Journal of Nematology 25(3):461–465. 1993.

Comparative Response of Alfalfa to *Pratylenchus penetrans* Populations[1]

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Abstract: Four populations of *Pratylenchus penetrans* did not differ ($P > 0.05$) in their virulence or reproductive capability on Lahontan alfalfa. There was a negative relationship ($r = -0.79$) between plant survival and nematode inocula densities at 26 ± 3 C in the greenhouse. All plants survived at an inoculum level (Pi) of 1 nematode/cm^3 soil, whereas survival rates were 50 to 55% at 20 nematodes/cm^3 soil. Alfalfa shoot and root weights were negatively correlated ($r = -0.87$; $P < 0.05$) with nematode inoculum densities. Plant shoot weight reductions ranged from 13% at Pi 1 nematode/cm^3 soil to 69% for Pi 20 nematodes/cm^3 soil, whereas root weight reductions ranged from 17% for Pi 1 nematode/cm^3 soil to 75% for Pi 20 nematodes/cm^3 soil. Maximum and minimum nematode reproduction (Pf/Pi) for the *P. penetrans* populations were 26.7 and 6.2 for Pi 1 and 20 nematodes/cm^3 soil, respectively. There were negative correlations between nematode inoculum densities and plant survival ($r = 0.84$), and soil temperature and plant survival ($r = -0.78$). Nematode reproduction was positively correlated to root weight ($r = 0.89$).

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

Pratylenchus spp. are among the most important plant-parasitic nematodes. The root-lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven, is pathogenic to alfalfa throughout most regions of North America (4,7,9). Plant growth is inhibited by the nematode, and stunted plants may appear (4). Damage caused by root-lesion nematodes is difficult to evaluate because other soil-borne microorganisms also invade and damage infected roots. Aboveground symptoms also are difficult to assess, because other pathogens may produce similar symptoms (10).

Genetic diversity and population variability are known to exist within nematode species (8,12–14), including *Pratylenchus penetrans* (11). A previous study has shown that a population of *P. neglectus,* apparently endemic to the western United States, was more virulent to alfalfa than were other geographically separated populations of *P. neglectus* (5). The limited distribution of *P. penetrans* in the western United States (4) suggests it may have been introduced with infected plant material or infested soil. Because race differences in *P. penetrans* have been introduced with infected plant material or infested soil. Because race differences in *P. penetrans* have been shown on tobacco (11), a study was made to determine if there are intraspecific differences in virulence and reproduction of four *P. penetrans* populations found associated with alfalfa in Utah and Colorado. Because temperature is known to affect the relationship between lesion nematodes and soybean (1), tomato (3), and alfalfa (5), the effect of temperature on the host–nematode relationship between *P. penetrans* and alfalfa was also considered.

Materials and Methods

Nematode inocula: Pratylenchus penetrans was obtained from alfalfa, *Medicago sativa* L., from southern Utah (UT1), central Utah (UT2), western Colorado (CO1), and central Colorado (CO2). Nematodes were cultured on Ranger alfalfa at 26 ± 3 C in a greenhouse. Nematode inocula were obtained from roots with a Baermann funnel and surface sterilized (5).

Greenhouse experiment: Lahontan alfalfa seeds were scarified, treated with *N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide, and germinated on filter paper in petri dishes for 48 hours. When radicles were 2–5 mm long, seedlings were planted into individual plastic containers (6-cm-d × 21-cm-deep) con-

Received for publication 9 September 1992.

[1] Cooperative investigation, USDA ARS, and the Utah Agricultural Experiment Station. Journal Paper No. 4334.

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taining 540-cm^3 steam pasteurized Kidman fine sandy loam (coarse-loamy mixed mesic Calcic Haploxeroll, 83% sand, 10% silt, 7% clay; pH 7.4, 1.0% OM). *Rhizobium meliloti* Dang. was applied around the roots of each seedling at planting to ensure nodulation.

