# Pathogenicity of Criconemoides xenoplax to Prune and Plum Rootstocks[1]

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*Abstract:* Elimination of *Criconemoides xenoplax* from a prune orchard soil by fumigation with ethylene dibromide at the rate of 42 μliter/liter of soil (equivalent to about 13 gal/acre) improved the growth of 'Myrobalan' plum. Addition of this nematode to Myrobalan seedlings or young 'Marianna 2624' plants propagated from cuttings resulted in destruction of cortical root tissue, darkening of roots, alteration of water stress, lowering of nutrient levels in leaves, and reduction in plant weight. *C. xenoplax* increased on all nine *Prunus cerasifera* varieties and hybrids tested, including those used commonly as rootstocks for prunes and plums. *Rhizoctonia solani* isolated from Myrobalan seedlings infected with *C. xenoplax* caused lesions on the hypocotyls of young Myrobalan seedlings in the laboratory, but had no effect on older seedlings in the greenhouse, and did not alter the effect of *C. xenoplax*. *Key Words:* soil fumigation, host range, *Rhizoctonia solani, Prunus persica.*

The ring nematode, *Criconemoides xenoplax* Raski, was originally described from specimens collected in a California vineyard (20). This nematode has been associated with poor growth of grape (14), peach (1, 16, 24), cherry (23), cranberry (2), and spruce (8). Ring nematodes, predominantly *C. xenoplax,* were found in 38% of California prune orchards, and in all four of the important prune-growing districts (17). Four experiments, described here, were conducted to determine the effect of *C. xenoplax* on rootstocks for prunes. A fifth experiment tested the host range of this nematode among *Prunus* species, and among varieties and hybrids of *Prunus cerasifera* Ehrh. which might be considered as rootstocks for prunes.

## MATERIALS AND METHODS

*General:* A loamy sand (4) from a prune orchard in Meridian, California, was used in the first four experiments, and another loamy sand from the banks of the American River at Sacramento, California, was used in the host range experiment. With the exception of the first experiment involving soil fumigation, the soil was steam-sterilized at one atmosphere (15 psi) for 3 h, and aerated at least 1 mo before use.

Seeds used in propagation of 'Myrobalan' plum (*P. cerasifera* Ehrh) and 'Lovell' peach (*Prunus persica* Batsch) were coated with thiram and kept wet at 5 C in cotton-plugged flasks until germination. Germinated seeds were grown 2 wk in a heated bed of sterilized sand, and 2 more wk in steam-sterilized soil before use in experiments. 'Myrobalan' seeds were obtained from Herbst Brothers, Brewster, N.Y. 'Lovell' peach seeds and cuttings of the other plants tested were obtained from Pomology Department plantings at the University of California, Davis. The cuttings were wounded and coated with a powder containing 1% indolebutyric acid, stratified in moist peat moss at room temperature until rooted, and then planted in steam-sterilized soil. Experimental containers were 12-liter cans in the first experiment, and 15- or 20-cm clay pots in all other experiments. Containers were immersed in wood shavings up to their collars to protect the nematodes from unfavorably high temperature. Plants were fertilized with half-strength Hoagland's solution (11) once a mo.

In the first experiment the *C. xenoplax* was from the Meridian, California, prune orchard. In the second, third, and fourth experiments we obtained *C. xenoplax* from a Rutherford, California prune orchard. For the fifth experiment, we used *C. xenoplax* obtained from a Rutherford, California, vineyard and maintained at Davis, California, on peach.

The Jenkins method (12) was used to obtain nematode inoculum and to assay nematode populations, except in the first experiment in which the Cobb method (6) was used. Before final population assay, the soil in each pot was mixed to obtain homogeneity and then nematodes were extracted from a 250-cc sample. Also, each root system was agitated in water, and the nematodes in a portion of this water were counted. From these samples, the

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total number of nematodes per replicate was calculated.

Student's *t*-test was used to evaluate differences between treatments.

*First experiment:* For this test of the effect of elimination of *C. xenoplax* from soil by fumigation, the infested soil was mixed thoroughly and divided into two portions. One portion was placed nontreated into 12-liter cans and kept moist. The other portion was fumigated with ethylene dibromide at the rate of 42 μliter/liter of soil (equivalent to about 13 gal/acre), aerated, and transferred to 12-liter cans. In April, 1 mo after the fumigation treatment, 'Myrobalan 29C' cuttings were planted in 14 cans of treated soil and 14 cans of nontreated soil, paired on a greenhouse floor. In October, the cans were embedded in wood shavings in a lathhouse so that the plants could go through the normal dormant cycle. After 16 mo of growth, the plants were weighed and nematode populations assayed.

