Effect of Ring and Pin Nematodes on the Development of Bacterial Canker and Cytospora Canker in Young French Prune Trees

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ABSTRACT

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The ring nematode, *Macroposthonia xenoplax*, decreased the growth of young French prune trees on Myrobalan 29C rootstock and increased their susceptibility to both bacterial canker (*Pseudomonas syringae*) and Cytospora canker (*Cytospora leucostoma*). Under similar conditions, the pin nematode, *Paratylenchus neoamblycephalus*, failed to inhibit tree growth or to increase susceptibility of the trees to either canker disease. The populations of both nematodes increased substantially during the experiment.

Bacterial canker (*Pseudomonas syringae* van Hall) and Cytospora canker (*Cytospora leucostoma* Sacc.) are two of the more serious diseases of prune (*Prunus domestica* L. 'French') in California. Soil factors such as texture, moisture, pH, nutrient status, and the presence of ring nematodes (*Macroposthonia xenoplax* (Raski) Loof and DeGrisse) have been reported to influence the incidence and severity of bacterial canker in stone-fruit trees (5,6,11,14). The development of Cytospora canker in prune trees has been associated with moisture stress and with soils high in clay content or unable to supply adequate potassium (2,3). Furthermore, Bertrand (1) presented evidence suggesting that the plant-parasitic nematodes *M. xenoplax* and *Paratylenchus neoamblycephalus* Geraert are implicated in the development of prune Cytospora canker. Both nematodes are common in prune orchard soils in California (12), and both are known pathogens of stone-fruit trees (4,12,13).

The present study was undertaken to provide information on the possible relation of ring (*M. xenoplax*) and pin nematodes (*P. neoamblycephalus*) to bacterial canker and Cytospora canker of French prune. An abstract covering a portion of this research has been published (6).

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MATERIALS AND METHODS

Experiment with *M. xenoplax*. Nursery-grown French prune trees on Myrobalan 29C (*Prunus cerasifera* Ehrh.) rootstock were planted in 12-L cans of autoclaved sand (91% sand, 7% clay, and 2% silt) on 30 January 1975. At planting, soil around the roots in each of 24 cans was inoculated with 20,000 *M. xenoplax*. A comparable group of 24 trees was not inoculated with nematodes. The *M. xenoplax* was originally obtained from a Sutter County, CA, prune orchard, and monocultures were increased on Myrobalan plum. The 48 planted cans were sunk in beds of wood shavings in a screenhouse at Davis, CA. The nematode-infested and uninfested cans were arranged in paired beds, and the trees were watered and fertilized in a uniform manner.

The bacterial inoculum used was our B-3 strain of *Pseudomonas syringae* originally isolated from an infected peach tree in Merced County, CA. A suspension containing 1×10^8 cells per milliliter was used either in spray inoculations (18 November 1976) onto the leaf scars or hypodermic injections (22 December 1976) into the bark of the trunk or branches. Inoculum preparation and inoculation procedures were similar to those previously described (10). Six trees in both nematode-infested and uninfested soil were used with each type of inoculation. Ten hypodermic inoculations were made into each tree. Comparable control inoculations were made with sterile distilled water. The lengths of the cankers and discolored xylem were recorded on 4 May 1977.

Inoculations with *C. leucostoma* were made on 13 December 1976 with culture F40, which was originally isolated from President plum. Inoculum consisted of 5-mm disks cut from near the margin of actively growing colonies on potato-dextrose agar. The trunk or scaffold branches were wounded by means of a 5-mm-diameter cork borer, the bark of the circumscribed area raised, and a disk of inoculum inserted. The inoculation site was covered with plastic film secured with adhesive tape. Five inoculations were made on each of six trees in both nematode-infested and uninfested soil. Comparable check inoculations were made with sterile potato-dextrose agar. Canker lengths were measured 5 May and 18 August 1977.

When the experiment was ended in summer 1977, sand was shaken from the

roots and fresh tree weights were obtained. The sand in each replicate was mixed for homogeneity, and *M. xenoplax* was extracted from 250 cc and counted. The Jenkins (9) extraction method was used to obtain nematode inoculum and to assay nematode population levels at the end of the experiment.

Experiment with *P. neoamblycephalus*. This test was started about a year later than the experiment using *M. xenoplax*. Twenty-thousand *P. neoamblycephalus* were added to each of 24 trees at planting (17 February 1976). An equal number of trees received no nematodes. The monoculture of *P. neoamblycephalus* was obtained from a Napa Valley, CA, prune orchard and was increased on Myrobalan plum. The growing medium was a silt loam (29% sand, 15% clay, and 56% silt).

As in the experiment with *M. xenoplax*, trees were both spray-inoculated (22 November 1976) and injection-inoculated (23 December 1976) with suspensions of *Pseudomonas syringae*. Trees were also inoculated (10 December 1976) with *C. leucostoma*. Cankers induced by *Pseudomonas syringae* and *C. leucostoma* were measured on 4 May 1977 and 18 August 1977, respectively.

