Citrus Tree Decline Caused by Pratylenchus coffeae

J. H. O'BANNON and A. T. TOMERLIN[1]

Abstract: The pathogenic effects of *Pratylenchus coffeae* on growth and yield of tangelo (*Citrus paradisi* × *C. reticulata*) scions grafted on rough lemon (*C. jambhiri*), sour orange (*C. aurantium*) and 'Cleopatra' mandarin (*C. reticulata*) rootstocks were evaluated under field conditions for 4 years. *Pratylenchus coffeae* on inoculated trees increased to significantly damaging population densities on rough lemon rootstock the second year, on sour orange the third and on Cleopatra mandarin the fourth year after planting. Mean growth reduction of *P. coffeae*-infected trees after 4 years was 80, 77 and 49%, respectively, for the three rootstocks. Noninoculated trees on rough lemon and sour orange rootstocks yielded significantly more fruit than comparable inoculated trees. Natural migration of *P. coffeae* occurred horizontally on roots for a distance of 4.5 m. *Key Words:* population densities, biology, nematode movement.

Pratylenchus spp. were first reported on citrus in Florida by Suit and DuCharme (12). Later investigations (1, 3) demonstrated under controlled conditions that one or more species were parasitic on citrus. *Pratylenchus* spp. have been reported associated with citrus from other citrus-growing areas (2, 5, 6).

Received for publication 25 May 1973.

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Pratylenchus coffeae (Zimmermann) Filipjev and Schuurmans-Stekhoven was reported to cause severe damage to citrus in the warmer regions of Japan (14). In India, Siddiqi (11) found that a nematode root-rot disease of citrus was caused by *P. coffeae*. He described symptoms of the disease, which include poor growth, general dieback and undersized fruits. From greenhouse-inoculation studies, he concluded that *P. coffeae* is pathogenic to citrus. In greenhouse studies in Florida, O'Bannon and Tomerlin (8) found that *P. coffeae* was pathogenic to rough lemon seedlings (*Citrus jambhiri* Lush.), and reduced growth by 22% after 1 year. In later studies (9), root weights of *P. coffeae*-infected citrus seedlings were reduced up to 47% compared to noninfected seedlings.

The relationship between the migratory endoparasitic nematode, *P. coffeae*, and the growth of a citrus tree is a complex situation which may be altered by various environmental factors in the field. Several greenhouse studies (1, 3, 8, 9, 11) have shown that *Pratylenchus* spp. are pathogenic to citrus. With a perennial host such as citrus, various ecological factors may influence either the parasite or the host at the expense of the other, particularly as related to time. This paper reports the pathogenicity of *P. coffeae* on three citrus rootstocks grown under field conditions for 4 years.

MATERIALS AND METHODS

Two-year-old citrus trees with tangelo (*C. paradisi* × *C. reticulata*) scion, grafted on each of three rootstocks, rough lemon (*C. jambhiri*), sour orange (*C. aurantium* L.) or 'Cleopatra' mandarin (*C. reticulata* Blanco), were transplanted into field plots in an Astatula fine sand (94-96% sand, 3-4% silt and clay; 1-2% organic matter; 5.5% moisture-holding capacity). *Pratylenchus coffeae* or other nematode parasites of citrus were not present in the sites selected for experimental plots. However, all tree sites were fumigated with methyl bromide at a rate of 0.45 kg/9m^2 before the trees were planted.

Six trees of each rootstock were planted randomly within each of eight plots in May 1969. Trees were set in rows 6 × 6 m apart. On 5 June 1969, four plots, selected at random, were inoculated with *P. coffeae*. About 4000 nematodes of all life stages were washed into holes in the soil placed around the root system of each tree. The trees in the remaining four plots served as controls.

