PATHOLOGICAL RELATIONSHIP OF MELOIDOGYNE HAPLA AND PHYTOPHTHORA MEGASPERMA F. SP. MEDICAGINIS IN MEDICAGO SATIVA L.: IMPORTANCE OF INOCULATION TIMING, SOIL TEXTURE, AND TEMPERATURE†

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ABSTRACT


The effects of inoculation timing, soil texture, and temperature on the interaction between Meloidogyne hapla and Phytophthora megasperma f. sp. medicaginis were studied on three alfalfa cultivars. Plant survival of cultivars 'Deseret' (susceptible to both pathogens) and 'Apollo II' (resistant only to the fungus) decreased, and plant growth was suppressed compared to uninoculated controls when inoculation with M. hapla preceded inoculation with P. m. f. sp. medicaginis. Survival and growth of 'Nev Syn XX' (resistant to both pathogens) were affected little or none by any inoculation treatment. Survival of Deseret inoculated with both pathogens was less in clay loam soil than in sandy loam soil, and shoot growth was suppressed in sandy loam, sandy-clay loam, and clay loam soils. Nematode reproduction and root galling induced by M. hapla were not affected when inoculation with M. hapla preceded inoculation with the fungus, but increased as a result of preinoculation with the fungus. When all three cultivars were inoculated with both pathogens and then grown at 16, 20, 24, and 28 °C, the greatest suppression of shoot growth and the smallest percentage of plants surviving occurred on Deseret at 28 °C. Growth of Apollo II was less affected by temperature than was Deseret, whereas temperature had little or no affect on Nev Syn XX.

Key words: alfalfa, Medicago sativa, Meloidogyne hapla, nematode resistance, Phytophthora megasperma f. sp. medicaginis, Phytophthora root rot, reproductive index, root-knot nematode, shoot weight, soil texture, survival, temperature.

RESUMEN


El efecto del tiempo de inoculación, textura de suelo y temperatura en la interacción entre Meloidogyne hapla y Phytophthora megasperma f. sp. medicaginis fueron investigados en tres cultivares de alfalfa. La sobrevivencia de cultivares 'Deseret' (susceptible a ambos patógenos) y 'Apollo II' (resistente solo al hongo) disminuyó y el crecimiento se inhibió cuando la inoculación de M. hapla estuvo anteccedida a inoculaciones con P. m. f. sp. medicaginis, en comparación a los testigos sin inocularse. Pocos efectos en la sobrevivencia y crecimiento fueron observados en el cultivar 'Nev Syn XX' (resistente a ambos patógenos) en todas las inoculaciones. La sobrevivencia de plantas de la variedad Deseret inoculadas con ambos patógenos fue menor en suelos arcillosos que en los franco arenosos y el desarrollo de rebrote inhibido en suelos franco arenoso, franco arcillo-arenoso y franco arcilloso. La reproducción de M. hapla e índice de agallamiento

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INTRODUCTION

Phytophthora root rot of alfalfa (Medicago sativa L.), caused by Phytophthora megasperma f. sp. medicaginis Kuan & Erwin (2) occurs in most areas of the United States where alfalfa is grown (18). It is particularly severe in flood-irrigated fields in the western United States (1,3, 10). The fungus attacks seeds, seedlings, mature plants, and Rhizobium nodules (4). Stand declines caused by Phytophthora root rot during the seedling year are characteristic in the midwest (16) and the Intermountain Region where stand losses of 75% have been observed (5).

Interactions between nematodes and other pathogens often have important economic effects on the survival and growth of plants in agricultural ecosystems, and diseases induced by the interaction between pathogens are well documented on various crops (14,17), including alfalfa (7,9,19). The northern root-knot nematode, Meloidogyne hapla, increased the severity of Fusarium wilt on alfalfa (8). There is recorded incidence of Fusarium wilt also in alfalfa in the presence of M. javanica (Treub) Chitwood, M. incognita (Kofoid & White) Chitwood, and M. arenaria (Neal) Chitwood (12). Meloidogyne hapla (7) and Ditylenchus dipsaci (9) are known to increase the incidence of bacterial wilt in alfalfa.

