Interrelationships Between *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *medicaginis* in Seedling Damping-off and Root Infection of Alfalfa

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Cooperative investigations of the Wyoming Agricultural Experiment Station, the U.S. Department of Agriculture-Agricultural Research Service, and the Utah Agricultural Experiment Station.

This research was supported in part by Wyoming Agricultural Experiment Station Hatch Project Number WYO 162-81 and by USDA grant 85-CRSR-2-2705.

Accepted for publication 31 August 1989.

ABSTRACT

Gray, F. A., Griffin, G. D., Johnson, D. A., Eckert, J. W., and Kazimir, J. E. 1990. Interrelationships between *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *medicaginis* in seedling damping-off and root infection of alfalfa. Phytopathology 80:228-232.

Interrelationships between *Phytophthora megasperma* f. sp. *medicaginis* and *Meloidogyne hapla* were studied in three alfalfa cultivars: Nevada Synthetic XX, resistant to *P. m. medicaginis* and highly resistant to *M. hapla*; Apollo II, resistant to *P. m. medicaginis* and susceptible to *M. hapla*; Deseret, susceptible to *P. m. medicaginis* and *M. hapla*. Post-emergence damping-off, attributed to *P. m. medicaginis*, was suppressed when both pathogens were applied in combination as compared with application of *P. m. medicaginis* alone at planting. Percent stand loss (percent of control) at 2 wk was 33 and 26, 45 and 21, and 87 and 35 with *P. m. medicaginis* alone and in combination with *M. hapla* for Apollo II, Nevada Synthetic XX, and Deseret, respectively. Phytophthora root rot was increased in surviving plants at 7 wk in the combination treatment as compared with the treatment of *P. m. medicaginis* alone. *P. m. medicaginis* with *M. hapla* resulted in less root galling and nematode reproduction in all three cultivars. Inoculation with both pathogens 7 wk after planting resulted in a 59% increase in infection of roots of Deseret by *P. m. medicaginis*, and an 87 and 13% increase in infection of nitrogen-fixing nodules of *Rhizobium meliloti* in Deseret and Apollo II, respectively, 6 mo after inoculation. The percentage of nodules per root system was less in the presence of *M. hapla* in Deseret (67%) and Apollo II (68%) and was less in Deseret (23%) in the presence of *P. m. medicaginis*, as compared with the uninoculated control. The benefit of dual resistance in preventing loss from Phytophthora root rot was shown in Nevada Synthetic XX, which had no detectable infection of nodules by *P. m. medicaginis* and only slight root infection when *P. m. medicaginis* was applied alone or in combination with *M. hapla* (11.3 and 10.3% infection, respectively).

Additional keywords: interaction, *Medicago sativa*, Northern root-knot nematode, reproduction.

Phytophthora root rot of alfalfa (*Medicago sativa* L.), incited by *Phytophthora megasperma* f. sp. *medicaginis* Kuan and Erwin (2,3), and root-knot, caused by *Meloidogyne hapla* Chitwood (7), are important diseases of dormant alfalfa in the intermountain region of the United States (5,6,9,10). Both pathogens often can be found in the same alfalfa fields in this region. When conditions in the field are optimum for infection and disease development, damage and even death can occur to germinating seeds and young seedlings of alfalfa from either *P. m. medicaginis* or *M. hapla* (6,8). Infection from either pathogen occurs at rot tips or at

bacterial nodules induced by *Rhizobium meliloti* (7). Earlier studies (17) showed that the incidence of Phytophthora root rot was increased and forage yield was decreased in alfalfa when plants were coinoculated with either *M. hapla* or *M. incognita* (Kofoid and White) Chitwood and *P. m. medicaginis* as compared with inoculation with either pathogen alone. Recently, Griffin et al (10) showed that the incidence of Phytophthora root rot and plant mortality were increased and forage yield was decreased when both pathogens were present as compared with either pathogen alone; this phenomenon was influenced by soil type, soil temperature, and the sequence of pathogen inoculation. Maximum disease occurred in the cultivar Deseret in a clay soil after 28 days at 28 C when plants were inoculated with *M. hapla* followed by *P. m. medicaginis*. Both of these studies (10,17) were conducted with established, 1-mo-old plants. When plants were inoculated, roots were well developed and nodulated with *R. meliloti*. Isolation of *P. m. medicaginis* from roots was not reported by either of these researchers.