*Second experiment:* The effects of a tap water suspension of 20,000 *C. xenoplax,* the same suspension with *C. xenoplax* removed (*C. xenoplax* was removed by seven passages through a sieve with 43-μm openings), and tap water alone on growth of Myrobalan seedlings were compared. The three kinds of inocula were added to the root zone of 2-mo-old Myrobalan seedlings when these were transplanted to the experimental containers in the greenhouse. After 11 wk, the plants were weighed, leaves were analyzed for N (5), P, and K (13), and nematode populations were determined.

*Third experiment:* Twenty thousand surface-sterilized *C. xenoplax* were added to rooted cuttings of 'Marianna 2624' plum. This plum, used commercially as a rootstock, is generally supposed to be an open-pollinated cross between *Prunus cerasifera* and a native Texas *Prunus* species, possibly *Prunus munsoniana* Wight and Hedr. (7). Control pots received equal volumes of sterile, distilled water.

*C. xenoplax* was surface-sterilized by washing once with a solution (2,000 μliter/liter) of zephiran chloride (benzalkonium chloride 17%), and three times with a solution (8,000 μg/ml) of 74% dihydrostreptomycin sulfate. Centrifugation was used to recover the nematodes after each wash. They were then shaken for 6 h in the dihydrostreptomycin sulfate, and washed three times in sterile water.

After 4 mo, plants were weighed, leaves were analyzed for N (5), P, and K (13), and nematode populations were assayed.

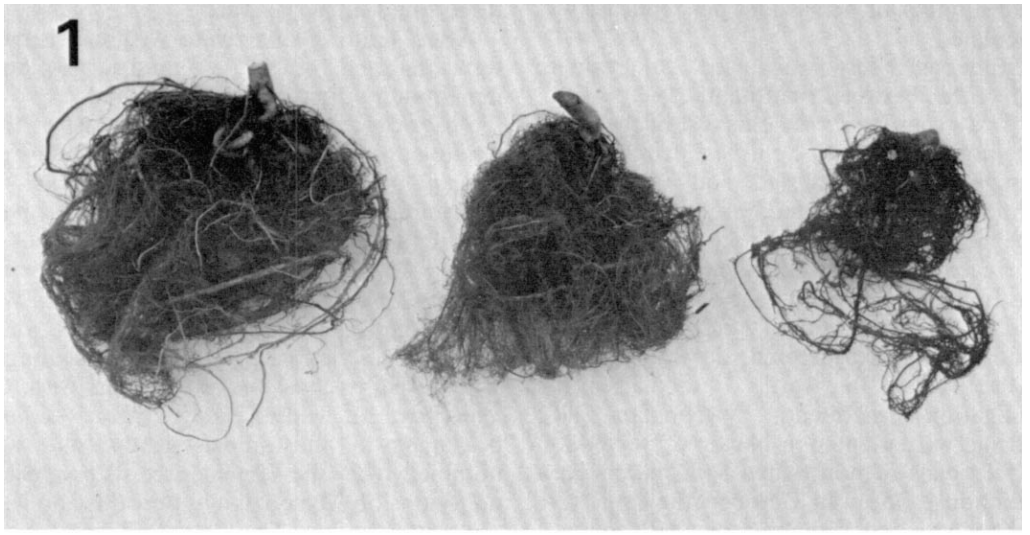
*Fourth experiment:* This was a test of the possible role of a brown (melanized) isolate of *Rhizoctonia solani* Kuhn belonging to anastomosis group two (19) which we had isolated using Bolkan and Butler's method (3) from Myrobalan roots infected with *C. xenoplax* in the second experiment. We found that this isolate would infect and cause lesions on cotyledons of Myrobalan seedlings, and tested its possible interaction with *C. xenoplax.* A white isolate of *R. solani* belonging to anastomosis group four was obtained from the same source. It produced no lesions on Myrobalan hypocotyls and was not tested further. The melanized isolate was cultured 10 days in a medium composed of 250 ml potato-dextrose broth in 500 g vermiculite. Then the colonized vermiculite was washed to remove nondigested nutrients (18) before use as inoculum.

