Ring Nematodes Increase Development of Bacterial Cankers in Plums

H. Mojtahedi, B. F. Lownsbery, and E. H. Moody

Department of Nematology, University of California, Davis 95616.

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ABSTRACT

In a lathhouse experiment, cankers developed on branches of Marianna 2624 plum trees injected with an aqueous suspension of the bacterium, *Pseudomonas syringae*. Cankers did not develop on control trees injected with sterile water. More extensive cankers developed on trees whose roots were infected with the ring nematode, *Criconemoides xenoplax*, than on control trees not infected by nematodes. *P. syringae* was isolated from the injection site and from necrotic tissue associated with the injection, but not outside the necrotic tissue, or from sites injected with water.

The nematode-infected trees, which were more susceptible to the bacteria, differed from the nematode-free control trees in other respects. Water stress was greater and nutrient levels lower in their leaves. They were smaller, lacked feeder roots, and suffered from waterlogging.

Progression of cankers to death of tops, as often occurs in the field, occurred in our experiment in only two trees, both inoculated with *C. xenoplax* and *P. syringae*. This indicates that additional factors are essential to full development of this disease.

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The ring nematode, *Criconemoides xenoplax* Raski occurs in all four of the important prune-growing districts in California (17). There is experimental evidence that this nematode is a root pathogen of prune and plum rootstocks (20).

Bacterial canker, a serious disease of the tops of prune trees in California (21), is believed to be incited by certain strains (22) of *Pseudomonas syringae* van Hall. Susceptibility to this bacterium is influenced by environmental factors (6, 7, 21), and physiological condition of the host plant (4, 9, 10). There is some evidence (16, 24) that *C. xenoplax* influences susceptibility of peach trees to bacterial canker.

The experiment described here was designed to test the effect of root damage by *C. xenoplax* on susceptibility of Marianna 2624 plum to bacterial canker. This plum, propagated vegetatively as a rootstock for prunes, is generally supposed to be the result of an open-pollinated cross between *Prunus cerasifera* Ehrh. and a native Texas *Prunus* species, possibly *Prunus munsoniana* Wight and Hedr. (8).

MATERIALS AND METHODS.—Marianna 2624 plants were propagated as described previously (20) and transplanted into 15-liter cans of steamed sandy loam (3) soil from a Meridian, California prune orchard. At the time of transplanting, the soil was infested with 10^3, 10^4, or 10^5 *C. xenoplax* by pouring 150 ml of tap water containing the nematodes over the roots. Controls received 150 ml of tap water. Plants were arranged in 10 randomized blocks in a lathhouse with cans sunk in a bed of wood shavings to protect nematodes from adverse environmental effects (19), and fertilized monthly with one-half strength Hoagland's solution (12).

Two isolates of *P. syringae* (B-3 and GS 28-16) were

supplied by W. H. English. Five of the 10 replicates of each treatment were inoculated with *P. syringae* by spraying leaf scars (16) with a suspension of isolate B-3 containing 10^8 cells/ml in October, 1972, and again in October, 1973. In January, 1974, these same five replicates were again inoculated with suspensions of this same concentration of each isolate of *P. syringae* by a stem-injection procedure (16). These injections were made into both 1- and 2-year-old branches on each replicate. At each inoculation time, the remaining five replicates were injected with sterile water. Trees receiving bacteria were separated from those receiving sterile water by a polyethylene partition. The experiment was terminated after 2 years. Extent of spread of the bacteria in the branches was determined at this time. Ten-cm lengths of the branches were disinfested by a 3-minute dip in 1% sodium hypochlorite, and pieces of tissue, taken aseptically from beneath the bark, were transferred to King's medium B (15). An oxidase test was also used to distinguish pathogenic pseudomonads from saprophytic ones (18).

Jenkins' method (13) was used to obtain nematode inoculum from plum cultures, and for assays of population levels at 6-month intervals. The final population level was determined from a sample taken after mixing all the soil in the can. Preharvest population levels were determined from a composite of three probes to the bottom of each replicate.

After 18 months, water stress in leaves was measured using a pressure chamber technique (23) and leaf nitrogen, phosphorus, and potassium content was determined (5, 14). In addition, the ability of the test plants to extract water from the soil was measured by soaking the sandy soil with water and determining water content of soil from the bottom of the cans after 48 hours.

For paired treatments, "Student's" *t*-test was used to judge the probability (*P*) that observed differences were a result of chance. Where more than two treatments were compared, Duncan's multiple range test was used.