To further understand this multiple pathogen-host system, the present studies were conducted to elucidate the interrelationships of *M. hapla* and *P. m. medicaginis* in seedling damping-off, root infection, and the fate of nodules caused by *R. meliloti* in alfalfa cultivars that differ in resistance and susceptibility to each pathogen.

MATERIALS AND METHODS

Experiments were conducted in a controlled-environment chamber at 24 ± 4 C, with a 14-hr light and 10-hr dark cycle. Seedling damping-off was studied in one experiment, and infection of roots and nodules by *P. m. medicaginis* and *M. hapla* was studied in another experiment. Pregerminated seeds of the alfalfa cultivars Apollo II (resistant to *P. m. medicaginis* and susceptible to *M. hapla*), Deseret (susceptible to both *P. m. medicaginis* and *M. hapla*), and Nevada Synthetic XX (resistant to *P. m. medicaginis* and highly resistant to *M. hapla*) were planted in a mixture of steam-sterilized sandy loam (70% sand, 17% silt, 13% clay, 2.6% O.M., pH 7.5) and sandy clay loam (62% sand, 17% silt, 21% clay, 2.4% O.M., pH 7.6) soil (1:1, v/v). Seeds were treated with inoculant of *R. meliloti* (The Nitragin Company, Milwaukee, WI) before germination. Seeds were placed in glass petri dishes containing moistened filter paper sprinkled with inoculant (0.1 g of inoculant/1 g of seed). Seeds or plants either were uninoculated or were inoculated with the following treatments: *M. hapla* alone, *P. m. medicaginis* alone, or *M. hapla* + *P. m. medicaginis*.

A population of *M. hapla* was obtained from diseased alfalfa plants from southeastern Wyoming. Inoculum was increased on roots of tomato, cultivar Rutgers, grown in the greenhouse. Inoculum was collected by extracting eggs with the NaOCl method (13). An isolate of *P. m. medicaginis* obtained from diseased alfalfa collected in northwestern Wyoming was maintained on cornmeal agar. Inoculum was prepared by placing two 5-mm-diameter plugs of mycelium from stock cultures into 1,000-ml roux culture bottles containing 160 ml of a sterilized, liquid V-8 juice medium (6). After 5 days, cultures were shaken vigorously to fragment mycelium and placed in the horizontal position for an additional 9 days. Inoculum was prepared by comminuting the mycelial mat from one culture bottle in 240 ml of distilled water.

Phytophthora root rot was rated on a scale of 1 to 5, with 1 = none, 2 = slight (fine roots destroyed or minor lesion on taproot), 3 = moderate (1 or more distinct lesions on taproot), 4 = severe (one-fourth to one-half of taproot destroyed), and 5 = very severe (nearly all of taproot destroyed). Root galling, caused by *M. hapla*, was rated on a scale of 1 to 4 in the first experiment, with 1 = none, 2 = 1–10 galls, 3 = 11–100 galls, and 4 = >100 galls, and 1 to 5 in the second experiment, with 1 = none, 2 = 1–5 galls, 3 = 6–20 galls, 4 = 21–50 galls, and 5 = >50 galls.

In the first experiment, seeds were planted in furrows in 10-cm^2 plastic pots (2 furrows/pot, 10 seeds/furrow). Inoculum was placed in furrows just before seeding. The treatment with *M. hapla* consisted of 2 ml of inoculum per seed (1,000 eggs/ml, 20 ml/furrow). In preliminary studies, the inoculum rate of *P. m. medicaginis* resulted in approximately 50% damping-off, which was suitable for expression of an interaction. Thus, the treatment with *P. m. medicaginis* consisted of 20 ml of the prepared inoculum (1:10 dilution, v/v, with distilled water) per furrow. The uninoculated check consisted of 20 ml of distilled water per furrow. If an interaction between *P. m. medicaginis* and *M. hapla* exists, seedling mortality could be increased if the interaction were synergistic, or decreased if the interaction were antagonistic. Pots were kept moist with daily irrigations. Stand counts were made at 1, 2, 4, 5, 6, and 7 wk after planting. The experiment was terminated after 7 wk, and shoot and root weight, Phytophthora root rot, nematode galling, and nematode reproduction were determined on surviving plants. Plants were removed, and roots were washed free of soil. Plants then were separated into shoots and roots, air dried at 50 C for 72 hr, and weighed.

