DIFFERENTIAL HOST RESPONSE OF ALFALFA TO *PRATYLENCHUS PENETRANS* AND *P. NEGLECTUS* POPULATIONS[†]

G. D. Griffin

Nematologist, USDA ARS, Forage and Range Research Laboratory, Utah State University, Logan, UT 84322-6300, U.S.A.

ABSTRACT

Griffin, G. D. 1993. Differential host response of alfalfa to *Pratylenchus penetrans* and *P. neglectus* populations. Nematropica 23:27–33.

In a 139-day greenhouse experiment at 24 ± 3 °C, *Pratylenchus penetrans* populations from alfalfa in southern (PP1) and central Utah (PP2) were more virulent to 'Lahontan' alfalfa than were *P. neglectus* populations from alfalfa in northern (PN1) and central Utah (PN2). PP1 and PP2 did not differ in virulence or reproduction, but PN1 was more virulent and had a higher reproductive index than PN2. For all populations, there were negative correlations between inoculum density and dry shoot weight, as well as between inoculum density and dry root weight. There were positive correlations between root growth and nematode reproduction. In environmental growth chambers at constant temperatures of 15, 20, 25, and 30 °C, differences between the effects of the populations on alfalfa in growth chambers at all temperatures were generally similar to those observed in the greenhouse. However, the optimum temperature for alfalfa growth was near 25 °C whereas the optimum temperature for nematode reproduction and pathogenicity in all cases was near 30 °C. At 30 °C, both *P. penetrans* populations and the *P. neglectus* population PN1 caused considerable damage to alfalfa.

Key words: alfalfa, *Medicago sativa, Pratylenchus neglectus, Pratylenchus penetrans*, reproductive index, root lesion nematode, shoot weight, temperature, virulence.

RESUMEN

Griffin, G. D. 1993. Respuesta comparativa de la alfalfa como hospedador a poblaciones de *Pratylenchus penetrans* y *P. neglectus*. Nematrópica 23:27–33.

En un experimento de invernadero de 139 días (24 = °C), poblaciones de *Pratylenchus penetrans* obtenidas de raíces de la alfalfa provenientes del sur (PP1) y la parte central (PP2) del estado de Utah de los EE.UU. fueron más virulentas en alfalfa que poblaciones de *P. neglectus* originarias del norte (PN1) y la parte central (PN2) del estado. No hubo diferencia en virulencia ni en la tasa de reproducción entre PP1 y PP2. En contraste, la PN1 fue más virulenta y tuvo un índice de reproducción más alto que la PN2. En todas las poblaciones se encontraron correlaciones negativas entre el nivel de inóculo y el peso seco de la planta y entre el nivel de inóculo y el peso de las raíces. Se observaron correlaciones positivas entre la reproducción del nematodo y el crecimiento de las raíces. Las diferencias relativas entre los efectos de las poblaciones sobre el crecimiento de la alfalfa en cámaras climáticas con temperaturas constantes de 15, 20, 25, y 30 °C fueron, en general, similares a las observadas en invernadero. Sin embargo, la temperatura óptima para el crecimiento de la alfalfa fue cerca de 25 °C, mientras que en todos los casos la temperatura óptima para reproducción y establecer patogenicidad de las poblaciones fue cerca de 30 °C. A esta temperatura, ambas poblaciones de *P. penetrans* y la población PN1 de *P. neglectus* causaron un daño significativo en alfalfa.

Palabras clave: alfalfa, *Medicago sativa, Pratylenchus neglectus, Pratylenchus penetrans*, índice de reproducción, nematodo lesionador, temperatura, virulencia.

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INTRODUCTION

Pratylenchus penetrans (Cobb) Filipjev & Schuurmans Stekhoven is the lesion nematode most commonly associated with poor growth and decline of alfalfa (*Medicago sativa* L.) in North America (2,7,8,10). However, *P. neglectus* (Rensch) Filipjev & Schuurmans Stekhoven and other *Pratylenchus* spp. have been found associated with poor growth of alfalfa (2,3,4).

