

Penetration and Development of *Meloidogyne hapla* in Resistant and Susceptible Alfalfa Under Differing Temperatures¹

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Abstract: Studies were conducted to examine under differing temperatures (12, 16, 20, 24, 28, and 32 C) the penetration and development of *Meloidogyne hapla* in resistant lines '298' and 'Nev. Syn XX', and susceptible 'Lahontan' and 'Ranger' hardy-type alfalfas. The results indicated that resistance to *M. hapla* was similar to that previously described for *M. incognita* in nonhardy alfalfa. Although initial penetration in resistant seedlings was similar to that of susceptible seedlings, nematode larvae failed to establish and develop in root tissues and nematode numbers subsequently declined. In susceptible seedlings, nematode development proceeded rapidly, and egg production began after 5 weeks. Temperature had little influence on the nematode development except to slow the response at the lower temperatures. Other studies were conducted to verify a previously reported immune (no penetration) reaction to *M. hapla* by the 'Vernal' selection 'M-4'. When compared to the resistant (penetration without nematode development) Vernal selection 'M-9' under differing temperatures (20, 24, 28, and 32 C), each selection was equally penetrated by *M. hapla* but at a lower level than in susceptible Ranger cuttings. Generally, no root galling was observed in either M-4 or M-9; however, very slight galling was found 35 days after inoculation on about 50% of these cuttings when grown at 32 C. **Key Words:** root-knot nematode, *Medicago sativa*, temperature.

The northern root-knot nematode *Meloidogyne hapla* Chitwood is prevalent in the cool areas of the U.S. where dormant or hardy alfalfa *Medicago sativa* L. is grown, whereas the southern root-knot nematode *M. incognita* Chitwood is found in the warm southwestern and southeastern areas where nondormant or nonhardy alfalfa is grown (4). Resistance to *M. incognita* was demonstrated in the alfalfa cultivar 'African' and other nonhardy cultivars in 1955 by Reynolds (6). Later, Reynolds, Carter, and O'Bannon (7) studied the penetration, development, and migration of *M. incognita* in resistant (African, Moapa, and Sonora) and susceptible (Lahontan) cultivars. They found that *M. incognita* larvae entered both resistant and susceptible seedlings in approximately the same numbers, but after 7 days there was a sharp reduction in the number of nematodes in resistant seedlings with no subsequent nematode development. In susceptible seedlings, larvae became sedentary and developed to maturity.

Resistance to *M. hapla* was discovered by Stanford et al. (8) in selections of the hardy cultivar 'Vernal'; and the develop-

ment of a root-knot nematode resistant line was described by Hunt et al. (5). Goplen and Stanford (1) characterized resistant S₁ plants of the Vernal selection 'M-4' as being immune to penetration by *M. hapla* when they were examined 7 days after inoculation. Later, Griffin (2) found that roots of another resistant Vernal selection, 'M-9', were slightly galled when it was grown at temperatures of 20 and 25 C for 60 days following inoculation. No attempt has been made to trace the development of *M. hapla* in resistant and susceptible hardy alfalfa from penetration to maturity as was done for *M. incognita* in nonhardy alfalfa (7).

In the research reported herein, we examined (under differing temperatures) the penetration and development of *M. hapla* in resistant and susceptible dormant alfalfa seedlings.

MATERIAL AND METHODS

Resistant cultivars used in this study were 'M-9', 'M-4', '298'—a resistant clone of M-9, and 'Nev. Syn XX'—a selection from 'M-7', and 'I-167'. M-9, M-7, and M-4 are selections from Vernal by Stanford et al. (8), and I-167 is a selection from Vernal by Carnahan (5). M-9, M-7, and I-167 are described as having simplex resistance to *M. hapla*.