In the second experiment, seeds were planted in plastic cone containers (4 × 21 cm) held in plastic racks (31 × 60 cm) and thinned to one plant per container. There were four racks; each rack contained 98 plants (28 plants of each cultivar, with end border rows of seven plants each). Plants in a given rack were inoculated with only one of the four inoculation treatments. Plants were grown for 7 wk before inoculation to avoid early mortality from damping-off and to insure nodule development. Single pathogen inoculations consisted of 2,500 eggs per plant in 20 ml of water for the treatment with *M. hapla* and 20 ml of undiluted inoculum for the treatment with *P. m. medicaginis*. The uninoculated control consisted of 20 ml of distilled water. Both inocula were applied in three 8-mm-diameter holes that extended to the root zone. Shoots were clipped back when 10% of the plants had flowered. The second experiment was terminated 4 mo after inoculation (6 mo after seeding). Plants were removed, and roots were washed free of soil and rated for galling and Phytophthora root rot. Infection incidence of roots and nodules by *P. m. medicaginis* was determined by placing root pieces and nodules in a Phytophthora-selective medium consisting of 19 g of cornmeal agar, 1.0 ml of pimaricin (Pimafucin, 2.5% sterile water suspension, Aldrich Chemical Company, Milwaukee, WI), 0.10 g of penicillin-G, and 0.10 g of streptomycin sulfate (Sigma Chemical Company, St. Louis, MO) per liter of distilled water. After 7 days, plates were observed for mycelial growth characteristic of *P. m. medicaginis*. The number of nodules per root system and the number of nodules galled by *M. hapla* were determined with a ×2 portable magnifying glass fitted with a fluorescent light.

All experiments were 3 × 4 factorials arranged in modified, randomized complete block designs. There were four replicates in the damping-off experiment and three replicates in the root infection experiment. Inoculation treatments in both experiments were blocked to avoid contamination. Cultivars were randomized within each inoculation treatment. Seedling survival data from the first experiment and all data from the second experiment were analyzed by analysis of variance (ANOVA), and mean separation was determined with Duncan's new multiple range test at $P \leq 0.05$. Data collected from surviving plants in the first experiment (shoot and root growth, root galling, egg reproduction, Phytophthora root rot rating) were analyzed by ANOVA, and mean separation was determined with the least significant difference test at $P \leq 0.05$ and $P \leq 0.10$. Both experiments were repeated once with similar results; data presented are from the second trial of each experiment.

RESULTS

Seedling damping-off. Differences among inoculation treatments and the uninoculated controls after 1 wk of incubation were not significant in either cultivar (data not presented), indicating little preemergence damping-off; however, seedling death was present by 2 wk (Table 1). Greater seedling death occurred in all cultivars in the treatments with *P. m. medicaginis* compared with plants inoculated with *M. hapla* alone and the uninoculated

control. Seedling death was suppressed in the combination treatment as compared with *P. m. medicaginis* alone in Nevada Synthetic XX and Deseret, but not in Apollo II. Deseret had greater seedling death than Nevada Synthetic XX or Apollo II (both resistant to Phytophthora root rot) in the treatment with *P. m. medicaginis* alone. There were no differences among cultivars in any of the other treatments. There was very little change in seedling stands among treatments or cultivars after 7 wk when the experiment was terminated. The greatest mortality (77% of the control) occurred in Deseret in the treatment with *P. m. medicaginis* alone.

For all three cultivars, severity of Phytophthora root rot in the surviving plants tended to be greater in the combined-pathogen treatment than in the treatment with *P. m. medicaginis* alone but was significant ($P \leq 0.05$) only in Deseret. Ratings for *P. m. medicaginis* alone and combined treatments were 1.9 and 2.2, 2.1 and 2.6, and 2.5 and 3.4 for Apollo II, Nevada Synthetic XX, and Deseret, respectively. In the treatment with *P. m. medicaginis* alone, Deseret had significantly ($P \leq 0.10$) more Phytophthora root rot than Apollo II; there was no significant difference between Deseret and Nevada Synthetic XX or between Nevada Synthetic XX and Apollo II. Deseret had significantly ($P \leq 0.05$) more Phytophthora root rot than either Nevada Synthetic XX or Apollo II in the combined treatment. Phytophthora root rot was not detected in the treatment with *M. hapla* alone or in uninoculated treatments.