After 30 days, soil in each container was infested with either 0, 1.0, 2.0, 10.0 or 20.0 *P. penetrans* (mixed stages) per cm^3 soil. Nematodes suspended in deionized water were poured into four 10-cm deep holes in the soil around the hypocotyl base of the plant. The experiment was a 4 × 5 factorial (4 nematode populations × 5 inocula densities) in a randomized complete block design with 20 replications (one plant per replicate). Uninoculated controls received deionized water. Containers were maintained at a greenhouse temperature of 26 ± 3 C. Supplemental light was provided for 19 hours by high-output fluorescent lamps. Plants were watered lightly after inoculation, and as needed thereafter. The experiment was terminated 120 days after inoculation, and plant survival and nematode reproductive indices (Pf/Pi = final nematode population [Pf] divided by initial nematode inoculum [Pi]) were determined. Shoots and roots were air dried and weighed. Nematodes were extracted from the soil by elutriation (2) and centrifugal-flotation (6). Nematodes were extracted from alfalfa roots using Baermann funnels (5). Data were analyzed using ANOVA and correlation analysis. Percentage data on plant survival were transformed using arcsine transformation prior to ANOVA. The experiment was repeated, and the data presented here are a combination of the two experiments.

Growth chamber experiment: Alfalfa seed was germinated, and seedlings were planted and inoculated in a manner similar to that described for the greenhouse experiment, except that Pi of 0, 2, and 10/cm^3 soil were used. The experiment was a 4 × 4 × 3 factorial (4 nematode populations × 4 temperatures × 3 inoculum densities) in a randomized complete block design with 20 replications (one plant per replicate). Plants were grown in chambers for 120 days after inoculation at 15, 20, 25, and 30 C (±1 C). A 19-hour daylength was provided by high-output fluorescent lamps. The experiment was repeated. Data from two experiments were collected, combined, and analyzed as described for the greenhouse study.

Results and Discussion

Greenhouse experiment: Virulence of the four *Pratylenchus penetrans* populations did not differ ($P > 0.05$) on Lahontan alfalfa. All plants survived at Pi 1 and 2 nematode/

Table 1. Effects of four different *Pratylenchus penetrans* populations from Utah (UT1, UT2), and Colorado (CO1, CO2) on survival, shoot, and root weight of Lahontan alfalfa, and nematode reproduction in the greenhouse.*

Inoculum† (nematodes/cm^3 soil)	UT1‡	UT2	CO1	CO2
	Percentage survival			
0	100 c§	100 c	100 c	100 c
1	100 c	100 c	100 c	100 c
2	95 c	95 c	90 c	95 c
10	70 b	75 b	70 b	70 b
20	50 a	50 a	55 a	50 a
	Shoot weight (g)			
0	3.13 e	3.18 e	3.20 e	3.15 e
1	2.66 d	2.75 d	2.70 d	2.79 d
2	2.16 c	2.12 c	2.07 c	2.16 c
10	1.66 b	1.55 b	1.60 b	1.50 b
20	1.05 a	0.98 a	1.12 a	1.08 a
	Root weight (g)			
0	1.23 e	1.20 e	1.18 e	1.25 e
1	1.04 d	0.98 d	1.00 d	1.01 d
2	0.84 c	0.78 c	0.79 c	0.87 c
10	0.57 b	0.63 b	0.61 b	0.55 b
20	0.32 a	0.36 a	0.32 a	0.37 a
	Nematode reproductive indices (Pf/Pi)¶			
1	23.8 d	26.7 d	22.5 d	24.6 d
2	17.2 c	20.9 c	18.9 c	18.6 c
10	10.8 b	13.3 b	12.6 b	11.5 b
20	5.3 a	4.6 a	6.2 a	4.1 a

* Plants were inoculated at 30 days after seeding and grown for 120 days after inoculation at 26 ± 3 C.

† Inoculum obtained from alfalfa from southern Utah (UT1), central Utah (UT2), western Colorado (CO1), and central Colorado (CO2).

‡ Values are the means of 20 replicates (1 plant/replicate). Means in columns not followed by the same letter differ significantly ($P > 0.05$).

§ The four nematode populations did not differ significantly ($P < 0.05$), and the letters are omitted.

