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Differential Pathogenicity of Four *Pratylenchus neglectus* Populations on Alfalfa[1]

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Abstract: A *Pratylenchus neglectus* population from Utah (UT3) was more virulent to Lahontan alfalfa than other *P. neglectus* populations from Utah (UT1, UT2) and Wyoming (WY). All alfalfa plants survived at 24 ± 3 C when inoculated with WY, UT1, or UT2 at initial populations (Pi) of 500, 1,000, and 5,000 nematodes per plant. At Pi 10,000 with WY, UT1, or UT2, plant mortality was 15, 15, and 20%, respectively; at Pi 5,000 and 10,000 with UT3, plant mortality was 10 and 40%. The WY, UT1, and UT2 populations reduced ($P \leq 0.05$) root growth at Pi 10,000 only, and UT3 reduced ($P \leq 0.05$) root growth at Pi 1,000, 5,000, and 10,000. At Pi 5,000, shoot dry weights were reduced by 10–23% by WY, 14–29% by UT1, 12–25% by UT2, and 20–48% by UT3 at 15–30 C. The UT3 population reduced ($P \leq 0.05$) root dry weight at 20–30 C at Pi 1,000 and 5,000. The WY, UT1, and UT2 populations did not reduce ($P \geq 0.05$) root growth at any temperature or Pi. The UT3 nematode reproductive indices were greater than those of the other nematode populations at all Pi and increased with temperature.

Key words: alfalfa, Lahontan, *Medicago sativa*, nematode, plant survival, *Pratylenchus neglectus*, reproduction, temperature, virulence.

Genetic diversity (1) and population variability exist within nematode species (17). Differences within a nematode species may be subtle; a host–parasite relationship may differ only in the ability of a nematode to adapt or acclimatize to environmental stimuli such as temperature (2,3,14,16). More pronounced genetic variability may be categorized as races and (or) pathotypes (4,5,8,10–12,17). This variability must be considered by scientists, plant breeders, and growers in developing cultivars resistant and tolerant to economically important plant-parasitic nematodes.

The root-lesion nematode, *Pratylenchus neglectus*, is endemic in the western United States, associated with both cultivated and native vegetation (15). A population of *P. neglectus* associated with poor growth of alfalfa, *Medicago sativa* L., in northern Utah was highly pathogenic to alfalfa in a recent controlled greenhouse study (4). The long association with alfalfa, or a genetic mutation or gene selection probably increased the virulence of this population of *P. neglectus* to alfalfa (9,13,17,18).

A recent study showed pathotypic differences between *Meloidogyne hapla* populations and how these differences affect nematode selection in alfalfa breeding programs (5). Hence, greenhouse bench and growth chamber studies were conducted to determine if there were pathotypic differences between *P. neglectus* populations that could directly affect future selection of nematode populations to be used in breeding programs.

Materials and Methods

Nematode inocula: Three endemic *Pratylenchus neglectus* populations were obtained from western wheatgrass, *Pascopyrum smithii* (Rydb.) Löve, from central Utah (UT1), northern Utah (UT2), and eastern Wyoming (WY), and a *P. neglectus* population, known to be virulent to alfalfa (4), was collected from northern Utah (UT3). Nematodes were cultivated on wheat, *Triticum aestivum* L. cv. Nugaines, in a temperature-controlled greenhouse. Nematode inocula were obtained from roots with a modified Baermann funnel and surface sterilized (4). Alfalfa cultivar Lahontan, which is resistant to the alfalfa stem nematode, *Ditylenchus dipsaci*, and susceptible to *P. neglectus* and the northern root-knot nematode, *Meloidogyne hapla*, was used in all experiments.

