Criconemoides xenoplax Experimentally Associated with a Disease of Peach

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ABSTRACT

Ring nematodes, *Criconemoides xenoplax*, added to 'Carolyn' peach on 'Lovell' rootstock at planting time, reduced growth of the trees and increased susceptibility to both *Pseudomonas syringae* and waterlogging. The full effect of the nematodes was evident only after three growing seasons. Addition of *Pythium* spp. at planting time increased susceptibility to *P. syringae* less than did inoculation with *C. xenoplax*. *Pythium* spp. did not reduce growth of peach trees significantly. No interaction between *C. xenoplax* and *Pythium* spp. was noted.

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The experiment described here was designed to learn more about the cause of bacterial canker and of peach decline, which are common and severe in the Central Valley of California (9), and the southeastern USA (7). Bacterial canker is first observed as a disease of the tree tops. Symptoms include cankers (which may exude gum), or rapid death of the entire top, accompanied by emission of a watery, fermenting liquid from branches (9). Roots often remain alive after the tops have died, and sprouts arise from the base of the trunk. *Pseudomonas syringae* can be isolated from affected tops, but the disease cannot be satisfactorily controlled by spraying the tops with bactericides (3). *Pythium* spp. are commonly recovered from around the roots of affected trees. Evidence from which the role of *Pythium* spp. might be deduced is conflicting (12, 22), and treatment of soil with fungicides active against *Pythium* has not prevented the disease (9, 11). Ring nematodes also commonly occur around affected trees (6, 9, 13), and soil treatment with nematicidal fumigants often prevents disease (6, 10, 11). This indicates that the ring nematodes may be weakening the trees and predisposing them to canker and decline. In previous tests (18, 19, 20) of the effect of *Criconemoides xenoplax* Raski on peach seedlings, no association between this nematode and disease was noted. However, those experiments were only 3 to 7 months in duration. In the field, bacterial canker and decline are not diseases of seedlings. Canker occurs most commonly on trees in their second to fourth growing season in the orchard. For this reason we conducted an extended experiment to measure effects of *P. syringae, Pythium* spp., and ring nematodes singly, and in combination, on peach trees.

MATERIALS AND METHODS.–In March 1970, *Prunus persica* (L.) Batsch 'Carolyn' trees on 'Lovell' rootstocks were planted in 15-liter cans containing steamed Madera sand from Merced County, California. At the time of planting, this soil was infested with either (i) 35,000 ring nematodes, (ii) three *Pythium* spp. (*P. debaryanum* Hesse, *P. irregulare* Buisman, and *P. ultimum* Trow), (iii) both ring nematodes and *Pythium* spp., or (iv) nothing. *Criconemoides xenoplax* was obtained from a Napa County, California vineyard soil chosen because it contained very few plant parasitic nematodes other than *C. xenoplax*. Jenkins' (14) method was used to recover the nematodes for inoculum and for later assays of population level. At 1- and 2-year intervals, nematode populations were estimated by recovery of nematodes from a composite of four probes to the bottom of each can. The final population was determined from a sample taken after mixing all the soil in the can. *Pythium* spp. were obtained from a Merced County, California peach orchard, and maintained several years on cornmeal agar. Before use in this experiment, each species was transferred to quart jars filled with a medium consisting of 250-ml Czapek-Dox broth per quart of vermiculite, and grown 1 week at 25 C. Twenty ml of infested medium for each *Pythium* species were added to the *Pythium*-infested treatments when peaches were planted. At the end of the experiment, probes were taken to the bottom of the cans, and samples of soil and roots were added to plates of Schmitthenner's (24) selective medium (E5S50) for *Pythium*.

Pseudomonas syringae, obtained from peach in Merced County, was maintained on nutrient agar. With the aid of a Klett-Summerson colorimeter, a suspension of *P. syringae* containing ca. 2×10^8 cells/ml was prepared from agar cultures 24 to 48 hr old, and used for inoculation. The colorimeter reading equivalent to this concentration had been determined previously by correlating colorimeter readings with cell concentrations determined by serial dilution. At the end of the experiment, peach branches were disinfested by a 1-min dip in 1% sodium hypochlorite, followed by two sterile-water rinses. Pieces of tissue were then transferred to King's medium B (17). Jones' (15) tests were used to distinguish *P. syringae* from the other fluorescent bacteria recovered.

Trees were arranged in randomized blocks in a lathhouse, with the cans sunk in a bed of wood shavings (Fig. 1). This insulated the soil in the containers from large temperature fluctuations, which are unfavorable to ring nematodes. The soil was kept moist, and the plants were fertilized every 2 weeks with an inorganic N, P, and K fertilizer.

Eight of the 16 replicates of each treatment were inoculated with *P. syringae* by stem injection in

January, 1971, and again by spraying leaf scars in November, 1971. Before inoculating by stem injection, a tangential tunnel, entirely through the bark, and adjacent to the cambium, was made with a dissecting needle. This hole was filled with an aqueous suspension of bacteria by means of a syringe. Inoculation by spraying was done when leaves were beginning to fall. Using a DeVilbiss atomizer ca. 50 μliters were sprayed around the point of leaf attachment. The leaf was then removed and another 50 μliters were applied.

