Comparative Influence of Radopholus similis and Pratylenchus coffeae on Citrus

J. H. O'BANNON, J. D. RADEWALD, A. T. TOMERLIN, and R. N. INSERRA[1]

Abstract: Pratylenchus coffeae was as pathogenic as *Radopholus similis* to commercial citrus rootstocks. No rootstock resistant to *R. similis* was resistant to *P. coffeae*. Both nematodes stunted citrus in three soil types. Seedling damage by *P. coffeae* and *R. similis* was greatest in fine- and coarse-textured soils, respectively. Reproduction and survival on citrus were greater for *P. coffeae* than for *R. similis*. Mixed inoculations with *R. similis* and *P. coffeae* resulted in lower populations of each species than did separate inoculations. *Key Words:* Burrowing nematode, lesion nematode, rootstocks, soil type.

Radopholus similis (Cobb) Thorne, the burrowing nematode, causes a "spreading-decline" disease of citrus in Florida. Symptoms of the disease are sparse foliage, defoliated branches, twig dieback, general unthriftiness, small fruit, and losses in yield. Spreading decline initially appears in a group of trees within a grove, and the decline area increases annually in size. Similar symptoms have been observed in citrus groves where the burrowing nematode was not found, but where *Pratylenchus coffeae* (Zimmerman) Filipj. & Shuur.-Stekh. was detected. An earlier study (6) showed *P. coffeae* to be a severe parasite of citrus in Florida.

The tissue most extensively invaded by *R. similis* is cortex, but phloem, cambium, apical meristem, xylem, parenchyma, and pericycle are commonly invaded (1). *Pratylenchus coffeae* also extensively invades cortex, but the endodermis is damaged only when high numbers enter the same site (8). This nematode does not cause hyperplasia or detectable cellular reaction and growth stimulus in pericycle and endodermis which are traits of *R. similis* infection. Failure of *P. coffeae* to enter vascular tissues may be due to chemical or physical barriers.

In groves, *R. similis* and *P. coffeae* produce similar disease symptoms. The effects of these two migratory endoparasitic nematodes on a common host have never been compared under controlled conditions. The objective of our investigations was to make this comparison.

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[1] First and third authors, Nematologist and Agricultural Research Technician, respectively, U.S. Horticultural Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Orlando, Florida 32803; second author, Extension Nematologist, University of California, Riverside 92502; and fourth author, Nematologist, Agricultural Nematology Laboratory, CNR, Bari, Italy.

MATERIALS AND METHODS

We conducted four greenhouse experiments using several *Citrus* cultivars inoculated with *R. similis* and *P. coffeae*. In one study, different soil types were used because soil type greatly influences the disease syndrome caused by the burrowing nematode (5).

Both nematode species were maintained on susceptible rough lemon seedlings grown in large greenhouse soil bins. We inoculated experimental seedlings by either transplanting them into nematode-infested soil bins for 2 months and replanting them into individual pots, or inoculating potted seedlings with juvenile and adult nematodes. The initial inoculum of seedlings transplanted from soil bins averaged 10 *R. similis* or 15 *P. coffeae* per gram of moist feeder roots. *Radopholus similis* and *P. coffeae* were extracted periodically and at harvest from 2-3 g of roots by incubation for 4 and 7 days, and numbers per gram of fresh root were recorded. After harvest, oven-dry root weights were obtained.

Seedlings were grown in Astatula fine sand unless otherwise noted. We used rough lemon seedlings in all experiments because they are highly susceptible to both nematodes. Plants were maintained in the greenhouse for 10-15 months at 20-35 C. All data were analyzed statistically.