Shoot growth was significantly ($P \leq 0.10$) suppressed in all cultivars when plants were inoculated with either pathogen alone or in combination, as compared with the uninoculated checks (Table 2). Shoot weights of all cultivars were lowest in the combined treatment as compared with either pathogen alone (significant [$P \leq 0.05$] only in Apollo II). Similar results were obtained when root weights were compared. The lowest shoot and root growth occurred in Deseret in the combined treatment.

Mean root gall ratings (mean of surviving plants per pot) for *M. hapla* and *M. hapla* + *P. m. medicaginis* were 1.6 and 1.4, 2.6 and 2.1, and 2.7 and 2.5 for Nevada Synthetic XX, Deseret, and Apollo II, respectively. There was significantly ($P \leq 0.05$) less root galling in Nevada Synthetic XX (highly resistant to *M. hapla*) than in Apollo II or Deseret in both treatments with *M. hapla*. Gall ratings were similar between Deseret and Apollo II (both susceptible to *M. hapla*) in the treatment with *M. hapla* alone, whereas Apollo II had a higher gall rating than Deseret in the combined treatment. Gall ratings of all cultivars were lower in the combined treatment, but differences were only significant ($P \leq 0.10$) in the Phytophthora root rot-susceptible cultivar Deseret. Root galling did not occur with the fungus alone or in uninoculated treatments. The most severe root galling occurred in Apollo II and Deseret in the treatment with *M. hapla* alone and in Apollo II in the combined treatment. A similar trend occurred in nematode reproduction, which was lowest in roots where both pathogens were present (significant [$P \leq 0.05$] difference only in Apollo II) as compared with *M. hapla* alone. Mean nematode eggs per pot for *M. hapla* and *M. hapla* + *P. m. medicaginis* were 1,568 and 701, 24,918 and 1,138, and 89,014 and 35,857 for Nevada Synthetic XX, Deseret, and Apollo II, respectively.

Root nodulation and infection. Root nodulation by *R. meliloti* was significantly less in Apollo II and Deseret, but not in Nevada Synthetic XX in both the *M. hapla* alone and combined nematode-fungus treatments when compared with the uninoculated check (Table 3). Nodulation was significantiy lowered compared with the uninoculated check by *P. m. medicaginis* alone in Deseret. However, the reduction in nodulation was significantiy less than that caused by *M. hapla* in the same cultivar. Nodule numbers in the combined treatment were similar in all cultivars to those in which *M. hapla* was used alone.

Infection of Rhizobium nodules by *P. m. medicaginis* was increased when *M. hapla* was present as compared with the fungus alone for Apollo II and Deseret (Table 4). No nodule infection occurred in Nevada Synthetic XX. The increase in infection was greater in the susceptible cultivar Deseret than in Apollo II. Infection among cultivars had not occurred in the treatment with *P. m. medicaginis* 4 mo after inoculation when the experiment was terminated. A similar trend occurred for root infection; however, the increase in infection in the combined treatment over the fungus alone was not significant ($P \leq 0.05$) in Apollo II. Overall, there was a higher percent root infection than nodule infection.

Visual symptoms of Phytophthora root rot were not significantly increased in any cultivar when *M. hapla* was added with *P. m. medicaginis* (Table 4). Deseret had significantly more Phytophthora root rot in both treatments than Apollo II or Nevada Synthetic XX except in the combination treatment where

TABLE 1. Effects of *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) and *Meloidogyne hapla* (Mh) applied at planting either alone or in combination on plant mortality in alfalfa

Alfalfa cultivar	Reaction to[a]		Live plants at 2 wk after planting[b]				Live plants at 7 wk after planting[b]			
	Pmm	Mh	Control	Pmm	Mh	Mh + Pmm	Control	Pmm	Mh	Mh + Pmm
Apollo II	R	S	18.0 aA	12.0 aB	19.0 aA	13.3 aB	18.5 aA	12.5 aBC	16.8 aAB	11.8 aC
Nevada Synthetic XX	R	HR	18.3 aA	10.0 aC	18.8 aA	14.5 aB	19.0 aA	10.5 aB	18.8 aA	15.0 aA
Deseret	S	S	19.0 aA	5.5 bC	19.0 aA	12.3 aB	18.8 aA	4.3 bC	14.5 aAB	10.5 aB

[a] R = resistant, HR = highly resistant, S = susceptible.

