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Biology and Pathogenicity of *Pratylenchus neglectus* on Alfalfa[1]

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Abstract: *Pratylenchus neglectus* reduced the growth of alfalfa cultivars in greenhouse and growth chamber studies. Inocula (1,000, 5,000 and 10,000 nematodes per plant) reduced shoot dry weights of Ranger by 16, 27, and 40%, of Lahontan by 16, 32, and 40%, and of Nevada Synthetic XX (Nev Syn XX) by 18, 26, and 37%, respectively, at 26 ± 2 C. *Pratylenchus neglectus* at 1,000 nematodes per plant reduced Ranger shoot dry weights by 5, 12, 18, and 27%, at 15, 20, 25, and 30 C, respectively, whereas 5,000 nematodes per plant reduced shoot dry weights by 12, 17, 26, and 38%, respectively, at similar temperatures. Reductions in dry root weights were directly related to reductions in shoot growth. At 1,000 nematodes per plant, Ranger root dry weights were reduced by 3, 14, 40, and 40%, whereas 5,000 nematodes per plant reduced root dry weight by 25, 31, 59, and 63%, respectively, at similar temperatures. Similar results were observed on Lahontan and Nev Syn XX at the same inoculum levels and soil temperatures. Nematode reproductive indices (final nematode population per plant divided by initial nematode inoculum per plant) were higher at 1,000 nematodes per plant than at 5,000 nematodes per plant, were positively correlated with temperature, and were unaffected by cultivar.

Key words: alfalfa, *Medicago sativa,* pathogenicity, plant mortality, *Pratylenchus neglectus,* reproduction, root weight, shoot weight.

Root lesion nematodes, *Pratylenchus* spp. attack and parasitize alfalfa, *Medicago sativa* L., in North America (1,3,5,8,9,12,13). *Pratylenchus* spp. severely damaged alfalfa sown in winter wheat fields, supposedly because of competition with wheat under moist field conditions, heavy nematode invasion, and destruction of alfalfa seedling roots; minimal damage occurred following summer fallow (1). *Pratylenchus pratensis* (de Man) Filipjev was associated with poor stands and low yields of alfalfa in Rhode Island (9). *Pratylenchus penetrans,* but not *Pratylenchus neglectus* reduced the growth of Washoe and Vernal alfalfa (7).

Host–parasitic relationships involving *Pratylenchus* spp. are affected by edaphic conditions, which affects the pathological potential. Soil temperature affected host–parasite relationships including those involving *Pratylenchus* spp. (3), and temperature limitations depended more on the host plant than on the nematode by affecting host susceptibility (2). The invasion of alfalfa roots by *P. penetrans* increased with soil temperature (5), and parasitism by root lesion nematodes affected cold hardiness of alfalfa (10). Plant age affected nematode invasion (6), and the efficiency of root penetration by lesion nematodes was dependent on the nematode stage (8).

Pratylenchus neglectus was associated with cultivated and virgin soil in the United States (11). Griffin (unpubl.), found *P. neglectus* associated with cultivated and native vegetation, including poor growth of alfalfa, grain, and rangeland grasses in the western United States; populations as high as 14,000 nematodes per root system were collected from alfalfa and wheat. In view of this, the objective of this study was to determine the relationship between *P. neglectus* and alfalfa under controlled laboratory and greenhouse conditions.

Materials and Methods

Inoculum of *P. neglectus* was obtained from an alfalfa field in northern Utah and cultivated on wheat, *Triticum aestivum* L. cv. Nugaines, in a temperature controlled greenhouse. A preliminary study showed no physiological differences in this population of *P. neglectus,* whether cultured on alfalfa or grain. Because grain is a better

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host for the nematode, it was used as a host-culture plant. Nematode inoculum was obtained with a modified Baermann funnel and was surface sterilized with solutions of streptomycin and gentamycin aretan, and rinsed through several rinses of distilled water.

Since no alfalfa cultivars are known to be resistant to *P. neglectus,* the following cultivars were chosen because they are resistant or susceptible to other plant-parasitic nematodes: Ranger, susceptible to the alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, and the northern root-knot nematode, *Meloidogyne hapla* Chitwood; Lahontan, resistant to *D. dipsaci* and susceptible to *M. hapla*; and Nevada Synthetic XX (Nev Syn XX), resistant to *D. dipsaci* and *M. hapla* (3).