Two-mo-old Myrobalan seedlings received 150 g of *R. solani*-infested vermiculite, 20,000 *C. xenoplax* plus the fungus-infested vermiculite, 20,000 *C. xenoplax* plus 150 g of uninfested vermiculite, or simply 150 g of uninfested vermiculite. One hundred ml of tap water was included in all treatments. The morning and afternoon water status of leaves of all plants was determined at three intervals after inoculation, using a pressure-chamber technique (21).

*Fifth experiment:* The host range of *C. xenoplax* was tested on several species and hybrids of *Prunus* by adding 5,000 *C. xenoplax* to each test plant and to fallow pots, and determining the number of nematodes after 9 mo.

## RESULTS AND DISCUSSION

*First experiment:* Treatment of the *C. xenoplax*-infested soil with ethylene dibromide reduced the number of those nematodes from 11,836 ± 1,584 to zero/replicate. Differences in plant growth were noted after 1 yr. Plum trees growing in treated soil were taller ($P = < 0.05$) than those in nontreated soil. When the experiment was terminated after 16 mo, plants from treated soil weighed 287 ± 13 g, and the untreated controls weighed 174 ± 7 g ($P = < 0.01$). Root systems from nontreated soil were smaller,



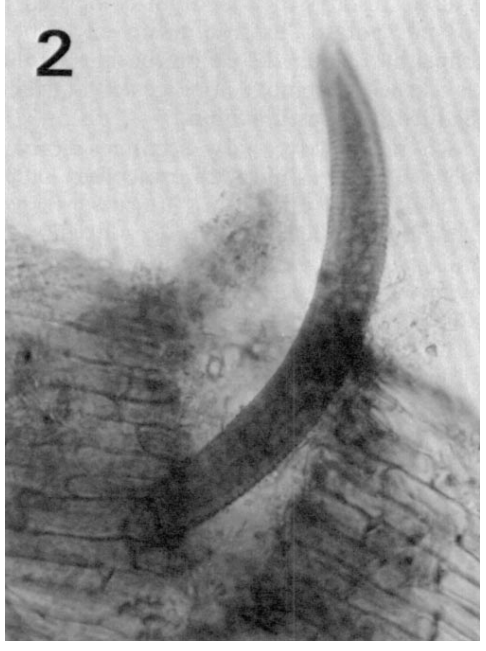




FIG. 1-2. **1)** Roots of Myrobalan plum seedlings treated as follows and grown for 11 weeks after treatment: left, tap water; center, suspension of *Criconemoides xenoplax* from which the nematodes had been removed; right, suspension of 20,000 *C. xenoplax*. **2)** *Criconemoides xenoplax* feeding on cortical tissues of Myrobalan plum root.

TABLE 1. Fresh plant weight, nutrient levels in leaves, and number of *Criconemoides xenoplax* per gram of root 11 weeks after inoculation of 'Myrobalan' plum seedlings with an aqueous suspension of 20,000 *C. xenoplax*, the same aqueous suspension with nematodes removed, or tap water.

| Treatment | Plant wt[a] (g) | Nutrient levels in leaves (%)[b] N | P | K | *C. xenoplax* per g root |
|---|---|---|---|---|---|
| 20,000 *C. xenoplax* | 13.2 ± 2.43 Y[c] | 1.64 ± .087 X | .100 ± .005 Y | 2.53 ± .202 Y y | 15,603 ± 3,125 |
| *C. xenoplax* removed | 30.7 ± 2.76 X | 1.73 ± .094 X | .163 ± .006 X | 3.33 ± .088 XY x | 0 |
| Tap water | 32.8 ± 1.69 X | 1.71 ± .037 X | .183 ± .008 X | 3.50 ± .057 X | 0 |

[a]Average of nine single seedling replicates and standard error.
[b]Average of three replicates, each a composite of leaves from three seedlings.
[c]Averages not followed by the same letter differ ($P = < 0.01$, capital letter; or $P = < 0.05$, small letter).