Interactions of plant parasitic nematodes and Phytophthora spp. have been reported on alfalfa (19). The incidence of Phytophthora root rot in alfalfa incited by P. megasperma f. sp. medicaginis increased, and forage yields decreased in the presence of M. hapla and M. incognita, indicating nematode-fungus interactions (5,19). Because P. megasperma f. sp. medicaginis and M. hapla are associated with alfalfa in the Intermountain Region of the United States (4,6), our study was designed to evaluate the effect of inoculation timing, soil texture, and temperature, on the interaction of M. hapla and P. megasperma f. sp. medicaginis on cultivars that are resistant or susceptible to one or both of these pathogens.

MATERIALS AND METHODS

Three alfalfa cultivars were used: ‘Nevada Synthetic XX’ (Nev Syn XX), resistant to P. megasperma f. sp. medicaginis and M. hapla (13); ‘Apollo II,’ resistant only to P. megasperma f. sp. medicaginis; and ‘Deseret,’ susceptible to both pathogens. Alfalfa plants were grown from seeds that had been scarified, treated with captan, and pregerminated on filter paper. Bacterial inoculum, Rhizobium meliloti Dang., was placed with the seed at planting to insure nodulation (5).

The M. hapla population used as inoculum was collected from a lettuce (Lactuca sativa) planting in northern Utah, and maintained in the greenhouse on ‘Rutgers’ tomato (Lycopersicon esculentum). Eggs were collected for inoculation by the sodium hypochlorite method (11). The
fungus used in all experiments was from an isolate of *P. megasperma* f. sp. *medicaginis* obtained from diseased alfalfa collected in northwestern Wyoming and maintained on cornmeal agar. Fungal inoculum was prepared by placing two 5-mm-diam plugs of hyphae from stock cultures into 1 000-ml roux culture bottles containing 160 ml of sterilized, liquid V-8 juice medium (4). After 5 days, cultures were shaken vigorously to fragment hyphae and placed in a horizontal position for an additional 9 days. Inoculum was prepared by blending the mycelial mat from a culture bottle in 238 ml of sterile distilled water (4).

In all experiments, each plant was inoculated with $10^3$ eggs of *M. hapla* per plant. Plants in the preliminary experiment were inoculated with a 12.5-ml suspension of fragmented hyphae of *P. megasperma* f. sp. *medicaginis*, whereas plants in all other experiments were inoculated with a 20-ml suspension of the fungus. In greenhouse bench studies, plants were grown at 26 ± 4 °C, with a 19-hr daylength provided by high-output fluorescent lamps, whereas plants in the growth chamber experiment were grown under artificial lights with a 14-hr light period. Soil used in all studies, except the soil texture experiment, consisted of a 1:1 (v:v) mixture of Kidman fine sandy loam (coarse-loamy mixed mesic Calcic Haploxeroll; 91% sand, 5% silt, 4% clay; pH 7.2; 1.0% organic matter) and Logan clay loam (mixed mesic Typic Calciaquolls; 27% sand, 31% silt, 42% clay; pH 7.8; 2.5% organic matter).

**Preliminary experiment on nematode-fungus interaction:** Four 12-day-old Apollo II or Deseret seedlings were transplanted into 15-cm-diam plastic containers containing 2 000 cm$^3$ of steam-pasteurized soil (four plants per container). Twenty-one days after transplanting, plants were treated as follows: plants in treatments 1 and 3 were inoculated with *M. hapla*; those in treatment 2 were inoculated with *P. megasperma* f. sp. *medicaginis*; those in treatment 4 served as the uninoculated control. Twenty-eight days after inoculation, plants in treatment 3 were inoculated with the fungus. Treatments were replicated 10 times (40 plants per treatment). The experiment was terminated 84 days after the final inoculation (145 days after seeding).