Intraspecific differences in pathogenicity have been found between *P. neglectus* populations on alfalfa (3), and between populations of *P. penetrans* on tobacco (9). It is not known, however, if *P. penetrans* populations differ in pathogenicity to alfalfa. This study was initiated to compare pathological differences between two populations of *P. penetrans* and two of *P. neglectus* on alfalfa. Since previous studies have shown that temperature can significantly alter the host-parasite relationship of lesion nematodes (1,3,7), growth chamber experiments at controlled temperatures as well as greenhouse experiments were conducted.

MATERIALS AND METHODS

Nematode inocula: Pratylenchus penetrans was obtained from alfalfa from southern (PP1) and central Utah (PP2). *Pratylenchus neglectus* was obtained from alfalfa in northern (PN1) and central Utah (PN2). Nematodes were cultured on 'Ranger' alfalfa in a greenhouse at 24 ± 3 °C. Nematodes were obtained from alfalfa roots with a Baermann funnel, then surface sterilized, and passed through several rinses of distilled water (4). Lahontan alfalfa was used in all experiments.

Greenhouse bench experiment: Alfalfa seeds were scarified, treated with captan, germinated on filter paper in petri dishes for 48 hr, and washed with deionized water. When radicles were 2–5 mm long, seedlings were planted into individual plastic containers (6 cm diam × 21 cm deep) containing 540 cm^3 of steam-pasteurized Kidman fine sandy loam (coarse-loamy mixed mesic Calcic Haploxeroll; 84% sand, 8% silt, 8% clay; pH 7.4; 1.0% organic matter). *Rhizobium meliloti* Dang. was applied around the seedling at planting to insure nodulation. After 21 days of plant growth, soil in each container was inoculated with a series of 0, 1, 2, 10, or 20 *P. penetrans* or *P. neglectus* (mixed stages) per cm^3 soil. Nematodes suspended in deionized water were poured into four holes 10 cm deep in the soil around the hypocotyl base. Uninoculated controls received deionized water alone. Containers were maintained in a greenhouse at 24 ± 3 °C.

The experiment was a 4 × 5 factorial (4 nematode populations × 5 inoculum densities) in a randomized complete block design with 20 replications, one plant per replicate. Supplemental light for a 19-hr day length was provided by high-output fluorescent lamps. Plants were watered daily and fertilized monthly with a complete nutrient solution. All plants were harvested 118 days after inoculation. Plant mortality, shoot and root weights, and nematode reproductive indices (Pf/Pi = final nematode population/initial nematode inoculum) were determined. Nematodes were extracted from the soil by elutriation followed by rapid centrifugal-flotation (6), and from alfalfa roots with a Baermann funnel (4). Each parameter measured was regressed against inoculum density. Differences between means were compared with the LSD at $P \leq 0.05$. Percentage data on plant survival was transformed to arcsin $\sqrt{X}$ prior to analysis of variance. The experiment was repeated. The data presented are the combined data from the two experiments.

Growth chamber temperature experiment: A study similar to the greenhouse exper-

iment was conducted in temperature controlled growth chambers, but only with inoculum densities of 0, 2, and 10 nematodes/cm³ soil. Plants were grown in four chambers at constant temperatures of 15, 20, 25, and 30 °C for 110 days following inoculation. The experiment was a 4 × 4 × 3 factorial (4 nematode populations × 4 temperatures × 3 inoculum densities) in a randomized complete block design with 10 replications, one plant per replicate. The experiment was repeated. Data were collected, recorded, and analyzed as described for the greenhouse bench study.

RESULTS

Greenhouse experiment: Both *P. penetrans* and *P. neglectus* reduced alfalfa growth. There were negative correlations between *P. penetrans* and P. neglectus inoculum densities and dry shoot weights ($r = -0.87$ and -0.79, respectively), and inoculum densities and dry root weights ($r = -0.84$ and -0.76). Between Pi = 1 and Pi = 20 nematodes/cm³ soil, dry shoot weights of plants inoculated with PP1, PP2, PN1, and PN2, respectively, ranged from 17 to 63%, 17 to 60%, 4 to 47%, and 0 to 30% (Table 1).