Greenhouse bench experiment: Alfalfa seeds of the three alfalfa cultivars were scarified, treated with captan, germinated on filter paper in petri dishes for 48 hours, and washed six times with deionized water. When radicles were 2–5 mm long, seedlings were planted into individual plastic containers (6 cm d × 21 cm deep) (Stuewe & Sons, Corvallis, OR) containing 540 cm^3 steam pasteurized sandy loam soil (83% sand, 10% silt, 7% clay; pH 7.2). Plants were inoculated after 28 days with mixed stages of *P. neglectus.* An aqueous suspension of nematodes in deionized water was poured into four 10-cm-deep holes in the root zone of each plant. Uninoculated controls received deionized water alone. Plants were maintained at a greenhouse temperature of 26 ± 2 C. Supplemental light for a 19-hour daylength was provided by high-output fluorescent lamps. Treatments were arranged in a randomized complete block design with 20 replications. Treatments were 1) 1,000 nematodes per plant, 2) 5,000 nematodes per plant, 3) 10,000 nematodes per plant, and 4) uninoculated controls. *Rhizobium meliloti* Dang. was applied around the seedling at planting in all experiments to insure nodulation. A replication consisted of one plant per container. Plants were watered lightly after inoculation, and as needed thereafter. The experiment was terminated 100 days after inoculation, and plant mortality, the nematode reproductive index R (Pf/Pi = final nematode population divided by initial nematode inoculum), and shoot and root weights were determined. Nematodes were extracted from the soil by elutriation and sugar flotation (4). Nematodes were extracted from alfalfa roots by the modified Baermann funnel technique. Data were recorded and analyzed using standard ANOVA and means separated using Duncan's new multiple-range test.

Growth chamber temperature experiment: A study similar to the greenhouse test was conducted in temperature controlled growth chambers, but the 10,000 nematodes per container treatment was not used. Plants were grown in chambers set at 15, 20, 25, and 30 C for 100 days after inoculation. Nematodes were extracted from soil and root tissue and the data were recorded and analyzed as described for the greenhouse study.

Experiments were repeated with similar results. Data presented here are from the first tests of each experiment.

Results

Greenhouse: Plant survival among the alfalfa cultivars did not differ ($P > 0.05$). At inoculum levels of 0, 1,000, 5,000, and 10,000 nematodes per plant, respectively, survival was 100, 100, 85, and 70% of Ranger plants; 100, 100, 80, and 70% of Lahontan plants; and 100, 100, 85, and 75% of Nev Syn XX plants.

Pratylenchus neglectus at 1,000 nematodes per plant did not affect shoot growth ($P > 0.05$) of any cultivar. However, shoot growth of all three cultivars was reduced ($P < 0.05$) by 5,000, or 10,000 nematodes per plant (Table 1). Alfalfa cultivars did not differ ($P > 0.05$) in their degree of susceptibility. Root growth was affected by *P. neglectus* similarly to shoot growth. There were differences ($P < 0.05$) among inoculum levels but not among cultivars.

Reproduction of *P. neglectus* on alfalfa varied in the same way as shoot and root growth of alfalfa. The reproductive index

TABLE 1. Effect of *Pratylenchus neglectus* on dry weight of three alfalfa cultivars at 26 ± 2 C.

Inoculum levels	Shoot weight (g)			Root weight (g)		
	Ranger	Lahontan	Nev Syn XX	Ranger	Lahontan	Nev Syn XX
0	2.97 cA	3.05 cA	2.94 cA	1.17 cA	1.21 cA	1.24 cA
1,000	2.50 cA	2.56 cA	2.41 cA	0.93 cA	0.95 cA	0.89 cA
5,000	2.17 bA	2.07 bA	2.18 bA	0.76 bA	0.70 bA	0.79 bA
10,000	1.79 aA	1.83 aA	1.86 aA	0.63 aA	0.60 aA	0.58 aA

Values are the means of 20 replicates (one plant per replicate). Means not followed by the same letter differ ($P < 0.05$) according to the Duncan's new multiple-range test (lower case letters for columns, CAPITAL letters for rows). Plants were inoculated at 28 days with *P. neglectus* and grown for 100 days.

was greater at an inoculum level of 1,000 nematodes per plant than at 5,000 and 10,000 nematodes per plant. Nematode reproduction did not differ among cultivars, however, differences ($P < 0.05$) were detected among inoculum rates, with the greatest reproduction occurring at 1,000 nematodes per plant. The reproductive indices on Ranger, Lahontan, and Nev Syn XX, respectively, were 19.5, 20.9, and 21.8, at a Pi of 1,000; 14.3, 15.2, and 15.8 at a Pi of 5,000; and 11.3, 12.2, and 11.6 at a Pi of 10,000. The LSD ($P < 0.05$) was 3.4 for differences among inoculum levels and 2.9 for differences among cultivars.

