Interrelationship of Meloidogyne hapla and Ditylenchus dipsaci on Resistant and Susceptible Alfalfa[1]

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Abstract: Simultaneous inoculations of alfalfa with *Meloidogyne hapla* larvae and *Ditylenchus dipsaci* at 16, 20, 24, and 28 C did not depress penetration of either nematode in 'Nev Syn XX'—a selection resistant to *M. hapla* and *D. dipsaci*, 'Vernal 298'—a selection resistant to *M. hapla* and susceptible to *D. dipsaci*, 'Lahontan'—a cultivar resistant to *D. dipsaci* and susceptible to *M. hapla*, and 'Ranger'—a cultivar susceptible to both *M. hapla* and *D. dipsaci*. Infection with *D. dipsaci* depressed growth of susceptible 'Vernal 298' and 'Ranger' at all soil temperatures, except for 'Vernal 298' at 16 C. Infection with *M. hapla* alone did not depress growth of any of the alfalfas. A combination of *M. hapla* and *D. dipsaci* resulted in a synergistic weight depression on 'Ranger' at all soil temperatures. Inoculation of the four alfalfas with *D. dipsaci* 2, 4, 6, and 8 wk before inoculation with *M. hapla* at 16, 20, 24, and 28 C did not influence the resistance or susceptibility of 'Nev Syn XX,' 'Lahontan,' or 'Ranger.' However, galling of 'Vernal 298' by *M. hapla* was affected by soil temperature, plant age, and inoculation with *D. dipsaci*. *Key Words:* concomitant species, root-knot nematode, alfalfa stem nematode, soil temperature, attraction, penetration, root galling.

The association of more than one species of nematode with the decline of a plant is not unusual, and host response to a plant-parasitic nematode may be altered by the presence of another nematode (6). One nematode may enhance or retard the development and reproduction of another nematode (2,4,14,15), and the effect may be reversed with the same two species of nematodes in a different host plant (3,4). Some nematode species act independently, and penetration by one nematode species may not be either hindered or aided by another species, although later development may be inhibited (5).

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, is found in all major alfalfa producing areas of the United States and is an important pathogen of alfalfa *Medicago sativa* L. (7). The northern root-knot nematode, *Meloidogyne hapla* