Influence of Pratylenchus vulnus and Meloidogyne hapla on the Growth of Rootstocks of Rose[1]

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Abstract: Pratylenchus vulnus is involved in a disease of *Rosa noisettiana 'Manetti'* rose rootstock characterized by darkening of roots, death of feeder roots, and stunting of entire plants. The disease is more severe when plants are grown in silt loam soil than when they are grown in sandy loam soil. The nematodes reproduce best in silt loam soil at 20 C. *Meloidogyne hapla* did not affect the growth of Manetti. *Rosa sp.* 'Dr. Huey', Manetti, and *R. odorata* rose rootstocks were found to be good hosts for *P. vulnus* whereas *R. multiflora* was less suitable. *M. hapla* reproduced well on *R. odorata*, Dr. Huey, and *R. multiflora*, but not on Manetti. *Key Words:* root-lesion nematode, root-knot nematode, reproduction, soil temperature, soil type.

Pratylenchus vulnus Allen and Jensen was first reported from rose roots in California in 1953 (14). A 1970 survey of commercial rose greenhouses in northern California shows that this nematode is now widely distributed (9), and Allen and Jensen (1) also report that *P. vulnus* is widely distributed on field-grown roses in southern California.

Sher (15) demonstrated that *P. vulnus* is associated with a disease of *Rosa* sp. 'Dr. Huey' rose rootstock. Plants infected with nematodes are stunted and chlorotic. The root systems are necrotic and have fewer feeder roots than noninfected plants. Sher and Bell (16) found that the disease is most severe in plants grown in light sandy soil at 29.4 C (85 F) or 32.2 C (90) F).

Meloidogyne hapla Chitwood commonly occurs and is widely distributed in commercial rose greenhouses in northern California (9). In 1969, severe economic losses in the production of 2-year-old field-grown roses (*Rosa multiflora japonica*) were attributed to *M. hapla* (6). Davis and Jenkins (5) showed that *M. hapla* causes extensive injury to roots of *R. multiflora*. In California, an increase in the production of cut roses was associated with a reduction in the population of *M. hapla* and *Xiphinema americanum* Cobb as a result of multiple applications of 1,2-dibromo-3-chloropropane (DBCP) (8).

Rosa noisettiana Thory 'Manetti' is the most popular rootstock used in growing greenhouse roses for cut flowers. *R. odorata* Sweet and *Rosa* sp. Dr. Huey (Schafter) rootstocks are used less frequently.

Because Manetti is an important rootstock and *P. vulnus* and *M. hapla* have been associated with diseases of roses, tests were conducted to determine their influence on the growth of Manetti. Tests using controlled temperature conditions and two types of soil were included in the experiments with *P. vulnus*. In addition, four rose rootstocks (Dr. Huey, Manetti, *R. multiflora, R. odorata)* were tested to compare their suitability as hosts for *P. vulnus* and *M. hapla*.

MATERIALS AND METHODS

Virus-free Manetti plants were obtained from the foundation block, Foundation Plant Material Service, University of California, Davis. Cuttings 20-cm long were rooted in a mixture of perlite and vermiculite (2:3). After 6 weeks, rooted cuttings were transplanted to soil in 10-cm diam clay pots and, 2 weeks later, they were transplanted to soil in 7.5-liter plastic pots (unless indicated differently). At this time, treatments were made and the plants were arranged in randomized blocks on a greenhouse bench or in temperature tanks.

Sandy loam soil (82% sand, 12% silt, 6% clay) amended with University California (UC) mix (1 part UC mix: 3 parts

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soil) (2) and/or silt loam soil (50% silt, 28% sand, 22% clay) amended with redwood shavings (1 part shavings: 4 parts soil) were used in the experiments. The silt loam soil mixture was obtained from a commercial rose grower's greenhouse (Enomoto and Company, Halfmoon Bay, California). All soils were steam-sterilized [3 h at 1.05 kg/cm^2 (15 psi)] at least 3 weeks before they were used.

The population of *P. vulnus,* originally isolated from prunes, was increased and maintained on carrot disks (12). *M. hapla,* isolated from roses, was increased and maintained on roots of tomato grown in sand. Nematodes were extracted by placing infected tissues under a heated intermittent mist for 48-72 h. Inoculations were made by transferring the desired number of nematodes into 50 ml of water and pouring this suspension around the roots of the plants.

In treatments testing effects of associated microorganisms, the nematodes from the highest inoculum level were removed by sieving and hand-picking. This treatment was necessary to prove nematode involvement (11).

Experiments were terminated after 4 months (unless otherwise indicated). Fresh weights of shoots and roots were determined, and nematode counts were made from soil and roots. Nematodes were extracted by placing root systems and a 50-cm^3 portion of the mixed soil in separate funnels under heated intermittent mist for 7 days (unless otherwise indicated). Using standard cultural procedures, root segments from each treatment were plated on PDA in an attempt to isolate any associated fungi.