Table 1. Effect of two *Pratylenchus penetrans* populations (PP1, PP2) and two *P. neglectus* populations (PN1, PN2) on Lahontan alfalfa dry shoot and dry root weights, and the reproductive index of each population 118 days after inoculation in greenhouse containers at 24 ± 3 °C.[x]

Inoculum (Nematodes/ cm³ soil)	Nematode populations[y]				LSD
	PP1	PP2	PN1	PN2	($P \leq 0.05$)
			Dry shoot weight (g)		
0	3.39	3.43	3.34	3.37	0.16
1	2.78	2.83	3.27	3.43	0.46
2	2.34	2.28	2.74	3.25	0.32
10	1.69	1.73	2.28	3.12	0.34
20	1.26	1.36	1.80	2.37	0.40
LSD ($P \leq 0.05$)	0.40	0.34	0.38	0.31	
			Dry root weight (g)		
0	1.41	1.44	1.38	1.40	0.13
1	1.26	1.21	1.35	1.39	0.28
2	0.87	0.93	1.19	1.30	0.23
10	0.69	0.64	1.04	1.26	0.34
20	0.48	0.52	0.72	0.97	0.16
LSD ($P \leq 0.05$)	0.32	0.38	0.29	0.24	
			Reproductive index (Pf/Pi)[z]		
1	25.6	27.2	18.6	7.5	3.8
2	19.4	18.2	13.9	6.6	3.2
10	12.8	14.1	9.3	4.5	2.3
20	4.7	4.6	4.2	3.1	2.0
LSD ($P \leq 0.05$)	2.8	3.5	3.2	1.8	

Values are the means of two experiments; 10 replicates (1 plant/replicate) per experiment.
[x]Plants inoculated at 21 days and grown for 118 days.
[y]PP1 from southern, PP2 from central, PN1 from northern, and PN2 from central Utah; all populations from alfalfa.
[z]Pf/Pi = final nematode population/initial nematode population.

Both *P. penetrans* populations (PP1 and PP2) were more virulent ($P \leq 0.05$) to Lahontan alfalfa than were the *P. neglectus* populations (PN1 and PN2); PN1 was more virulent than PN2 ($P \leq 0.05$). Mortality rates of plants parasitized by PP1 and PP2, respectively, were 0 and 0% at Pi = 1, 10 and 15% at Pi = 2, 30 and 25% at Pi = 10, and were 45 and 50% at Pi = 20 nematodes/cm^3 soil. This compared to mortality rates for PN1 and PN2 of 0 and 0%, 0 and 0%, 10 and 0%, and 30 and 15%, at the same inoculum densities.

Reproduction of both *P. penetrans* populations was greater ($P \leq 0.05$) than that of both *P. neglectus* populations, and reproduction of PN1 was greater ($P \leq 0.05$) than that of PN2 (Table 1). There were positive correlations between root growth and nematode reproduction ($r = 0.86$ for both populations of *P. penetrans* combined; $r = 0.89$ for *P. neglectus* population PN1; $r = 0.62$ for PN2). In each case, the greatest reproductive index (Pf/Pi) occurred on plants with greatest root growth and the lowest nematode inoculum level.

