Effects of Temperature and Inoculation Timing on the Meloidogyne hapla/Corynebacterium insidiosum Complex in Alfalfa[1]

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Hunt et al. (2) and Norton (4) showed that the northern root-knot nematode, *Meloidogyne hapla* Chitwood, increased the incidence of bacterial wilt in alfalfa. Hunt et al. (2) also found the bacterial wilt organism, *Corynebacterium insidiosum* (McCull) H. L. Jens., in plant tissue that had been inoculated only with the bacteria. This would indicate that the role of *M. hapla* is something more than that of aiding the entry of bacteria through nematode-induced wounds.

This study was designed (i) to determine bacterial wilt incidence with *M. hapla* inoculation before and after inoculation with *C. insidiosum*; and (ii) to determine the effect of temperature on susceptibility of alfalfa to bacterial wilt.

Twelve-week-old 'Vernal,' 'Ranger,' and 'Moapa' alfalfa plants were transplanted (one plant/pot) into Provo sand in 15-cm diameter clay pots, and segregated into six equal 25-plant treatment groups. Treatments consisted of: (N) = plants inoculated with 200 *M. hapla* larvae/plant; (B) = plants inoculated with 10 cc *C. insidiosum* suspension/plant (2); (NB_1) = plants inoculated with 200 *M. hapla* larvae plus 10 cc *C. insidiosum* suspension/plant; (NB_2) = plants inoculated with 200 *M. hapla* larvae; and after 30 days the plants were carefully washed free of soil, surface sterilized in 100 ppm streptomycin sulfate solution, washed in distilled water, transplanted into sterile soil and inoculated with 10 cc *C. insidiosum* suspension; (NB_3) = plants inoculated with 10 cc *C. insidiosum* suspension, and after 30 days the plants were carefully washed free of soil, surface sterilized in 100 ppm streptomycin sulfate solution, washed in distilled water, transplanted into sterile soil and inoculated with 200 *M. hapla* larvae/plant; and (C) = untreated control.

TABLE 1. Interaction of *Meloidogyne hapla* and *Corynebacterium insidiosum* on three alfalfa cultivars.

Alfalfa cultivars	Percent of plants† showing bacterial wilt symptoms					
	N‡	B	NB_1	NB_2	NB_3	C
'Moapa'	0	12	60	76	84	0
'Ranger'	0	4	36	52	56	0
'Vernal'	0	0	12	12	16	0

Least Square Test

LSD (cultivars) 0.05 = 20.6
LSD (treatments) 0.05 = 29.1

† Pathogen exposure treatments (25 replicate plants)
‡ N = Meloidogyne hapla alone (200 larvae/rep plant)
B = Corynebacterium insidiosum alone (10 cc suspension/rep plant)
NB_1 = M. hapla + C. insidiosum
NB_2 = M. hapla + C. insidiosum (bacteria added 30 days after nematodes)
NB_3 = M. hapla + C. insidiosum (nematodes added 30 days after bacteria)
C = untreated control

After 120 days growth in greenhouse culture at 22 ± 4 C, all plants were harvested and the percentage of plants showing vascular discoloration was determined.

There were significant differences in the incidence of vascular discoloration between cultivars when plants were inoculated with a combination of *M. hapla* and *C. insidiosum* (Table 1). There were no significant differences between treatments NB_1, NB_2, and NB_3, and all were significantly greater than *C. insidiosum* alone in 'Moapa' and 'Ranger'. The occurrence of the bacterial wilt organism in root tissue of the NB_3 treatment is apparently a result of normal root injury, supposedly from lateral root branching and splitting. This can create sufficient infection courts for the bacteria, but infection of *C. insidiosum*-infected root tissue by *M. hapla* enhanced the expression of bacterial wilt. The mode of predisposition by

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M. hapla is not known. However, Owens and Specht (5) found an increase in organic acids, free amino acids, protein, RNA, DNA, and lipids in root-knot galls of tomato roots. Johnson and Powell (3) found apparent *Pseudomonas solanacearum* E. F. Smith masses in *Meloidogyne incognita* (Kofoid and White) Chitwood-induced galls in tobacco and speculated that bacteria reproduction was associated with an increase in available nutrients found in the giant cells. Since these nutrients were not available to bacteria in non-galled tissue, little bacterial wilt developed.

To determine the effect of soil temperature on the bacterial wilt/root-knot nematode interaction in alfalfa, 12-week-old 'Moapa' alfalfa plants were inoculated with: (i) *M. hapla* and *C. insidiosum*; (ii) *C. insidiosum* with cut rootball (1); (iii) *C. insidiosum* with uncut rootball. Five plants/container were grown in one quart polyethylene containers at soil temperatures of 16, 20, 24, and 28 C (10 containers/treatment for each temperature). After 3 months growth, plants were harvested, and the degree of vascular discoloration was determined. Increased soil temperature caused no significant increase in vascular discoloration at temperatures above 16 C. There were no differences in the incidence of vascular discoloration between plants inoculated with a combination of *M. hapla* and *C. insidiosum*, and cut rootball inoculated with *C. insidiosum* (Table 2). Severing of the root tissue also causes an apparent increase in bacterial nutrients.

TABLE 2. Effect of temperature on interaction of *Meloidogyne hapla* and *Corynebacterium insidiosum* on 'Moapa' alfalfa.

Temperature (C)	Percent of plants showing bacterial wilt symptoms†		
	A‡	B	C
16	64	62	12
20	72	76	16
24	76	80	18
28	80	76	12

Least Square Test

LSD (temperature) 0.05 = 8.2
LSD (treatments) 0.05 = 7.1

† Plants 12-weeks old at initial inoculation.
‡ A = plants inoculated with 200 M. hapla larvae and 10 cc C. insidiosum suspension
B = C. insidiosum (rootball cut; 10 cc C. insidiosum suspension)
C = C. insidiosum (rootball uncut; 10 cc C. insidiosum suspension)

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