Effect of Single and Interplantings on Pathogenicity of *Pratylenchus penetrans* and *P. neglectus* to Alfalfa and Crested Wheatgrass

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**Abstract:** Alfalfa is a host of *Pratylenchus penetrans* and *P. neglectus*, whereas crested wheatgrass is a host of *P. neglectus* but not of *P. penetrans*. In a 120-day greenhouse experiment at 24 ± 3°C, *P. neglectus* inhibited the growth of 'Lahontan' alfalfa and 'Fairway' crested wheatgrass. There were no differences in persistence and plant growth of alfalfa and crested wheatgrass, or reproduction of *P. neglectus*, in single plantings of alfalfa (AO) or crested wheatgrass (CWO), or in interplanted alfalfa and crested wheatgrass (ACW) treatments. On alfalfa, *P. penetrans* inhibited growth and reproduced more than did *P. neglectus*. Inhibition of plant growth and reproduction of *P. penetrans* was greater on alfalfa in AO than in ACW treatments. *Pratylenchus penetrans* did not reproduce on crested wheatgrass, but inhibited growth of crested wheatgrass in interplanted treatments and was avirulent in single planted treatments. Results were similar in a controlled growth chamber experiment at 15, 20, 25, and 30°C. Both nematode species inhibited alfalfa growth at all temperatures, and *P. penetrans* was more virulent than was *P. neglectus* to alfalfa at all temperatures and treatments. Plant growth inhibition and reproduction of *P. penetrans* on alfalfa in single and interplanted treatments were similar at 15–20°C, but were greater in single than in interplanted treatments at 25–30°C. *Pratylenchus penetrans* was avirulent to crested wheatgrass in the single planted treatments at all temperatures, but inhibited growth of crested wheatgrass in interplanted treatments at 20–30°C. Plant growth and reproduction of *P. neglectus* on crested wheatgrass was similar in single and interplanted treatments at 20–30°C and 15–30°C, respectively.

**Key words:** *Agropyron cristatum*, alfalfa, crested wheatgrass, *Medicago sativa*, nematode, *Pratylenchus neglectus*, *P. penetrans*, reproductive index, root lesion nematodes, root weight, shoot weight, survival, virulence.

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Rangelands in the western United States consist mainly of mixtures of grasses, forbs, and shrubs. Less than optimum usage of rangelands has resulted in an increase in shrubs associated with a loss of grasslands (20). It is estimated that more than 86% of the rangelands are in poor condition, and productivity is less than 60% of their natural potential (22). The effort to upgrade rangeland quality includes the breeding of palatable grass cultivars and germplasm, including wheatgrasses that provide high quality forage grasses in western North America during the spring and early summer (1,2).


Interplanting of host and nonhost plants (4) into soil infested with plant-parasitic nematodes may affect plant growth and longevity. The Columbia root-knot nematode, *Meloidogyne chitwoodi*, Golden, O'Bannon, Santo, & Finley, reduced plant growth less in uniform plantings of alfalfa than in interplantings of alfalfa and grass (13). The lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjév & Schuurmans Stekhoven, is pathogenic to alfalfa throughout most regions of North America and inhibits alfalfa growth and stand persistence (7,17). Grasses are nonhosts of *P. penetrans* (10). However, *P. neglectus* (Rensch) Filipjév & Schuurmans Stekhoven, endemic to the western United States (7,11,14), parasites and reproduces on both alfalfa and grasses (7,8,10).
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Interplanting alfalfa and grasses affects the virulence of M. chitwoodi and plant persistence (13). The current study was initiated to determine the host–parasite relationships of P. penetrans and P. neglectus on alfalfa alone and interplanted with crested wheatgrass, and how these relationships are affected by inoculum density and temperature.

MATERIALS AND METHODS

Nematode inoculum: Pratylenchus penetrans and P. neglectus were obtained originally from alfalfa in separate fields from central Utah. Nematodes were cultured on Ranger alfalfa in a greenhouse at 26 ± 3 C. Nematodes were collected from alfalfa roots in a Baermann funnel, then surface sterilized, and passed through several rinses of distilled water (11).

