Effects of Pratylenchus coffeae—Tylenchulus semipenetrans Interactions on Nematode Population Dynamics in Citrus

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Abstract: The distributions of *Pratylenchus coffeae* and *Tylenchulus semipenetrans* in a central Florida citrus grove were mutually exclusive. In a challenge experiment conducted in the grove, indigenous populations of either species did not preclude infection by the other species. Inoculation with either *T. semipenetrans* or *P. coffeae* tended to reduce the population size of the other nematode species. In greenhouse tests, individual feeder roots were parasitized predominantly by one or the other of the two species. Host response to parasitism in dual infections did not differ from response to single infection by either species. *Key words:* nematode distribution, infection, parasitism, histology.

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The citrus pathogens, *Pratylenchus coffeae* (Zimmerman), Filipjev and Schuurmans Stekhoven and *Tylenchulus semipenetrans* Cobb cause citrus slump (12) and slow decline (16), respectively. Their biology (6, 8, 9,12,15), mode of parasitism (8,9), and distribution in Florida citrus are quite different. *Pratylenchus coffeae* is a migratory endoparasite which destroys root cells as it feeds (8), thus reducing tree vigor and causing serious decline of trees. *Tylenchulus semipenetrans* is a sedentary semi-endoparasitic nematode which does not induce necrosis, but does alter root physiology (9). Tree appearance is not seriously affected in central Florida, but high citrus nematode populations reduce yield and fruit size. *Tylenchulus semipenetrans* is present in about 50% of Florida citrus groves (4), whereas *P. coffeae* has been detected in only seven commercial Florida citrus groves (total area 40.5 ha) (unpublished Division of Plant Industry records).

In 1979–80, the Division of Plant Industry (DPI), Florida Department of Agriculture and Consumer Services, extensively sampled a grove to delimit a *P. coffeae* infestation. From maps of that grove, we associated poor tree vigor with *P. coffeae*, but not with *T. semipenetrans* infestations. Sampling by DPI and our preliminary sampling suggested that *P. coffeae* and *T. semipenetrans* rarely coexisted, but no quantitative data were available. Thus, this study was initiated to determine the relationship between *P. coffeae* and *T. semipenetrans* under grove conditions, the reactions of established field populations of each nematode to challenge inoculations, and their interaction on individual roots.

MATERIALS AND METHODS

Experiments were conducted in a central Florida citrus grove near Auburndale (Polk County) which was infested with *P. coffeae* and *T. semipenetrans*. Initially, the grove was surveyed 1) to verify and quantify the relationship between the two nematodes, 2) to identify trees to serve as inoculum sources, and 3) to select test trees for a challenge experiment. Trees sampled were selected on the basis of previous nematode detection by DPI and by tree vigor.

Roots from 0–50 cm deep were collected from mature 'Valencia' sweet orange trees (*Citrus sinensis* [L.] Osb.) on rough lemon (*C. limon* [L.] Burm. f.) rootstock for quantitative studies of the distribution of the two nematodes. The area under the skirt of each of 26 trees was divided into eight sectors. Root samples, one per sector on 10 March 1980, three per sector on 26 March 1980, and two per sector on 24 February 1981, were collected at random. Roots were gently rinsed free of debris, weighed, and incubated in jars for 7 days at 26 C (17). Nematodes were counted and data expressed as the number of *P. coffeae* and/or *T. semipenetrans* per gram of root (fresh weight).

Sectors were selected for challenge inoculation experiments to determine whether the observed *T. semipenetrans-P. coffeae* population distribution resulted from nematode-nematode antagonism. Inoculum was prepared by aerating for 7 days roots

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which were collected from field trees in sectors infested by only one nematode species. Concentrations of nematodes of each species selected for inoculations were based on the naturally occurring nematode populations observed in the grove in the previously described survey. To inoculate each sector, two soil cores (2.5 × 30 cm) were removed at random with soil augers. The *T. semipenetrans*-infested sectors were inoculated with 1.6×10^4 *P. coffeae* per core; the *P. coffeae*-infested sectors were inoculated with 1.75×10^5 *T. semipenetrans* per core. Thirteen noninfested sectors were inoculated with each nematode at the above concentrations. Markers were placed in the holes and to distribute the inoculum uniformly with depth aqueous nematode suspensions (250 ml) were poured in as the holes were filled with soil. Each inoculation site was watered weekly for 2 months to prevent dessication.