RESULTS

Comparative pathogenicity: To compare the pathogenicity of *R. similis* and *P. coffeae* on citrus, three commercially available rootstocks, rough lemon [*Citrus limon* (L.) Burm. f.], sour orange (*C. aurantium* L.), and 'Cleopatra' mandarin (*C. reticulata* Blanco) were used. Equal numbers of

6-month-old healthy seedlings were transplanted into three soil bins; one was infested with *R. similis,* the second with *P. coffeae,* and the third was kept free of nematodes. After 2 months, seedlings were removed and selected for uniformity, and five replicates of each rootstock were transplanted into 20-cm diam clay pots. Plants were randomly placed on benches and grown for 15 months.

Nematodes were extracted from roots at 5, 11, and 15 months. Numbers of *P. coffeae* extracted from all seedlings were greater than those of *R. similis* ($P \leq 0.01$) in the first and second sampling (Fig. 1). Sour orange and Cleopatra rootstock had more *P. coffeae* than *R. similis* ($P. \leq 0.01$) at final sampling, but this was not true for rough lemon.

Compared to noninfected controls, both nematodes retarded root growth ($P \leq 0.05$) in rough lemon and sour orange rootstocks (Table 1). *R. similis* did not significantly suppress Cleopatra rootstock growth but *P. coffeae* did ($P \leq 0.05$). Inhibition of growth in rough lemon, sour orange, and Cleopatra rootstocks, as compared with controls, was 42, 31, and 20% respectively for *R. similis,* and 52, 67, and 57% respectively for *P. coffeae.*

Evaluation of rootstocks for resistance to Pratylenchus coffeae: Seven rootstocks were evaluated for resistance to *P. coffeae.* Four of these, 'Algerian' navel (*C. sinensis* (L.) Osbeck), 'Carrizo' citrange (*C. sinensis* X *Poncirus trifoliata* (L.) Raf.), 'Milam' lemon (*Citrus* sp.), and 'Ridge Pineapple' orange (*C. sinensis*) are resistant to *R. similis* because populations disappeared or remained in low numbers. Three, 'Estes' lemon, rough lemon, and sour orange maintained high populations of *R. similis* and are considered susceptible, although Estes is reported tolerant, because growth is allegedly not suppressed more than 20% (7). Six-month-old seedlings were transplanted into infested and noninfested soil bins. After 2 months, 10 replicates of each treatment were transplanted into 20-cm diam clay pots and grown for 15 months.

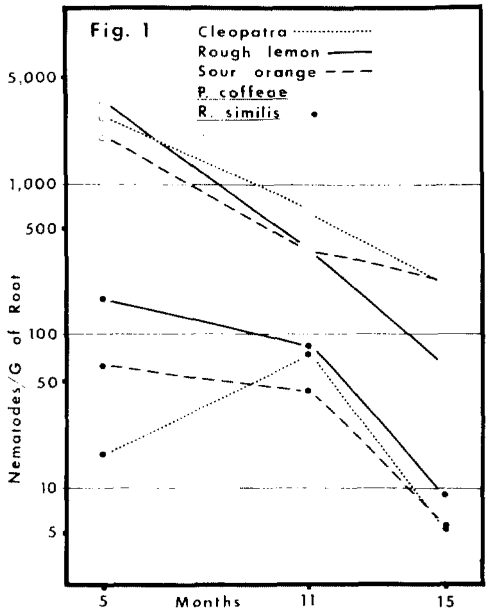
Numbers of *P. coffeae* extracted at sampling dates of 5, 9, and 15 months were greater ($P \leq 0.01$) than numbers of *R. similis* (Fig. 2). No rootstocks resistant to *R. similis* showed resistance to *P. coffeae.* Milam showed the highest resistance to *R. similis* ($P \leq 0.01$); Algerian navel and Ridge Pineapple also were resistant ($P \leq 0.05$).