The *P. coffeae* inoculum came from roots collected in an infested citrus nursery. The nematodes were extracted from roots and surface-sterilized in 4 ppm ethoxyethylmercury chloride and rinsed in sterile distilled water. All nematode-data determinations were based on nematodes per gram of moist feeder roots. To have comparable initial nematode counts, feeder roots were removed from similar noninfected trees and weighed moist. The number of nematodes to be used as inoculum for the test trees was calculated on the basis of number of nematodes per gram of root tissue. The initial inoculum averaged 20 *P. coffeae* per gram of moist feeder roots.

Usual orchard cultural practices were maintained for the duration of the test (4 years). Feeder-root samples for nematode evaluation were taken periodically from beneath the drip line of trees. Root samples were washed in running water and placed moist in 453-ml jars for nematode extraction by the Young root-incubation method (15). After 4 and 7 days, 50 ml of water was added to each jar, the contents were roiled, and the water was decanted into a 50-ml beaker. Aliquot samples of 5 ml were placed in counting dishes, and the number of *P. coffeae* counted. After the second count, the moist roots were weighed, and the nematodes per gram of root were calculated.

As a measure of tree growth, canopy height and width of trees were measured and foliar volume was determined by the formula:

$$V = \frac{H \times W^2}{4},$$

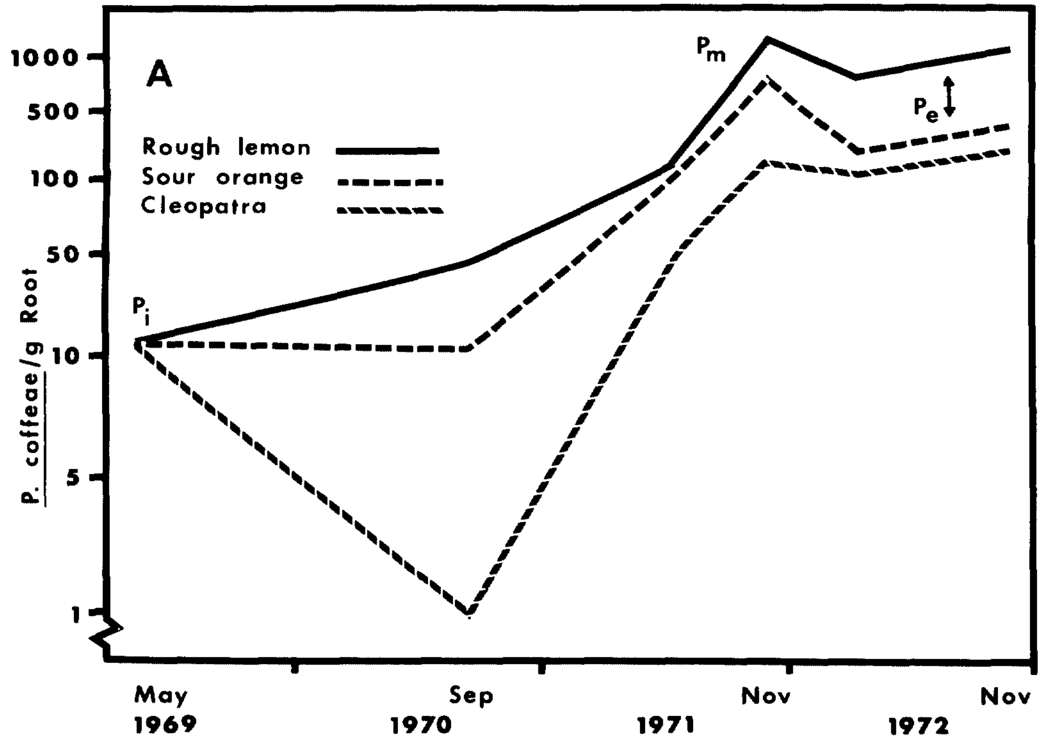
where V = volume, H = tree height, W = tree width taken at widest part, 4 = a constant.

Nematode and tree-growth data were obtained for 3.5 years, and fruit yields were measured in the first (1971) and second (1972) bearing years.

The lateral movement of nematodes from inoculated to noninoculated trees was determined from root samples taken in areas between adjacent inoculated and noninoculated trees. All data were subjected to statistical analysis. At the completion of the experiment, soil and root samples were taken and assayed for the foot rot fungus, *Phytophthora parasitica*, a major disease of citrus in Florida.