Penetration and development of M. hapla in roots of resistant and susceptible alfalfas under differing temperatures:

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Fourteen-day-old alfalfa seedlings of the resistant line 298 and the susceptible cultivar Ranger were planted singly into 20 x 70-mm vials containing Provo sand (a fine, sandy loam soil; moisture holding capacity=17%) previously fumigated with methyl bromide. Each seedling was inoculated with 200 *M. hapla* larvae which were collected from a Utah population cultured on tomato plants (*Lycopersicon esculentum* Mill.). Following inoculation, plants were grown in growth chambers at 12, 16, 20, 24, 28, and 32 C. Ten replicates (vials) of each entry were harvested from each temperature after 2, 4, 6, 8, 12, and 16 days. The roots were stained in acid fuchsin-lactophenol solution, and the numbers of root-knot larvae/plant were determined by using a stereomicroscope.

In a second experiment, germinated seed of either Nev. Syn XX or Lahontan were planted into eighteen, 10-cm plastic pots of steamed, very fine, sandy loam soil (10 seeds/pot). Each seed was inoculated with 500 *M. hapla* larvae of a Washington State population cultured on tomato, and the pots were placed in a growth chamber at 20 C. Two pots of each entry were removed weekly for 9 weeks, and nematode penetration and development were determined by the method described in the previous experiment.

Nematode penetration of M-4 under differing temperatures: Three-week-old cuttings of immune M-4, M-9 [reported as resistant but not immune to penetration (2)], and the susceptible cultivar Ranger were inoculated with 200 *M. hapla* larvae. Plants were grown singly in fumigated Provo sand in 1-liter polyethylene containers in a greenhouse temperature (22 ± 4 C). Twenty plants of each selection were harvested after 14 and 28 days and number of nematodes/plant was determined by the method previously described.

In an experiment using different temperatures, 3-week-old cuttings of M-4, M-9, and Ranger plants were transplanted singly in 1-liter polyethylene containers of Provo sand. Each plant was inoculated with 200 *M. hapla* larvae and grown in greenhouse water baths at 20, 24, 28, and 32 C. Six plants of each selection were harvested from each temperature after 4, 8, and 16 days, and the numbers of nematodes/plant were

determined. This experiment was repeated except that twenty 8-week-old cuttings of each alfalfa type were each inoculated with 1,000 *M. hapla* larvae. The plants were harvested after 35 days and root-knot galling was determined.

RESULTS

Penetration and development of M. hapla in roots of resistant and susceptible cultivars under different temperatures: At day 2, nematode penetration of both 298 and Ranger alfalfa increased as temperature increased (Fig. 1), with maximum penetration at 24 and 28 C, slightly lower at 20 and 32 C, and lowest at 12 and 16 C. Penetration was slightly but not significantly higher in the Ranger seedlings than in the 298 seedlings. Nematode numbers continued to increase slowly in the two entries until day 6; subsequently, nematode numbers at 20, 24, 28, and 32 C in resistant line 298 declined rapidly. At 12 and 16 C, the decline was more gradual, a circumstance indicating a slower response to the lower temperatures. Nematode numbers in the Ranger seedlings maintained the initial penetration level or continued to increase slightly until day 16 at all temperatures except at 32 C where a very slight decline was noted. Until day 8, there were no significant differences in nematode penetration of 298 and Ranger at any temperature with the exception of 12 C where significant differences did not occur until day 12.

In the second experiment, *M. hapla* penetration was observed in both resistant Nev. Syn XX and susceptible Lahontan roots 1 week after inoculation. An occasional undeveloped larva was found in the Nev. Syn XX roots after 2 weeks and no nematodes could be found thereafter. In the Lahontan roots, larval development occurred between 2 and 6 weeks. Egg production was first observed at 5 weeks and many eggs were observed in the 6-9th weeks.

Nematode penetration of M-4 under differing temperatures: The Utah population of *M. hapla* penetrated M-4 as readily as it did M-9 cuttings in the greenhouse experiment at 22 ± 4 C; there were no significant differences in nematode penetration of the two cultivars (Table 1). Although a high percentage of M-4 and