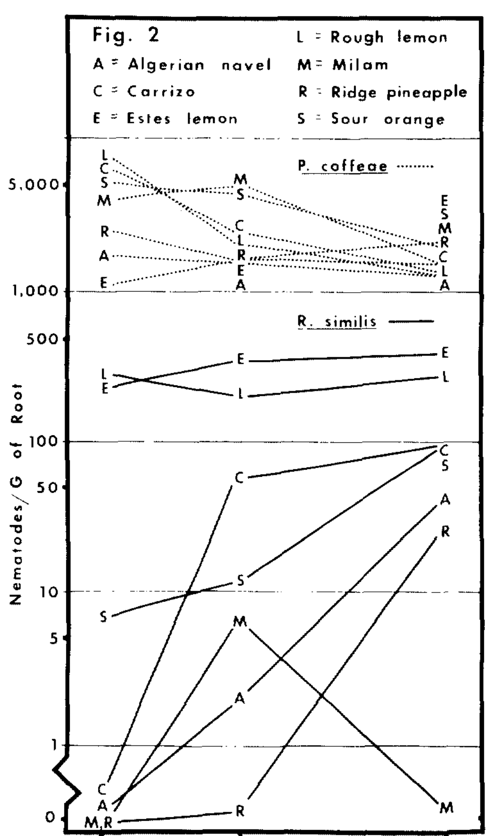
Compared with the controls ($P \leq 0.05$) and with *R. similis* on those rootstocks which are resistant to *R. similis* (except Carrizo), *Pratylenchus coffeae* caused the most severe infections and greatest stunting (Table 2). Growth did not differ significantly for rootstocks resistant to *R. similis* and controls, but *R. similis* retarded growth of other cultivars ($P \leq 0.05$).

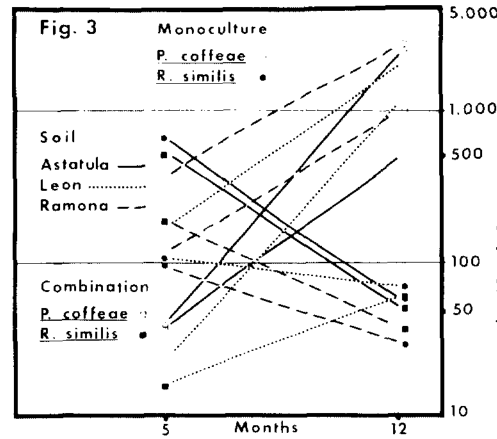
Influence of soil type of nematode reproduction: To evaluate the effects of soil types on host-parasite relations, a study was made on the influence of the nematodes separately and in combination in three soils. The soils and their physical characteristics are shown in Table 3. The soils were steam pasteurized and stored 3 months before use. One-year-old, noninfected rough lemon seedlings were transplanted into 120 twenty-cm diam pots. After 1 month, one-fourth of the seedlings in each soil type were inoculated with 100 *R. similis* per pot, one-fourth with 100 *P. coffeae,* and one-fourth with 100 *P. coffeae* plus 100 *R. similis.* The remaining seedlings served as noninoculated controls. Each combination, replicated 10 times, was randomized on greenhouse benches and grown for 15 months.

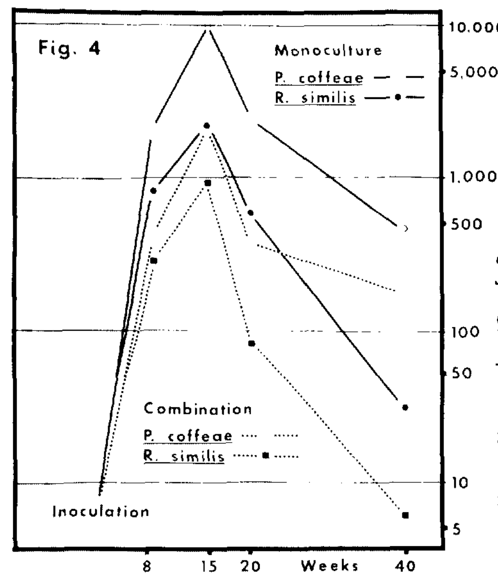
Numbers of *R. similis* and *P. coffeae* were extracted from seedlings grown in these soils for 5 and 12 months after inoculation. Populations from the first sampling did not differ; however, numbers of *R. similis, P. coffeae,* and the mixed population significantly segregated in the second sampling with *R. similis* < 100 and *P. coffeae* > 500 ($P \leq 0.05$). Recovery of *R. similis* and *P. coffeae* in the combined population was less than it was in the respective monoinoculations, except for seedlings in Ramona soil infested with *R. similis.* These seedlings yielded the fewest nematodes.