[b] Values are the number of live plants per 20 seeds planted and are means of four replicates. Means not followed by the same letter (lowercase letters for columns and uppercase letters for rows) differ ($P \leq 0.05$) according to Duncan's new multiple range test.

TABLE 2. Influence of *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) and *Meloidogyne hapla* (Mh) applied at planting either alone or in combination on shoot and root growth of alfalfa

Cultivar	Reaction to:[a]		Shoot weight (mg)[b]				Root weight (mg)[b]			
	Pmm	Mh	Check	Pmm	Mh	Mh + Pmm	Check	Pmm	Mh	Mh + Pmm
Apollo II	R	S	980 aD	762 aC	356 bB	86 bA	601 aB	585 cB	222 bA	110 abA
Nevada Synthetic XX	R	HR	1077 aC	412 bA	601 aB	286 aA	649 aC	303 bAB	330 bB	188 bA
Deseret	S	S	791 bC	212 cB	27 cA	8 bA	545 aC	174 aB	84 aAB	46 aA

[a] R = resistant, HR = highly resistant, S = susceptible.

[b] Values are the total dry weight of remaining plants (shoots or roots) after 7 wk and are means of four replicates. Shoot weight means with differences of 180 and 217 mg or greater and root rot means with differences of 130 and 157 mg or greater are significantly different at $P \leq 0.10$ and $P \leq 0.05$, respectively, according to the Fisher's least significant difference test. Means (shoots or roots) not followed by the same letter (lowercase letters for columns and uppercase letters for rows) differ at $P \leq 0.10$.

there was no difference between Deseret and Apollo II.

There was no significant effect of *P. m. medicaginis* on nodule galling caused by *M. hapla* in either cultivar (Table 5). Nodule galling was less in the treatment with *P. m. medicaginis* in Deseret, but the difference was not significant. Nevada Synthetic XX had the fewest nodules with galls in either treatment. Similar results occurred in the percent roots galled, with the exception that galling was less in Apollo II (17%) rather than in Deseret. Root galling was lower in all three cultivars (significant only in Apollo II) when *P. m. medicaginis* was present. Nevada Synthetic XX had lower gall indexes than Apollo II or Deseret in both treatments. Only limited mortality had occurred when the study was discontinued, and differences in the mean number of plants surviving between treatments were not significant.

DISCUSSION

Our previous studies with established plants showed that the incidence of Phytophthora root rot and resultant plant mortality were increased when *P. m. medicaginis* and *M. hapla* were present as compared with the fungus alone (10). In this study, root infection by *P. m. medicaginis* was shown to be increased in the presence of *M. hapla* as compared with *P. m. medicaginis* alone, which supports our previous findings. More than twice as much root tissue from Deseret was infected in the combined treatment as compared with *P. m. medicaginis* alone (61.3 and 25.3%, respectively). The presence of *M. hapla* also increased infection of Rhizobium nodules in Deseret and Apollo II. *M. hapla* was extremely destructive to either the formation or retention of Rhizobium nodules on susceptible cultivars, resulting in 68 and 70% less nodules in Apollo II and Deseret, respectively, as compared with the uninoculated check. The experiment was not designed to determine if lower nodule number was a result of suppression of nodules to form, or destruction of nodules after formation. However, previous work (4,15) would indicate the latter to be the most plausible explanation. Although limited work has been done on the interaction between nematodes and nodules caused by Rhizobium on roots of leguminous plants, only one nematode (*Heterodera glycines* Ichinohe) has been shown to result in binding of fewer rhizobia (12). Surprisingly, soybean plants infected with species of *Meloidogyne* and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans SteKhoven showed improved nodulation, although nodules usually were smaller than those on plants free of nematodes (14). Nodules on different legumes appear to vary considerably in their susceptibility to nematodes (1). Although only limited work has been done with nodules on roots of alfalfa, it appears that they may be quite susceptible. Nodule numbers were significantly lower in Deseret inoculated with *P. m. medicaginis* but to a lesser degree than when inoculated with *M. hapla*, compared with the uninoculated check. Although nodulation was not significantly lower in the combined-pathogen treatment over the single-pathogen treatments when the experiment was terminated, such a decrease may be expected to occur. Although infection by *P. m. medicaginis* was increased in the combined treatment, a corresponding increase in root rot did not occur. The experiment was terminated 4 mo after inoculation before plant death occurred so that root infection could be studied. Had the experiment been allowed to continue, an increase in root rot, as well as root infection, might have occurred.