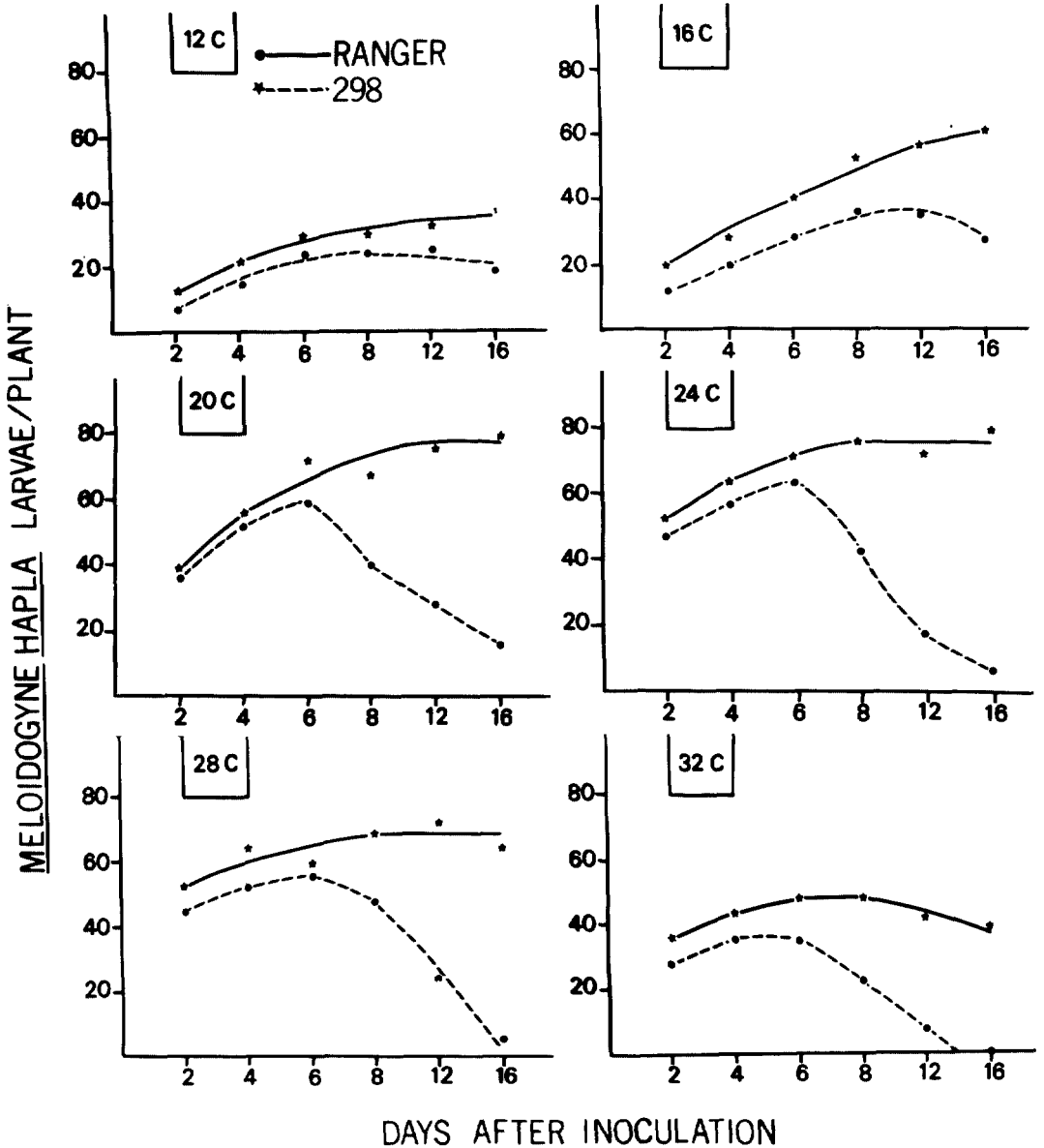


FIG. 1. Effects of soil temperature on penetration of 14-day-old resistant 298 and susceptible Ranger alfalfa seedlings by *Meloidogyne hapla* larvae (in 20 x 70-mm vials). Differences in penetration of 298 and Ranger: after 12 days at 12 C and 8 days at 16 C ($P=0.05$), and after 8 days at 20, 24, 28, and 32 C ($P=0.01$).

M-9 cuttings (80 and 85%, respectively) was penetrated, numbers of nematodes per infected plant at 14 days were significantly lower for these selections than for Ranger. There was a slight decrease of the nematode numbers in Ranger and a marked decrease in the resistant selections M-4 and M-9 between 14 and 28 days. The percentage of sexually nondifferentiated larvae at 14 days was similar for both M-4 and M-9, but

significantly greater than for Ranger (Table 1).