Greenhouse bench experiment: Alfalfa seeds were scarified, treated with captan, germinated on filter paper in petri dishes for

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48 hours, and washed six times with deionized water. When radicles were 2–5 mm long, seedlings were planted in individual plastic containers (6 cm d × 21 cm deep) containing 540 cm^3 steam-pasteurized sandy loam soil (81% sand, 10% silt, 9% clay; pH 7.2, OM 0.8%). Plants were inoculated after 28 days with a suspension of mixed stages of *P. neglectus* in deionized water, pipetted into four 10-cm-deep holes in the root zone of each plant. Uninoculated controls received deionized water alone. Plants were maintained in a greenhouse at 24 ± 3 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 (one plant per container). Treatments were 500, 1,000, 5,000, or 10,000 nematodes (Pi) per inoculated plant and uninoculated controls. *Rhizobium meliloti* Dang. was applied around the seedling at planting to insure nodulation. Plants were watered lightly after inoculation and as needed thereafter. The experiment was terminated 120 days after inoculation, and plant mortality, the nematode reproductive index (Pf/Pi = final nematode population divided by initial nematode inoculum), and shoot and root dry weights were determined. Nematodes were extracted from the soil by elutriation and sugar flotation (6). Nematodes were extracted from alfalfa roots by the modified Baermann funnel technique. Data were analyzed using standard ANOVA, and means were 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 with treatments of Pi 1,000 and 5,000. Plants were grown in chambers for 120 days after inoculation at 15, 20, 25, and 30 C. Nematodes were extracted from soil and root tissue, and the data were analyzed as described for the greenhouse study.

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

Results

Greenhouse bench experiment: Virulence of the WY, UT1, and UT2 *Pratylenchus neglectus* populations did not differ. However, the UT3 population of *P. neglectus* was more virulent ($P \leq 0.05$) to Lahontan alfalfa than the WY, UT1, and UT2 populations. Plant survival was not affected by WY, UT1, and UT2 populations of *P. neglectus* at Pi 500 to 5,000. With UT3, plant mortality was 10 and 40% at Pi 5,000 and 10,000, respectively. At Pi 10,000, plant mortality was 15, 15, and 20% with WY, UT1, and UT2, respectively.

The WY, UT1, and UT2 populations reduced shoot dry weights ($P \leq 0.05$) at Pi 10,000 only, whereas UT3 reduced shoot dry weight ($P \leq 0.05$) at all Pi except 500 (Table 1). Root growth of plants inoculated with WY, UT1, and UT2 at Pi 10,000 was less ($P \leq 0.05$) than that of uninoculated controls (Table 1). At Pi 1,000 and above, UT3 reduced ($P \leq 0.05$) root dry weights below those of the other nematode populations.

The reproductive index of UT3 was greater than that of WY, UT1, and UT2 (Table 1). The indices of all nematode populations were greatest at Pi 500 and smallest at Pi 10,000.

Growth chamber experiment: At Pi 1,000, all plants survived all *P. neglectus* populations at 15–25 C and WY at 30 C. At Pi 1,000 and 5,000 at 30 C, mortality of plants inoculated with UT1 and UT2 was 10%. This corresponded to UT3 mortality rates of 10% at 30 C for Pi 1,000 and 10 and 20% at 25 and 30 C, respectively, for Pi 5,000.

Temperature and inoculum density were negatively correlated with shoot dry weights. The WY, UT1, and UT2 populations of *P. neglectus* at Pi 5,000 reduced shoot dry weights at 30 C, whereas Pi 1,000 did not reduce shoot dry weights at any temperature (Fig. 1). At Pi 1,000 and 5,000, UT3 did not affect alfalfa shoot growth at 15 C, but it reduced ($P \leq 0.05$) shoot dry

TABLE 1. Effect of different *Pratylenchus neglectus* populations† from Wyoming and Utah on Lahontan alfalfa shoot and root dry weights and nematode reproductive index‡ at a greenhouse temperature of 24 ± 3 C.