Five months after inoculation, soil and root samples were taken from two challenge-inoculated and two noninoculated sites within each sector. Samples were taken from the 0–25-cm and 25–50-cm depths at each site with a 7.6-cm-d auger and stored in plastic bags at 18 C ± 2 until processed. After mixing the samples thoroughly, the roots were removed and incubated at 26 C for 7 days (17). A 400-cm^3 subsample from each soil sample (1,134 cm^3) was processed in a flotation can. The effluent was drained through a stack of 250-μm, 105-μm, 90-μm, 63-μm, and three 44-μm sieves for 1 min. The water flow (17 l/min) was turned off and the remaining water in the tank was poured through the sieves. The material remaining in the top three sieves was discarded. Material in the bottom four sieves was backwashed into a beaker with 40 ml water and the nematodes extracted by sucrose centrifugation. Samples were stored at 18 C until read. Data were expressed as nematodes/g of root (fresh weight) or nematodes/l of soil. All data were transformed to log (x + 1) prior to statistical analyses.

The distribution of *T. semipenetrans* and *P. coffeae* in sour orange (*Citrus aurantium*) feeder roots was determined in a greenhouse study. Seedlings were grown in 20-cm-d clay pots containing Astatula fine sand, peat moss, and vermiculite (2:1:1; v/v/v), pH 6.7. Root systems were drenched with an aqueous suspension of *P. coffeae* (5.0×10^3) and *T. semipenetrans* (5.0×10^4). Ten months later, roots were harvested, stained in cold acid fuchsin in lactophenol for 5 days, cleared in lactophenol for 5 days, and placed in glycerin. Roots ($\bar{x}$ = 80 mm) were dissected at 40 × magnification to determine the average distance between infection sites of the two nematode species, the location of *P. coffeae* with respect to lesions, and the frequency of concomitant parasitism in the 43 randomly selected root segments containing 150 nematode infection sites.

Feeder roots were stained, sectioned, and observed to determine whether cellular response to infection differed when both species were present. Feeder roots infected by *T. semipenetrans* and/or *P. coffeae* were fixed in Perfix (Fisher Scientific) for 65 h at 34 C under vacuum (52 kPA). The samples were then dehydrated in ethanol/xylene series and embedded in parafin (60 C, 52 kPA). Sections (12 μm) were stained with safranin and fast green and examined at 200 and 500 ×.

RESULTS

A significant negative correlation ($P \leq 0.001$) existed between *P. coffeae* and *T. semipenetrans* populations each time the established grove populations were sampled (Table 1).

In the challenge experiment, our initial nematode-characterization of sectors appeared accurate and no nematode movement between sectors was detected at completion of the experiment (Table 2). For example, where *T. semipenetrans* or *P. coffeae* was added to noninfested sectors, only significant numbers of *T. semipenetrans* or *P. coffeae,* respectively, were found (Table 2, treatment 3 + 4). Inoculation of previously noninfested sites with *P. coffeae* resulted in root (0–25, 25–50 cm) and soil (0–25 cm) populations which were comparable to natural field populations (Table 2, treatment 2 vs. 4). *Pratylenchus coffeae* was detected in roots, but not soil, from the 25–50 cm-depth in treatment 4. Where *T. semipenetrans* was inoculated on noninfested sites, the populations in roots and

Table 1. Incidence of *Pratylenchus coffeae* and *Tylenchulus semipenetrans* in a survey of a Florida citrus grove.

Date	No. of samples	Sector†	Nematode/g root		r*
			P. coffeae	*T. semipenetrans*	
		1‡	1688	10	
10-3-80	58	2	23	3041	−0.81
		1	272	17	
26-3-80	117	2	32	2483	−0.69
		1	337	8	
24-2-81	160	2	0	1759	−0.92

*Correlation coefficient significant at $P \leq 0.001$.