There were no differences in initial nematode penetration and subsequent nematode numbers in M-4 and M-9 at 20-32 C (Fig. 2). Nematode numbers in M-4 and M-9 were similar but significantly lower than those of Ranger at all temperatures at each of the three dates following inoculation. An increase in nematode

TABLE 1. Differences in infection of resistant-alfalfa selections and a susceptible-alfalfa cultivar by *Meloidogyne hapla*.

Plant selections and cultivar	Plants infected ^a (%)	Nematodes per infected plant ^b		Sexually nondifferentiated larvae after 14 days (%)
		14 days	28 days	
Ranger	100	72	66	29
M-9 ^c	85	23	13	74
M-4 ^c	80	27	16	78
LSD ($P=0.05$)		13	8	23

^aForty plants of each selection inoculated with 200 larvae/plant and grown at 22 ± 4 C.

^bNematode number determined for 20 plants of each selection at 14 and 28 days.

^cResistant Vernal selections.

numbers occurred between 4 and 16 days after inoculation for the three entries at all soil temperatures. Although nematode numbers were low for M-4 and M-9 selec-

tions when compared to those for Ranger, the increase between 4 and 16 days was approximately a multiple of 2-3 for all three entries.

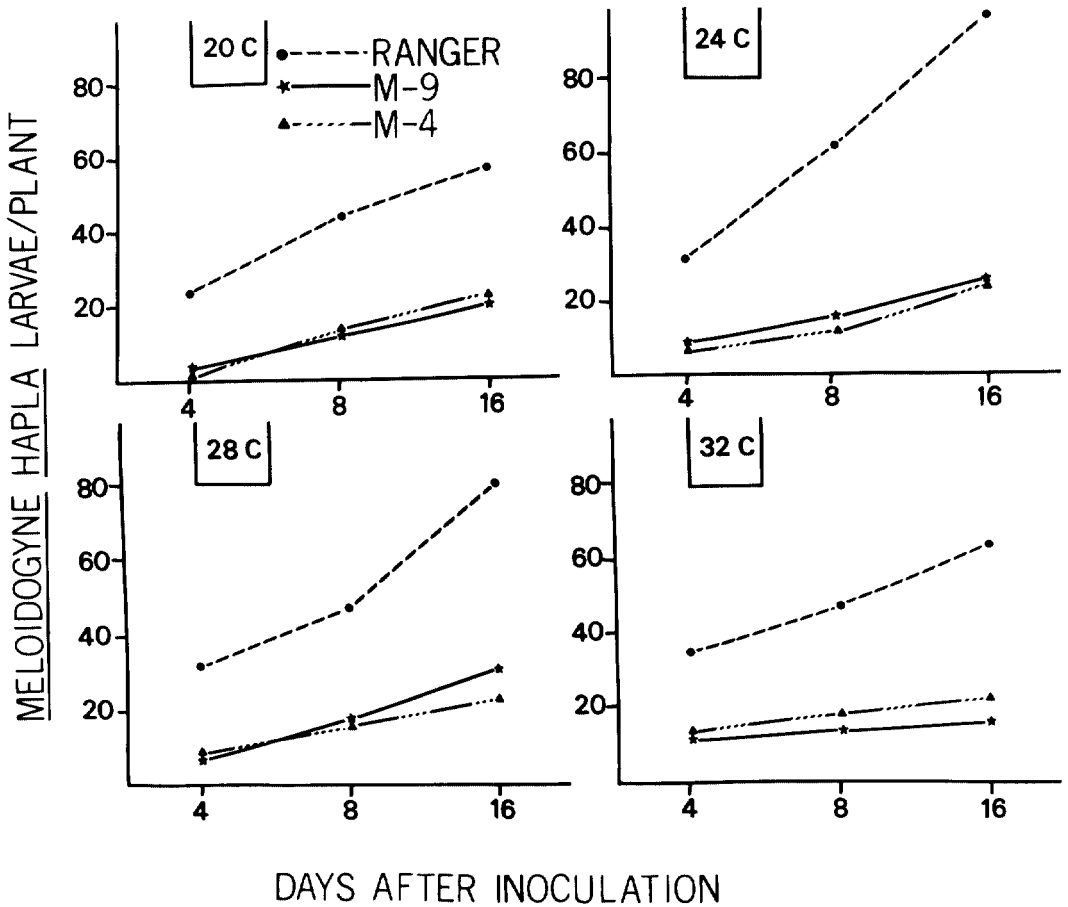


FIG. 2. Effects of soil temperature on penetration of resistant M-4 and M-9, and susceptible Ranger alfalfa cuttings by *Meloidogyne hapla* larvae (3-week-old cuttings inoculated with 200 larvae in 1-liter containers). [Penetration of 'M-4' and 'M-9' similar at all temperatures ($P=0.01$); penetration of 'M-4' and 'M-9' different from that of 'Ranger' after 4 days ($P=0.01$)].

When this experiment was repeated by using 1,000 larvae/plant and when galling ratings were made after 35 days, severe to very severe galling was observed in 100% of the cuttings of Ranger at temperatures from 20 to 32 C. No galling was observed in M-4 and M-9 cuttings at 20, 24, and 28 C, but very slight galling was evident on 40 to 50% of the respective cuttings at 32 C (Table 2).

DISCUSSION AND CONCLUSIONS

Results of our studies of *M. hapla* infection on resistant and susceptible dormant alfalfa generally paralleled those found for *M. incognita* on nondormant alfalfa (7). Following initial penetration (6 days) at temperatures above 20 C, nematode larvae failed to establish and develop in roots of resistant selection 298 and nematode numbers subsequently declined. At temperatures below 20 C, a slower, although similar, response was observed. In susceptible alfalfa roots, no decline in numbers was observed and nematode development occurred rapidly. The resistance mechanism in nondormant and dormant alfalfa is apparently similar for both *M. incognita* and *M. hapla*.