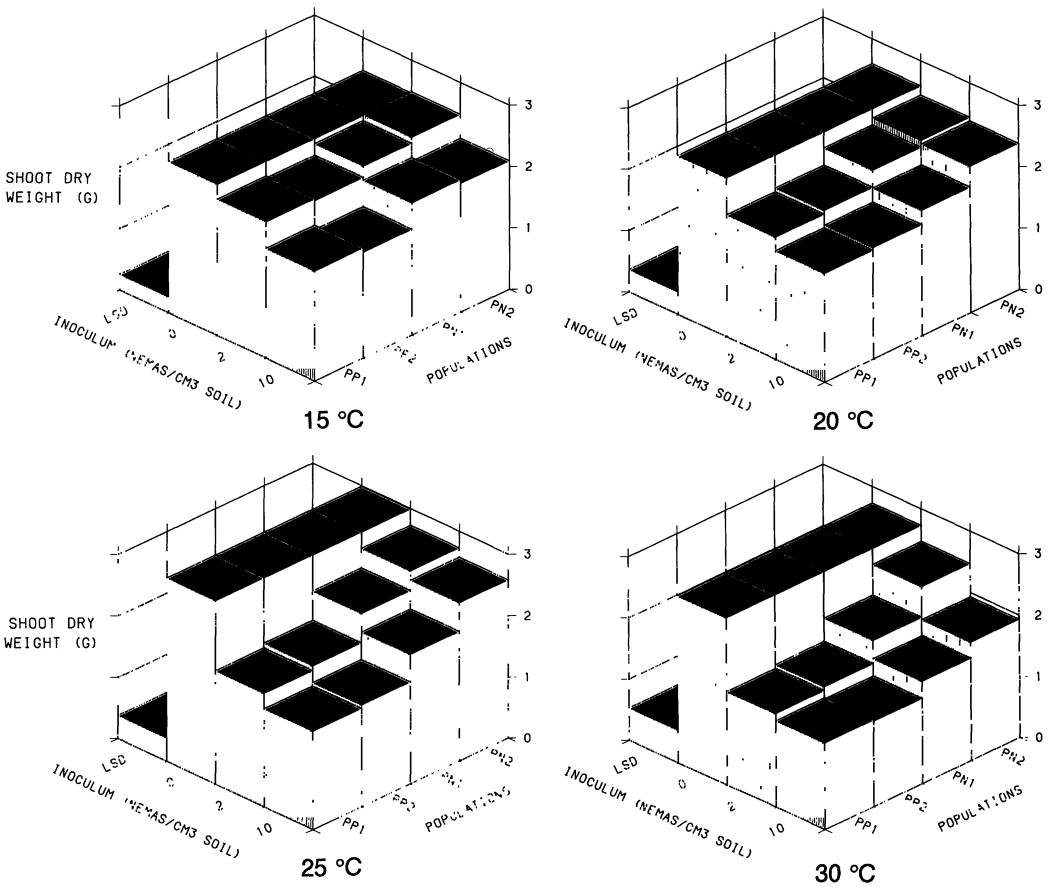

Fig. 1. Effects of *Pratylenchus penetrans* populations from alfalfa in southern (PP1) and central Utah (PP2), and *P. neglectus* populations from alfalfa in northern (PN1) and central Utah (PN2), on the dry shoot weight of Lahontan alfalfa after 110 days at four constant growth chamber temperatures. The LSD at $P < 0.05$ for all data at each temperature is given in the PN2 row of each 3-dimensional histogram.