Growth chamber: All plants of the three cultivars survived at all temperatures except 30 C. At inoculum levels of 0, 1,000, and 5,000 nematodes per plant at 30 C, respectively, survival was 100, 85, and 80% of Ranger plants; 100, 85, and 75% of Lahontan plants; and 100, 85, and 75% of Nev Syn XX plants.

Shoot growth, inoculum density, and temperature were directly related (Fig. 1). *Pratylenchus neglectus* did not affect shoot growth at 15 C, but reduced ($P < 0.05$) shoot growth of all cultivars at inoculum levels of 1,000 and 5,000 nematodes per plant at 20–30 C. The greatest reduction in shoot growth was observed on plants inoculated with 5,000 nematodes at 30 C. The three alfalfa cultivars did not differ in degree of resistance and susceptibility to *P. neglectus.*

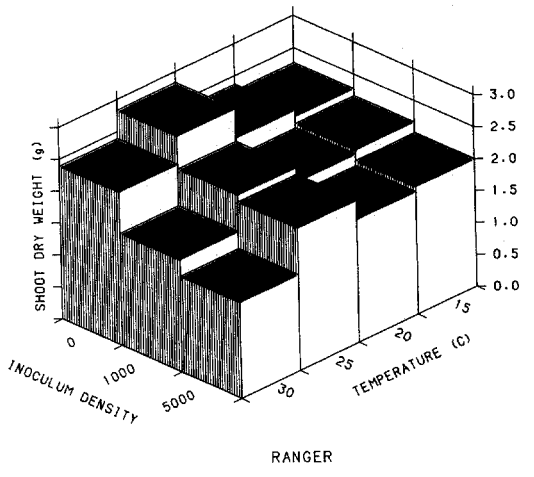
The effect of *P. neglectus* on root growth was similar to its effect on shoot growth (Fig. 2). The greatest reduction in root growth was with 5,000 nematodes per plants at 30 C, but root growth of the three cultivars was not different ($P > 0.05$).

Nematode reproduction and temperature were directly related in all cultivars (Fig. 3). The highest reproduction occurred at 30 C at the 1,000 and 5,000 *P. neglectus* inoculum levels. The only significant differences occurred between temperatures, with increased nematode reproduction being positively associated with increased temperature.

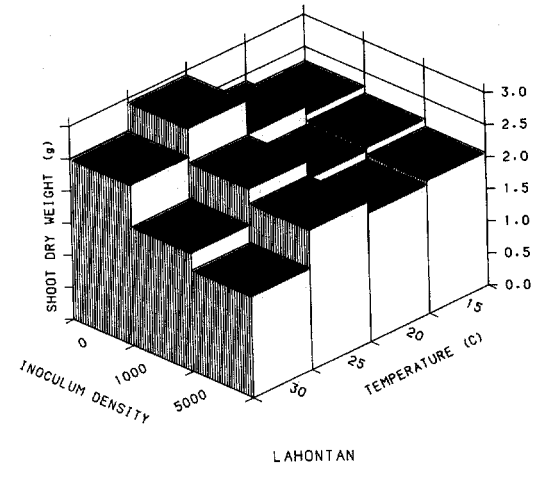
DISCUSSION

Pratylenchus neglectus is supposedly an endemic species, because it is associated with both cultivated and native vegetation throughout the western United States (11). Its ability to parasitize and reproduce on alfalfa emphasizes the importance of *P. neglectus* on alfalfa and possibly other native and introduced plants. Alfalfa growth was not reduced in the greenhouse at an inoculum level of 1,000 nematodes per plant which agrees with the findings of Santo et al. (7) who showed no reduction in root and crown weight of Washoe, Thor, and Vernal alfalfa inoculated with 540 *P. neglectus* per pot. We had significant nematode reproduction, whereas Santo et al. observed little or no reproduction. This difference in reproduction may be due to variability in nematode populations, differences in alfalfa cultivars, inoculum densities, or experimental conditions.