RESULTS AND CONCLUSIONS.—Injection of either strain of *P. syringae* into the plum branches resulted in the formation of small cankers. Injection of sterile water only caused discoloration and callus formation at the immediate injection site. Cankers were easily seen on the surface of 1-year-old branches, and these superficial symptoms could be graded as to degree of severity (Table 1). Few superficial symptoms resulted from injection of 2-year-old branches. When longitudinal cuts were made into these branches however, dark, necrotic xylem tissue could be seen extending from the injection point. After cutting longitudinally through all injection sites on either 1- or 2-year-old branches it was possible to measure the extent of necrosis (Table 2). The superficial symptoms on 1-year-old branches and the extent of necrosis in the wood of both ages were greatest in trees whose roots had been inoculated with nematodes, and whose tops had been inoculated with bacteria.

P. syringae was isolated from the injection site and throughout the necrotic tissue, but could not be isolated from tissue beyond the necrosis, or from sites injected with water. These results support the view (7) that *P. syringae* is not systemic in the plant.

By the end of the experiment, the average population of *C. xenoplax* in nematode-inoculated trees was between one and two million per replicate. After 18 months, some of the uninoculated controls had become contaminated with *C. xenoplax*, but these contained less than 1,000 nematodes when the experiment was terminated.

Some of the differences between nematode-infected plants and nematode-free plants which we observed may be related to the difference in susceptibility to bacterial canker. *C. xenoplax* destroyed feeder roots (Fig. 1), producing trees with high top/root ratios (Table 3). The reduction in root surface reduced the ability of the root

TABLE 1. Canker ratings at sites of winter injection of *Pseudomonas syringae* into 1-year-old branches of Marianna 2624 plum trees whose roots had been inoculated 2 years earlier with *Criconemoides xenoplax*

Bacterial isolate	Canker rating[a]			
	No nemas	10^3 nemas	10^4 nemas	10^5 nemas
B-3	2.3±.37 V	3.4±.14 W	3.9±.17 W	3.4±.34 WY
GS 28-16	1.2±.14 VZ	2.8±.07 XY	2.0±.24 XY	2.5±.28 Y
Water	1.1±.01 Z	1.0±.06 Z	1.1±.14 Z	1.1±.06 Z

[a]Rated as follows: 1 = no necrosis at injection site; 2 = injection point blackened; 3 = injection point blackened, water-soaked, and sunken; 4 = canker about 10 mm long around injection point; 5 = canker longer than 10 mm around injection point. Ratings are averages of 15 readings and standard error. Averages not followed by the same letter in each row or column differ ($P = < 0.01$).

TABLE 2. Extent of xylem necrosis following winter injection of *Pseudomonas syringae* or sterile water into branches of Marianna 2624 plum trees whose roots had been inoculated 2 years earlier with *Criconemoides xenoplax*

C. xenoplax (no.)	Extent of xylem necrosis (cm)	
	Sterile water[a]	*P. syringae*[b]
0	0	5 ± 0.7 x
10^3	0	9 ± 1.0 y
10^4	0	12 ± 2.2 y
10^5	0	14 ± 3.9 y

[a]Average of five single-tree replicates, each replicate the average of the readings from five injection sites per tree.

[b]Average of five single-tree replicates, each replicate the average of the readings from 10 injection sites per tree–two bacterial strains × five injection sites (three in 1-year-old wood, and two in 2-year-old wood); averages not followed by the same letter differ ($P = \leqslant 0.05$).

TABLE 3. Fresh plant weight, top/root ratios, and moisture content[a] of soil around roots of Marianna 2624 plum trees grown 2 years after inoculation with *Criconemoides xenoplax*

C. xenoplax	Plant weight[b]	Top/root ratio[b]	Soil moisture[b] (%)
0	1,129 ± 49 X	2.64 ± .15 x	5.1 ± 0.2 x
10^3	1,131 ± 48 X	3.02 ± .09 y	14.9 ± 0.9 y
10^4	970 ± 54 Y	3.04 ± .18 y	16.1 ± 0.5 y
10^5	788 ± 79 Z	3.03 ± .16 y	17.1 ± 1.1 y

[a]The % moisture of this soil at field capacity was 14.5.

[b]Average of 10 replicates and standard error. Averages not followed by the same letter differ ($P = \leqslant 0.01$, capital letter; or $P = \leqslant 0.05$, small letter). All replicates were flooded 48 hours before the soil moisture determination.