¶ Pf/Pi = final nematode population/initial nematode population.

cm^3 soil. Plant persistence was reduced ($P = 0.05$) with Pi 10 and 20 nematodes/cm^3 soil (Table 1), and there was a negative correlation between plant survival and nematode inoculum density ($r = -0.79$). *Pratylenchus penetrans* reduced ($P < 0.05$) shoot and root growth of Lahontan alfalfa (Table 1). There were negative correlations between shoot weight and nematode inoculum density ($r = -0.87$) and root weight and nematode inoculum density ($r = -0.82$). Shoot weight reductions ranged from 13% for 1 nematode/cm^3 soil to 69% for 20 nematodes/cm^3 soil, while root weight reductions ranged from 17% for 1 nematode/cm^3 soil to 75% for 20 nematodes/cm^3 soil. Nematode reproduction was positively correlated with root weight ($r = 0.84$) and negatively correlated with inoculum density ($r = -0.93$). Maximum and minimum Pf/Pi were 26.7 and 4.1 for 1 and 20 nematodes/cm^3 soil, respectively.

Growth chamber experiment: Results similar to the greenhouse findings were observed in the growth chamber experiment at 15–30 C. There were negative correlations between nematode inoculum densities and plant survival ($r = -0.84$) and soil temperature and plant survival ($r = -0.78$). At Pi 2 nematodes/cm^3 soil, survival rates of Lahontan plants inoculated with the four *P. penetrans* populations were 90–100, 80–90, 70–80, and 60–70% at 15, 20, 25, and 30 C, respectively. At Pi 10 nematodes/cm^3 soil, survival rates were 80–90, 80–90, 70–80, and 50–60% over the same temperature range. Survival of uninoculated plants was 100%. *Pratylenchus penetrans* reduced ($P < 0.05$), plant growth (shoot and root weights) at all soil temperatures (Figs. 1,2). Shoot weights and root weights were equally suppressed ($P < 0.05$) by the four nematode populations. There were negative correlations between