"Student's" *t-test* was used to judge the probability (*P*) that observed differences were a result of chance.

RESULTS.–Populations of *C. xenoplax* increased six times in the first year, 60 times in 2 years, and then declined in the final 4 months (Table 1). The final decline was probably related to the nematode effect on root systems (Fig. 2). After 2 years, some noninoculated replicates had become contaminated with ring nematodes (Table 1), but the nematode population had not reached a high level in these replicates. Reductions in trunk diameter of 8, 13, and 20% after 1, 2, and 2.3 years, respectively, were associated with ring nematode infection. (*P* for comparisons at all three time periods was less than 1%). Final mean weight of peach roots inoculated with ring nematodes (211 ± 15 g) was conspicuously (Fig. 2) less ($P = < 0.01$) than that of trees not receiving nematodes (314 ± 17 g). Root systems supporting ring nematodes were practically devoid of feeder roots.

Fifteen of the cans inoculated with *Pythium* spp. were checked for presence of *Pythium* after 2 years. One or more of the *Pythium* spp. originally added was recovered from 14 of the 15 cans. The fungi *P. dissotocum* Drechsler and *P. paroecandrum* Drechsler, presumably from natural infestation, were also recovered from some cans. *Pythium ultimum* and *P. dissotocum* were also recovered from two of three cans which had not been inoculated with *Pythium* spp. No reduction in trunk diameter resulted from inoculation with the *Pythium* spp. Final mean weight of roots of *Pythium*-inoculated trees (249 ± 20 g) did not differ significantly from those of trees not inoculated with *Pythium* (270 ± 18 g), but there was slightly more necrosis in fine roots of *Pythium*-inoculated trees.

In the spring following the first inoculation with *P. syringae*, we found no statistically significant differences in lengths of cankers developing in trees with different soil infestations. However, two trees died of typical bacterial canker, and both of these were infested with ring nematodes. In the spring following the second bacterial inoculation, more cankers developed on trees growing in soil infested with ring nematodes or *Pythium* than on trees in noninfested soil (Table 2). These cankers ranged from small (1 to 8 mm) to large (32 to 193 mm) but there was little of the rapid canker development which kills trees in the field.

In July, 1972, leaf-drop suddenly appeared in many of the experimental trees, and some trees died. There was some tissue shrinkage, darkening, and gummosis associated with the dead and dying trees. We graded the trees on a scale of 0 (healthy) to 6 (dead), with intermediate grades representing degrees of leaf-drop and dieback. We found that all the necrosis and leaf-drop occurred in ring nematode-infected trees, whose mean grade was 2.7 ± 0.7. The mean grade for trees not inoculated with ring nematodes was 0.6 ± 0.2. When the trees were harvested, the roots of the trees parasitized by ring nematodes were as shown in Fig. 2. The most obvious characteristics of these root systems were lack of

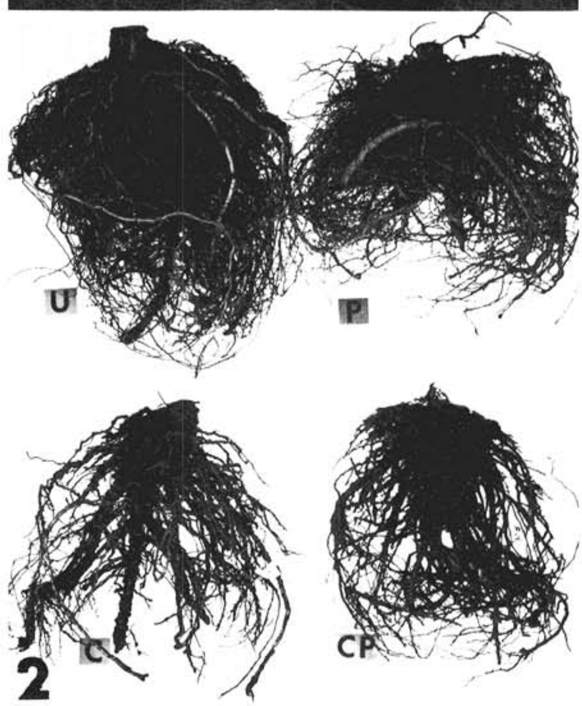

Fig. 1-2. 1) Experimental setup used for study of role of ring nematodes, *Criconemoides xenoplax*, *Pythium* spp., and *Pseudomonas syringae* in bacterial canker and decline of peach. **2)** 'Lovell' peach roots grown 2.3 years in steamed soil: uninoculated (U), inoculated with three *Pythium* spp. (P), 35,000 *C. xenoplax* (C), both *C. xenoplax* and *Pythium* spp. (CP). Roots photographed were those nearest the average weight for each treatment.