†Sector = 1/8 of area under the tree canopy.

‡1 = Sectors predominantly infested by *P. coffeae*. 2 = sectors predominantly infested by *T. semipenetrans*.

soil were lower than those occurring naturally in the field (Table 2, treatment 3 vs. 6). When established *P. coffeae* populations were challenged by *T. semipenetrans*, no significant differences in the number of *P. coffeae* in roots were noted when compared to nonchallenged *P. coffeae* populations (Table 2, treatment 1 vs. 2). However, *T. semipenetrans* inoculations did significantly reduce the number of *P. coffeae* in soil (0–25 cm) compared to established *P. coffeae* (Table 2, treatment 1 vs. 2). Established *T. semipenetrans* populations in roots and soil (0–25 cm) declined in response to *P. coffeae* inoculation compared to nonchallenged populations (Table 2, treatment 5 vs. 6). However, no significant differences were observed in samples from the 25–50 cm depth (Table 2, treatment 5 vs. 6).

Neither nematode precluded the establishment of the other species. Significant numbers of *T. semipenetrans* became established in *P. coffeae*-infested sectors (Table 2, treatment 1). *Tylenchulus semipenetrans* populations in *P. coffeae*-infested sectors were not significantly different from those in which *T. semipenetrans* was inoculated in noninfested sites (Table 2, treatment 1 vs. 3). *Pratylenchus coffeae* became established in *T. semipenetrans*-infested sectors and were not significantly different from *P. coffeae* populations developing in *P. coffeae*-inoculated sites which were previously noninfested (Table 2, treatment 4 vs. 5).

Over all treatments, 97.5% of feeder roots were in the 0–25 cm samples with <3% at the lower depth (Table 3). All

Table 2. Effect of inoculation of rough lemon (*Citrus limon* [L.] Burm. f.) rootstock with *Tylenchulus semipenetrans* and *Pratylenchus coffeae* on established populations of the two nematodes at two soil depths.

Treatment	Established	Inoculated	*P. coffeae*				*T. semipenetrans*			
			Nematodes/g root fresh wt		Nematodes/l of soil		Nematodes/g root fresh wt		Nematodes/l of soil	
			Depth (cm)				Depth (cm)			
			0–25	25–50	0–25	25–50	0–25	25–50	0–25	25–50
1	Pc*	Ts	128a†	211a	40b	62a	21cd†	49b	13c	4b
2	Pc	N	166a	282a	159a	44ab	0e	24c	0c	0b
3	N	Ts	2b	0b	0c	0c	63c	98b	19c	25b
4	N	Pc	60a	113a	109ab	0c	6de	0c	0c	16b
5	Ts	Pc	92a	33ab	47b	26bc	933b	2081a	413b	439a
6	Ts	N	1b	0b	10bc	0c	2206a	1401a	1793a	676a

*Pc = *P. coffeae*, Ts = *T. semipenetrans*, and N = no nematode.

†Column means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.10$). (Based on 22 replicate samples.)

Table 3. Influence of established and inoculated *Pratylenchus coffeae* and *Tylenchulus semipenetrans* on root weight of rough lemon (*Citrus limon* [L.] Burm. f.) rootstock in field samples.

Treatment	Established	Inoculated	Root wt (g)/l of soil, Depth (cm) 0–25	25–50
1	Pc*	Ts	154 bc	2.9 a
2	Pc	N	114 c	5.2 a
3	N	Ts	138 bc	4.3 a
4	N	Pc	156 bc	4.0 a
5	Ts	Pc	111 c	4.7 a
6	Ts	N	182 ab	4.5 a
7	N	N	281 a	4.5 a

*Pc = *P. coffeae*, Ts = *T. semipenetrans* and N = no nematode detected or inoculated.

†Column means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.10$).

nematode treatments except established *T. semipenetrans* populations (Table 3, treatment 6) significantly reduced root quantity in the top 25 cm of soil when compared with noninfested sectors. Total nematode populations were largest in roots from the 0–25 cm samples (98% of the *T. semipenetrans* and 93% of the *P. coffeae*). Soil populations were also greatest in the top 25 cm (73% of the *T. semipenetrans* and 78% of the *P. coffeae*).