Plants were watered with distilled water and fertilized with one-half strength Hoagland's nutrient solution every 2 weeks.

Pratylenchus vulnus: The first experiment was conducted to determine the pathogenicity of *P. vulnus* on Manetti. A second experiment was run to determine the effects that soil temperature and soil type have on the population of *P. vulnus* and the subsequent effect on growth of Manetti plants.

In the first experiment, silt loam soil was used in five treatments: (i) complete control (50 ml water); (ii) control testing the effect of microorganisms associated with the nematodes; (iii) 100±7 *P. vulnus;* (iv) 1,000±22 *P. vulnus;* and (v) 10,000±216 *P. vulnus.* Each treatment was replicated eight times. The greenhouse temperature during the experiment ranged from 12.8 to 24.4 C, with a mean of 20.2 C.

In the second experiment, temperature-controlled water tanks were used to maintain two temperatures (20 and 25 C). Two soil types (sandy loam and silt loam) were used in 15-cm diam clay pots. For each temperature and soil type, treatments were: (i) complete control (50 ml water); (ii) control testing the effect of microorganisms associated with the nematodes; and (iii) 10,000±192 *P. vulnus.* Each treatment was replicated six times. The soil temperature during the experiment fluctuated between 19 and 23 C in one regime and 24 and 27 C in the other with the higher temperatures occurring during the day.

Meloidogyne hapla: Silt loam soil was used in five treatments: (i) complete control (50 ml water); (ii) control testing the effect of microorganisms associated with the nematodes; (iii) 100±8 *M. hapla;* (iv) 1,000±15 *M. hapla;* and (v) 10,000±148 *M. hapla.* Each treatment was replicated 10 times. The soil temperature during the experiment ranged from 11.1 to 33.3 C, with a mean of 23.8 C. After 7 months, the plants were harvested in the same manner as in the experiments with *P. vulnus* but only second-stage larvae were counted.

Host suitability of rose rootstocks to P. vulnus and H. hapla: Four rose rootstocks (*R. multiflora,* Dr. Huey, Manetti, *R. odorata*) were tested for their suitability as hosts for *P. vulnus* and *M. hapla.* Plants were grown in sandy loam soil in 15-cm clay pots to which 1,000 nematodes were added. Each treatment was replicated four times. The soil temperature during the test ranged from 13.9 to 26.7 C, with a mean of 20 C. After 4 months, nematode counts were made from the soil and roots. The centrifugal-flotation method (7) and incubation under heated intermittent mist were used to extract the nematodes from the soil and roots, respectively. Only second-stage larvae of *M. hapla* were recovered.

RESULTS

Pratylenchus vulnus: In the pathogenicity test with *P. vulnus,* final fresh weights

TABLE 1. Reproduction of *Pratylenchus vulnus* and its influence on growth of *Rosa noisettiana* 'Manetti.'

Treatment	Fresh weight of plants[a] (g)	Final number of nematodes/pot[a] (in 1,000's)
Complete control	49.4 a	0
CMAN[b]	48.2 ab	0
100[c]	34.2 b	333
1,000	26.3 bd	398
10,000	17.4 c	212

[a]Mean of eight replicates after 4 months growth in silt loam soil. Values in each column not followed by the same letter differ significantly, $P=0.01$, according to Duncan's Multiple Range Test.
[b]Control testing the effect of microorganisms associated with the nematodes.
[c]Differs from CMAN, $P=0.02$.

of plants were inversely proportional to the number of *P. vulnus* added (Table 1). Plants exposed to each level of nematodes weighed less than plants with no nematodes. The control testing the effect of associated microorganisms did not differ significantly in fresh plant weight from the complete control. Stunting of tops was also proportional to the number of *P. vulnus* added (Fig. 1). Root systems of infected plants were smaller, darker, and had fewer feeder roots (Fig. 1). Sloughing off of the cortex was evident on feeder roots.

The final number of *P. vulnus* recovered was highest in the pots receiving 1,000 nematodes (Table 1). The rate of reproduction, however, was inversely proportional to the number of nematodes added.

Cephalosporium, Trichothecium, and *Monilia* species were isolated from the roots of plants in all treatments.

In the experiment involving the effects of soil type and soil temperature, all plants infected with *P. vulnus* weighed less ($P=0.01$) than those in the complete control or the control testing the effects of microorganisms associated with the nematodes (Table 2). Nematode reproduction was greater at 20 C and in the silt loam soil. The plants grew better in the sandy loam soil, but they were not affected by temperature.

Regardless of soil temperature, more nematode damage to the roots occurred in silt loam soil. The disease symptoms observed were similar to those in the previous experiment.