Greenhouse bench experiment: Seedlings (3–5 mm radicle) of 'Lahontan' alfalfa and 'Fairway' crested wheatgrass were planted singly or interplanted into individual 6-cm-d plastic containers containing 540 cm³ of steam-pasteurized Kidman fine sandy loam (coarse-loamy mixed mesic Calcic Haploxeroll; 86% sand, 7% silt, 7% clay; pH 7.2; 1.0% organic matter). Rhizobium meliloti Dang. was applied around the roots of alfalfa to ensure nodulation. At 28 days after planting, soil in each container was inoculated with an initial density (Pi) of 0 (uninoculated control), 2, 4, or 8 P. penetrans or P. neglectus (mixed states) per cm³ soil. Nematodes suspended in deionized water were poured into four holes 10 cm deep in the soil around the hypocotyl base of the plant. Uninoculated controls received deionized water alone. Containers were maintained in a greenhouse at 24 ± 3 C.

The experiment was a 2 × 3 × 4 factorial (2 nematodes species × 3 plant combinations × 4 inoculum densities) in a randomized block design with 30 replications, four plants per replicate or single pot (four alfalfa plants, or four crested wheatgrass plants, or two alfalfa plus two crested wheatgrass plants). Supplemental light for a 19-hour day length was provided by high-output fluorescent lamps. Plants were watered daily and fertilized biweekly with a complete nutrient solution to offset any effect of an increase in nitrogen from R. meliloti. Ten replicates of each treatment were harvested 14 days after inoculation, roots were washed free of soil, stained, and nematode invasion determined under a stereomicroscope. The remaining 20 replicates were harvested 120 days later, and plant persistence, shoot and root weights, and nematode reproductive indices (Pi/Pf = final nematode population/initial nematode inoculum) were determined. Nematodes were extracted from the soil by elu-triation (3) followed by rapid centrifugal-flotation (15), and from alfalfa and grass roots with a Baermann funnel (11). Each parameter measured was correlated against inoculum density. Data were analyzed with ANOVA and analyzed as two 2 × 2 × 4 factorials, since data for each plant species were analyzed separately. Percentage data on plant survival were transformed using an arcsine transformation, and differences among means were compared at P < 0.05 using LSD or Duncan's Multiple-Range test. The experiment was repeated, and the data presented here are means of a combination of the two experiments.

Growth chamber temperature experiment: A study similar to the greenhouse experiment was conducted in temperature-controlled growth chambers, but only with inoculum densities of 0 (uninoculated control) and 4 nematodes/cm³ soil. Plants were grown in four chambers at constant temperatures of 15, 20, 25, and 30 C. The experiment was a 4 × 3 × 3 factorial (4 temperatures × 3 plant combinations × 3 nematode treatments and analyzed as two 4 × 2 × 3 factorials) in a randomized block design with 12 replications, four plants per replicate. Four replicates of each treatment were harvested 14 days after inoculation and nematode invasion was determined. The remaining eight replicates were harvested 106 days later, and data were collected, recorded, and analyzed as described for the greenhouse bench study.
The experiment was repeated; the data presented here are means of a combination of the two experiments.

**RESULTS**

Greenhouse bench experiment—alfalfa: All alfalfa in single and alfalfa-crested wheatgrass interplant treatments survived *Pratylenchus penetrans* and *P. neglectus* at Pi of 0 and 2 nematodes/cm$^3$ soil, whereas survival rates were 89 and 93% for single and 79 and 88% for interplanted treatments at Pi of 4 and 8 *P. penetrans*, respectively. Comparable survival rates for *P. neglectus* were 94 and 97%, and 97 and 98%. Inoculum density was positively correlated with the invasion by *P. penetrans* ($r = 0.89$) and *P. neglectus* ($r = 0.86$). Invasion by *P. penetrans* at all Pi exceeded ($P < 0.05$) that of *P. neglectus* for both single and interplanted treatments (Table 1). Invasion of alfalfa root tissue by *P. neglectus* was similar for single and interplant treatments at all Pi, whereas more ($P < 0.05$) *P. penetrans* invaded single than interplantings of alfalfa at Pi of 8.