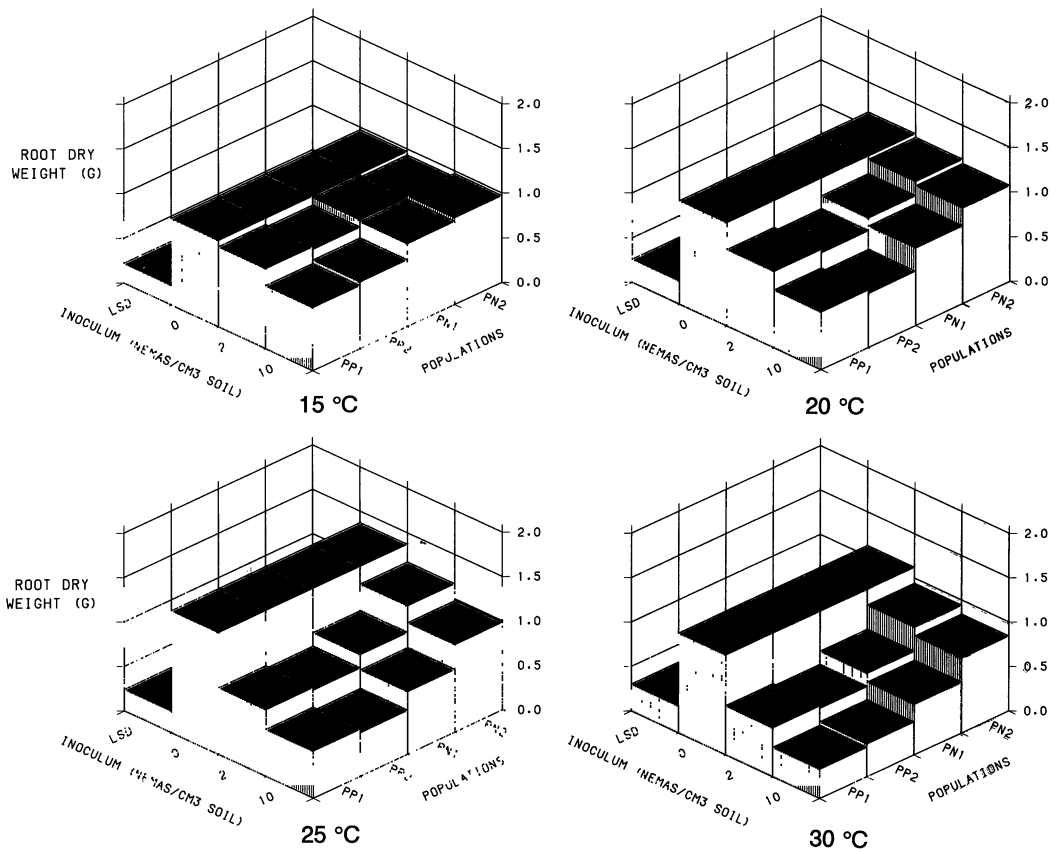

Fig. 2. Effects of *Pratylenchus penetrans* populations from alfalfa in southern (PP1) and central Utah (PP2), and *P. neglectus* from alfalfa in northern (PN1) and central Utah (PN2), on the dry root weight of Lahontan alfalfa after 110 days at four constant growth chamber temperatures. The LSD at $P < 0.05$ for all data at each temperature is given in the PN2 row of each 3-dimensional histogram.

Growth chamber experiment: At all temperatures, the ranking of the nematode populations tested with regard to nematode reproduction and with regard to effects of nematodes on plant survival and growth were the same as observed in the greenhouse experiment. However, temperature altered the magnitudes of differences appreciably. The greatest plant growth of uninoculated plants occurred at 25 °C, with dry shoot weights 13 and 9% lower and dry root weights 15 and 18% lower at 20 and 30 °C (Fig. 1,2). For all nematode populations, however, detrimental effects of nematode parasitism were greater ($P \leq 0.05$) at 30 °C than at 20 °C. Nematode reproductive indices, similarly were 54 to 69% greater for *P. penetrans* and 53 to 101% greater for *P. neglectus* at 30 °C than at 20 °C. Therefore, the optimum temperature for plant growth at a constant temperature was near 25 °C, whereas the optimum temperature for nematode reproduction and pathogenicity in both nematode species was higher, and probably near 30 °C.

