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Interaction of *Ditylenchus dipsaci* and *Meloidogyne hapla* on Resistant and Susceptible Plant Species[1]

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Abstract: Numbers of *Ditylenchus dipsaci* or *Meloidogyne hapla* invading Ranger alfalfa, Tender crop bean, Stone Improved tomato, AH-14 sugarbeet, Yellow sweet clover, and Wasatch wheat from single inoculations were not significantly different from numbers by invasion of combined inoculations. *D. dipsaci* was recovered only from shoot and *M. hapla* only from root tissue. Combined inoculations did not affect reproduction of either *D. dipsaci* or *M. hapla*. *D. dipsaci* suppressed shoot growth of all species at 15–30 C, and *M. hapla* suppressed shoot growth of tomato, sugarbeet, and sweet clover at 20, 25, and 30 C. There was a positive correlation ($P < 0.05$) between shoot and root growth suppression by *D. dipsaci* on all cultivars except wheat at 20 C and tomato at 30 C. *M. hapla* suppressed ($P < 0.05$) root growth of sugarbeet at 20–30 C and wheat at 30 C. Growth suppression was synergistic in combined inoculations of sweet clover shoot growth at 15 C and root growth at 20–30 C, wheat root growth at 15 and 20 C, and tomato root growth at 15–30 C ($P < 0.05$) *D. dipsaci* invasions caused mortality of alfalfa and sweet clover at 15–30 C and sugarbeet at 20–30 C. Mortality rates of alfalfa and sweet clover increased synergistically ($P < 0.05$) from combined inoculations.

Key words: alfalfa, alfalfa stem nematode, bean, *Ditylenchus dipsaci*, growth suppression, interaction, *Meloidogyne hapla*, mortality, soil temperature, sugarbeet, sweet clover, tomato, wheat.

Two or more species of plant-parasitic nematodes are often associated with the growth of a given plant (3,5,8,10,13–16). The alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, and the northern root-knot nematode, *Meloidogyne hapla* Chitwood, are found throughout the western United States, often in the same field (4,7). Although the host range of the alfalfa stem race of *D. dipsaci* is small (17,18), it invades and suppresses growth of nonhost plants (9). A combination of *D. dipsaci* and *M. hapla* affects the host–parasite relationship between the nematodes and resistant and susceptible alfalfa (8).

Since both resistant and susceptible plant cultivars are often grown in fields infested with both nematode species, this study was to determine the influence of these two nematode species on the growth of selected host and nonhost plants as affected by temperature.

Materials and Methods

Plant cultivars used in each experiment of this study were alfalfa, *Medicago sativa* L. 'Ranger'; bean, *Phaseolus vulgaris* L. 'Tender Crop'; tomato, *Lycopersicon esculentum* Mill. 'Stone Improved'; sugarbeet, *Beta vulgaris* L. 'AH-14'; sweet clover, *Melilotus indica* (L.) All. 'Yellow'; and wheat, *Triticum durum* Desf. 'Wasatch'. Alfalfa is the only species susceptible to *D. dipsaci*, whereas all species except wheat are susceptible to *M. hapla*.

D. dipsaci and *M. hapla* inocula were originally obtained from alfalfa and lettuce, respectively, in northern Utah. *D. dipsaci* was cultured on Ranger alfalfa, and *M. hapla* was cultured on Stone Improved tomato under controlled greenhouse conditions. To obtain *D. dipsaci* inoculum, nematode-infected alfalfa stems were cut into 10-mm lengths, split, and placed on a Baermann funnel filled with deionized water for 24 hours. Nematodes were collected on a 0.043-mm-pore screen and washed six times with deionized water. *M. hapla* inoculum was obtained by extracting eggs and juveniles (J2) from infected tomato roots by a NaOCl method (12).

The following experimental technique was used in all tests: Seeds of alfalfa and sweet clover were scarified, and all seeds were treated with captan (cis[{Trichloromethyl}thio]-4-cyclohexene-1,2-dicarbox-

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TABLE 1. Invasion of resistant and susceptible plant selections by *Ditylenchus dipsaci* and *Meloidogyne hapla*.