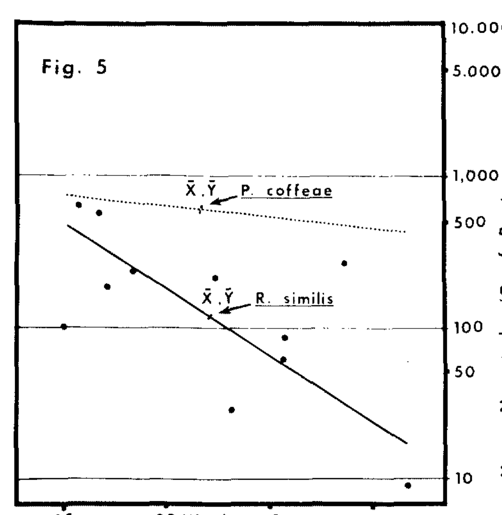
Stunting of seedlings infected with *P. coffeae* in Ramona soil was more severe ($P \leq 0.05$) than that of seedlings infected with *R. similis* (Table 4). Growth of all infected seedlings in all soil types was less ($P \leq 0.05$) than that of controls. The deleterious effects of *P. coffeae* were most pronounced in











TIME OF SAMPLING

TABLE 1. Effects of *Radopholus similis* and *Pratylenchus coffeae* on the growth of selected citrus rootstocks.

	Oven-dry root weights (g)		
Treatment	Rough lemon	Sour orange	Cleopatra mandarin
Radopholus similis	8.3[a]	10.3[a]	7.3
Pratylenchus coffeae	7.3[a]	4.9[ab]	3.9[ab]
Control	14.2	14.9	9.1

[a]Denotes stunting comparing infections with control ($P \leq .05$), using Tukey's Honestly Significant Differences (THSD).
[b]Denotes stunting comparing infections only ($P \leq .05$), using THSD.

the finer textured Ramona and Leon series. Damage by *R. similis* was greatest in the coarser-textured Astatula soil (Fig. 6-8).

Population dynamics: To study population variation, 4-month-old noninfected rough lemon seedlings were transplanted into fifteen 20-cm diam pots. After 1 month, one-third of the seedlings were inoculated with 50 *R. similis* per pot, one-third with 50 *P. coffeae* per pot, and one-third with 50 *P. coffeae* plus 50 *R. similis* per pot. We took nematode population counts from five replicate root samples at 8, 15, 20, and 40 weeks after inoculation.

Numbers of *P. coffeae* and *R. similis* rapidly increased to peak populations in excess of 10,000 and 2,500/g of root, respectively (Fig. 4). Populations recorded at final sampling, 40 weeks after inoculation, showed at least a 15-fold difference between numbers of *P. coffeae* and *R. similis* for both the separate and combined inoculations. Suppression of the combined nematode populations indicated an antagonistic effect, with *R. similis* the poorer competitor.

To determine if population numbers differed, we combined data from experiments on two nematode populations from rough lemon seedlings grown in Astatula fine sand from 15 to 65 weeks and tested the linearity of regression of the log. The linear regression of population samples of *R. similis* was highly significant, but population samples of *P. coffeae* showed poor linearity. The slopes of the lines of best fit for the mean populations for these nematodes at each sampling were significantly different (Fig. 5). At 40 weeks, populations of *P. coffeae* showed a 5-fold difference from populations of *R. similis*.