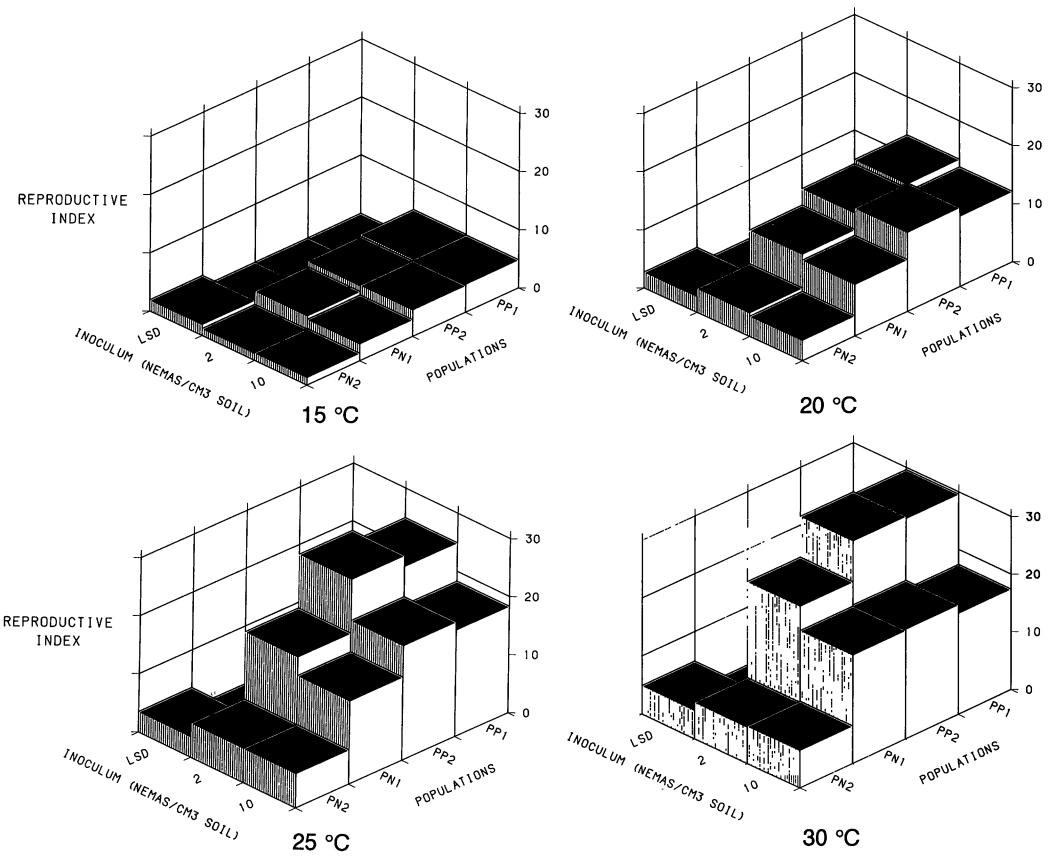

Fig. 3. Reproductive indices (Pf/Pi) of *Pratylenchus penetrans* populations from alfalfa in southern (PP1) and central Utah (PP2), and *P. neglectus* populations from alfalfa in northern (PN1) and central Utah (PN2), on Lahontan alfalfa after 110 days at four constant growth chamber temperatures. The LSD at $P < 0.05$ for all data at each temperature is given in the PN2 row of each 3-dimensional histogram.

DISCUSSION

Pratylenchus neglectus is found throughout the western United States in virgin soils as well as in cultivated soils, suggesting it is endemic to this region. In the vast Intermountain Region, east of the Sierra Nevada range of North America, *P. penetrans* has a limited distribution compared to that of *P. neglectus* and occurs primarily in cultivated soils (G. D. Griffin, unpublished data). This suggests that *P. penetrans* has been introduced into the Intermountain Region. Intraspecific differences have been observed between populations of *P. penetrans* (9); the absence of differences in pathogenicity to alfalfa among the populations of *P. penetrans* from the Intermountain Region that have been studied suggests that those populations may have originated from a recent common origin. *Pratylenchus neglectus*, however, is more likely native to the Intermountain region, or has apparently been present in the region long enough for differences in host preference, or reproductive potential on the same host to evolve

among geographically isolated populations. Intraspecific divergence of nematode populations may have developed from association with a single host favoring certain gene combinations while suppressing others (12). This may be the situation with *P. neglectus* on alfalfa. Since population and race differentiation have commonly been observed in nematodes, including root lesion nematodes, (3,5,9, 11), additional pathological as well as molecular systematic comparisons are needed to further test this hypothesis.

Results of these experiments are in agreement with data from previous studies where *Pratylenchus* spp. were found to have thermal optima for reproduction and pathogenicity near 30 °C (3,4,7). Although soil temperatures may approach 30 °C only during the warmest months of the year in the Intermountain Region of the western United States, it is apparent that during those months some populations of *P. neglectus* may economically damage alfalfa crops. The possible economic impact of *P. neglectus* populations, such as PN1, that can reproduce rapidly on alfalfa indictates a need for further study under field conditions.

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