Pi was negatively correlated with alfalfa shoot and root growth for *P. penetrans* (shoot $r = -0.90$; root, $r = -0.85$), and for *P. neglectus* (shoot, $r = -0.91$; root, $r = -0.82$); the greatest reductions ($P < 0.05$) in shoot and root weights were at a Pi of 8 (Table 1). *Pratylenchus penetrans* was more virulent to alfalfa than *P. neglectus* at all Pi and treatments. Virulence of *P. neglectus* was similar in single and interplanted treatments, whereas *P. penetrans* was more virulent to shoot growth in single than in interplanted treatments.

There were significant differences in reproduction of *P. penetrans* in single and interplantings at Pi of 2 and 4, whereas there were no differences in reproduction of *P. neglectus* on alfalfa in single or interplantings. Nematode reproduction was positively correlated with root growth for *P. penetrans* ($r = 0.83$) and *P. neglectus* ($r = 0.88$). The Pi was negatively correlated with reproduction of *P. penetrans* ($r = -0.85$) and *P. neglectus* ($r = -0.80$).

Crested wheatgrass: All ‘Fairway’ crested wheatgrass plants survived exposure to all

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**Table 1.** Invasion, pathogenicity, and reproduction of *Pratylenchus penetrans* and *P. neglectus* on ‘Lahontan’ alfalfa from single and interplantings of alfalfa and ‘Fairway’ crested wheatgrass at 24 ± 3 C.

<table>
<thead>
<tr>
<th>Inoculum level (Nematodes/cm$^3$ soil)</th>
<th><em>P. penetrans</em></th>
<th><em>P. neglectus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Interplant</td>
</tr>
<tr>
<td></td>
<td>Nematodes/g root tissue‡</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>55 cA</td>
<td>56 cA</td>
</tr>
<tr>
<td>4</td>
<td>110 bA</td>
<td>123 bA</td>
</tr>
<tr>
<td>8</td>
<td>162 aB</td>
<td>210 aA</td>
</tr>
<tr>
<td></td>
<td>Dry shoot weight (g)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.48 aA</td>
<td>3.42 aA</td>
</tr>
<tr>
<td>2</td>
<td>2.25 bC</td>
<td>2.53 bB</td>
</tr>
<tr>
<td>4</td>
<td>1.34 cC</td>
<td>2.07 cB</td>
</tr>
<tr>
<td>8</td>
<td>1.12 dB</td>
<td>1.73 dA</td>
</tr>
<tr>
<td></td>
<td>Dry shoot weight (g)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.75 aA</td>
<td>1.73 aA</td>
</tr>
<tr>
<td>2</td>
<td>1.33 bA</td>
<td>1.44 bB</td>
</tr>
<tr>
<td>4</td>
<td>0.61 cB</td>
<td>0.93 cA</td>
</tr>
<tr>
<td>8</td>
<td>0.40 dB</td>
<td>0.78 dA</td>
</tr>
<tr>
<td></td>
<td>Reproductive indices (Pi/Pf)§</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23.4 aA</td>
<td>17.4 aB</td>
</tr>
<tr>
<td>4</td>
<td>14.7 bA</td>
<td>12.0 bB</td>
</tr>
<tr>
<td>8</td>
<td>7.2 cA</td>
<td>5.5 cA</td>
</tr>
</tbody>
</table>

Values are the means of 20 replicates (four plants/replicate). Means not followed by the same letter differ ($P < 0.05$) according to Duncan's Multiple-Range Test (lower case letters for columns, CAPITAL letters for rows).

$\dagger$ Susceptible to *P. penetrans* and *P. neglectus*.

$\ddagger$ Nematode invasion determined 14 days after inoculation. Plants inoculated at 28 days with Pi 0 and 4 nematodes/cm$^3$ soil and grown for 120 days.

