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Spatial Patterns of *Belonolaimus spp.* Among and Within Citrus Orchards on Florida's Central Ridge[1]

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Abstract: A survey was initiated to determine the incidence of *Belonolaimus spp.* (sting nematodes) in citrus orchards in the central ridge region of Florida, following widespread damage by these nematodes to young trees replanted after freezing weather in 1989–90. Sting nematodes were detected in 50% of 210 samples and in 64% of 84 orchards surveyed. More orchards in Polk County were infested with sting nematodes (82%) than in counties to the north (36%) or south (48%). Principal component analysis of morphometric data separated six of seven sting nematode populations in northeastern Polk County from six populations in adjacent regions. Stylet:tail ratio for nematodes in northeastern Polk County tend to be >1.0 and were ≤1.0 for all other populations. Patchiness of nematodes within an orchard was associated with stunted trees (23% smaller), reduced root mass density (25% lower), and low fruit yield (57% reduction). Soil texture did not vary among trees of different size in the orchard, but soil water potential between irrigation events was highest beneath small trees with low root mass density. Results of the survey indicate that the incidence of sting nematodes in orchards on the central ridge is much higher than previously estimated and that sting nematodes can cause substantial damage in replanted orchards. Further research is needed to evaluate the significance of sting nematode population variability and its relationship to citrus crop loss in Florida.

Key words: *Belonolaimus longicaudatus*, citrus, crop loss assessment, ecology, nematode, nematode survey, soil moisture, spatial distribution, spatial pattern, sting nematode.

Sting nematodes, *Belonolaimus longicaudatus* Rau and *B. euthychilus* Rau, occur in light-textured soils throughout Florida's citrus production area (4). *Belonolaimus longicaudatus* was detected recently in 7% of 1,147 samples taken from citrus orchards in 35 counties (5), whereas *B. euthychilus* was detected in only one of those samples. *Belonolaimus longicaudatus* was highly pathogenic to citrus seedlings in pots (1,13) and to young trees in nurseries (8). Few studies have been conducted to determine how these nematodes may affect older trees growing in citrus orchards (2).

Belonolaimus longicaudatus feeds on tips of citrus fibrous roots causing cessation of growth and characteristic stubby-root symptoms (8,13). Growth of nematode-infected seedlings in greenhouse experiments can be reduced by 40% compared to noninfected controls (1), and stunting of field-grown trees in infested nurseries is similarly severe (8). Bistline et al. (2) found that young trees replanted in old-orchard sites infested by *B. longicaudatus* yielded twice as much fruit during the first 5 years of production when planted in fumigated, compared to nonfumigated, soil. Trees began to exhibit decline symptoms in the form of reduced root systems and thinning foliage as nematodes recolonized fumigated soil. Similar observations associating decline of mature trees with the presence of sting nematodes indicate a need for further study of the economic importance of this nematode in Florida's citrus (4).

Freezing weather in the winter of 1989–90 killed large numbers of citrus trees in central Florida. The central ridge region of this part of the state is characterized by deep-sandy soils conducive to population development of sting nematodes (9). As trees were replanted, an increasing number of soil and root samples submitted by growers for nematode assay exhibited symptoms of damage by sting nematodes. The majority of the samples were from a discrete area within northeastern Polk

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County. Observation of several orchards suggested that damage by the nematode was widespread in that area. A research program was initiated to assess crop loss on young trees due to the nematode and to evaluate methods to manage nematode population density. The research reported herein is a portion of those studies.

The objectives of this research were to (i) estimate the incidence of orchards on the central ridge infested by sting nematodes, (ii) examine patterns of tree quality and fruit production relating to nematode infestation levels within an orchard, and (iii) evaluate morphometric similarity of nematode populations from citrus for evidence of variability related to regional differences in problems caused by the nematode.

Materials and Methods

Incidence of sting nematodes among groves: Two hundred and ten samples were collected in 82 orchards along a 145-km-long span of the central ridge, between Clermont and Lake Placid, Florida. Samples were collected between June 1994 and October 1995. One hectare in each 4 ha of each orchard was sampled by systematically obtaining a single core of soil (110 cm^3 × 15 cm deep) beneath the canopies of 30 citrus trees. Soil cores were bulked and mixed and 500-cm^3 subsamples were processed by sieving (2-mm pore openings over 44-μm pore opening) and centrifugal flotation (7) to extract nematodes. A second subsample was processed if no sting nematodes were detected in the first. Orchards were resampled if no sting nematodes were detected in the original sample(s).