TABLE 1. Numbers of ring nematodes, *Criconemoides xenoplax*, at several time intervals after peach trees were inoculated with different combinations of organisms

Inoculum			Ring nematodes per replicate in thousands			
			Time after inoculation (yr)			
Ring nematodes	*Pythium* spp.	*Pseudomonas syringae*	0 (initial)	1	2	2.3 (final)
–[a]	–	–	0		0.8 ± 0.8	0.1 ± 0.1
–	–	+	0		0.5 ± 0.5	0 ± 0
+	–	–	35	202 ± 80	2,026 ± 377	386 ± 48
+	–	+	35	301 ± 77	1,891 ± 307	380 ± 97
–	+	–	0		4.5 ± 4.1	
–	+	+	0		1.8 ± 1.3	
+	+	–	35	188 ± 59	2,722 ± 618	1,185 ± 150
+	+	+	35	173 ± 103	1,936 ± 320	680 ± 131

[a] (+) indicates presence, and (–) indicates absence of the organism in the inoculum.

feeder roots and swollen lenticels on the larger roots. The enlarged lenticels were present on roots from all trees, but were particularly conspicuous on the trees parasitized by ring nematodes, partly because of the lack of feeder roots. At harvest, *P. syringae* was recovered from 2 of 14 trees originally inoculated with the bacterium, and from 0 of 14 noninoculated trees. None of our measurements indicated that ring nematodes and *Pythium* affected each other, or had a different effect in combination than singly on tree growth.

DISCUSSION.–The effects of *C. xenoplax* on peach trees in this experiment were much more serious than had been observed previously (18, 19, 20). We believe that the earlier failures of *C. xenoplax* to limit growth of peach in experiments occurred because of the short duration (less than a year) of the tests. In addition, the experimental conditions used in two of the earlier tests (pots on a greenhouse bench) were less favorable for the nematodes than the more natural conditions provided in this test.

The failure to isolate *P. syringae* from the inoculated trees at the end of the experiment was not unexpected. The attempt was made in July, and it is usually difficult to isolate this bacterium in midsummer.

We do not believe that the ring nematodes were the sole cause of the final rapid decline of the nematode-infected trees, and we do not think that the trees died of bacterial canker, despite the presence of some gummosis and dieback. The swollen root lenticels on these trees indicated oxygen deficiency, or waterlogging, of the soil (2). Peach is very susceptble to disease resulting from this condition (4, 5, 23), and the fact that the rapid decline of our trees occurred in midsummer is in agreement with the finding by Rowe & Catlin (23) that susceptibility increases with temperature. Top symptoms were similar to those which have been associated with waterlogging in peach (1). We had noticed that the nematode-infected trees required less frequent watering than did the other trees. Apparently nematode-infected trees received more water than the impaired root systems could absorb, causing an oxygen deficiency, which further limited the ability of the roots to supply water and nutrients to the tops (1), when midsummer needs were at a high level. Tensiometers were not used to guide watering.

In this experiment, the primary pathogen was probably *C. xenoplax*, with oxygen deficiency around the roots imposing a secondary, aggravating stress. We

TABLE 2. Numbers of infected leaf scars[a] on peach trees grown in steamed soil infested with *Criconemoides xenoplax*, *Pythium* spp., both, or neither, at five intervals after inoculation with *P. syringae*

	Soil infested with			
Weeks after inoculation	*C. xenoplax*	*Pythium* spp.	*C. xenoplax* and *Pythium* spp.	Nothing
1	7.9 ± 0.7[b]	7.0 ± 0.7[c]	7.3 ± 0.4[b]	4.7 ± 0.6
4	7.4 ± 0.6[b]	7.4 ± 0.5[b]	6.6 ± 0.5[c]	4.9 ± 0.4
9	7.8 ± 0.4[b]	5.9 ± 0.9	7.0 ± 0.4[b]	4.3 ± 0.8
14	6.0 ± 0.6[b]	5.0 ± 0.8	5.1 ± 0.6	3.5 ± 0.5
18	7.4 ± 0.6[b]	6.4 ± 0.7[b]	5.7 ± 0.8	4.0 ± 0.3

[a] Means of seven replicates, each with 10 subreplicates.
[b] Higher than noninfested ($P = < .01$).
[c] Higher than noninfested ($P = < .05$).

believe that the kind of disease with which this nematode is associated in the field will vary with the kind of secondary stress to which trees are exposed.

Susceptibility of plants to bacterial disease can be influenced by a wide variety of conditions; e.g., nutrition (16), presence of other pathogens (21), temperature, growth regulators (8), and date of pruning (7). The field association of *C. xenoplax* with bacterial canker, the control of canker with nematicidal fumigants, and the increased susceptibility to infection by *P. syringae* of nematode-infected trees in this experiment, lead us to believe that bacterial canker is one kind of secondary stress placed on ring nematode-infected trees in the orchard, and that ring nematodes are one of the agents predisposing peach trees to bacterial canker.

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