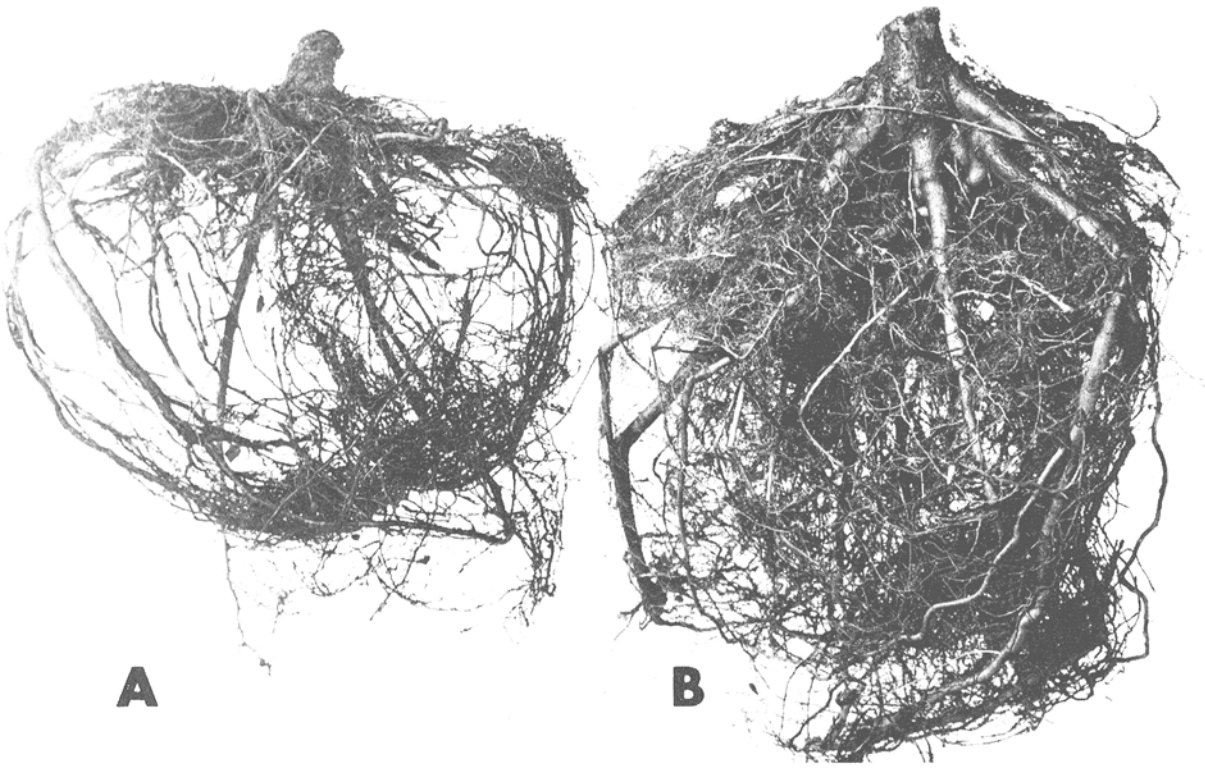

Fig. 1-(A, B). Roots of Marianna 2624 plum trees 2 years after inoculation with **A)** 150 ml of a tap water containing 10^5 *Criconemoides xenoplax*, or **B)** 150 ml of tap water.

TABLE 4. Nutrient levels in leaves 18 months after inoculation of Marianna 2624 plum roots with *Criconemoides xenoplax*

	Nutrient levels (%)[a]		
C. xenoplax	N	P	K
0	1.25 ± .05 X	.16 ± .01 X	3.60 ± .16 X
10^3	1.03 ± .03 Y	.17 ± .01 X	3.10 ± .19 XY
10^4	.96 ± .03 Y	.11 ± .01 Y	2.82 ± .14 Y
10^5	.95 ± .04 Y	.11 ± .01 Y	2.25 ± .16 Z

[a]Average of 10 replicates and standard error; averages not followed by the same letter differ ($P = \leqslant 0.01$).

TABLE 5. Water stress in leaves of Marianna 2624 plum trees 18 months after addition of tap water, or tap water containing 10^5 *Criconemoides xenoplax* to root systems

	Water stress (atm)[a]	
Inoculum	1 AM	1 PM
Tap water	9.95 ± 0.34 X	14.30 ± 0.82 X
10^5 *C. xenoplax*	10.10 ± 0.84 X	19.50 ± 1.36 Y

[a]Average of 10 replicates and standard error; averages not followed by the same letter differ ($P = \leqslant 0.01$).

system to absorb nutrients and water. This lowered nutrient levels in leaves of trees infected with *C. xenoplax* (Table 4) and increased soil moisture around their roots (Table 3), giving rise to the oxygen deficiency symptoms known as waterlogging (1). Water stress was higher in leaves of nematode-infected plants than in uninfected plants (Table 5).

Earlier field and experimental observations (11, 25) suggested that nutrition and soil moisture influenced the course of bacterial canker to some extent. Moisture content of plant tissue may be influential also. High moisture content in the bark of willow and cottonwood inhibits canker development by certain fungi (2).

In the field, bacterial cankers often enlarge rapidly until much or all of the top of the tree is killed. For unknown reasons, this rarely happens at Davis, California, where our experiment was conducted (21). In our experiment, this rapid development occurred in only two trees, both inoculated with *C. xenoplax* and *P. syringae*. Because this happened in only two of the ten replicates inoculated with both organisms, we concluded that factors other than these organisms are essential to pathogenesis.

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