Soil texture was determined (14) for 10 random samples each from Lake, Polk, and Highland counties, representing the northern, central, southern regions of the survey, respectively. Rootstock (7 cultivars), scion (4 cultivars), tree age (2 to >30 years), method of weed management (mowing or disking), and nematicide use (positive or negative in the past 2 years) were obtained for each orchard in the survey. Qualitative variables were coded for statistical analysis.

Relationships between the incidence of sting nematode(s) and orchard characteristics were analyzed (Minitab, State College, PA) by linear correlation analysis, analysis of variance, and principal components analysis. Patterns in the incidence of sting nematode(s) on the central ridge were examined using *t*-tests of the binomial probabilities that the incidence in Polk County was different from those in Highlands, Lake, or Orange counties.

Patterns of sting nematodes within an orchard: Seventy-two trees in a 1.9-ha area infested by sting nematodes were selected in September 1993, based on tree height. The orchard, in northeastern Polk County, consisted of 3-year-old microirrigated Valencia orange trees on Carrizo citrange rootstock growing in sandy soil (98% sand, 0% silt, 2% clay). Soil samples comprised of 16 cores (2-cm diam. × 30 cm deep) were taken beneath 12 trees in each of six height categories ranging in 30-cm increments from 91 to 274 cm tall. Samples were processed as in the survey, except that fibrous root fragments were sieved (2-mm pore opening) from the soil and weighed. Soil texture was determined for the 10 plots with lowest and highest nematode population densities (excluding plots in which sting nematodes were not detected). Fruit yield was recorded on 28 February 1994. Soil water potential beneath the eight smallest (mean trunk diam. = 72 cm) and eight largest (mean trunk diam. = 122 cm) trees was monitored with tensiometers (Irrometer, Riverside, CA) between 5 to 12 December 1995.

Nematode numbers were transformed with $\log_e (x + 1)$ before analysis. All data were ranked by nematode population density and pooled by half log units (6) to investigate the relationships between nematode infestation levels and plant variables.

Population morphometrics: Twelve orchards and two fallow fields of primarily mixed grasses were sampled to recover female sting nematodes for morphometric analysis. Ten orchards were selected from the original survey and two were selected outside the central ridge (Manatee and Pasco counties). Nematodes were extracted as described previously. Females were mounted in water on slides beneath glass coverslips and heated over a flame. Lengths of the body, stylet, and tail were measured immediately using an ocular micrometer at ×400.

Principal component analysis, analysis of variance, and *t*-tests were used to analyze morphometric similarity of populations from different geographic areas. Allometry between nematode body length and lengths of stylets or tails was investigated using simple linear regression of the latter two variables on body length.

Results

Incidence of sting nematode(s) among groves: Sting nematodes were detected in 50% of the 210 samples and 64% of the citrus orchards in the survey (Fig. 1). Polk County had a higher ($P = 0.05$) incidence of sting nematodes than any other county with detections in 65% of 135 samples and in 83% of the orchards. Lake and Orange counties in the north had 27% of 33 samples and 36% of orchards positive for sting nematodes. Sting nematodes were detected in 29% of 42 samples and in 48% of the orchards in Highlands County, the southernmost part of the survey.

Average soil texture in the southern region of the survey was comprised of slightly more sand and less clay (99% sand, <1% silt, <1% clay) than in those in the central (96% sand, 1% silt, 3% clay) and northern (97% sand, 1% silt, 2% clay) regions ($P = 0.05$). Method of weed control was the only variable that was correlated with incidence of sting nematodes. Samples from orchards in which row middles were disked had a lower incidence of sting nematodes (27% positive, $n = 48$) than those from orchards in which row middles were mowed (58% positive, $n = 162$) ($P = 0.05$). Similarly, method of weed management was the only orchard variable useful