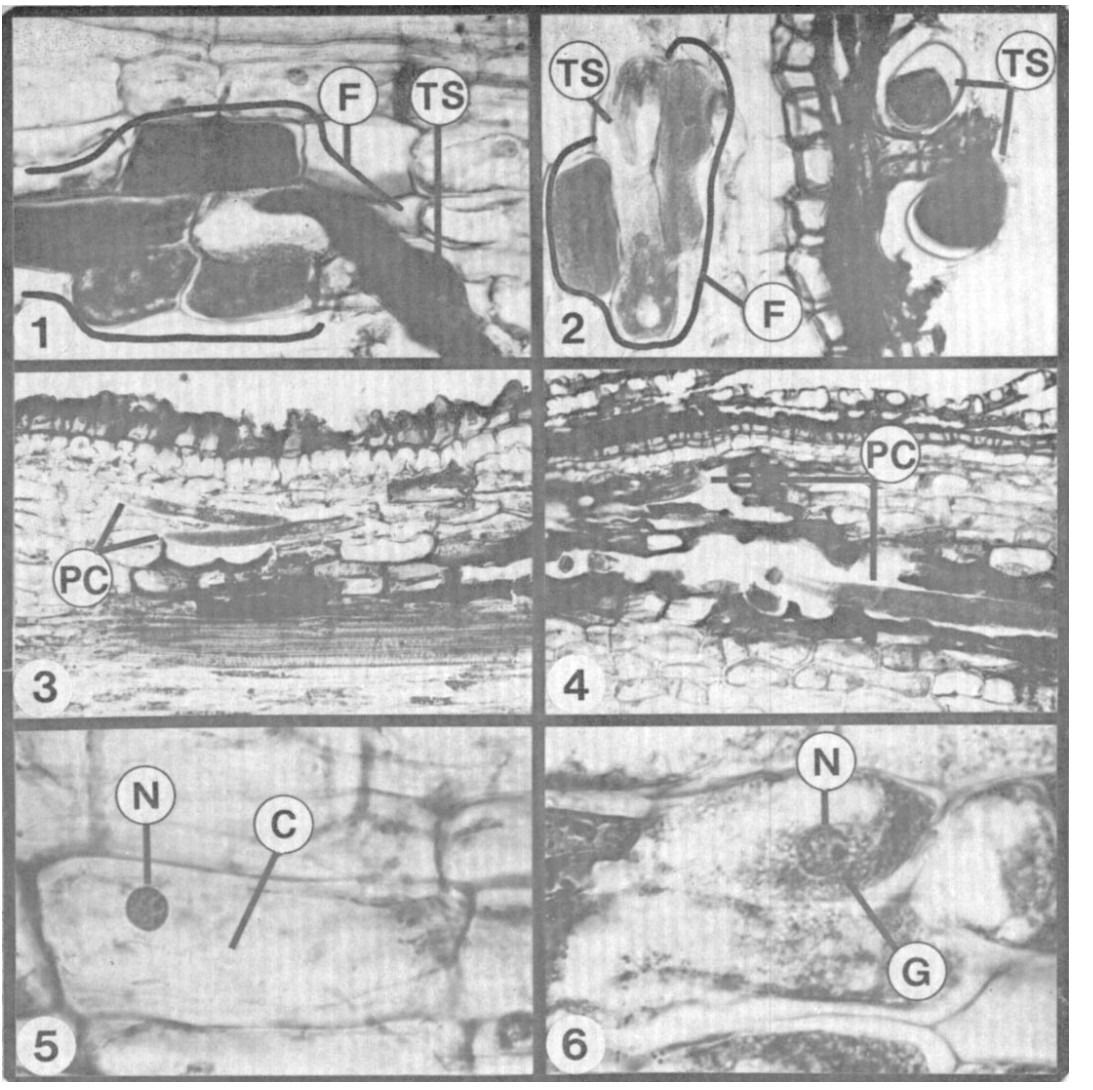
The distribution of *P. coffeae* and *T. semipenetrans* in stained feeder roots reflected the gregarious nature of the two species. Citrus nematode females were generally found in groups, while darkly stained lesions contained numerous *P. coffeae* larvae and adults. The 43 root segments selected at random contained 150 infection sites. Thirty-four of the 43 segments were parasitized by either *P. coffeae* or *T. semipenetrans*. In seven instances, both species were found parasitizing the same feeder roots with an average distance of 10.25 mm (± 3 mm) between infection sites of the two species. At two *T. semipenetrans* infection sites, *P. coffeae* males were associated with *T. semipenetrans* egg masses. The observed frequency of concomitant parasitism was significantly lower than that expected according to a nonparametric sign test ($P = 0.05$).