TABLE 3. Mean numbers of Rhizobium nodules per root system after single and dual inoculations of *Meloidogyne hapla* (Mh) and *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) in alfalfa

	Reaction to[a]		Number of Rhizobium nodules per root system[b]			
Cultivar	Pmm	Mh	Check	Mh alone	Pmm alone	Mh + Pmm
Apollo II	R	S	24.4 bA	7.9 bB	24.6 aA	11.0 bB
Nevada Synthetic XX	R	HR	25.8 abA	21.7 aA	23.4 aA	23.1 aA
Deseret	S	S	30.2 aA	10.0 bC	23.4 aB	11.0 bC

[a] R = resistant, HR = highly resistant, S = susceptible.
[b] Values are means of three replicates. Means not followed by the same letter (lowercase letters for columns and uppercase letters for rows) differ ($P \leq 0.05$) according to Duncan's new multiple range test.

TABLE 4. Mean root rot rating and percent root and Rhizobium nodule infection after single and dual inoculation with *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) and *Meloidogyne hapla* (Mh) in alfalfa

	Reaction to[a]		Percent nodules infected[b] with Pmm		Percent roots infected[b] with Pmm		PRR rating[b]	
Cultivar	PMM	Mh	Pmm	Pmm + Mh	Pmm	Mh + Pmm	Pmm	Mh + Pmm
Apollo II	R	S	0.0 aA	13.3 bB	10.3 aA	25.3 aA	1.6 aA	2.1 abA
Nevada Synthetic XX	R	HR	0.0 aA	0.0 aA	11.3 aA	10.3 aA	1.7 aA	1.6 aA
Deseret	S	S	2.3 aA	17.3 cB	25.3 aA	61.3 bB	2.5 bA	2.6 bA

[a] R = resistant, HR = highly resistant, S = susceptible.
[b] Values are means of three replicates. Means not followed by the same letter (lowercase letters for columns and uppercase letters for rows) differ ($P \leq 0.05$) according to Duncan's new multiple range test. Phytophthora root rot (PRR) was rated on a scale of 1–5, with 1 = none, 5 = very severe.

TABLE 5. Mean root gall ratings and percent galling of roots and Rhizobium nodules after single and dual inoculations with *Phytophthora megasperma* f. sp. *medicaginis* (Pmm) and *Meloidogyne hapla* (Mh) in alfalfa

	Reaction to[a]		Percent nodules galled[b]		Percent roots galled[b]		Root gall rating[b]	
Cultivar	PMM	Mh	Mh	Mh + Pmm	Mh	Mh + Pmm	Mh	Mh + Pmm
Apollo II	R	S	40.0 aA	41.7 aA	100.0 aA	83.0 aB	4.4 aA	3.8 aB
Nevada Synthetic XX	R	HR	3.3 bA	1.3 bA	37.7 bA	26.3 bA	2.1 bA	1.4 bB
Deseret	S	S	31.7 aA	16.7 bA	100.0 aA	94.3 aA	4.4 aA	3.7 aB

[a] R = resistant, HR = highly resistant, S = susceptible.
[b] Values are means of three replicates. Means not followed by the same letter (lowercase letters for columns and uppercase letters for rows) differ ($P \leq 0.05$) according to Duncan's new multiple range test. Root galling was rated on a scale of 1–5, with 1 = none, 5 = very severe.