TABLE 2. Fresh plant weight, nutrient levels in leaves, and number of *Criconemoides xenoplax* per gram of root 4 months after inoculation of 'Marianna 2624' plum cuttings with an aqueous suspension of 20,000 surface-sterilized *C. xenoplax*, or sterile tap water.

| Treatment | Plant wt[a] (g) | Nutrient levels in leaves (%)[a] N | P | K | *C. xenoplax* per g root |
|---|---|---|---|---|---|
| Tap water | 84.9 ± 7.9 x | 1.34 ± .017 X | .140 ± .008 X | 2.46 ± .037 X[b] | 0 |
| *C. xenoplax* | 53.3 ± 4.6 y | .96 ± .030 Y | .077 ± .007 Y | 1.82 ± .059 Y | 11,830 ± 1,964 |

[a]Average of four replicates, and standard error.
[b]Averages not followed by the same letter differ ($P = < 0.01$, capital letter; or $P = < 0.05$, small letter).

TABLE 3. Root weight of 'Myrobalan' plum seedlings and numbers of *Criconemoides xenoplax* 18 weeks after inoculation with this nematode, melanized *Rhizoctonia solani*, both the nematode and the fungus, or neither.

| Inoculum | Root wt (g)[a] | Number of *C. xenoplax*/pot[a] |
|---|---|---|
| 20,000 *C. xenoplax* | 8.8 ± 0.8 Y | 1,059,870 ± 222,580 Y |
| *R. solani* | 22.7 ± 2.9 Z | 0 ± 0 Z |
| 20,000 *C. xenoplax* + *R. solani* | 10.5 ± 1.5 Y | 823,600 ± 80,090 Y |
| Tap water control | 17.5 ± 1.5 Z | 0 + 0 Z |

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

and had fewer feeder roots than those from treated soil. At the end of the experiment, *C. xenoplax* had reached a level of 133,637 ± 7,339 per can in untreated soil, and was absent from the treated soil. Thus, control of *C. xenoplax* with ethylene dibromide was associated with improved growth of Myrobalan 29 C. This chemical has effects other than nematicidal, but at the dosage used here it is most effective as a nematicide (25).

*Second experiment:* Inoculation of Myrobalan plum with a suspension of 20,000 *C. xenoplax* reduced the fresh weight of plants and levels of phosphorus and potassium in leaves (Table 1). Nematode-infected roots were generally dark and devoid of feeder roots (Fig. 1), and cortical tissue was partially disintegrated and separated from the stele. Cortical tissues at the feeding sites of *C. xenoplax* were darkened (Fig. 2), presumably because of oxidation of phenolic compounds. Mass feeding of thousands of nematodes caused darkening of entire root systems. Plants receiving the suspension from which nematodes had been removed did not differ from the controls, which received tap water only. The results of this experiment indicate that *C. xenoplax* is involved in disease. They do not rule out the possibility that other organisms, small enough to pass through the sieve with 43-μm openings, or airborne organisms, or organisms introduced with the seedlings may have a secondary role. At harvest, *Rhizoctonia solani* was isolated from roots of plants in this experiment. Its role was tested in the fourth experiment.

*Third experiment:* Surface-sterilized *C. xenoplax* produced the same effects as the nonsurface-sterilized nematodes used previously. In this instance, levels of nitrogen, phosphorus, and potassium in the Marianna 2624 plum leaves were reduced by the nematodes (Table 2). Nitrogen deficiency was severe enough to result in reddening of leaves. Reduction in phosphorus and potassium was highly significant ($P = < 0.01$), but not severe enough to cause deficiency symptoms. The supply of nitrogen in the soil was probably nearer a limiting level than the supply of phosphorus and potassium.

*Fourth experiment:* The melanized isolate of *Rhizoctonia solani* did not affect Myrobalan plant weight (Table 3), did not affect water stress in Myrobalan leaves, and did not alter the pathogenicity of *C. xenoplax*. Water stress in nematode-infected plants followed a pattern different from that in noninfected plants. *C. xenoplax* first reduced, but finally increased, this stress (Table 4). The initial reduction in water stress could be a defense mechanism of a host whose roots are damaged by nematodes. The root damage might have triggered a shift in balance of hormones affecting stomatal closure (15). Epstein and Bravdo (9) reported increased resistance to water loss in roses infected with *Meloidogyne hapla*. The gradual increase in water stress in leaves of *C. xenoplax*-infected Myrobalan plum until the stress was finally higher than in noninfected controls probably resulted from failure of the defense mechanism as disease progressed. Other kinds of root pathogens

TABLE 4. Water stress in 'Myrobalan' plum leaves after inoculation of the seedlings with 20,000 *Criconemoides xenoplax* or tap water.

