Pathogenicity of Macroposthonia xenoplax to Walnut

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Abstract: Preplanting treatment of soil naturally infested with *Macroposthonia xenoplax* with 1,2-dibromoethane (ethylene dibromide) significantly increased the growth rate of *Juglans hindsii* seedlings. When seedlings of *J. hindsii, J. regia* CV "Serr" and *J. regia* CV Eureka were inoculated with *M. xenoplax,* their growth was significantly less than that of nematode-free controls. This retarded growth rate was accompanied by feeder root necrosis, longitudinal cracks in the older roots, and distinct lesions in the secondary phloem. *Key Words: Juglans hindsii, Juglans regia,* lesion formation, feeder root necrosis, phenolic compounds.

In California, *Macroposthonia xenoplax* (Raski) Loof and De Grisse is common and widely distributed in orchards and vineyards and is most abundant around grapes and walnuts (16). In 1968, *M. xenoplax* was found in 26 of 29 declining walnut orchards surveyed in San Joaquin County. The role of this nematode is difficult to assess in those orchards, however, because 24 of them are also infested by *Pratylenchus vulnus* Allen and Jensen, a known walnut pathogen (5, 12). However, lesions were observed on roots of trees in orchards infested with *M. xenoplax,* even when *P. vulnus* was not found. *M. xenoplax* has been shown to limit growth of peach (7, 8) and prune, and predispose these plants to bacterial canker disease (8, 10). This study was undertaken to determine whether this nematode has similar effects on Northern California black walnut, *Juglans hindsii* Jeps., and Persian walnut, *J. regia* L.

MATERIALS AND METHODS

General procedures: Three experiments were conducted. The first measured the effect on *J. hindsii* growth of nematicidal fumigation of an orchard soil naturally infested with *M. xenoplax.* The second tested the effects on *J. hindsii* of inoculation with *M. xenoplax.* The third compared the effects of *M. xenoplax* on *J. hindsii* and two varieties of *J. regia.* Soil textures (2), measured by Boyoucos' method (1), were loamy sand in the first experiment and sandy loam in the second and third. The walnut seed-

lings were obtained by stratifying seeds in sand. This sand, and the sandy loam used in the second and third experiments, were autoclaved (118 C; 1 atm, 3 h) and aerated for 1 mo. The plants, grown singly in 12-liter cans embedded in pine-wood shavings in a lathhouse, were supplied with a complete water-soluble fertilizer and insecticides as needed. The *M. xenoplax* used in the second and third experiments, originally from a vineyard near Rutherford, California, had been used in experiments with peaches prior to this use. Nematode inoculum was obtained, and nematode populations assayed by sieving (3) in the first experiment and a centrifugal-flotation method (4) in the second and third. Final population densities were determined from a 250-cc soil sample taken after jarring soil from roots and mixing it. The total number of nematodes in the can was calculated after counting the number in an aliquot of the extract. Differences between treatments were evaluated with Student's *t*-test, except where indicated otherwise.

Effect of killing M. xenoplax: In March an untreated portion of a Meridian, California, prune orchard soil was stored and kept moist in 12-liter cans. Another portion was fumigated with 1,2-dibromoethane (ethylene dibromide) at 100 liters/ha (11 gal/acre), left 1 mo for escape of fumigant, and transferred to 12-liter cans. In April, *J. hindsii* seedlings were transplanted to 14 cans of fumigated soil and 14 of untreated soil. The experiment was terminated after 14 mo, when the plants were weighed. Nematode populations were determined at planting and when the experiment was terminated.

Effect of adding M. xenoplax: Treatments were 500, 5,000, and 50,000 *M. xenoplax* per can, an untreated control, and

a control for microorganisms which might be associated with the nematodes in the water suspension. The latter control was obtained by removing *M. xenoplax* from the 50,000-nematode concentration by repeated passage of the suspension through a sieve with 0.043-mm openings (325 meshes/inch). The removal was possible because the original centrifugal-flotation extraction of the sandy soil yielded a clean extract of *M. xenoplax* from which the smaller nematodes were largely eliminated by allowing them to pass through a sieve with 0.074-mm openings (200 meshes/inch). The treatments were added around the roots when the seedlings were planted in May. Experimental units were arranged in four randomized blocks. Plants were examined and weighed, and representative ones were photographed after 1 year's growth.

Fresh hand-cut sections of primary and secondary root were immersed and mounted in diazotized p-nitroanaline. Control sections were mounted in distilled water for comparison (17). The amount of phenolic compounds present was judged by the intensity of orange to brown color.