The benefit of dual resistance was shown in Nevada Synthetic XX, which had no detectable infection of nodules by *P. m. medicaginis* and only slight root infection, even when both pathogens were present. Apollo II, which is resistant only to *P. m. medicaginis*, displayed good resistance to the fungus in the absence of *M. hapla*. However, when *M. hapla* was present, a significant increase in nodule infection occurred. This agrees with previous studies in which species of *Meloidogyne* have altered resistance in crops to root-infecting fungi. Tobacco plants resistant to *Phytophthora parasitica* var. *nicotianae* Tucker are transformed into susceptible hosts after infection with *M. incognita* (16). Several possible explanations for this breakdown in resistance have been suggested. One possible explanation is that, after the nematodes establish feeding sites in susceptible root tissue, they transform parenchyma cells into elaborate giant cells rich in nutrients. This altered tissue is often more susceptible to invasion and parasitism by other organisms present in the rhizosphere (15). A second possible explanation for the breakdown in resistance is the increased exudation from nematode-infected tissue. Increased root rot in tomato and okra caused by *Rhizoctonia solani* Kühn was shown to be directly associated with increased root exudates from galls produced by *M. incognita* (4). A possible reason for increased root rot in alfalfa from *P. m. medicaginis* in the presence of *M. hapla* may be a combination of altered tissue and increased root exudates. Because Rhizobium nodules play a vital role in the overall health and productivity of the alfalfa plant, it is important to understand their role and fate in root disease development. Huang (11) recently pointed out that, although our knowledge of biological N_2 fixation at the genetic, biochemical, and physiological levels has expanded rapidly during the last two decades, numerous obstacles that limit maximum nitrogen fixation still remain unresolved. He stated that, among those obstacles, suppression of nodulation by plant pathogens is a major threat to grain legume production. From our results, it is obvious that nodules appear to be readily attacked by root pathogens. A greater emphasis needs to be placed on nodule health in pathogenicity studies and in the selection for disease resistance in alfalfa.

The major finding in the seedling damping-off study was that postemergence damping-off caused by *P. m. medicaginis* was suppressed in the presence of *M. hapla* when both pathogens were present at planting. Disease suppression occurred in both Phytophthora root rot-susceptible and resistant cultivars. This finding differs from results of the root infection experiment and from results of previous studies (10,17), in which an increase in disease was observed with multiple-pathogen inoculations. However, in the previous studies, pathogens were applied to older plants (≥1-mo-old) when root tissue had differentiated. One possible explanation for the disease suppression is that nematodes feeding on immature roots may have interfered with infection by *P. m. medicaginis*. Damping-off caused by *P. m. medicaginis* is an extremely dynamic event that is influenced by many factors, of which soil moisture and soil temperature are paramount. Our experiments were designed where these factors were only moderately favorable, thus allowing the interaction to be expressed. Under these suboptimal conditions, *M. hapla* suppressed damping-off caused by *P. m. medicaginis*. More favorable conditions would have resulted in total seedling death. If a similar suppression in damping-off does occur in the field, identical conditions, as provided in the experiment, would have to occur. Although *M. hapla* + *P. m. medicaginis* resulted in less damping-off, the same treatment resulted in greater Phytophthora root rot than *P. m. medicaginis* alone in surviving 7-wk-old plants. Statistical separation of Phytophthora root rot and egg production means in the treatments with *P. m. medicaginis* alone and *M. hapla* + *P. m. medicaginis* could be shown only in one of the three cultivars. However, an overall trend of increased root rot and lower egg reproduction in the combined treatment was evident and is consistent with previous work (10,17).

Our studies were not aimed at developing a multipathogen screening technique for use in selecting for resistance to both pathogens. However, because damping-off caused by *P. m. medicaginis* was suppressed in the presence of *M. hapla* when both were applied at planting, this obviously would not be the optimal time to inoculate for plant selection. Further studies would be required to determine the optimal plant age for inoculation to obtain the highest level of resistance to both pathogens in alfalfa. Although multipathogen screening should shorten the time required for cultivar development, single-pathogen screening has produced the desired results, as exemplified in Nevada Synthetic XX. It is obvious from our studies that, regardless of what type of screening method is used, resistance to *M. hapla* and probably resistance to *M. incognita* are essential to the stability of Phytophthora root rot resistance in alfalfa when both pathogens are present.

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