Plant	Nematodes per plant			
	Single		Combined	
	D. dipsaci	*M. hapla*	*D. dipsaci*	*M. hapla*
Bean	36 a	58 c	38 a	57 c
Sweet clover	38 a	58 c	40 a	58 c
Alfalfa	42 a	61 c	44 a	64 c
Wheat	35 a	60 c	34 a	56 c
Sugarbeet	38 a	46 b	37 a	47 b
Tomato	40 a	59 c	36 a	58 c

Data are averages of 10 replicates (four plants per replicate). Numbers followed by the same letter are not significantly different ($P < 0.05$) according to split plot analysis of variance and Duncan's multiple-range test.

Each pregerminated seed inoculated with 50 *D. dipsaci* and 100 *M. hapla* (J2) and grown on greenhouse bench at 22 ± 4 C for 14 days.

imide), pregerminated on filter pads in petri dishes for 48 hours, washed six times with deionized water, and planted into methyl bromide fumigated sandy loam soil (91% sand, 5% silt, 4% clay; pH 7.2).

Experiment 1: Pregerminated seeds were planted into 15-cm-d plastic containers (four per container). Immediately after planting, 50 *D. dipsaci* adults and juveniles or 100 *M. hapla* juveniles (J2), or both, per seed were poured over the pregerminated seed. Containers, including noninoculated controls were replicated 10 times and maintained on a greenhouse bench at 22 ± 4 C. Plants were harvested after 14 days growth and stained in hot lactoglycerol (1:1:1 lactic acid : glycerol : distilled water) and acid fuchsin, and invasion of plants by each nematode species was determined.

Experiment 2: In the second experiment, pregerminated seeds were planted into 15-cm-d plastic containers (one seed per container) and inoculated with either 50 *D. dipsaci* adults and juveniles or 500 *M. hapla* J2, or both, per seed. Treatments, including noninoculated controls, were replicated 10 times and grown on a greenhouse bench at 22 ± 4 C. Plants were harvested 30 days after inoculation. Percentage of mortality and shoot and root growth of inoculated plants were compared with noninoculated plants, and nematode reproduction was determined as described for experiment 1.

Experiment 3: Pregerminated seeds were planted into 10-cm-d plastic containers (one seed per container) which were placed in controlled baths at 15, 20, 25, and 30 C ± 1 C. Treatments included 1) 50 *D. dipsaci* adults and juveniles per pregerminated seed, 2) 100 *M. hapla* J2 per pregerminated seed, 3) 50 *D. dipsaci* adults and juveniles per pregerminated seed followed 14 days later by 200 *M. hapla* J2 per plant, 4) noninoculated controls. Treatments were replicated six times. The experiment was terminated 30 days after final inoculation. Percentage of mortality, plant growth, and nematode reproduction were determined in a manner similar to that described.

Data from all experiments were analyzed using analysis of variance and Duncan's multiple-range test.

RESULTS

Experiment 1: There were no significant ($P < 0.05$) differences in the numbers of *D. dipsaci* invading any of the plant species either from single or combined inoculations. Only sugarbeet had less *M. hapla* infection than the other plant species (Table 1). Tissue invasion by either nematode was not affected ($P < 0.05$) by combined inoculations; *D. dipsaci* was recovered only from shoot tissue and root-knot juveniles were recovered only from root tissue. This agrees with data obtained from a previous study (8).

Experiment 2: Inoculation with *D. dipsaci,* suppressed shoot growth ($P < 0.05$) of alfalfa (the only host plant), tomato, sugarbeet, and sweet clover and root growth of alfalfa, sugarbeet, and sweet clover in the greenhouse at 22 ± 4 C. *M. hapla* reduced shoot growth ($P < 0.05$) of all plants except bean, and wheat (the only nonhost), but reduced root growth of only sugarbeet. Inoculations with both *D. dipsaci* and *M. hapla* reduced ($P < 0.05$) shoot growth only of tomato and root growth only of tomato and sweet clover below that of inoculations with either nematode alone (Table 2).

The plant mortality rate from inocula-

TABLE 2. Plant weights as a percentage of controls after single and combined inoculations with *Ditylenchus dipsaci* and *Meloidogyne hapla*.