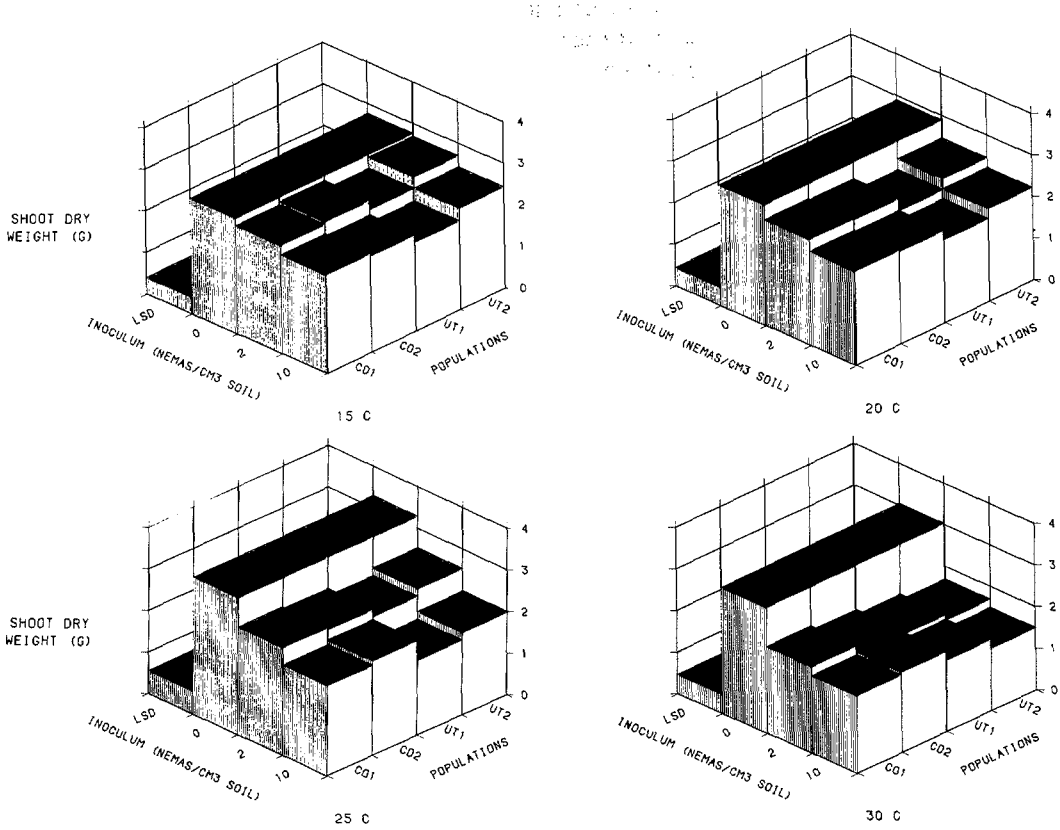

FIG. 1. Effect of *Pratylenchus penetrans* from alfalfa from southern Utah (UT1), central Utah (UT2), western Colorado (CO1), and central Colorado (CO2) on the shoot weight of Lahontan alfalfa after 120 days at four different growth chamber temperatures. LSD (0.05).

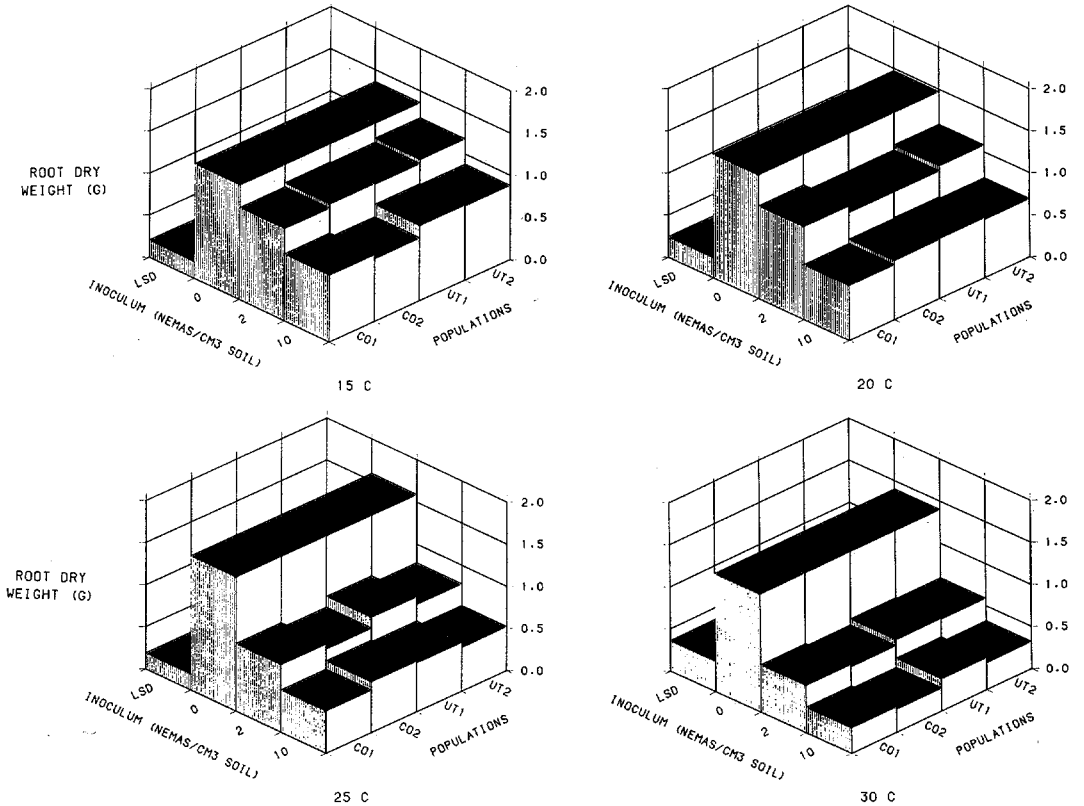

FIG. 2. Effect of four *Pratylenchus penetrans* from alfalfa from southern Utah (UT1), central Utah (UT2), western Colorado (CO1), and central Colorado (CO2) on the root weight of Lahontan alfalfa after 120 days at four different growth chamber temperatures. LSD (0.05).

