of SN to 49 C for 2.5 minutes inhibited motility (Table 3). The only treatment that eliminated SN from root systems was the hot water treatment of 49 C for 5 minutes (Table 4). Although an ectoparasite, SN appears to be closely associated with citrus roots and may be spread even on bare-rooted trees from infested nurseries.

DISCUSSION

The occurrence of SN in commercial citrus nurseries poses a significant threat to the citrus industry because SN may be dispersed during transplanting and can adversely affect young citrus tree growth at relatively low densities (40 SN per liter soil). The intimate association of SN with citrus roots was emphasized by the failure of rinsing or shaking to eliminate the pest from root systems. SN can infest even bare root trees and thus be spread to groves by planting infested nursery trees.

Management of SN in citrus nurseries or in groves will require the integration of sound cultural practices that prevent nematode introduction into noninfested areas with the use of nematicides. At transplanting, nursery tree root systems are often cut 30 cm below the soil surface, the trees removed, and most of the soil left behind. The bare root systems are then dipped in water in the field to remove excess soil, and water from the root systems is allowed to drain into the field as the trees are carried by truck or tractor to a processing area. This practice leads to rapid dispersal of SN within the nursery.

Ideally, the use of SN-infested sites for citrus nurseries should be avoided. The Florida Department of Agriculture and Consumer Services, Division of Plant Industry, conducts a statewide nursery site certification program which has effectively limited the spread of several economically important endoparasitic nematode pathogens of citrus. The findings presented here suggest that nursery site analysis should include evaluation for biotypes of SN pathogenic to citrus. Should a site become infested, the bare root trees can be effectively disinfested of SN by exposing the root systems to water at 49 C for 5 minutes. This treatment does not adversely affect subsequent plant growth and should interface well with current nursery practices. In addition, before replanting, soil should be fumigated or treated with a nematicide.

The relatively low SN population den-

TABLE 1. Vertical distribution of *Belonolaimus longicaudatus* in a central Florida citrus nursery.

Depth (cm)	Nematodes/liter soil*	Root weight (g/liter soil)
0–15	147.5	3.7
15–30	107.9	2.9
30–45	41.5	0.02
45–60	13.3	0.0
60–75	4.7	0.0
75–90	4.6	0.0
90–105	5.1	0.0

* Nematode population densities were negatively correlated with depth ($r = 0.88$; $P = \leq 0.01$) but positively correlated with root weight ($r = 0.97$; $P = \leq 0.01$).

TABLE 3. Survival of *Belonolaimus longicaudatus* following exposure to different temperatures in vitro.

Temperature (C)	Time (minutes)					
	0	2.5	5.0	7.5	10.0	12.5
23	184					
27		147	205	157	220	202
38		187	157	161	132	166
49		0	0	0	0	0
60		0	0	0	0	0

Mean number of nematodes per test vial which migrated through a Baermann funnel following temperature-×-time exposure. (Based on five replicate samples.)

sities, SN's ectoparasitic feeding habit (12), the apparent absence of permanent feeding sites, similar root damage, and poor tree growth among popular (widely planted) and experimental citrus rootstocks suggest that identification of rutaceous germplasm resistant to sting nematodes will be difficult. *Poncirus trifoliata,* the only germplasm source of citrus nematode (*Tylenchulus semipenetrans* Cobb) resistance currently incorporated into commercially acceptable rootstocks, was damaged by sting nematodes. However, a great deal of rutaceous germplasm has not been evaluated for SN resistance, and some may be useful to the development of future SN-resistant rootstocks.

The gross symptoms in citrus roots attributed to SN damage were similar to those associated with copper toxicity (11). Analyses for copper in soils from SN-infested and noninfested soils (asymptomatic) within the same test field, however, contained comparable concentrations of copper (6.5–12.0 ppm) which were not considered excessive (D. T. Kaplan and H. K. Wutscher, unpubl.).

Sting nematode damage to citrus roots recently was described as irregular swelling of the root epidermis which sloughed easily (7). The present histological examination of damaged roots indicated that gross changes in root anatomy did not involve changes in epidermal cells. In addition, sloughing of cortical and epidermal tissues could not be conclusively attributed to nematodes alone because fungi, such as *Phytophthora parasitica* Dast. which is often associated with citrus, can cause root tissue sloughing and was isolated from symptom-

TABLE 2. Persistence of *Belonolaimus longicaudatus* on roots of Carrizo citrange nursery tree root systems.

Treatment*	Nematodes removed by treatment	Total nematodes per root system	Percentage of original population remaining on roots
Shake	67.0	124.5	46 a†
Dip	112.1	136.7	18 b
Rinse	115.2	127.8	13 b

* Treatments described in text.

† Column means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$). (Based on 10 replicate samples.)

TABLE 4. *Belonolaimus longicaudatus* population densities on nursery tree Carrizo citrange root systems in the greenhouse 3 months after treatment.

Treatment*	Nematodes/liter soil
None	54.0 a†
Inoculated	17.0 b
Dip	3.8 c
Shake	3.5 c
Rinse	3.0 c
Heat	0.0 d
Healthy	0.0 d

* None = transplanted in infested field soil. Inoculated = nematodes (280/liter soil) added to soil around base of healthy nursery tree. Dip = tree removed from infested site, roots dipped twice in water at 25 C, planted in noninfested soil. Shake = tree removed from infested site, root system shaken free of loose soil, planted in noninfested soil. Rinse = tree removed from infested site, roots rinsed with tap water (flow rate = 17 liters/minute), planted in noninfested soil. Heat = tree removed from infested site, excess soil removed, roots incubated in water at 49 C for 5 minutes, planted in noninfested soil. Healthy = tree removed from noninfested site, planted in noninfested soil.

† Column means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$). (Based on 10 replicate samples.)

atic roots. Such fungi may enter the roots directly or through wounds caused by SN feeding, colonize root tissue, and cause root destruction.

The histopathology of SN-damaged citrus roots from the field differs from that described previously (12) for roots grown in the greenhouse. Previous observations reflect the initial response of roots to SN feeding over a relatively short (3 months) period of time (12). Initial nematode feeding inhibits root elongation, and, with the exception of the actual feeding site, cells enlarge normally. Development of new meristems and disorganized hyperplastic tissues within the root terminal are responsible for the grossly enlarged root tips on plants damaged by SN in the field. Parasitized roots had 3–5 apical meristems. The generation of enlarged root terminals reflects the continued feeding of nematodes on apical meristems as they arise. Apical dominance appears to be lost. When removed from SN, normal roots emerged from the swollen terminals. Lesions contiguous with the root surface were observed in SN-damaged plants grown in both the field and greenhouse. Cavities within the apical meristems, which were prevalent in this study were pictured previously (12—Fig. 5) but were not mentioned. Occasionally large cells were found within these cavities, but their relationship to SN feeding requires further study.

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