Cultivars	Plant weights (% control)						Control wt (g)	
	Shoots			Roots				
	A	B	C	A	B	C	Shoots	Roots
Alfalfa	25 a	65 c	35 a	50 ab	95 d	35 a	0.26	0.23
Bean	85 d	95 e	85 de	90 d	95 d	85 d	5.61	7.05
Sugarbeet	65 c	60 c	50 b	70 c	70 c	65 bc	2.72	1.25
Sweet clover	35 a	70 cd	30 a	65 bc	80 cd	45 a	0.22	0.28
Tomato	65 c	65 c	40 ab	80 cd	90 d	50 ab	2.13	1.09
Wheat	85 de	95 e	80 d	80 cd	85 d	75 c	1.44	3.18

Data are averages of 10 replicates. Numbers followed by the same letter are not significantly different ($P < 0.05$) according to split plot analysis of variance and Duncan's multiple-range test.

Each pregerminated seed inoculated with 50 *D. dipsaci* adults and juveniles (A); 500 *M. hapla* J2 (B); 50 *D. dipsaci* adults and juveniles and 500 *M. hapla* J2 (C). Plants grown for 30 days at a greenhouse temperature of 22 ± 4 C.

tion with *M. hapla* at 22 ± 43 C was 5% for alfalfa and 0% for all other species. This compared with 20, 0, 0, 10, 15, and 0%, for alfalfa, bean, tomato, sugarbeet, sweet clover, and wheat, respectively, from inoculations with *D. dipsaci*. Plant mortality from combined inoculation of the two nematodes was similar to that caused by *D. dipsaci*.

Combined inoculations did not affect reproduction of either nematode on host plants. Reproduction rates for *D. dipsaci* were 2.7 and 2.4 ($\times 10^3$) for single and combined inoculations, respectively. This compared with *M. hapla* reproduction rates of 6.4, 4.3, 6.8, 4.8, and 5.0 ($\times 10^3$) for single and 7.2, 4.5, 6.5, 4.4, and 5.1 ($\times 10^3$) for combined inoculations of alfalfa, bean, tomato, sugarbeet, and sweet clover, respectively.

Experiment 3: Soil temperature affected the host–parasite relationship of single and combined inoculations of *D. dipsaci* and *M. hapla*. *D. dipsaci* suppressed ($P < 0.05$) shoot growth of alfalfa, sweet clover, sugarbeet, and tomato at all soil temperatures (Fig. 1), and the greatest shoot growth suppression was at 15 C. *D. dipsaci* suppressed ($P < 0.05$) shoot growth of wheat at 15 C, but not at higher temperatures, and the nematode had no effect on shoot growth of bean. *M. hapla* suppressed ($P < 0.05$) shoot growth of alfalfa, tomato, sugarbeet, and sweet clover at temperatures of 20, 25, and 30 C and bean at 30 C; it had no effect on shoot growth of wheat (Fig. 1). Combined inoculations suppresssed ($P < 0.05$) shoot growth of all species except bean at one or more temperatures. Reductions in top growth were additive, except for sweet clover at 15 C where a synergistic growth suppression was observed. There was a direct relationship between shoot and root growth in all species except wheat at 20 C and tomato at 30 C (Fig. 1). *M. hapla* suppressed root growth of only sugarbeet at 20–30 C and wheat at 30 C. There was, however, synergistic root growth suppression from combined inoculations of *D. dipsaci* and *M. hapla* in wheat at 15 and 20 C, sweet clover at 20–30 C, and tomato at all soil temperatures. Reduction in root growth of the other cultivars was additive.

No plant died from single inoculations of *M. hapla* on any species nor from single inoculations with *D. dipsaci* on bean, tomato, and wheat at any temperature. *D. dipsaci* inoculation resulted in mortality rates of 20, 25, 15, and 10% on alfalfa; 20, 15, 10, 0% on sugarbeet; and 10, 20, 15, and 5% on sweet clover at 15, 20, 25, and 30 C, respectively. Combinations of the two nematodes resulted in synergistic mortality rates on alfalfa at all temperatures and on sweet clover at 15 and 20 C. Mortality rates were 40, 45, 20, and 20% at 15, 20, 25, and 30 C for alfalfa and 20 and 30% for sweet clover at 15 and 20 C (Fig. 2).