Comparison of J. hindsii and J. regia: In California the commercial walnut, *Juglans regia,* is commonly grafted to rootstocks of *J. hindsii* to control oak root fungus disease (14). Use of *J. regia* as a rootstock is being considered currently because of the increasing incidence of a lethal stock-scion union failure known as blackline (13). For that reason we tested *J. regia* as well as *J. hindsii.* Seedlings of *J. hindsii* and *J. regia,* CV 'Serr' and CV 'Eureka,' were transplanted to cans in July and inoculated around the roots with 10,000 *M. xenoplax,* or left uninoculated. The inoculated plants were grouped in one-half of the experimental design, and nematode-free cans in the other half to avoid contamination. The eight replicates of each variety x treatment combination were arranged in eight systematic blocks. The experiment was terminated after 2 years, when the plants were measured and symptoms were examined.

RESULTS

Effect of killing M. xenoplax: At planting, *M. xenoplax* was recovered from untreated soil at the rate of about 12,000

per can. No nematodes were recovered from the fumigated soil. Plants grown 14 mo in fumigated soil (336 $\pm$ 8 g) were greater (P < 0.01) in fresh wt than plants grown in the untreated soil (127 $\pm$ 5 g). Roots had considerable new growth and little necrosis in fumigated soil, but had no new growth and much necrosis in untreated soil.

Effect of adding M. xenoplax: Plants inoculated with the suspensions of *M. xenoplax* weighed less than the controls (Table 1). Plants inoculated with the suspension minus nematodes did not differ from complete controls. Growth suppression was as severe with 5,000 nematodes as with 50,000. Fine feeder roots were killed on nematode-infected plants. Externally, larger infected roots were darker than uninfected roots, and longitudinal cracks extended through the periderm into the secondary phloem (Fig. 1). When the phellum and phellogen of these roots were cut away, black necrotic areas were found in the secondary phloem of nematode-infected roots but not in controls or controls for associated microorganisms (Fig. 1), which had secondary phloem of the normal light-yellow color. Secondary phloem of inoculated or uninoculated roots was high in phenols. Tips of nematode-infected roots had no living primary root tissue that could be tested for phenols. The cortex in uninoculated root tips was low in phenols.

Comparison of J. hindsii and J. regia: Seedlings used in this experiment were transplanted from stratification flats to 1-liter paper milk cartons, and again to the cans used in this experiment. Shock from

TABLE 1. Fresh weight of California black walnut seedlings and numbers of *Macroposthonia xenoplax* 13 mo after inoculation.

Treatment	Plant wt (g)[a]	Final no. of nematodes (thousands[a])
Complete control	91	0.2 $\pm$ 0.2
Associated microorganisms	89	4 $\pm$ 1
500 *M. xenoplax*	62	98 $\pm$ 16
5,000 *M. xenoplax*	48[b]	66 $\pm$ 47
50,000 *M. xenoplax*	50[b]	45 $\pm$ 15

[a]Average of four replicates and standard error.
[b]Differ from the complete control at the 1% level using analysis of variance and the LSD test.
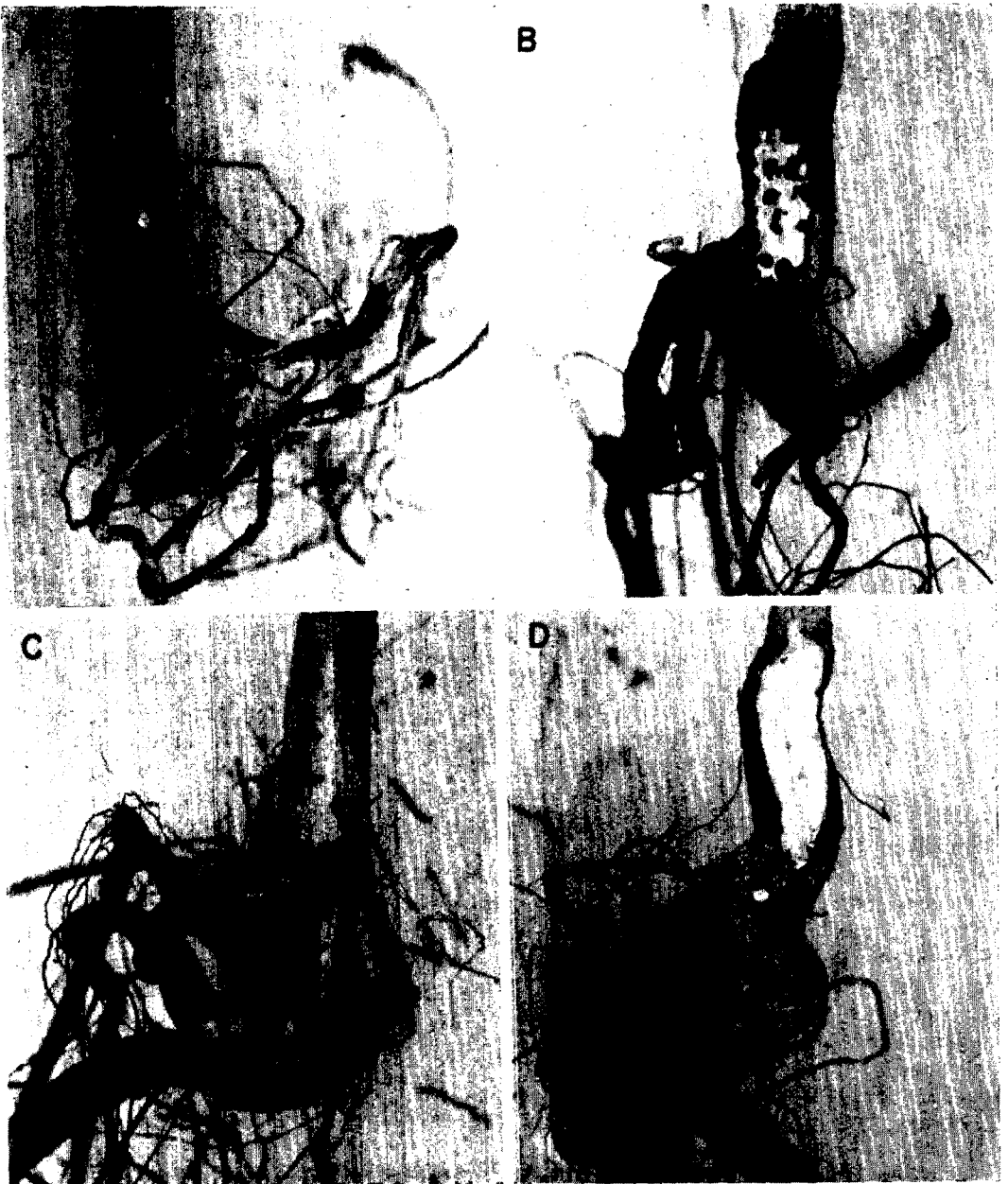
FIG 1 (A-D). Roots of Northern California black walnut 1 year after inoculation with 5,000 *Macroposthonia xenoplax (A & B)*, or in absence of nematodes (C & D). The surface of roots B and D has been cut away to reveal the condition of the secondary phloem.