| Weeks after inoculation | Treatments | Water stress (atm)[a] 0100 hours | 1300 hours |
|---|---|---|---|
| 11 | tap water | 7.65 ± 0.45 X | 12.64 ± 0.55 X[b] |
| | 20,000 *C. xenoplax* | 4.59 ± 0.50 Y | 9.75 ± 0.55 Y |
| 14 | tap water | 7.31 ± 0.39 X | 15.93 ± 0.97 X |
| | 20,000 *C. xenoplax* | 6.24 ± 0.62 X | 15.25 ± 1.47 X |
| 18 | tap water | 8.84 ± 0.58 X | 14.40 ± 1.36 Y |
| | 20,000 *C. xenoplax* | 10.26 ± 1.24 X | 23.30 ± 2.34 X |

[a]Average of six replicates, and standard error.
[b]Averages not followed by the same letter in each measuring date differ ($P = < 0.01$).

TABLE 5. Number of *Criconemoides xenoplax* nine months after inoculation of *Prunus* species and hybrids with 5,000 nematodes per plant.

| Experimental host | Number of Replicates | Final root wt (g) | *C. xenoplax*/pot[a] |
|---|---|---|---|
| 'Corotto Marianna' (*Prunus cerasifera*[b]) | 4 | 23 ± 3 | 134,842 ± 11,995 X |
| 'Myrobalan 3J' (*P. cerasifera*) | 5 | 26 ± 4 | 124,228 ± 14,139 XY |
| 'Myrobalan' Herbst Bros (*P. cerasifera*) | 5 | 9 ± 2 | 120,986 ± 16,807 XY |
| 'Etter's Best' (*P. subcordata* × *P. domestica*) | 3 | 83 ± 19 | 105,003 ± 13,579 XY |
| *Prunus moseri* (*P. cerasifera* var. *atropurpurea*) | 4 | 17 ± 1 | 90,215 ± 11,761 XY |
| 'Myrobalan 29C' (*P. cerasifera*) | 5 | 30 ± 4 | 86,880 ± 14,335 XY |
| 'Marianna F' (*P. cerasifera* × *P. munsoniana*) | 5 | 30 ± 4 | 79,318 ± 11,045 XY |
| 'Marianna 2624' (*P. cerasifera* × *P. munsoniana*) | 5 | 24 ± 6 | 72,648 ± 13,743 XY |
| 'Lovell' peach (*P. persica*) | 4 | 4 ± 1 | 72,445 ± 5,302 Y |
| 'Marianna 2623' (*P. cerasifera* × *P. munsoniana*) | 5 | 22 ± 2 | 67,512 ± 10,879 Y |
| 'Marianna 4001' (*P. cerasifera* × *P. munsoniana*) | 5 | 69 ± 11 | 66,528 ± 14,780 Y |
| Peach × almond hybrid (*P. persica* × *P. amygdalus*) | 4 | 14 ± 2 | 64,192 ± 8,651 Y |
| Fallow (no plant) | 4 | | 94 ± 48 Z |

[a]Averages not followed by the same letter differ ($P = < 0.01$).
[b]or a *P. cerasifera* hybrid.

also cause water stress in leaves (10). The ultimate high water stress and low nutrient levels in leaves of *C. xenoplax*-infected Myrobalan plum are probably both a result of root malfunction.

*Fifth experiment:* All the species or hybrids of *Prunus* tested were suitable hosts for *C. xenoplax* (Table 5). 'Etter's Best' (*P. subcordata* Benth. × *P. domestica* L), the Marianna selections (probably *P. cerasifera* × *P. munsoniana*), and the peach-almond cross (*P. persica* × *P. amygdalus*) are additions to the list of known hosts for *C. xenoplax*. The ratio of the final number of nematodes recovered from 'Lovell' peach to the number recovered from Myrobalan 3J plum was greater in this test than in one by Seshadri (22). Nematodes used in the present study were taken from peach, whereas Seshadri's were from grape. This agrees with results of other experiments (Mojtahedi and Lownsbery, *unpublished*) in which we found that the previous host influences the ability of *C. xenoplax* to increase on subsequent hosts.