DISCUSSION

Recovery of alfalfa stem nematode from epicotyl tissue and recovery of root-knot

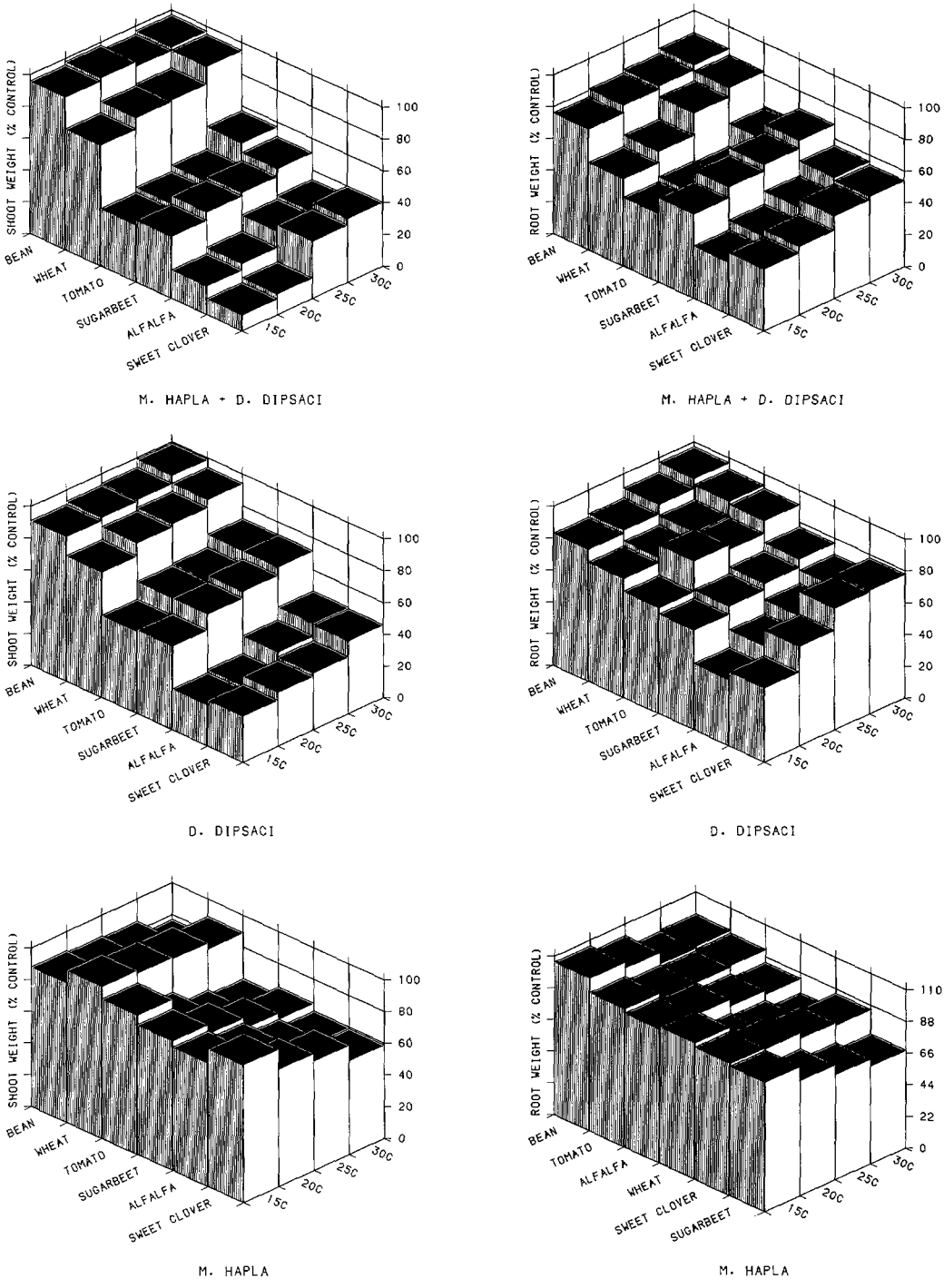

FIG. 1. Shoot and root weights, as percentages of controls, of plants inoculated with *Ditylenchus dipsaci* and/or *Meloidogyne hapla*.