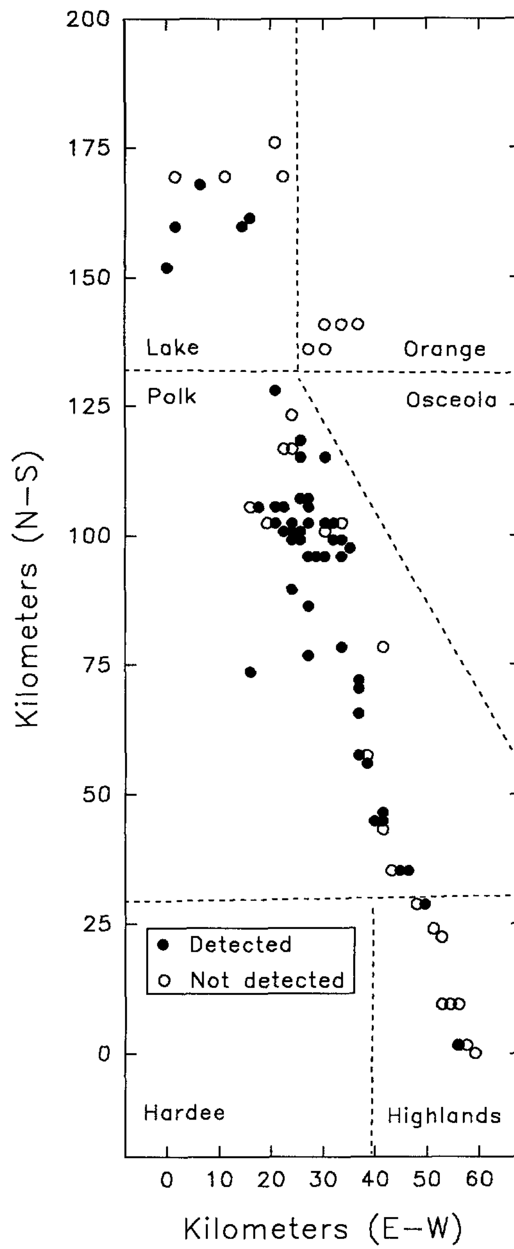

FIG. 1. Incidence of sting nematodes in the central ridge citrus production region of Florida. County lines are not drawn to scale.

to distinguish infested from noninfested orchards using principal components analysis (data not shown).

Patterns of sting nematode(s) within an orchard: Sting nematodes were aggregated in patches that were visually detectable based on the heights of trees (Fig. 2). The height

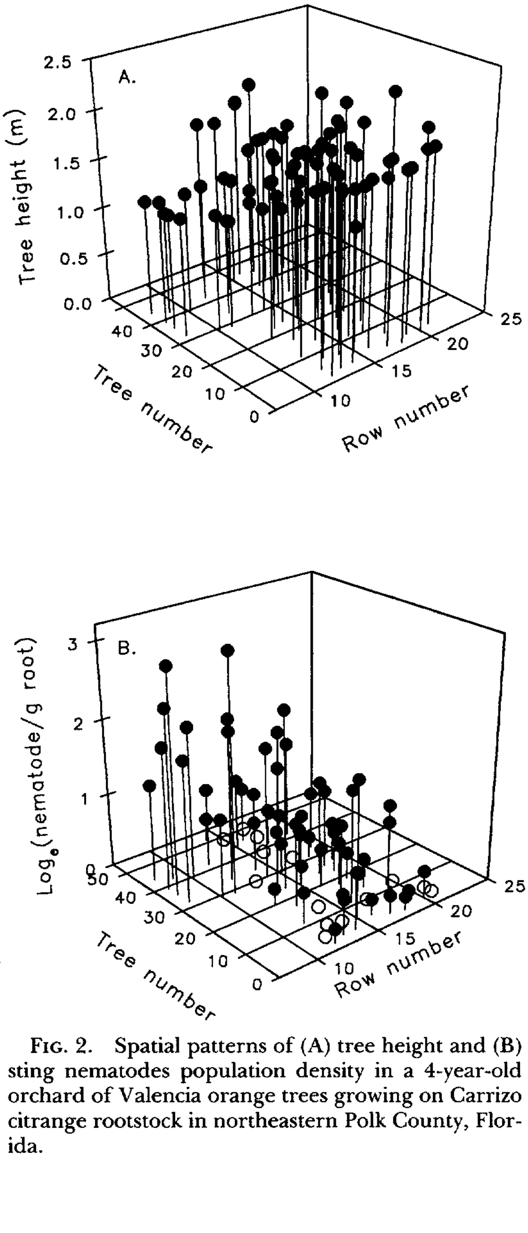

FIG. 2. Spatial patterns of (A) tree height and (B) sting nematodes population density in a 4-year-old orchard of Valencia orange trees growing on Carrizo citrange rootstock in northeastern Polk County, Florida.

of 3-year-old trees was inversely related to numbers of sting nematodes in the soil (Fig. 3A). This inverse relationship persisted at a significant level through October 1995 (data not shown). Average height of trees with the highest nematode population densities was 77% that of trees with non-detectable levels of sting nematodes. A similar, but more pronounced, relationship existed between fruit yield and nematode population density (Fig. 3B). The mean fruit weight from heavily infested trees was 43% of that from trees with nondetectable population levels of the nematode. Average root mass density was reduced by 25% on heavily infested compared to noninfested trees (Fig. 3C).