DISCUSSION

Under conditions favorable to *R. similis*, *P. coffeae* is more deleterious and can cause greater damage to citrus. Although differences were detected between numbers initially invading citrus roots, *P. coffeae* pop-

TABLE 2. Influence of *Radopholus similis* or *Pratylenchus coffeae* on the growth of seven citrus rootstocks.

	Oven-dry root weights (g)						
Treatment	Rough lemon	Estes lemon	Milam[c] lemon	Sour orange	A. navel[c] orange	R. Pine.[c] orange	Carrizo[c] citrange
Radopholus similis	2.9[a]	2.7[a]	7.0	7.8[a]	8.0	8.6	3.7
Pratylenchus coffeae	4.8[a]	4.7[a]	2.2[ab]	4.5[ab]	3.1[ab]	3.7[ab]	1.5[a]
Control	14.1	15.6	7.2	14.3	7.4	6.7	5.7

[a]Denotes stunting comparing infections with control ($P \leq .05$), using Tukey's Honestly Significant Differences (THSD).
[b]Denotes stunting comparing infections only ($P \leq .05$), using THSD.
[c]Rootstocks resistant to *R. similis*.

←

FIG. 1-5. *1)* Numbers of *Pratylenchus coffeae* and *Radopholus similis* recovered from three rootstocks at three sampling times. *2)* Numbers of *P. coffeae* and *R. similis* recovered from seven rootstocks at three sampling times. *3)* Separate and combined populations of *P. coffeae* and *R. similis* recovered from rough lemon seedlings growing in each of three soils. *4)* Comparative population development of *P. coffeae* and *R. similis* separately and in combination from rough lemon seedlings growing in Astatula fine sand. *5)* Regression analysis comparing populations of *P. coffeae* with *R. similis* from four experiments. *Radopholus similis* significantly separate ($P \leq .05$) from *P. coffeae*.