Inoculum level	WY	UT1	UT2	UT3	LSD ($P < 0.05$)
		Shoot dry weight (g)			
0	3.51 bA	3.51 bA	3.51 bA	3.51 dA	
500	3.47 bA	3.44 bAB	3.56 bA	3.32 dB	0.13
1,000	3.46 bA	3.37 bcA	3.33 bA	2.73 cB	0.23
5,000	3.22 bA	3.15 bA	3.22 bA	2.37 bB	0.38
10,000	2.82 aA	2.67 aA	2.70 aA	1.92 aB	0.34
LSD ($P < 0.05$)	0.38	0.32	0.37	0.27	
		Root dry weight (g)			
0	1.79 bA	1.79 bA	1.79 bA	1.79 cA	
500	1.85 bA	1.75 bA	1.77 bA	1.72 cA	0.22
1,000	1.67 bA	1.63 bA	1.60 bA	1.23 bB	0.25
5,000	1.45 bA	1.49 bA	1.53 bA	0.93 abB	0.28
10,000	1.05 aA	0.96 aA	0.98 aA	0.65 aB	0.19
LSD ($P < 0.05$)	0.38	0.34	0.29	0.41	
		Reproductive indices (Pf/Pi)			
500	6.5 bA	8.5 bA	8.3 bA	16.2 cB	3.3
1,000	6.8 bA	7.8 bA	7.0 bA	14.2 cB	3.8
5,000	4.2 aA	5.7 aA	5.3 aA	9.7 bB	2.5
10,000	3.7 aA	4.4 aA	4.0 aA	4.6 aA	1.2
LSD ($P < 0.05$)	1.2	1.4	1.6	2.3	

Plants inoculated at 28 days and grown for 120 days.

Values are the means of 20 replicates (one plant per replicate). Means not followed by the same letter differ ($P < 0.05$) according to Duncan's new multiple-range test (lower case letters for columns, CAPITAL letters for rows).

† WY from western wheatgrass in eastern Wyoming; UT1 from western wheatgrass in central Utah; UT2 from western wheatgrass in northern Utah; UT3 from alfalfa in northern Utah.

‡ Reproductive index (Pf/Pi) = final nematode population/initial nematode population.

weights at 20–30 C. At Pi 1,000 and 5,000 at 20–30 C, the shoot dry weight of plants inoculated with UT3 was lower ($P \leq 0.05$) than the shoot dry weight of plants inoculated with the other nematode populations.

The UT3 population had similar effects on the growth of roots and shoots. At 20–30 C with Pi 1,000 and 5,000, root dry weight of plants inoculated with UT3 was lower ($P \leq 0.05$) than the root dry weight of uninoculated plants. At Pi 1,000 and 5,000, however, WY, UT1, and UT2 did not reduce ($P \geq 0.05$) Lahontan root growth at any temperature.

Nematode reproductive indices decreased as inoculum density (Pi) increased, but the ratio increased with an increase in temperature (Fig. 2). Reproductive indices of all nematode populations were largest at Pi 1,000 at 25–30 C. There were no differences ($P = 0.05$) in nematode reproduction among any of the four populations at 15 C. At 20–30 C the reproductive index of UT3 was greater ($P \leq 0.05$) than indices of the other populations; however, there were no differences in reproduction among WY, UT1, and UT2 populations at these temperatures.

DISCUSSION

There may be important biological differences in individuals within animal populations, and any population of an organism continually undergoes genetic change (1,9). Even neighboring populations may differ from each other, and no two demes (local populations) or spatially separated populations are identical (9). In this study the pathological relationship of the UT3 *P. neglectus* population showed a distinctive physiological difference from the other nematode populations in relation to its virulence on alfalfa. Formation of physiological races of nematodes may result from monoculture of a given host favoring cer-