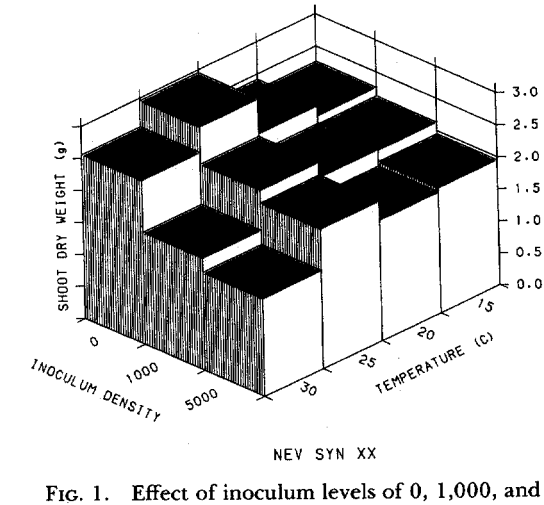
The increased pathogenicity of *P. neglectus* at the higher soil temperature (30 C) indicates the adaptation of this nematode over a long association with plants in the hot arid regions of the western United States (Griffin, unpubl.). Soil temperatures in new alfalfa plantings in the arid western United States often approach 30 C which can result in a high plant mortality rate



RANGER

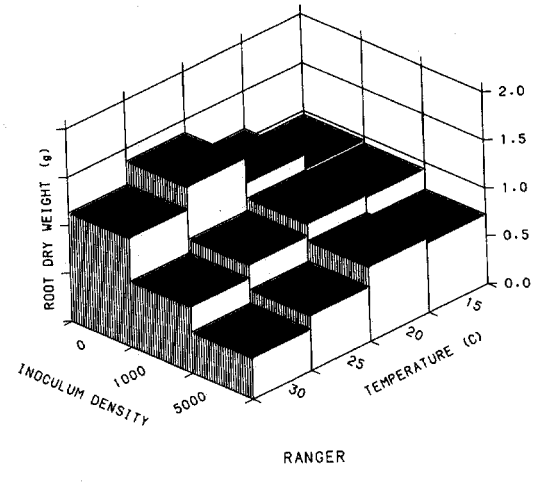


LAHONTAN

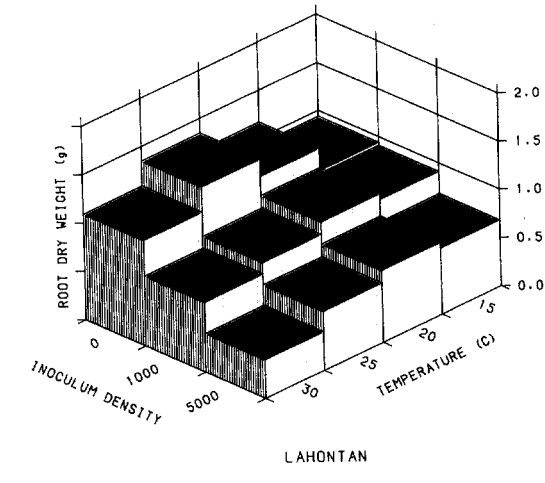


NEV SYN XX

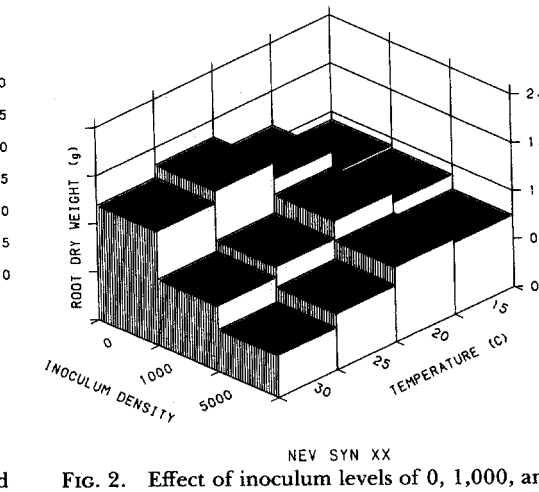
FIG. 1. Effect of inoculum levels of 0, 1,000, and 5,000 *Pratylenchus neglectus* per plant on shoot dry weights of Ranger, Lahontan, and Nevada Synthetic XX (Nev Syn XX) alfalfa plants after 100 days growth at different soil temperatures. LSD 0.05 = 0.14 for inoculum level × temperature, 0.20 for inoculum level × cultivar, and 0.21 for temperature × cultivar.



RANGER



LAHONTAN



NEV SYN XX

FIG. 2. Effect of inoculum levels of 0, 1,000, and 5,000 *Pratylenchus neglectus* per plant on root dry weights of Ranger, Lahontan, and Nevada Synthetic XX (Nev Syn XX) alfalfa plants after 100 days growth at different soil temperatures. LSD 0.05 = 0.11 for inoculum level × temperature, 0.16 for inoculum level × cultivar, and 0.19 for temperature × cultivar.