RESULTS

Populations: Fig. 1-A illustrates the mean numbers of *P. coffeae* extracted from roots of three rootstocks during the experimental period. Mean numbers of *P. coffeae* extracted from rough lemon and sour orange roots in September 1970 were 45 and 14 nematodes per gram of root, respectively. *P. coffeae* on Cleopatra rootstock were nearly below the limits of detection with an average of only one nematode per gram of root. *P. coffeae* extracted during 1971 continued the population trend of rough lemon > sour orange > Cleopatra. In November, these populations were 1847, 722 and 210/g of root, respectively. Differences in nematode numbers between all rootstocks were highly significant ($P = 0.01$) at



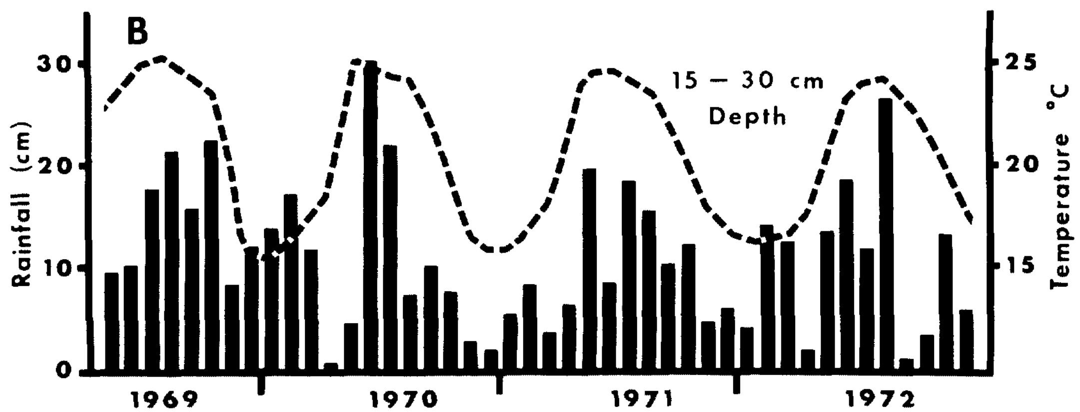


FIG. 1. **A.** *Pratylenchus coffeae* populations from three citrus rootstocks during 4 years (logarithmic scale). Initial inoculum averaged 20 nematodes per gram of root. P_m = maximum population density; P_e = population-equilibrium density on rough lemon and sour orange rootstocks. **B.** Seasonal rainfall (bar) and temperature ranges (line) in the years 1969-72. Soil temperature depths 15-30 cm.

this stage. This was the maximum population density (P_m) for rough lemon and sour orange rootstocks. During 1972, an unsteady equilibrium (P_e) was established on these two rootstocks as evidenced by population reduction from Pm. Nematode numbers were greater (P = 0.01) on rough lemon, but differences were not significant between sour orange and Cleopatra rootstocks. Final *P. coffeae* counts from Cleopatra rootstocks indicated that maximum densities had not yet been reached.

Growth: Fig. 2-A, B and C illustrate tree growth for the duration of the experiment, 1969-72 for the three rootstocks. Regression analysis conclusively demonstrates reduction in foliar volume growth to be concurrent with *P. coffeae* infectivity. During these years, reduction in growth of infected rough lemon and sour orange rootstock was highly significant compared to controls. Tree growth closely paralleled nematode density. Annual analysis of tree growth showed the first significant growth reduction in *P.*