There were no differences in average soil texture (98% sand, 0% silt, 2% clay) between plots with high and low population densities of sting nematodes. However, different transpiration rates due to differences in root mass density resulted in water potentials substantially higher in soil beneath small compared to large trees (Fig. 4).

Population morphometrics: Most populations of sting nematodes from orchards in northeastern Polk County differed consistently from those in other parts of the state, whether orchards were located on (southern Polk, Lake, and Highlands counties) or off (Manatee and Pasco counties) the central ridge (Fig. 5). Stylet length tended to be greater than 132 μm in populations from northeastern Polk County and less for all other populations (Table 1). Tail measurements tended to be less than 133 μm for populations in northeastern Polk County and greater for all others. The stylet:tail ratios for all but one population from northeastern Polk County were greater than 1.0, whereas those of all other populations were significantly less than unity. Two populations of sting nematodes from fields of predominantly mixed grass species had stylet:tail ratios consistent with their geographic origins.

Body length was directly related to the length of the tail and stylet (Table 2). Nevertheless, there was no consistent difference in body length between populations with high or low stylet:tail ratios that could explain the differences in these characters. The slope of the linear relationship between body length and each of the other variables was identical for high- and low-ratio populations (Table 2). Only the intercept values of the regressions differed, suggesting fundamental differences in lengths of tails and stylets for nematodes of a given size among the two groups of nematodes. Although rootstock cultivar

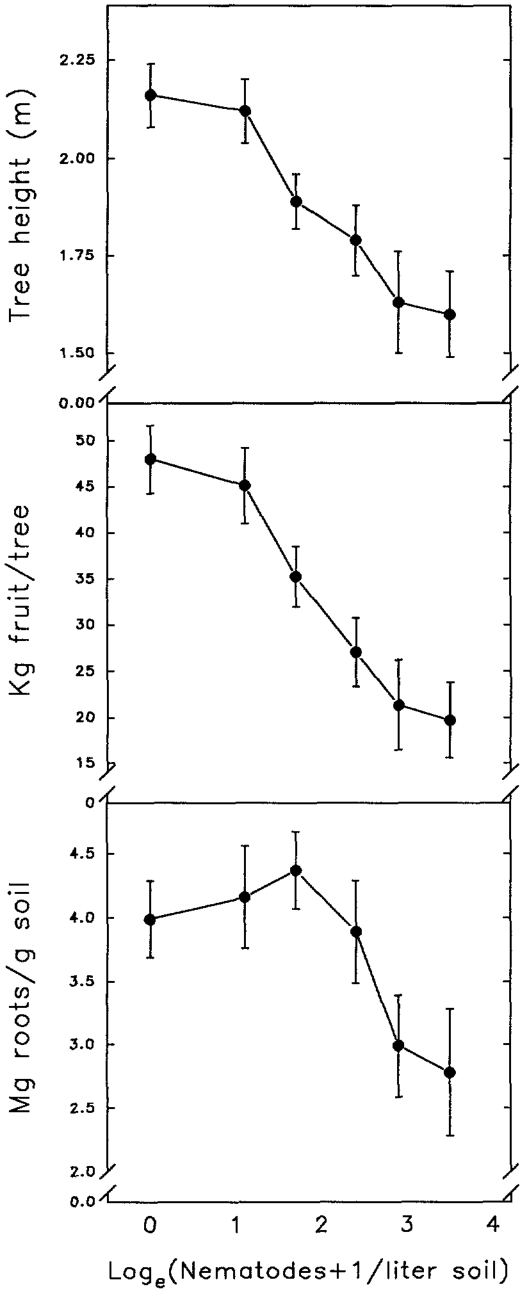

FIG. 3. Relationships between (A) tree height, (B) fruit yield, and (C) root mass density and population density of sting nematode(s) in a 4-year-old orchard of Valencia orange trees growing on Carrizo citrange rootstock in northeastern Polk County, Florida.

was not balanced between the different regions, nematode morphometrics on a given rootstock were related more closely to region than to the host (Table 1, Fig. 5).