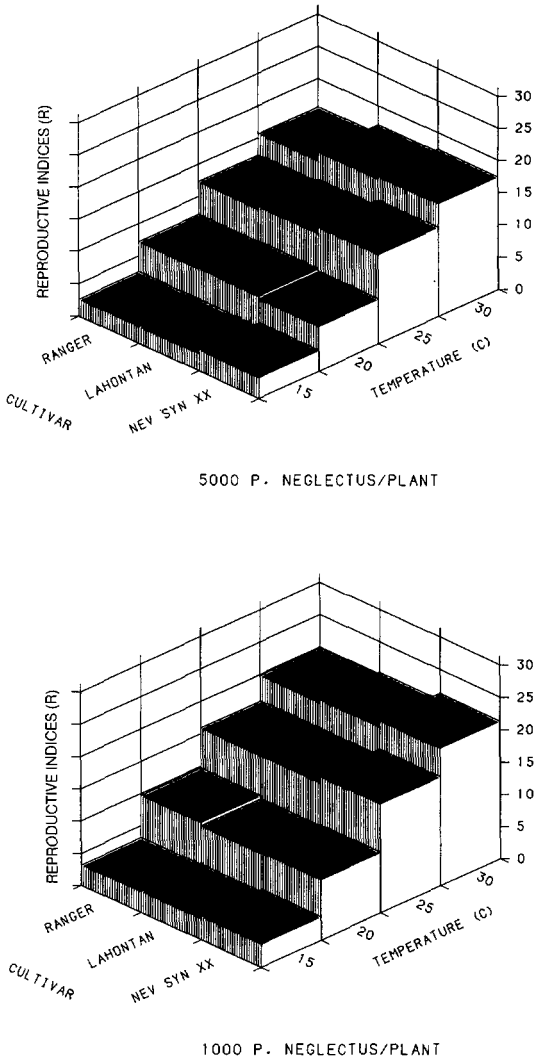


FIG. 3. Effect of inoculum levels of 0, 1,000 and 5,000 *Pratylenchus neglectus* per plant on nematode reproduction R (Pf/Pi) on Ranger, Lahontan, and Nevada Synthetic XX (Nev Syn XX) alfalfa plants after 100 days growth at different soil temperatures. LSD 0.05 = 3.7 for inoculum level × temperature, 2.3 for inoculum level × cultivar, and 1.9 for temperature × cultivar.

from *P. neglectus*. As the alfalfa canopy develops, there will be a reduction in soil temperature resulting in supposedly less nematode damage. There is also a potential importance of *P. neglectus* in southern climates and its possible adaptation to cooler alfalfa temperatures in the northern climates.

The effect of temperature on reproduction of *P. neglectus* on alfalfa is similar to results obtained with a Canadian population of *P. penetrans* by Kimpinski and Willis (5), although Canadian soils are usually cooler than those encountered in most arid regions of the Western United States. They found that 30 C was optimum for *P. neglectus* reproduction on Iroquois alfalfa. Kimpinski and Willis, however, found that reproduction of *Pratylenchus crenatus* Loof was greatest at 10 C, whereas Dickerson (2) showed the optimum temperature for reproduction of *P. penetrans* on corn and potato was a function of plant growth. Differences in *P. neglectus* reproduction at the different inoculum levels apparently is due to the availability of a greater root mass per nematode at 1,000 nematodes per plant; the suppression of plant growth at inoculum levels of 5,000 and 10,000 nematodes per plant probably adversely affected nematode reproduction.

The effect of *P. neglectus* inoculum density on survival of alfalfa plants is similar to findings obtained by Townshend with *P. penetrans* (13), in which a greater mortality of alfalfa seedlings occurred at high inoculum levels. Townshend, however, showed a greater mortality rate and a greater nematode reproduction of *P. penetrans* on alfalfa than was observed with *P. neglectus*. Our study verifies previous findings, that *P. neglectus* is less virulent than other root lesion nematodes such as *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven and *Pratylenchus thornei* Sher & Allen (3,7). However, reductions ($P < 0.05$) in alfalfa growth in the presence of this population of *P. neglectus* emphasizes the potential importance of this nematode and underscores the need for additional investigation to determine its importance on alfalfa and on native and introduced plants. Further study is needed to determine the geographical importance and variability of different *P. neglectus* populations on alfalfa as well as other plant cultivars.

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