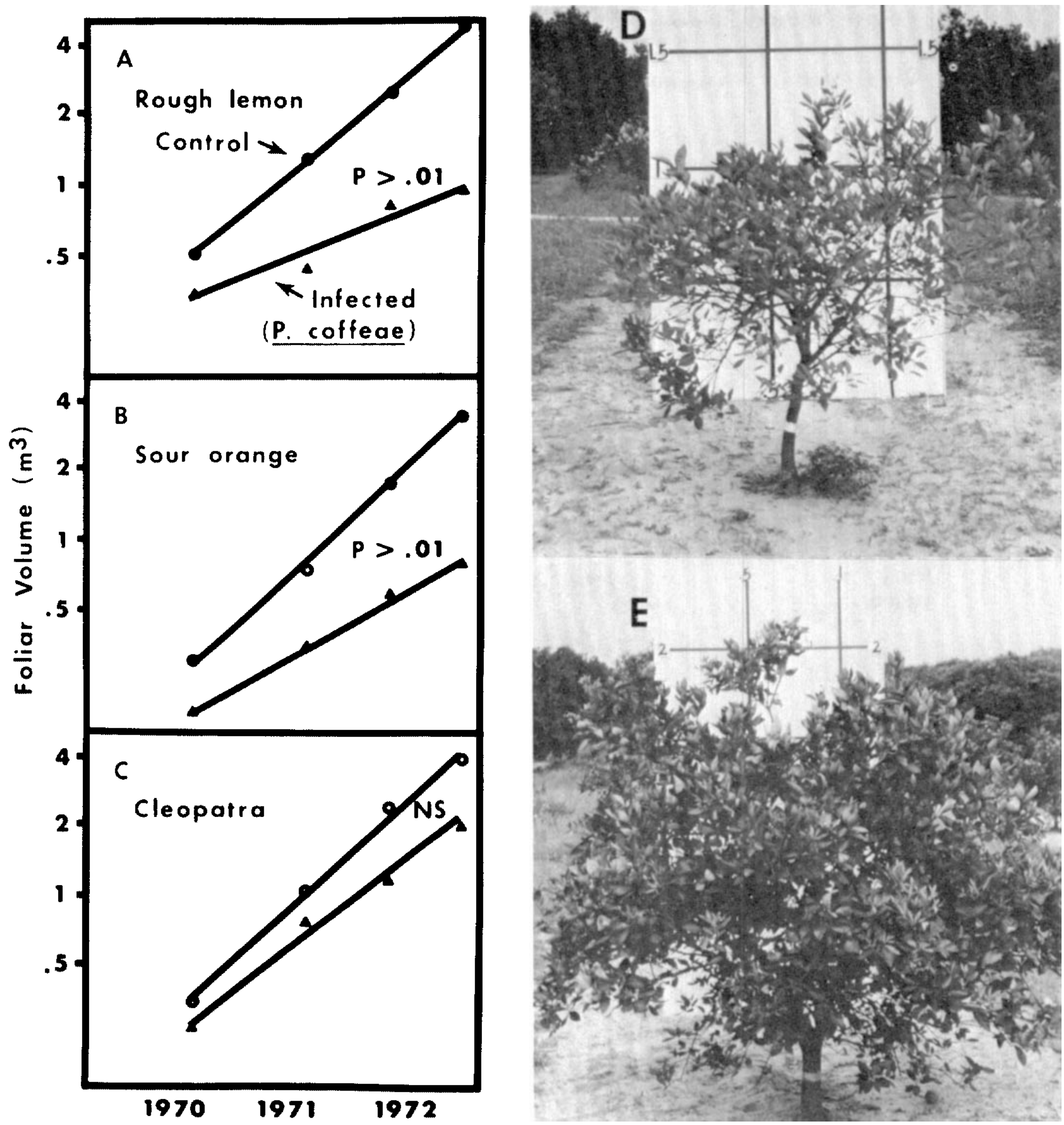


FIG. 2. Influence of *Pratylenchus coffeae* on tree growth of tangelo scion. Growth reduction, as measured by foliar volume, of infected trees compared to control trees. A. Rough lemon, B. sour orange, C. Cleopatra, D. five-year-old *P. coffeae*-infected tangelo tree, showing decline symptoms and E. five-year-old control tree. Both trees on sour orange rootstock.