**Effect of sequential inoculations:** Pregerminated seeds of Deseret, Apollo II, and Nev Syn XX alfalfa cultivars were planted into plastic containers (6-cm-diam × 21-cm-deep) containing 540 cm$^3$ of steam-pasteurized soil (one seed per container). After 28 days, inoculations were as follows: plants in treatments 1 and 3 were inoculated with *M. hapla*; plants in treatments 2 and 5 were inoculated with *P. megasperma* f. sp. *medicaginis*; plants in treatment 4 were inoculated with both pathogens; plants in treatment 6 served as the uninoculated controls. After an additional 28 days, plants in treatment 3 were inoculated with the fungus, and plants in treatment 5 were inoculated with *M. hapla*. Each treatment had 34 replicates (plants). The experiment was terminated 68 days after the final inoculation (124 days after planting). Percentage plant survival, shoot growth, and Phytophthora root rot severity were recorded. Phytophthora root rot was rated on a 1–6 scale with 1 = none; 2 = slight (fine roots destroyed or minor lesions); 3 = moderate (1–2 distinct lesions per root); 4 = severe (many elongated lesions or 25–50% tap root rotted); 5 = very severe (more than 50% tap root rotted, plant nearly dead); 6 = plant dead. A nematode reproduction index (PR/Pi = final population per plant/initial population per plant) was also recorded.

**Effect of soil texture:** Pregerminated seeds (one seed per container) of Deseret
were planted in 6-cm-diam × 21-cm-deep containers containing the sandy loam soil, clay loam soil, or a 1:1 mixture of sandy loam and clay loam soil. After 28 days growth, inoculations were performed as follows: plants in treatments 1 and 3 were inoculated with M. hapla; those in treatment 2 were inoculated with P. megasperma f. sp. medicaginis; and plants in treatment 4 served as uninoculated controls. Twenty-eight days after inoculation, plants in treatment 3 were inoculated with the fungus. Each treatment had 30 replicates (plants). Plant shoots were trimmed to evaluate forage yields at first bloom and over four additional cuttings. The experiment was terminated 120 days after final inoculation (176 days after planting). Percentage plant survival, accumulated shoot dry weights, Phytophthora root rot severity, galling of alfalfa roots by M. hapla, and nematode reproduction were recorded. Root galling by M. hapla was rated on a scale of 1–6 based on the percentage of total root system galled: 1 = none; 2 = 1–10%; 3 = 11–20%; 4 = 21–50%; 5 = 51–80%; 6 = 81–100%.

Effect of temperature: Germinated seeds of Deseret, Apollo II, and Nev Syn XX were planted into 6-cm-diam × 21-cm-deep containers (one seed per container). After 35 days, inoculations were as follows: plants in treatments 1 and 3 were inoculated with M. hapla; those in treatment 2 were inoculated with P. megasperma f. sp. medicaginis; and plants in treatment 4 served as uninoculated control. After an additional 28 days, plants in treatment 3 were inoculated with the fungus. Each treatment was replicated 20 times. Plants were maintained in growth chambers at 16, 20, 24, and 28 ºC. Shoot growth was clipped at first blossom, and dry weights recorded over four cuttings. The experiment was terminated 100 days after the final inoculations (163 days after planting), and data on plant survival, shoot growth, root rot, root-knot galling, and nematode reproduction were collected.

All greenhouse and growth chamber experiments were repeated and data presented are means of the combined data of the two experiments. Data were analyzed by factorial analyzes of variance and differences between means were compared with the LSD at \( P < 0.05 \). Percentage data on plant survival were transformed using arcsine transformation.

RESULTS

Preliminary examination of nematode-fungus interactions: No uninoculated control plant died, whereas the survival rates of plants inoculated with the fungus alone, M. hapla alone, and M. hapla plus the fungus, respectively, were 83, 75, and 42% for Deseret, and were 100, 92, and 75% for Apollo II. Mean dry shoot weights for the same treatments were 0.24, 0.42, and 0.01 g for Deseret, and 0.43, 0.24, and 0.24 g for Apollo II. The mean dry shoot weights of uninoculated Deseret and Apollo II plants were 0.41 and 0.42 g, respectively. (LSD = 0.04).