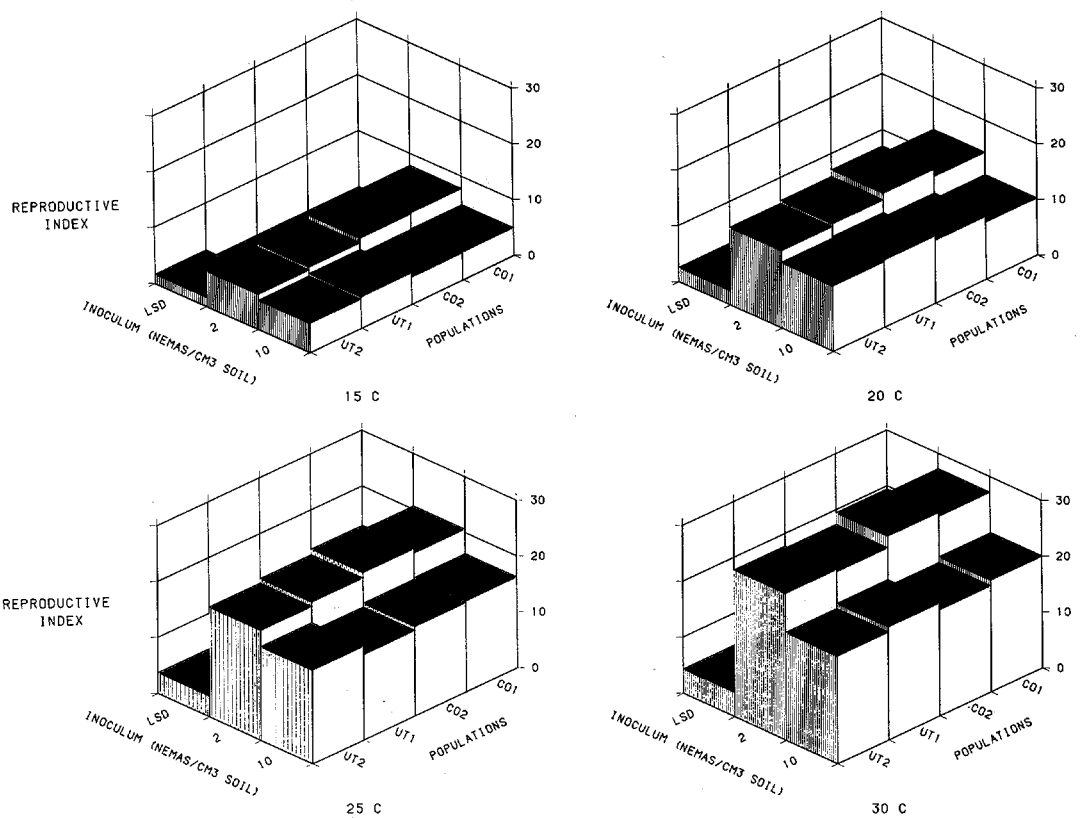

FIG. 3. Reproductive indices (Pf/Pi) of *Pratylenchus penetrans* from alfalfa from southern Utah (UT1), central Utah (UT2), western Colorado (CO1), and central Colorado (CO2) after 120 days at four different growth chamber temperatures. LSD (0.05).

inoculum densities and shoot weights ($r = -0.84$) and root weights ($r = -0.87$).

Temperature affected ($P < 0.05$) nematode reproduction (Fig. 3). The greatest nematode reproduction occurred at 30 C with a Pi of 2 nematodes/cm^3 soil. Nematode reproduction was positively correlated with root weights ($r = 0.89$). This corresponded to a negative relationship between nematode inoculum density and nematode reproduction ($r = -0.77$). The overall average Pf/Pi of the four nematode populations at all temperatures were 17.3 at Pi 2.0 and 13.0 at Pi 10 nematodes/cm^3 soil.

It appears from this study that there may not be the degree of intraspecific divergence in *Pratylenchus penetrans* populations as was shown for *P. neglectus* (5). The limited distribution of *P. penetrans* indicates that it was introduced into the Intermountain Region of the western United States. The possibility exists, however, that further study of a larger sample size may document intraspecific differences among *P. penetrans* populations attacking alfalfa as have been found for tobacco (11).

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