$§$ (Pi/Pf) = final nematode population/initial nematode population.
Pratylenchus penetrans treatments and P. neglectus at Pi of 2. Survival rates in response to P. neglectus were 95 and 89% for single and 96 and 88% for interplanted treatments at Pi of 4 and 8 P. neglectus, respectively. Invasion of crested wheatgrass increased with inoculum density for P. penetrans (r = 0.73) and P. neglectus (r = 0.77). Invasion by P. neglectus at all Pi exceeded (P < 0.05) invasion by P. penetrans in both single and interplanted treatments (Table 2). Invasion of crested wheatgrass by P. penetrans and P. neglectus was similar in both single and interplanted treatments at all Pi.

Pratylenchus penetrans did not affect (P > 0.05) shoot growth of crested wheatgrass in the single-planted treatment, but it did cause a reduction (P < 0.05) in shoot growth in the interplanted treatment at Pi of 4 and 8 (Table 2). Crested wheatgrass root growth was not affected in any treatment by P. penetrans. Pratylenchus neglectus did not reduce crested wheatgrass shoot growth at Pi of 2, but at Pi of 4 and 8 it reduced (P < 0.05) shoot and root growth in both single and interplanted treatments. There were negative correlations between Pi and crested wheatgrass growth in single and interplanted treatments with P. neglectus (shoot, r = -0.79; root, r = -0.74).

Pratylenchus penetrans did not reproduce on crested wheatgrass, whereas reproduction of P. neglectus did not differ between single and interplanted treatments. There was, however, a positive correlation (r = 0.80) between root growth and nematode reproduction, and a negative correlation (r = -0.73) between Pi and reproduction of P. neglectus.

Growth chamber experiment—alfalfa: Alfalfa survived single and interplanted treatments (Pi = 4.0) of P. penetrans and P. neglectus at 15 and 20 C, whereas survival rates were 90 and 96% for single treatments, and 83 and 90% for interplanted treatments of P. penetrans at 25 and 30 C, respectively. Comparable survival rates were 95 and 96% for single treatments and 93 and 95% for interplanted treatments with P. neglectus at 25 and 30 C, respectively.

Significantly (P < 0.05) more P. pen-
than *P. neglectus* invaded alfalfa at all temperatures (Fig. 1). Similar numbers of *P. penetrans* invaded alfalfa in single and interplanted treatments at 15–20 C, whereas more (*P < 0.05*) nematodes invaded single than interplanted treatments at 25–30 C. There were no differences (*P < 0.05*) between the invasion of alfalfa by *P. neglectus* in single and interplanted treatments. Invasion by both nematode species increased with temperature (*r* = 0.91), and was greatest at 30 C.

Both *P. penetrans* and *P. neglectus* reduced (*P < 0.05*) alfalfa shoot growth in single and interplanted treatments at all soil temperatures, and reduced root growth at 20–30 C. Soil temperature was negatively correlated with shoot and root growth for *P. penetrans* (*r* = −0.82; *r* = −0.79), and for *P. neglectus* (*r* = −0.74; *r* = −0.70).

Soil temperature was positively correlated with nematode reproduction (*r* = 0.90) for *P. penetrans* and (*r* = 0.83) for *P. neglectus*. Reproduction of *P. neglectus* was not affected by single and interplanted treatments, but reproduction of *P. penetrans* was greater (*P < 0.05*) on alfalfa in both treatments at 20–30 C. There were no differences in *P. neglectus* reproduction in single and interplanted treatments.

*Crested wheatgrass:* All crested wheatgrass plants survived exposure to *P. penetrans* at 15–30 C and *P. neglectus* at 15 and 20 C. Survival rates were 95 and 92%, and 85 and 87% of plants from single and interplantings inoculated with *P. neglectus* at 25 and 30 C, respectively. Invasion of ‘Fairway’ crested wheatgrass was similar in single and interplanted treatments at all temperatures for both *P. penetrans* and *P. neglectus* at all temperatures (Fig. 1). A greater number of *P. neglectus* than *P. penetrans* invaded crested wheatgrass in both single and interplant treatments.