Sequential inoculations: Simultaneous inoculation of Deseret and Apollo II with M. hapla + P. megasperma f. sp. medicaginis increased disease incidence and decreased plant survival and shoot growth compared to inoculations with either pathogen alone. Inoculation of plants with M. hapla 28 days before introducing the fungus resulted in the smallest survival rate and the greatest suppression of plant growth of both Deseret and Apollo II, but did not affect the survival of Nev Syn XX plants (Fig. 1). Deseret alfalfa had the lowest survival of the three alfalfa cultivars for like treatments.

Inoculation with P. megasperma f. sp. medicaginis suppressed shoot growth.
Further decreases in growth were not obtained through simultaneous or subsequent inoculations with *M. hapla*. Inoculation with *M. hapla* followed by the fungus 28 days later increased Phytophthora root rot severity in Deseret over the fungus alone. Root rot ratings on Deseret, Apollo II, and Nev Syn XX as a result of single inoculation with *P. megasperma* f. sp. *medicaginis* were 1.1, 1.0, and 1.0, respectively. The corresponding ratings, when *M. hapla* preceded *P. megasperma* f. sp. *medicaginis* by 28 days were 3.6, 1.2, and 1.2. (LSD = 0.6). Root rot ratings for all other treatments receiving *P. megasperma* f. sp. *medicaginis* ranged from 1.0 to 1.3 and did not differ significantly.

Greatest nematode reproduction occurred on Desert, followed by Apollo II. *Meloidogyne hapla* did not reproduce on Nev Syn XX (Table 1). Compared to the *M. hapla* alone treatment, nematode reproduction was not affected by *P. megasperma* f. sp. *medicaginis* when *M. hapla* preceded inoculation with *P. megasperma* f. sp. *medicaginis*. However, the nematode reproductive index (PfPi) increased for Deseret and Apollo II when inoculation with *P. megasperma* f. sp. *medicaginis* preceded inoculation with *M. hapla* (Table 1).

**Soil texture:** Growth of Deseret alfalfa was suppressed by *M. hapla* in all soils tested, but was suppressed by *P. megasperma* f. sp. *medicaginis* only in clay loam. Growth reduction and mortality were highest when inoculation with *M. hapla* preceded inoculation with the fungus, and plant survival for this inoculation treatment was smallest in the clay loam soil (Fig. 2).

Plants parasitized by *M. hapla* before inoculation with *P. megasperma* f. sp.

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**Table 1.** Effect of time of inoculation with *Meloidogyne hapla* (MH) and *Phytophthora megasperma* f. sp. *medicaginis* (PMM) on the reproductive index of MH on the alfalfa cultivars Deseret, Apollo II, and Nevada Synthetic XX (Nev Syn XX).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Deseret</th>
<th>Apollo II</th>
<th>Nev Syn XX</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH</td>
<td>5.8</td>
<td>4.2</td>
<td>0.4</td>
</tr>
<tr>
<td>MHPMM1</td>
<td>10.0</td>
<td>8.1</td>
<td>0.6</td>
</tr>
<tr>
<td>MHPMM2</td>
<td>12.2</td>
<td>8.4</td>
<td>0.7</td>
</tr>
<tr>
<td>MHPMM3</td>
<td>6.1</td>
<td>4.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

LSD (P < 0.05): 2.5.

*MHPPM1 = simultaneous inoculation with MH and PMM; MHPMM2 = PMM preceded MH by 28 days; MHPMM3 = MH preceded PMM by 28 days.*
medicaginis had the greatest incidence of Phytophthora root rot. Soil texture did not influence the incidence of root rot. Root rot indices in clay loam, sandy clay loam, and sandy loam soil were 1.1, 1.0, and 1.2 for plants inoculated with P. megasperma f. sp. medicaginis alone, and were 1.4, 1.4, and 1.4 for plants inoculated with both M. hapla and the fungus.