juveniles only from root tissue agrees with data obtained from a previous study (8). Both nematode species exhibited a greater attraction to susceptible than to resistant alfalfa when the inoculum was placed at a distance from the seed (11). However, introduction of *D. dipsaci* and *M. hapla* immediately over the pregerminated seed ne-

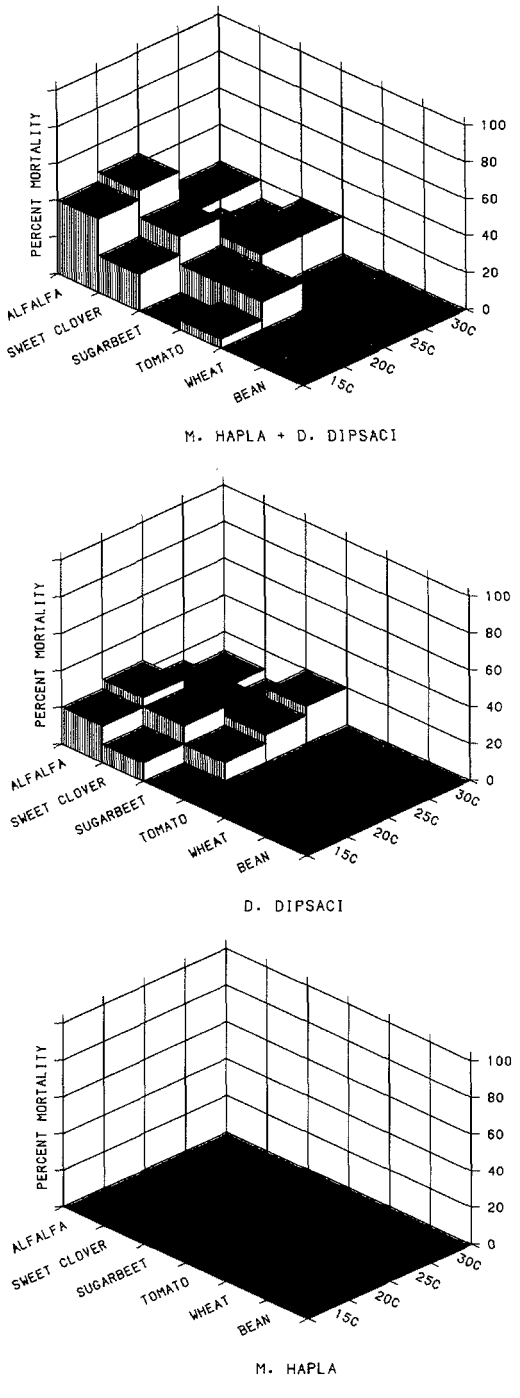

FIG. 2. Mortality of plants inoculated with *Ditylenchus dipsaci* and (or) *Meloidogyne hapla.*

gated any effect of plant attraction or multiple nematode inoculation.

Pathogenesis of nonhost plant species by *D. dipsaci* agrees with a previous study (9) that showed the alfalfa stem nematode race of *D. dipsaci* causes symptoms similar to those of *D. dipsaci* races that categorize these plants as host plants (1, 2).

Differences in the growth of plants inoculated with either or both *D. dipsaci* and *M. hapla* in the greenhouse bench and soil temperature studies may be due to differences in plan tolerance and the fact that *D. dipsaci* and *M. hapla* are more virulent at 15 and 25 C, respectively. This is especially notable on suppression on the growth of wheat, a nonhost of both nematodes. Combinations of two plant-parasitic nematodes may suppress plant growth and increase plant mortality regardless of plant susceptibility, resistance, or tolerance, and plant growth may be suppressed by cohabitating nematodes even if they infect distinctly different plant organs. We may become more aware of such relationships as we learn more about the host–parasite relationships between nematodes and plants.

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