Meloidogyne hapla: In the pathogenicity test with *M. hapla,* no differences in fresh weight of plants were found among treatments. The numbers of nematodes recovered per pot were low: 147, 480, and 1,401 nematodes were recovered from pots receiving 100, 1,000, and 10,000 nematodes, respectively; and only a few inconspicuous galls were observed on the roots of plants receiving 10,000 nematodes.

Host suitability of rose rootstocks to P. vulnus and H. hapla: Dr. Huey, Manetti, *R. odorata,* and, to a lesser degree, *R. multiflora* are good hosts for *P. vulnus* (Table 3). *M. hapla* reproduced well on *R. odorata,* Dr. Huey, and *R. multiflora,* but poorly on Manetti.

Dr. Huey, Manetti, and *R. odorata* root systems infected with *P. vulnus* were darker and had fewer feeder roots than *R. multiflora.* Similar symptoms were observed on roots of *R. odorata* and Dr. Huey infected with *M. hapla* but not on roots of *R. multiflora* and Manetti. In addition, the roots of *R. odorata* and Dr. Huey had more and larger galls than those of *R. multiflora.* No galls were observed on Manetti.

DISCUSSION

The results show that *P. vulnus* is involved in a disease of Manetti rose rootstocks. Sher (15) reported results similar to ours with *P. vulnus* on Dr. Huey rootstock.

In the experiment using different numbers of *P. vulnus* as inoculum, final weights of plants were inversely related to numbers of nematodes added. The rate of reproduction was greatest in the lower nematode inoculum levels. This relationship was expected, since there was less competition for feeding sites. A decrease in the rate of reproduction would be expected over a longer period as the competition for feeding sites increases.

Pratylenchus vulnus reproduced best in silt loam soil at 20 C. Nematode damage to the roots was also more severe in silt loam than in sandy loam soil. Poorer plant growth in silt loam soil without nematodes could accentuate damage caused by nematodes because plants growing under suboptimal conditions are generally more sus-