Root galling by M. hapla was not affected by P. megasperma f. sp. medicaginis or soil texture. Gall ratings of treatments receiving M. hapla with or without the fungus ranged from 2.6 to 3.1 and did
not differ significantly. The nematode reproductive index (PI/PI) also was not affected by soil texture, but there was greater nematode reproduction in treatments receiving both pathogens than in those receiving *M. hapla* alone. Reproductive indices of *M. hapla* in clay loam, sandy clay loam, and sandy loam soils were 12.4, 17.7, and 13.5 in the *M. hapla* treatment, and were 18.5, 15.3, and 20.4 in the *M. hapla* + fungus treatment (LSD = 3.8).

**Temperature:** Inoculation with *M. hapla* + *P. megasperma* f. sp. *medicaginis* markedly decreased the survival of Deseret over the uninoculated controls at all temperatures, and all plants died at 28 °C (Fig. 3). Effects on Apollo II were less pronounced. No Apollo II plants died following a single inoculation with *P. megasperma* f. sp. *medicaginis*, 18% died following a single inoculation with *M. hapla* at 24 and 28 °C, and the death rate increased from inoculation with *M. hapla* plus the fungus at 20, 24, and 28 °C (Fig. 3). Single inoculations with *M. hapla* or *P. megasperma* f. sp. *medicaginis* had minimal effect on the survival of Nev Syn XX, and inoculation with both pathogens did not decrease the survival of Nev Syn XX over that of the fungus alone.

Shoot growth of Deseret following inoculation with *M. hapla* + *P. megasperma* f. sp. *medicaginis* was suppressed at 20, 24, and 28 °C (Fig. 4). *Meloidogyne hapla* was most suppressive to Deseret at 28 °C, while plant growth suppression by *P. megasperma* f. sp. *medicaginis* was greatest at 16 °C. Temperature had minimal effect on the interaction of *M. hapla* and *P. megasperma* f. sp. *medicaginis* on Nev Syn XX, and combined inoculation with the two pathogens did not affect survival or shoot growth when compared with single inoculations with either pathogen.

Gall ratings at 16, 20, 24, and 28 °C for plants inoculated with *M. hapla* alone

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Fig. 3. Effects of temperature and inoculation with *Meloidogyne hapla* (MH) and *Phytophthora megasperma* f. sp. *medicaginis* (PMM) on survival of the alfalfa cultivars Apollo II (resistant only to PMM), Nevada Synthetic XX (Nev Syn XX, resistant to both pathogens), and Deseret (susceptible to both pathogens). PMM and MH were added to pots on the same date except in the MH-PMM treatment, in which PMM introduction was delayed 28 days.
were, respectively: 2.6, 4.7, 5.5, and 5.3 for Deseret; 1.9, 4.2, 3.9, and 5.0 for Apollo II; and 1.1, 1.7, 1.1, and 1.9 for Nev Syn XX. These were comparable to gall ratings from inoculations with *M. hapla + P. megasperma* f. sp. *medicaginis*, which were respectively: 2.5, 4.3, 5.0, and 6.0 for Deseret; 1.9, 3.2, 3.5, and 3.9 for Apollo II; and 1.4, 1.1, 1.3, and 2.4 for Nev Syn XX. The *M. hapla* plus the fungus treatment suppressed root galling on Deseret below that of the *M. hapla* alone treatment at 20 °C. The same effect was observed on Apollo II at 20, 24, and 28 °C. On Nev Syn XX root gall indices were low and unaffected by the presence of the fungus.

Phytophthora root rot from single inoculation of *P. megasperma* f. sp. *medicaginis* was not affected by temperature. Root rot ratings of Deseret, Apollo II, and Nev Syn XX from the single inoculation with *P. megasperma* f. sp. *medicaginis*, respectively, were as follows: 2.6, 1.4, and 1.7 at 16 °C; 2.1, 1.3, and 1.4 at 20 °C; 1.8, 1.6, and 1.7 at 24 °C; and 1.6, 1.6, and 1.5 at 28 °C. *Meloidogyne hapla* increased the incidence of Phytophthora root rot on Deseret and Apollo II, but not Nev Syn XX. Root rot ratings of Deseret, Apollo II, and Nev Syn XX plantings receiving combined inoculations with *M. hapla + P. megasperma* f. sp. *medicaginis* were: 2.8, 1.6, and 1.7 at 16 °C; 2.6, 1.8, and 1.4 at 20 °C; 3.9, 2.8, and 1.7 at 24 °C; and 6.0, 3.6, and 1.9 at 28 °C (LSD = 1.0).