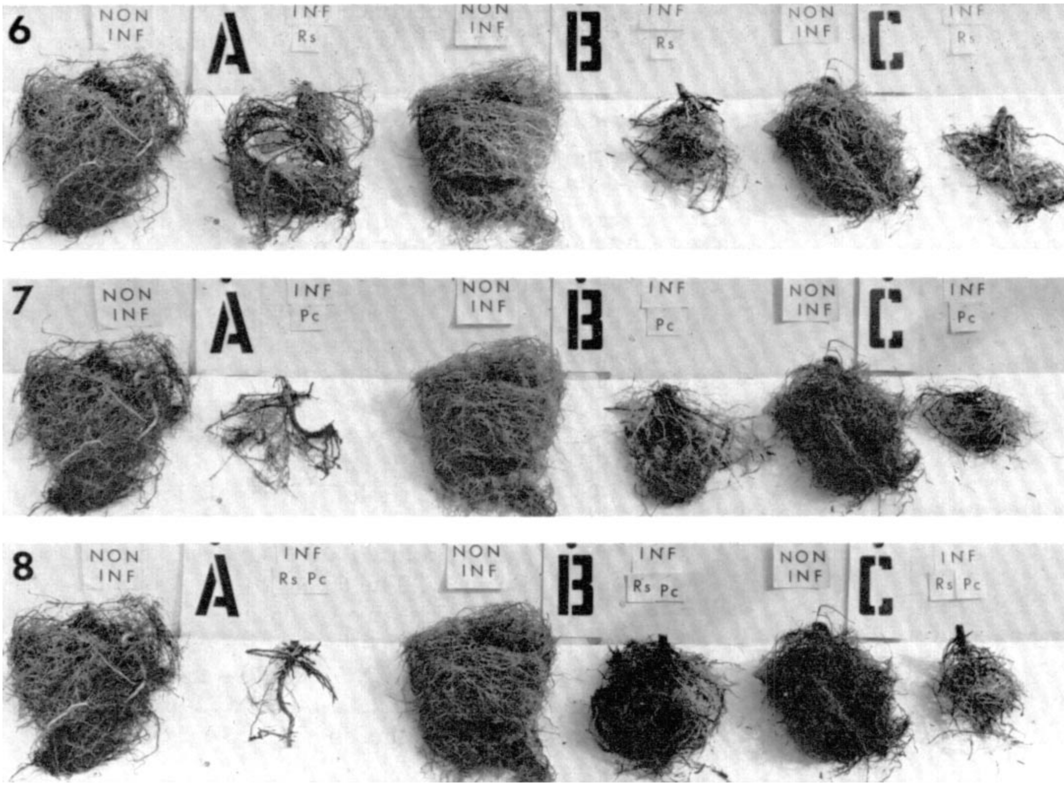


FIG. 6-8. Comparision of effects of separate and combined populations of *6) Radopholus similis* (Rs), *7) Pratylenchus coffeae* (Pc), and *8)* Rs plus Pc on roots of rough lemon seedlings growing in three soils. (A = Ramona sandy clay loam soil; B = Astatula fine sand; C = Leon fine sand; "NON-INF" = non-infected controls; and "INF" = infected).

ulations increased faster and maintained higher numbers than *R. similis* populations, regardless of whether in monoculture or in combination. However, combined populations of *R. similis* and *P. coffeae* were lower with either nematode indicating a mutual inhibitory effect. Similar observations have been made with other nematodes. Estores and Chen (3), who studied interactions of *P. penetrans* and *Meloidogyne incognita* on tomato, indicate primary inhibitory effects to be competition for feeding sites. Miller and Wihrheim (4) suggest antagonism with their studies of

TABLE 3. Physical and chemical characteristics of soils utilized.

Soil type	pH Range	Clay (<2μm)	Silt (2-50μm)	Sand (50μm-2mm)	Organic matter	0.1 bar percentage[d]
		(%)	(%)	(%)	(%)	
Astatula fine sand[a]	5.6-6.1	1.7	2.1	96.2	0.25	6.0
Leon fine sand[b]	6.2-6.5	7.2	6.2	86.6	2.42	10.0
Ramona sandy clay loam[c]	7.6-7.9	27.6	26.7	45.7	0.20	18.0

[a]Soil from the central ridge section of Florida.
[b]Soil from the Atlantic Coastal Plain near Vero Beach.
[c]Soil from Citrus Experiment Station, Riverside, California.
[d]Percentage moisture by volume at 100-millibar soil-water pressure.

TABLE 4. Growth of rough lemon as affected by *Radopholus similis* and *Pratylenchus coffeae* and soil type.

Treatment	Oven-dry root weights (g) per soil type		
	Astatula	Leon	Ramona
Radopholus similis	7.4[a]	9.6[a]	13.7[a]
Pratylenchus coffeae	6.5[a]	5.8[a]	4.4[ab]
Radopholus similis + *Pratylenchus coffeae*	10.6[a]	5.8[a]	5.5[ab]
Control	17.7	14.5	23.6

[a]Denotes stunting comparing infections with control ($P \leq .05$), using Tukey's Honestly Significant Differences (THSD).
[b]Denotes stunting comparing infections only ($P \leq .05$), using THSD.

nematodes on tobacco. In these studies, we feel that inhibitory effect was a combination of competition for feeding sites and antagonism. Earlier work by DuCharme and Price (2) implies that the population limit of *R. similis* in the roots of a citrus tree is predetermined by the available food supply and competition with other microorganisms for these same feeding sites.

All rootstocks resistant to *R. similis* were highly susceptible to *P. coffeae;* thus the possibility of dual resistance from a single gene is eliminated.

Coarse-textured soil favors *R. similis* at the expense of the citrus host. By measuring population increases and severe growth retardation, we have shown that fine-textured soils favor *P. coffeae* activity on citrus. However, plant damage and root deterioration were significant with all soils tested.

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