Tylenchulus semipenetrans feeding sites and/or lesion formation associated with *P. coffeae* did not appear to differ in roots parasitized by one or both species (Fig. 1–6).

DISCUSSION

The mutually exclusive distribution of *P. coffeae* and *T. semipenetrans* within a Florida citrus grove suggests that the two nematodes are incapable of concomitant parasitism of citrus roots for prolonged periods of time (years). Although some nematode-nematode interactions (2,3,14) adversely affect infection, in this study, lesion and citrus nematode infected roots in sectors already infested by the other nematode. Greenhouse studies suggested that a single nematode species would dominate a feeder root, but prevention of infection may not be a primary limiting factor since complete and uniform infection of roots has not been observed. The challenge experiment indicated that *P. coffeae* could gradually reduce established *T. semipenetrans* populations and vice versa. This experiment was conducted for only a 5-month period. However, if the observed trends were projected over several years, populations would probably approximate the mutually exclusive distribution of *P. coffeae* and *T. semipenetrans* observed in the grove.

The distribution of *P. coffeae* in Florida citrus is limited and contrasts to the wide distribution of *T. semipenetrans*. Factors responsible for the mutually exclusive distribution of the two nematodes within the grove might also play a role in the limited distribution of *P. coffeae* in the state. In support of this hypothesis, *Pratylenchus vulnus* infestations of citrus nurseries in Italy disappeared when *T. semipenetrans* invaded (R. Inserra, personal communication). Thus, it would appear that lesion nematodes are poor competitors. However, under experimental conditions, *P. coffeae* invaded *T. semipenetrans*-infested and noninfested sites and thereby increased its relative distribution. Florida's program of nursery stock certification for freedom from nematodes has undoubtedly contributed to limiting the spread of *P. coffeae*. However, other unidentified factors are probably also involved. These might include differences



Figs. 1–6. Cellular responses in sour orange (*Citrus aurantium*) feeder roots to infection by *Tylenchulus semipenetrans* and/or *Pratylenchus coffeae* (F = feeding site, TS = *T. semipenetrans*, PC = *P. coffeae*, N = nucleus, C = cytoplasm, G = granular cytoplasm). 1, 2) *T. semipenetrans* associated with feeding site composed of darkly stained nurse cells within the cortex of a root parasitized solely by *T. semipenetrans* (Fig. 1) and in a root parasitized by both species (Fig. 2). 3, 4) Lesion and cavity formation associated with infection of the root cortex by *P. coffeae* (Fig. 3) and by both nematode species (Fig. 4). 5) Normal cortical cell with small nucleus and thin cytoplasm. 6) Cortical cell adjacent to lesions had enlarged nuclei, nucleoli and dense granular cytoplasm.

in relative nematode reproduction potential (6,7,15), differences in the effect of nematodes on root physiology (8,9), differences in modes of parasitism (8,15), and environmental and cultural factors. Biological control (10) and/or the development of nematode biotypes (5,11,13) might also contribute to this limited distribution. Once the biology of *P. coffeae* is more completely understood, then *P. coffeae* as a pathogen of Florida citrus can be identified as either a neospecies (1), increasing abundance and expanding distribution, or as a telospecies, scarce with narrow geographic distribution. The relative importance of this species to Florida's citrus industry can then be better defined.

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