In our examination of *M. hapla* infection of M-4 and M-9, we found a low level of penetration in both selections at temperatures between 20 and 32 C. Thus, immunity was not demonstrated in M-4. The reasons for differences between these

and previously described findings (1) may be attributed to physiological differences between nematode populations or to the conditions under which observations were made. The partial loss of resistance in both M-4 and M-9 at 32 C, as evidenced by the slight root galling after 35 days, is an indication that host-plant resistance may be altered by high temperatures. Our results are in agreement with previous findings on the host-parasite relationship between *M. hapla* and alfalfa (2).

Temperature effects on initial penetration and subsequent nematode numbers, including a decline after 6 days in populations for the resistant entry 298 but not for M-4 and M-9, can best be explained by differences in experimental techniques. It has been shown that *M. hapla* larvae are attracted to alfalfa root tissue and attraction is affected by the proximity of the nematode to the root(s) (3). Since seedlings of selection 298 were grown in small vials and M-4 and M-9 cuttings were grown in large (1-liter) containers, the greater confinement in the vials resulted in a more rapid nematode penetration and eventually in migration from the 298 seedlings.

Since Nev. Syn XX and 298 both received resistance to *M. hapla* from simplex clones, we can also assume that the same reaction to nematode penetration would occur among the two selections if similar inoculation studies, regardless of plant age, plant size, container size, or temperature, were made.

TABLE 2. Effects of soil temperature on pathogenicity of *Meloidogyne hapla* on resistant-alfalfa selections and a susceptible-alfalfa cultivar.^a

Soil temperature (C)	Alfalfa selections					
	Ranger ^b		M-9 ^c		M-4 ^c	
	% Galled	Root-knot index ^d	% Galled	Root-knot index	% Galled	Root-knot index
20	100	4.8	0	—	0	—
24	100	5.0	0	—	0	—
28	100	5.0	0	—	0	—
32	100	4.5	50	1.0	40	1.0

^aEight-week-old cuttings were inoculated with 1,000 *M. hapla* larvae/plant and readings were made after 35 days.

^bSusceptible cultivar.

^cResistant Vernal selections.

^d0 = no galling; 5 = very severe galling.

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