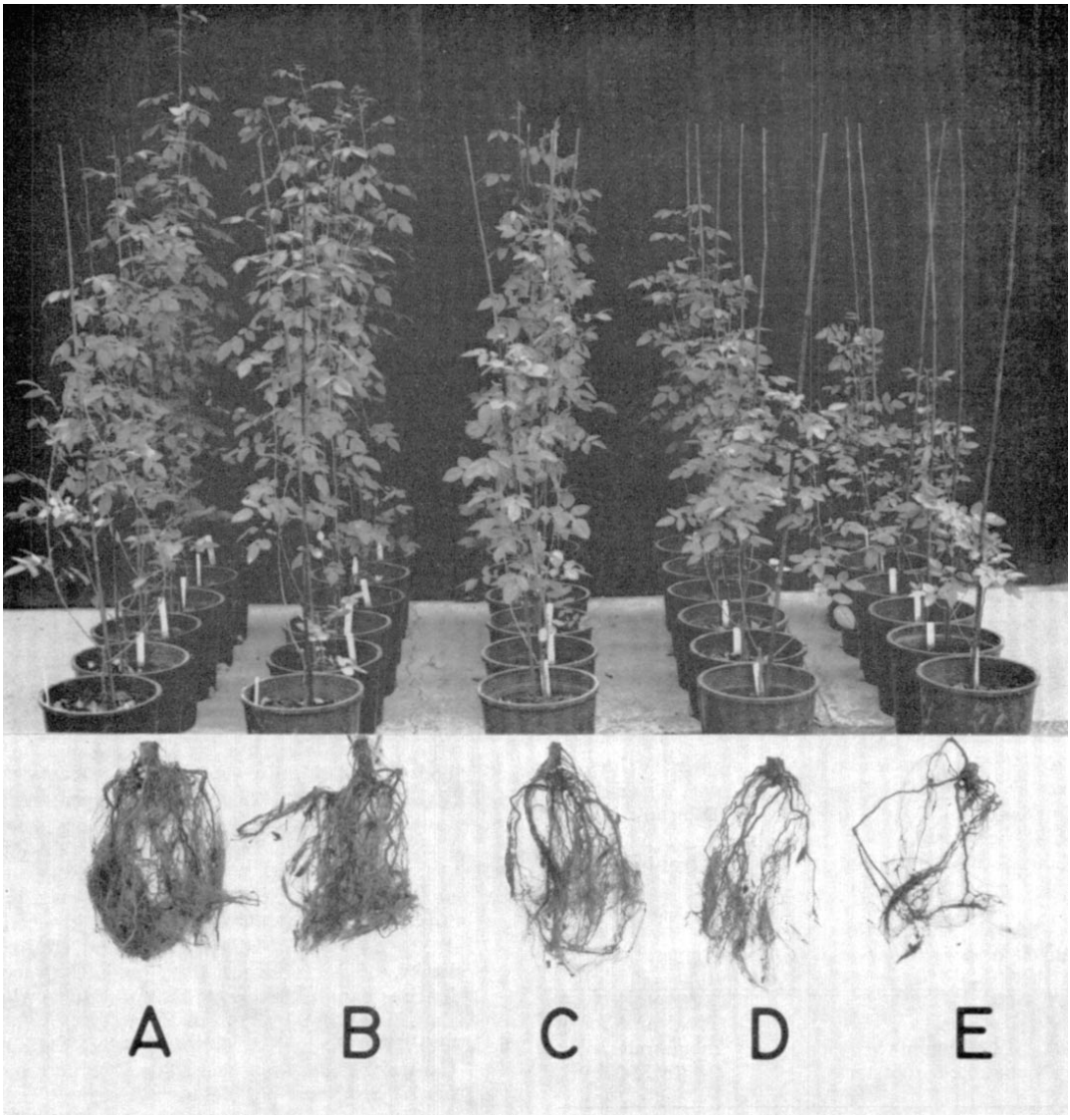

FIG. 1 (A-E). Influence of *Pratylenchus vulnus* on the growth of *Rosa noisettiana* 'Manetti' in silt loam soil. (A) No nematodes. (B) Control testing the effect of microorganisms associated with the nematodes. (C) 100 nematodes. (D) 1,000 nematodes. (E) 10,000 nematodes.

ceptible to nematode damage. These results are significant because silt loam soil and soil temperatures of 20 C are present in some greenhouse-rose ranges in northern California.

Our results are contrary to those found by Sher and Bell (16) with *P. vulnus* on Dr. Huey rootstock. They found that rose plants grew better in heavier soil and that nematodes reproduced best in lighter soil at higher temperatures (29.4 and 32.2 C). Lownsbery et al. (10), working with alfalfa callus tissue, found that *P. vulnus* increased more rapidly at 25 C than at 20 C and that no increase occurred at 30 or 35 C.

Manetti was not a good host for *M. hapla*. Berge (3), in France, found Manetti growing in moist soil to be a good host for *M. hapla*, although under dry conditions *M. hapla* failed to maintain itself.

The results of the host-suitability test indicate that *P. vulnus* may be an important pathogen on *R. odorata* rootstock as well as on Manetti and Dr. Huey. Damage to the root systems of these three rootstocks was similar. Likewise, *M. hapla* may

TABLE 2. Influence of soil type and temperature on reproduction of *Pratylenchus vulnus* and on growth of *Rosa noisettiana* 'Manetti.'

Treatment[a]	Fresh weight of plants[b] (g)	Final number of nematodes/pot[b] (in 1,000's)
20 C (L):		
Complete control	97.9 a	0
CMAN[c]	90.1 a	0
10,000	54.4 b	278
20 C (H):		
Complete control	74.1 a	0
CMAN	84.6 a	0
10,000	40.3 b	699
25 C (L):		
Complete control	87.1 a	0
CMAN	89.6 a	0
10,000	44.1 b	57
25 C (H):		
Complete control	78.1 a	0
CMAN	73.6 a	0
10,000	32.7 b	428

[a]L=sandy loam soil; H=silt loam soil; P_i of 10,000 nematodes where indicated.
[b]Mean of six replicates after 4 months. Values in each column not followed by the same letter differ significantly, $P=0.01$, according to Duncan's Multiple Range Test.
[c]Control testing the effect of microorganisms associated with the nematodes.

TABLE 3. Reproduction of *Pratylenchus vulnus* and *Meloidogyne hapla* on four rose rootstocks.

Treatment[a] (Nematode & rootstock)	Final number of nematodes/pot[b] (in 1,000's)
Pratylenchus vulnus:	
Rosa sp. 'Dr. Huey'	196
R. noisettiana 'Manetti'	162
R. odorata	151
R. multiflora	31
Meloidogyne hapla:	
Rosa sp. 'Dr. Huey'	129
R. noisettiana 'Manetti'	0.2
R. odorata	170
R. multiflora	78

[a]1,000 nematodes were added initially to each pot.
[b]Mean of four replicates after 4 months.

be important on *R. odorata* and Dr. Huey and, to a lesser extent, on *R. multiflora*. Therefore, these rootstocks should be avoided in fields infested with either of these nematodes.

The fungi (*Cephalosporium, Trichothecium, Monilia* species) isolated from the *P. vulnus* experiments are not known pathogens on roses. Although the probability that these fungi are involved with *P. vulnus* in causing a disease of Manetti is low, it should not be overlooked as a factor in nematode-fungus interactions. Several workers have reported that fungi which seem incapable of invading and damaging roots do so when roots are predisposed by nematodes (4, 13).

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