coffeae-infected trees compared with control trees occurred on rough lemon rootstock in the second year (1970), and highly significant differences (P = 0.01) were evident the third and fourth years (Fig. 2-A). There was no significant growth difference between infected and control trees on sour orange or Cleopatra rootstock the first or second year. However, as populations increased, trees on infected sour orange rootstock showed significant (P = 0.05) tree growth reduction after the third (1971) and highly significant (P = 0.01) the fourth (1972) year (Fig. 2-B). Regression analysis showed growth reduction of trees on Cleopatra rootstock were not significant over the entire growth period, 1969-72 (Fig. 2-C). However, trees on Cleopatra rootstock did show adverse effects from nematode attack, but it was not until the end of the fourth growing year that populations increased to levels sufficient to cause damage that was significant (P = 0.05), as revealed by annual analysis of variance.

Growth reduction of *P. coffeae*-infected trees after 4 years was 80, 77 and 49%, respectively, for rough lemon, sour orange and Cleopatra rootstocks compared to control trees. Infected trees showed dieback, sparse growth and unthriftiness (Fig. 2-D) compared to control trees (Fig. 2-E).

Yield: Fruit yields are shown in Table 1. Control trees on rough lemon and sour orange rootstocks yielded 143% and 231% more fruit, respectively, than infected trees the first bearing year, and 2031% and 273% more the second year. There were no significant differences in yields between control trees and infected trees on Cleopatra rootstock in either the first or second bearing year.

Spread: Root samples taken from various rootstocks in January 1973 showed that horizontal root spread of inoculated and noninoculated trees was overlapping at 3 m. *P. coffeae* were found on roots of all previously noninoculated trees adjacent to the original infection. Tarjan (13) reported that *P. coffeae* was capable of migrating 0.86 m in 19 months on citrus seedlings in Astatula (formerly Lakeland) fine sand. In these studies, *P. coffeae* were found on roots of previously noninoculated trees 4.5 m from the original inoculum source, and 1.5 m past where roots first began to overlap 4 years after inoculation (Table 2).

TABLE 1. Yields expressed as number of fruit per tree of *Pratylenchus coffeae*-infected (Inf.) and noninfected tangelo (*Citrus paradisi* X *C. reticulata*) scion on three rootstocks.

	1971		1972	
Rootstock[a]	Control	Inf.	Control	Inf.
Rough lemon	90**[b]	37	138**	6
Sour orange	53**	16	78*	21
'Cleopatra' mandarin	43NS	28	67NS	24

[a]Mean of eight trees on each rootstock.
[b]* = significant at the 5% level, ** = significant at the 1% level, NS = not significant.

TABLE 2. Lateral movement of *Pratylenchus coffeae* from infected to noninfected trees. Tree spacing six meters apart. Final sampling, January 1973.

		Distance from infected tree (m)		
		P. coffeae per g root		
Rootstock	Sample depth (cm)	1.5 m	3.0 m	4.5 m
Rough lemon	15-30	21	20	49
	60-90	15	- [a]	0
Sour orange	15-30	68	112	12
	60-90	17	-	0
'Cleopatra' mandarin	15-30	13	15	8
	60-90	206	-	1

[a]- = no roots found in sampling zone.

DISCUSSION

Previous data (7) have shown that in older trees *P. coffeae* populations that had reached an unsteady equilibrium were affected to some extent by environmental stress such as low moisture and excessive temperatures. Figure 1-B shows precipitation and temperature for the duration of this experiment. Supplemental irrigation was provided during periods of low rainfall; consequently, trees were not adversely affected by drought conditions. Thus, the external conditions were suitable for nematodes to increase to equilibrium densities, as illustrated by the nearly sigmoid population curves (Fig. 1-A). These data indicate a lower rate of nematode multiplication on Cleopatra rootstock. A study of feeder-root distribution of the three noninfected rootstocks in 5- to 9-year-old trees (4) showed a similar root development, but sour orange roots were found to grow the slowest.