the double transplanting proved to be more important than *M. xenoplax* in limiting growth in the first growing season after inoculations, so no differences in shoot growth were apparent between inoculated and uninoculated plants. In the second growing season, however, *M. xenoplax* limited the growth of all cultivars (Table 2). In terms of growth reduction and lesion formation, *M. xenoplax* appeared to have more severe effects on *J. hindsii* than on the two *J. regia* cultivars, although the two cultivars of *J. regia* were better hosts than *J. hindsii* for *M. xenoplax* (Table 2).

TABLE 2. Fresh weights of three kinds of walnut seedlings and number of *Macroposthonia xenoplax* 2 years after inoculation.

Walnut	Nema-todes added	Plant wt (g)[a]	Final no. of nematodes in thousands[a]
J. regia Serr	0	593 w	0 x
J. regia Eureka	0	547 wx	0 x
J. hindsii	0	453 xy	0 x
J. regia Serr	5,000	398 xwz	837 y
J. regia Eureka	5,000	343 yz	602 y
J. hindsii	5,000	203 z	263 z

[a]Averages of eight replicates. Those followed by the same letter do not differ at P = 0.01.

DISCUSSION

Lesions and longitudinal cracks caused in roots by *M. xenoplax* are also caused by the root-lesion nematode, *Pratylenchus vulnus* (12). *M. xenoplax* produces smaller lesions than does *P. vulnus,* but in time they coalesce to become quite similar. We presume that the nematodes cause lesions by increasing phenol oxidase activity. *P. vulnus* probably causes larger lesions because it is a migratory endoparasite, whereas *M. xenoplax* is a sedentary ectoparasite. Accumulation of phenolic compounds, observed repeatedly in nematode-infected tissues, appears to be a plant defense reaction in some instances (11). If lesion formation is too slow to cause hypersensitivity, it fails as a defense reaction and is correlated with susceptibility. It is probably a less damaging effect on the plant than the death of feeder roots caused by both *M. xenoplax* and *P. vulnus.* Longitudinal cracking was caused by continued growth of lateral meristems adjacent to necrotic phelloderm.

Less severe effects of *M. xenoplax* on *J. regia* than on *J. hindsii* were an expression of tolerance rather than resistance, because final nematode populations were higher on *J. regia.* We doubt that the tolerance shown in this short experiment is sufficient to protect *J. regia* from this nematode over the life span of a walnut tree.

These experiments used a very sandy soil, in which *M. xenoplax* reproduces best (15). Most walnuts in California are grown in soils with a higher silt and clay content, where *M. xenoplax* will increase more slowly and may be less pathogenic. Nevertheless, we expect that *M. xenoplax* limits walnut growth in the orchard in proportion to its population level. It may be responsible for some disease attributed to *P. vulnus.*

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