*Pratylenchus penetrans* reduced (*P < 0.05*) crested wheatgrass shoot growth only in the interplanted treatment at 30 C, and did not affect root growth in either single or interplanted treatments. *Pratylenchus neglectus* reduced (*P < 0.05*) crested wheatgrass shoot and root growth at 25–30 C; there were no differences in shoot and root growth between single and interplanted treatments. Temperature was negatively correlated with shoot growth (*r* = −0.75) and root growth (*r* = −0.70).

*Pratylenchus penetrans* did not reproduce on crested wheatgrass, and *P. neglectus* reproduction did not differ between single and interplanted treatments. Reproduction of *P. neglectus* was greatest at 20–30 C.

**DISCUSSION**

Plant-parasitic nematodes can invade and parasitize resistant cultivars. The alfalfa stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjév, and the northern root-knot nematode, *Meloidogyne hapla* Chitwood, are attracted to and invade nematode-resistant cultivars, including alfalfa (6,9,12). These previous findings are in agreement with the findings in this study. Although fewer nematodes may invade resistant than susceptible cultivars, and nematodes may not reproduce on resistant cultivars, nematodes may have adverse physiological effects on resistant cultivars. Invasion by the alfalfa strain of *D. dipsaci* caused a hypersensitive reaction that killed all resistant onion seedlings (9) and 74% of resistant alfalfa seedlings (6). Thus, a plant-parasitic nematode can inhibit the growth of a plant that is resistant to the nematode (4), as was evidenced by the invasion of crested wheatgrass by *P. penetrans*. While *P. neglectus* negatively affected the growth of crested wheatgrass, decrease in the number of nematodes invading alfalfa in the interplanted treatment resulted in an increase in the persistence and growth of an alfalfa. An increase in the duration of exposure to nematode invasion would probably increase the nematode pressure on the resistant cultivar. This may be accompanied by a reduction in the invasion of the susceptible cultivar. Hence, each successive nematode generation would invade the resistant cultivars, further reducing nematode invasion of the susceptible cultivar. Alfalfa has considerable value in range-
**Pratylenchus penetrans** and **P. neglectus** on Alfalfa and Grass: Griffin

**Fig. 1.** Invasion, pathogenicity, and reproduction of **Pratylenchus penetrans** and **P. neglectus** on 'Lahontan' alfalfa and 'Fairway' crested wheatgrass in single (PP-S = **P. penetrans**; PN-S = **P. neglectus**) and interplantings (PP-I = **P. penetrans**; PN-I = **P. neglectus**) of alfalfa and crested wheatgrass grown at four different temperatures. Plants inoculated at 28 days and invasion and reproduction determined 14 and 106 days after inoculation. (LSD values ($P < 0.05$): Nematodes/g root tissue: (alfalfa) 15 C = 17, 20 C = 21, 25 C = 29, 30 C = 24; (crested wheatgrass) 15 C = 6, 20 C = 10, 25 C = 18, 30 C = 17; dry shoot weight: (alfalfa) 15 C = 0.36, 20 C = 0.38, 25 C = 0.27, 30 C = 0.26; (crested wheatgrass) 15 C = 0.16, 20 C = 0.12, 25 C = 0.28, 30 C = 0.23; dry root weight: (alfalfa) 15 C = 0.20, 20 C = 0.38, 25 C = 0.17, 30 C = 0.20; (crested wheatgrass) 15 C = 0.11, 20 C = 0.12, 25 C = 0.28, 30 C = 0.24; reproductive index: (alfalfa) 15 C = 1.4, 20 C = 2.7, 25 C = 3.6, 30 C = 4.1; (crested wheatgrass) 15 C = 0.1, 20 C = 1.1, 25 C = 1.4, 30 C = 1.3.
land improvement and it has increased forage yields (20). Interplanting alfalfa with grasses improves annual growth of grasses (19) and increases forage yields by increasing available soil nitrogen. Although *P. penetrans* may adversely affect the growth of grass in alfalfa–grass interplantings, the addition of grass should increase forage yields due to the greater persistence of alfalfa. An increase in available nitrogen from *Rhizobium* nodulation of alfalfa should also increase the growth of grass and have a buffering effect on nematode virulence.

**Literature Cited**