## LITERATURE CITED


1. BARKER, K. R., and C. N. CLAYTON. 1973. Nematodes attacking cultivars of peach in North Carolina. J. Nematol. 5:265-271.
2. BIRD, G. W., and W. R. JENKINS. 1964. Occurrence, parasitism, and pathogenicity of nematodes associated with cranberry. Phytopathology 54:677-680.
3. BOLKAN, H. A., and E. E. BUTLER. 1974. Studies on heterokaryosis and virulence of Rhizoctonia solani. Phytopathology 64:513-522.
4. BUCKMAN, H. O., and N. C. BRADY. 1960. The nature and properties of soils. 6th ed. Macmillan, New York, N.Y. 567 p.
5. CHAPMAN, H. D., and P. H. PRATT. 1961. Methods of analysis for soils, plants, and waters. Univ. Calif., Div. Agric. Sci., Berkeley. 309 p.
6. COBB, N. A. 1918. Estimating the nema population of soil. U.S. Dep. Agric., Agric. Technol. Circ. 1. 48 p.
7. DAY, L. H. 1953. Rootstocks for stone fruits. Calif. Agric. Exp. Stn. Bull. 736. 76 p.
8. EPSTEIN, A. H., and G. D. GRIFFIN. 1962. The occurrence of Cytospora canker on spruce in the presence of Xiphinema americanum and Criconemoides xenoplax. Plant Dis. Rep. 46:17.
9. EPSTEIN, E., and B. BRAVDO. 1973. Effects of three nematicides on the physiology of rose

infected with Meloidogyne hapla. Phytopathology 63:1411-1414.
10. HELMS, J. A., F. W. COBB, JR., and H. S. WHITNEY. 1971. Effect of infection by Verticicladiella wagenerii on the physiology of Pinus ponderosa. Phytopathology 61:920-925.
11. HOAGLAND, D. R., and D. I. ARNON. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 32 p.
12. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
13. JOHNSON, C. M., and A. ULRICH. 1959. II. Analytical methods for use in plant analysis. Calif. Agric. Exp. Stn. Bull. 766. 78 p.
14. KLINGLER, J., and B. GERBER. 1972. Wachstumsdepressionen an Reben durch wurzelparasitische Nematoden. Schweiz. Z. Obst. Weinbau 108:217-223.
15. LIVNE, A., and Y. VAADIA. 1972. Water deficits and hormone relations. Pages 255-275 *in* T. T. Kozlowski, ed. Water deficits and plant growth. Vol. III. Academic Press, New York, N.Y. 368 p.
16. LOWNSBERY, B. F., H. W. ENGLISH, E. H. MOODY, and F. J. SCHICK. 1973. Criconemoides xenoplax experimentally associated with disease of peach. Phytopathology 63:994-997.
17. LOWNSBERY, B. F., E. H. MOODY, and A. BRAUN. 1974. Plant-parasitic nematodes in California prune orchards. Plant Dis. Rep. 58:633-636.
18. MIRCETICH, S. M., and H. W. FOGLE. 1969. Role of Pythium in damping-off of peach. Phytopathology 59:356-358.
19. PARMETER, J. R., JR., R. T. SHERWOOD, and W. D. PLATT. 1969. Anastomosis grouping among isolates of Thanatephorus cucumeris. Phytopathology 59:1270-1278.
20. RASKI, D. J. 1952. On the morphology of Criconemoides Taylor, 1936, with descriptions of six new species (Nematoda: Criconematidae). Proc. Helminthol. Soc. Wash. 19:85-99.
21. SCHOLANDER, P. F., H. T. HAMMEL, E. D. BRADSTREET, and E. A. HEMMINGSEN. 1965. Sap pressure in vascular plants. Science 148:339-346.
22. SESHADRI, A. R. 1964. Investigations on the biology and life cycle of Criconemoides xenoplax Raski, 1952 (Nematoda: Criconematidae). Nematologica 10:540-562.
23. THORNE, G. 1955. Nematodes associated with slow decline, or dieback of orchards in Idaho. Pages 11-12 *in* Trans. Idaho State Hortic. Soc. 1955.
24. WEAVER, D. J., E. J. WEHUNT, and W. M. DOWLER. 1974. Association of the tree site, Psuedomonas syringae, Criconemoides xenoplax and pruning date with short life of peach trees in Georgia. Plant Dis. Rep. 58:76-79.
25. WENSLEY, R. N. 1953. Microbiological studies of the action of some selected soil fumigants. Can. J. Bot. 31:277-308.