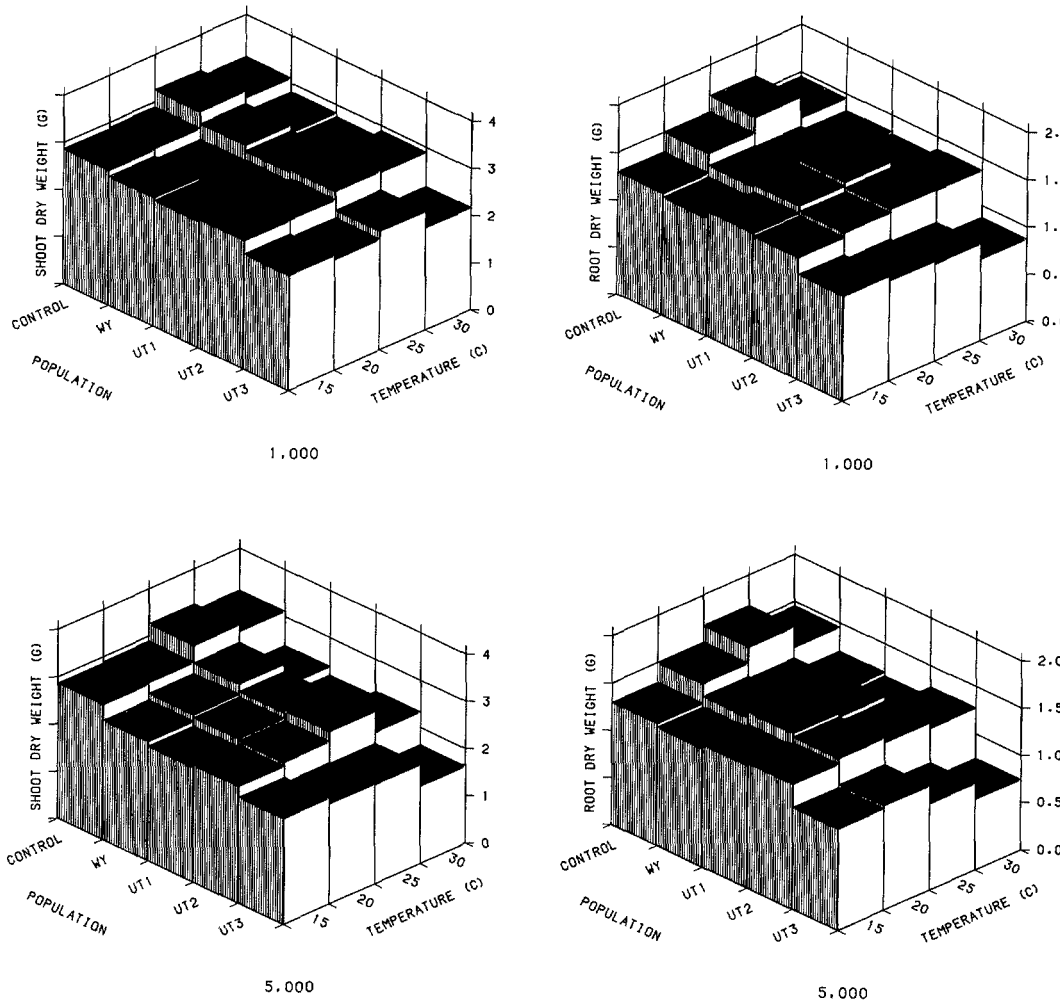

FIG. 1. Effect of different *Pratylenchus neglectus* populations from Wyoming (WY) and Utah (UT1, UT2, UT3) on shoot and root dry weights of Lahontan alfalfa at four different growth chamber temperatures. A) LSD ($P \leq 0.05$) = 0.50 and 0.71 for differences between nematode populations on shoot dry weight at the same temperature, and 0.34 and 0.40 for a given nematode population at different temperatures, at Pi of 1,000 and 5,000 eggs per plant, respectively. B) LSD ($P \leq 0.05$) = 0.32 and 0.39 for differences between nematode populations on root dry weight at the same temperature, and 0.27 and 0.34 for a given nematode population at different temperatures, at Pi of 1,000 and 5,000 eggs per plant, respectively.