During the first 18 months after inoculation, nematode populations on

Cleopatra rootstock decreased rapidly, even though the food supply in the form of available root growth was not limiting. It would appear that this rootstock might be resistant. However, subsequent population increases resulted in tree damage. Seinhorst (10) suggested that equilibrium densities become higher than tolerance limits for plants. We feel, while equilibrium density had not yet been reached, a parallel condition existed.

This experiment has demonstrated that *P. coffeae* is capable of causing severe damage to young citrus trees under field conditions. We have shown that this nematode can readily migrate from infected to noninfected roots, especially when the roots overlap. Cultures of soil and root samples analyzed for *Phytophthora parasitica* gave evidence of infection in only one tree from an original noninfected plot. We feel this positive sample did not alter the results of this experiment.

Surveys in Florida suggest that *P. coffeae* is not yet widely distributed in citrus groves in the State. If all nursery stock is examined, and the planting of infected stock is prohibited, the distribution of this nematode can be controlled and the spread of the disease limited.

LITERATURE CITED

1. BROOKS, T. L. and V. G. PERRY. 1967. Pathogenicity of *Pratylenchus brachyurus* to citrus. Plant Dis. Rep. 51:569-573.
2. EDWARD, J. C., S. L. MISRA, Z. NAIM and R. A. SINGH. 1963. Survey of plant parasitic nematodes of farm soils of the Allahabad Agricultural Institute. Allahabad Farmer 37:1-7.
3. FELDMESSER, J. and C. I. HANNON. 1969. Susceptibility of two citrus rootstocks to *Pratylenchus* spp. Plant Dis. Rep. 53:603-607.
4. FORD, H. W. 1954. The influence of rootstock and tree age on root distribution of citrus. Proc. Amer. Soc. Hort. Sci. 63:137-142.
5. GOTAH, A. and Y. OHSHIMA. 1963. The root-lesion nematodes, *Pratylenchus* spp., and their geographical distribution in Japan (Nematoda: Tylenchida). Rev. Plant Prot. Res. 1:118-121.
6. HUNG, Y. P. 1969. Occurrence of plant parasitic nematodes in Taiwan citrus plantations. First Int. Citrus Symp. Proc. 2:1007-1011.
7. O'BANNON, J. H., J. D. RADEWALD and A. T. TOMERLIN. 1972. Population fluctuation of three parasitic nematodes in Florida citrus. J. Nematol. 4:194-199.
8. O'BANNON, J. H. and A. T. TOMERLIN. 1969. Population studies on two species of *Pratylenchus* on citrus. J. Nematol. 1:299-300 (Abstr.).
9. RADEWALD, J. D., J. H. O'BANNON and A. T. TOMERLIN. 1971. Temperature effects on reproduction and pathogenicity of *Pratylenchus coffeae* and *P. brachyurus* and survival of *P. coffeae* in roots of *Citrus jambhiri*. J. Nematol. 3:390-394.
10. SEINHORST, J. W. 1968. Population dynamics, its relation to ecology and its importance for research on the economic importance and control of plant parasitic nematodes. C. R. 8th Symp. Nematol., Antibes, 1965, 58-62.
11. SIDDIQI, M. R. 1964. Studies on nematode root-rot of citrus in Uttar Pradesh, India. Proc. Zool. Soc., Calcutta 17:67-75.
12. SUIT, R. F. and E. P. DU CHARME. 1953. The burrowing nematode and other parasitic nematodes in relation to spreading decline of citrus. Plant Dis. Rep. 37:379-383.
13. TARJAN, A. C. 1971. Migration of three pathogenic citrus nematodes through two Florida citrus soils. Proc. Soil Crop Sci. Soc. Fla. 31:253-255.
14. YOKOO, T. and Y. IKEGEMI. 1966. Some observations on growth of the new host plant, Snapdragon (*Antirrhinum majus* L.) attacked by the root lesion nematode, *Pratylenchus coffeae*, and control-effect of some nematicides. Agr. Bull. Saga Univ. 22:83-92.
15. YOUNG, T. W. 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Dis. Rep. 38:794-795.