DISCUSSION

Sting nematodes are more widely distributed on the central ridge of Florida than previously estimated. The 64% of orchards infested by these nematodes in this study is nearly an order of magnitude greater than an 8% statewide estimate from a recent survey (5). Greater sampling intensity and restricting this survey to regions with soil texture conducive to the nematode probably account for the discrepancy.

The reasons for a higher incidence of sting nematodes beneath citrus trees in Polk County than in the other counties are unknown. The positive correlation between nematode incidence and mowing for weed control is suggestive; however, the few disked orchards in the survey were concentrated in southern Highlands County, where few mowed orchards exist for comparison. During the past decade, mowing has largely replaced the use of disking and herbicides to manage weeds in row middles in parts of the central ridge region. The availability of food for these nematodes increased in mowed orchards because many grasses and other weeds are good hosts for sting nematodes (9). Freezing temperatures that killed large numbers

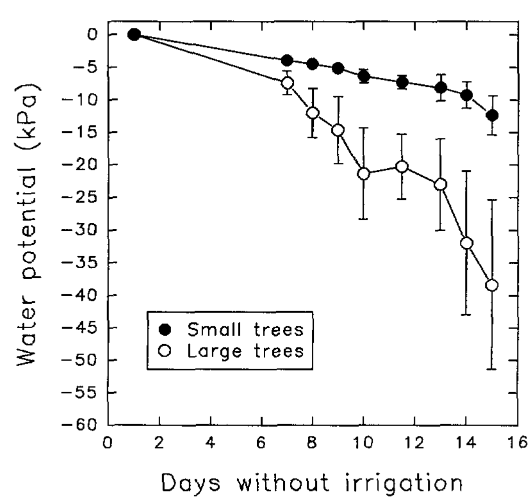

FIG. 4. Soil water potential in the root zone (10 cm deep) of small and large trees, in a 4-year-old orchard of Valencia orange trees growing on Carrizo citrange rootstock in northeastern Polk County, Florida.

TABLE 1. Morphometric (μm) comparison of 14 populations of sting nematodes from citrus orchards and fields of mixed grasses in central Florida.

N	Plant host	County	Body length	Stylet length	Tail length	Stylet:tail ratio
20	Carrizo citrange	Polk (NE)[a]	2,483 (43)[b]	133 (1)	132 (3)	1.02 (0.02)
23	Carrizo citrange	Polk (NE)	2,521 (32)	136 (1)	132 (3)	1.04 (0.02)
17	Swingle citrumelo	Polk (NE)	2,411 (46)	133 (1)	129 (3)	1.04 (0.01)
15	Swingle citrumelo	Polk (NE)	2,545 (52)	142 (2)	131 (4)	1.10 (0.04)
15	Swingle citrumelo/ Ridge pineapple	Polk (NE)	2,466 (41)	133 (1)	124 (3)	1.08 (0.03)
8	Milam lemon	Polk (SE)	2,592 (55)	127 (2)	146 (2)	0.86 (0.02)
13	Carrizo citrange	Polk (SE)	2,519 (36)	130 (2)	145 (4)	0.90 (0.02)
5	Carrizo citrange	Pasco	2,372 (55)	118 (2)	136 (2)	0.86 (0.02)
18	Swingle citrumelo	Polk (NE)	2,538 (52)	124 (2)	150 (3)	0.83 (0.01)
16	Sour orange/Rough lemon	Manatee	2,589 (54)	128 (1)	147 (3)	0.88 (0.02)
7	*Citrus spp.*	Lake	2,394 (60)	126 (1)	144 (4)	0.88 (0.03)
4	Cleopatra mandarin/ Milam lemon	Highlands	2,503 (90)	126 (1)	163 (5)	0.78 (0.02)
20	Grass	Polk (NE)	2,484 (34)	135 (1)	135 (3)	1.01 (0.02)
10	Grass	Hillsborough	2,557 (49)	121 (2)	156 (4)	0.79 (0.03)
		Pooled citrus populations				
N	Stylet:tail ratio type	County	Body length	Stylet length	Tail length	Ratio
90	High ratio	Polk (NE)	2,487 (19)	135 (1)	130 (1)	1.05 (0.01)
71	Low ratio	Many	2,526 (22)	126 (1)	148 (2)	0.86 (0.01)

[a] NE = northeast; SE = southeast.
[b] Mean and standard error.

of trees in all but the southern part of the central ridge resulted in widespread planting of young susceptible trees (8) into orchards likely to have high population densities of the nematode. The increase in damage by sting nematodes in these areas may be due in part to recent changes in weed management practices as well as widespread replanting.