Appreciable nematode reproduction occurred only on Deseret and Apollo II and only at 20, 24 and 28 °C. Overall, *M. hapla* reproduced less on plants that were inoculated with the fungus than on those that were not. (Fig. 5). *Meloidogyne hapla* reproduced on Nev Syn XX only at 28 °C and reproduction at that temperature was only a fraction of that which occurred on the other two cultivars.

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**Fig. 4.** Effects of temperature and inoculation with *Meloidogyne hapla* (MH) and *Phytophthora megasperma* f. sp. *medicaginis* (PMM) on shoot dry weight of the alfalfa cultivars Apollo II (resistant only to PMM), Nevada Synthetic XX (Nev Syn XX; resistant to both pathogens), and Deseret (susceptible to both pathogens). PMM and MH were added to pots on the same date except in the MHPMM treatment, in which PMM introduction was delayed 28 days.
Fig. 5. Effect of temperature and inoculation with Meloidogyne hapla and Phytophthora megasperma f. sp. medicaginis (PMM) on the reproduction of M. hapla (MH) on the alfalfa cultivar Apollo II (resistant only to PMM), Nevada Synthetic XX (Nev Syn XX; resistant to both pathogens), and Deseret (susceptible to both pathogens). In the MHPMM treatment, PMM was introduced 28 days after inoculating with MH. Note that the Nev Syn XX reproductive index axis is 0–8.

DISCUSSION

Although Apollo II is considered resistant to P. megasperma f. sp. medicaginis, resistance to the fungus was decreased when plants were infected with M. hapla. By comparison, resistance to the fungus was not decreased in nematode-inoculated Nev Syn XX, which is resistant to the nematode as well. Nematode resistance should be considered when breeding for resistance to P. megasperma f. sp. medicaginis and other pathogens that attack alfalfa.

The ability of M. hapla to increase the aggressiveness of P. megasperma f. sp. medicaginis in alfalfa that was observed in this study, agrees with previous reports involving nematode/fungal complexes (8,14,17). Results from this study, however, conflict with those of Gray et al. (4), who found that survival of inoculated seedlings was smaller following a single inoculation with P. megasperma f. sp. medicaginis than following inoculation with M. hapla plus P. megasperma f. sp. medicaginis. Differences in the results of the two studies could be due to the nematode and fungus competing for undifferentiated tissue in radicles of the newly germinated seed utilized in the Gray et al. study, limiting infection by both pathogens. Such competition may not have occurred in the present study since plants were 28 days old at time of inoculation and considerable differentiated root tissue had developed. Hence, the nematodes would be expected to have invaded and parasitized the vascular tissue while the fungus invaded the cortical tissue. This hypothetical sequence of events needs clarification by further experimental studies.

Greater reproduction of M. hapla on Deseret alfalfa in the growth chamber than in the greenhouse studies may have resulted from less fluctuation in tempera-
ture and soil moisture in the growth chamber. Nematode reproduction was apparently suppressed by *P. megasperma* f. sp. *medicaginis* in the temperature study due to increased destruction of plant tissue by the two pathogens. The increase in nematode reproduction from preinoculation with *P. megasperma* f. sp. *medicaginis* in the greenhouse may be due to a partial breakdown of the cortical tissue by *P. megasperma* f. sp. *medicaginis* and a subsequent increase in the ability of the nematode to invade the vascular root tissue. Alternatively, *P. megasperma* f. sp. *medicaginis* may cause a physiological change in the root tissue that enhanced invasion and reproduction of *M. hapla*. This agrees with the findings of Ross (15), who found significantly higher populations of the soybean cyst nematode, *Heterodera glycines*, developed on soybean in soil infested with *Fusarium oxysporum* than in soil without the fungus.

**LITERATURE CITED**


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