In all other methodology, this experiment was similar to the one with *M. xenoplax* described above.

RESULTS

Insufficient leaf-scar infection to provide worthwhile information resulted from the spray inoculations with *Pseudomonas syringae* in both the *M. xenoplax* and *P. neoamblycephalus* tests. The hypodermic injections, however, resulted in a high incidence of infection and the development of measurable cankers in both tests.

M. xenoplax reduced fresh weight of the trees and increased their susceptibility to bacterial canker (Table 1). Canker length, as expressed in terms of either bark or xylem necrosis, was greater in trees grown in soil infested with this nematode than in those grown in uninfested soil. The population of *M. xenoplax* in the infested soil increased about 40-fold during the 30-mo period of the experiment. This nematode also increased the susceptibility of French prune trees to Cytospora canker, as evidenced by measurements taken about 5 and 8 mo after inoculation (Table 2).

Contrary to the results obtained with *M. xenoplax*, *P. neoamblycephalus* neither decreased tree growth nor increased tree susceptibility to either bacterial canker or Cytospora canker. This lack of effect, however, was not caused by the failure of *P. neoamblycephalus* to increase its numbers markedly during the 18-mo experiment. There was, in fact, an approximate 18-fold increase in the population of this nematode during the test.

DISCUSSION

The reduction in growth of French prune on Myrobalan 29C rootstock in soil infested with *M. xenoplax* is not surprising. Earlier studies have shown that the growth of peach (11), Myrobalan plum (4), and Marianna 2624 plum (13) is adversely affected by this nematode. The ability of *M. xenoplax* to increase the susceptibility of French prune to bacterial canker is consistent with results of previous experiments with peach (11) and Marianna plum (13). Our data also provide at least a partial explanation for the efficacy of nematicidal soil fumigation in the field control of this disease (6).

Although reports have suggested that certain plant-parasitic nematodes increase the severity of Cytospora canker in trees (1,7), our research with *M. xenoplax* provides the first experimental evidence for such a relationship. Because a number of stress factors are known to increase the susceptibility of fruit trees to Cytospora canker (1–3,8), it is not surprising that a root-feeding nematode also should have this effect. Although this nematode is known to occur commonly in California prune orchards (12), especially in sandier soils, its importance in the development of Cytospora canker in the field has not been thoroughly investigated.

The inability of *P. neoamblycephalus* to affect adversely the growth of French prune trees on Myrobalan 29C rootstock was surprising in view of the marked increase in nematode populations during the test and the reported susceptibility of Myrobalan seedlings to this organism (4). It is possible that some other condition may have limited growth of trees in this experiment. They were in relatively heavy soil, and inadequate drainage was sometimes observed. It is also possible that the Myrobalan 29C selection was more resistant than Myrobalan seedlings to this nematode. Because *P. neoamblycephalus* did not adversely affect tree growth, it is not surprising that this nematode had no effect on bacterial canker or Cytospora canker.

Table 1. Effect of *Macroposthonia xenoplax* on the growth of French prune trees and on their susceptibility to bacterial canker

Soil inoculation	Tree inoculation[v]	Fresh tree wt (g)[w,x]	Mean canker length (mm)[x,y]			*M. xenoplax* per 250 cc of soil (mean no.)[w]
			Bark	Outer xylem	Inner xylem	
M. xenoplax	*P. syringae*	1,056 a	20.1 a	14.0 a	21.3 a	17,700
None	*P. syringae*	...[z]	7.6 b	3.1 b	15.2 b	...
M. xenoplax	None	1,221 a	0.0 c	0.7 b	4.9 c	15,512
None	None	1,571 b	0.0 c	0.7 b	4.9 c	4

[v] Trees were inoculated with *Pseudomonas syringae* on 22 December 1976.
[w] Data taken when the experiment was terminated in summer 1977. The initial nematode population was 417/250 cc of soil.
[x] Within each column, means followed by different letters differed significantly at the 5% level according to Duncan's multiple range test.
[y] Lengths of bark canker and xylem discoloration were recorded 4 May 1977.
[z] Missing data.

Table 2. Effect of *Macroposthonia xenoplax* on the susceptibility of French prune trees to Cytospora canker

Soil inoculation	Tree inoculation[a]	Mean canker length (mm)[b]		*M. xenoplax* per 250 cc of soil (mean no.)[c]
		5 May 77	18 August 77	
M. xenoplax	*C. leucostoma*	40.3 x	65.2 x	...[d]
None	*C. leucostoma*	32.8 y	55.0 y	...
M. xenoplax	None	0.0 z	0.0 z	15,512
None	None	0.0 z	0.0 z	4

[a] Trees were inoculated with *Cytospora leucostoma* on 13 December 1976.
[b] Within each column, means followed by different letters differed significantly in both May ($P = 0.01$) and August ($P = 0.05$) according to Duncan's multiple range test.
[c] Data taken when the experiment was terminated in summer 1977. The initial nematode population was 417/250 cc of soil.
[d] Missing data.

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