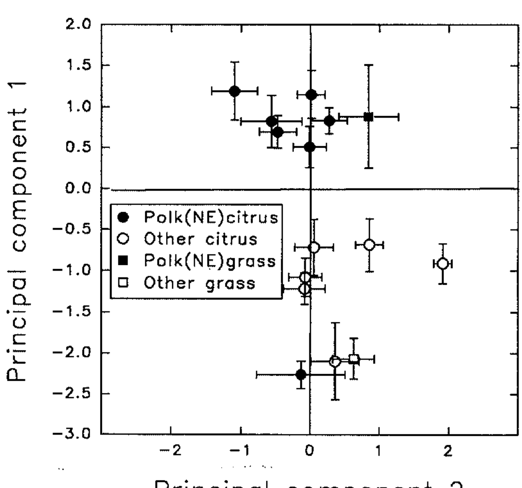

FIG. 5. Principal component analysis of morphometric characteristics of 15 populations of sting nematode(s) from citrus or mixed grasses in Florida. Nematode populations were from northeastern (NE) Polk County and from adjacent regions.

Patchiness of sting nematodes within an orchard corresponded to patches of small, low-yielding trees with reduced root systems. No other cause of tree decline was identified other than the obvious symptoms of sting nematode damage to the root systems. Many of the smallest trees in the orchard had few fibrous roots, and most existing roots were stunted by nematode feeding. While those observations are consistent with the possibility that the sting nematode is a major pathogen on these trees, it is unknown why stunted trees with few roots supported higher population levels of sting nematodes than large trees with abundant fibrous roots. An opposite relationship generally exists between tree quality (root availability) and nematode population levels in perennial crops (3,10). Soil moisture may be responsible for this anomaly. Some trees planted in orchards with high patchiness of sting nematode levels escape early damage by the nematode and develop root systems with higher

TABLE 2. Allometry between sting nematode body length and length of stylet or tail for populations with average stylet-to-tail ratios greater than or less than unity.

Type[a]	Intercept[b]	Slope[b]	r^2	p
		Stylet		
High ratio	93.3	0.017	0.18	0.0001
Low ratio	83.6	0.017	0.24	0.0001
		Tail		
High ratio	65.9	0.026	0.12	0.0010
Low ratio	82.0	0.026	0.10	0.0080

[a] Ninety and 71 female nematodes measured from five and seven populations of nematodes with ratios >1.0 and <1.0, respectively.
[b] Intercepts and slopes from simple linear regression of variable against body length.

mass density than occurs on heavily infected trees. Soil water potential at the end of irrigation cycles can become low in the upper soil profile, which contains the greatest root mass density. Differences in soil water potential beneath trees of different size may regulate sting nematode population densities when trees are young and help to conserve the patterns of sting nematodes that exist in fields when groves are planted. Higher soil moisture levels beneath small trees also may explain why sting nematode damage is more evident on young than on mature trees.

Our study on citrus in the field and other research (2,8) have been conducted in a limited area of northeastern Polk County. The high incidence and damage to trees by sting nematodes in Polk County found in this survey are consistent with other research conducted in this region. Moreover, populations of sting nematodes in northeastern Polk County tended to have stylets longer than tails, unlike all other populations. Stylet:tail ratio has been used to differentiate *B. longicaudatus* from other species of this genus (12). Two previous studies demonstrated variability in the morphometrics of *B. longicaudatus* populations, which was related to reproductive incompatibility (11) and host range and pathogenicity (1). The authors of both studies suggested the need for further evaluation of the taxonomic status of *B. longicaudatus,* based on their findings. Additional study of the populations from northeastern Polk County is ongoing to clarify the taxonomic relationship of these nematodes to *B. longicaudatus* and to compare host-parasite relationships between citrus and morphometrically distinct populations of sting nematodes from orchards in Florida.

In conclusion, sting nematodes infest a high proportion of citrus orchards on Florida's central ridge. The nematode is associated with severe damage to young trees in the region. Our results demonstrate a need for microplot and field experiments to determine the economic impact of sting nematodes on citrus trees of various age and methods for nematode management. Population differences require further investigation to determine whether variability exists in the economic impact of different sting nematode populations.

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