tain gene combinations while suppressing others (19). The long association with alfalfa or a genetic mutation or gene selection may have increased the virulence of this population of *P. neglectus* to alfalfa (9,13,17,18). Another study, however, demonstrated that UT3 was more virulent than the other three populations on western wheatgrass and Nugaines wheat, although the three populations were originally collected from wheatgrass and increased on Nugaines wheat (Griffin, unpubl.); this would tend to discount the monoculture hypothesis. Hence, differences in virulence could have resulted from mutation or natural selection or long-term association of UT3 with alfalfa.

A temperature of 30 C favored both pathogenicity and nematode reproduction, which is consistent with data from

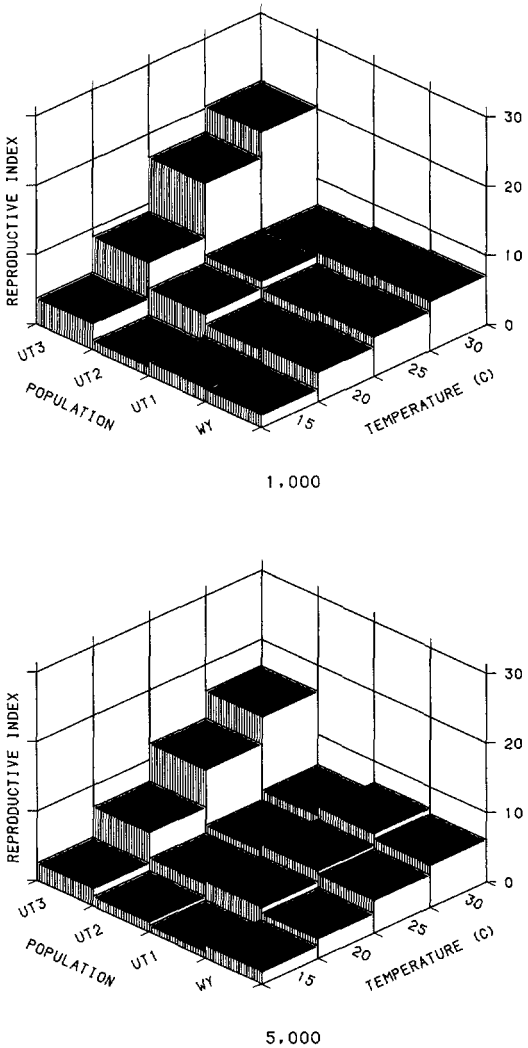

FIG. 2. Effect of four different growth chamber temperatures on the reproductive index (Pf/Pi) of *Pratylenchus neglectus* populations from Wyoming (WY) and Utah (UT1, UT2, UT3) on Lahontan alfalfa. LSD ($P \leq 0.05$) = 2.9 and 1.8 for differences between nematode populations at the same temperature and 4.4 and 3.8 for a given nematode population at different temperatures, at Pi of 1,000 and 5,000 eggs per plant, respectively.

other studies with lesion nematodes, including *P. neglectus* (4,7). Soil temperatures may be lower than 30 C in areas of the intermountain region of the United States. However, only nematodes that have adapted, through natural selection or mutational changes, may have survived periods of drought or severe moisture stress and high soil temperatures, thus perpetuating a temperature-adapted population.

The pathotypic differences observed in the different *P. neglectus* populations indicate that variability in geographically and environmentally separated populations may be greater than once thought. These differences may occur in overlapping populations of similar or different species or in an isolated deme.

A recent study showed pathotypic differences between *Meloidogyne hapla* populations (5) that directly affect breeding and screening programs. These findings with *M. hapla* and *P. neglectus* would indicate the possible existence of similar relationships between geographically separated populations as well as other nematode species, indicating comparable factors must be considered in nematode resistant breeding and screening programs.

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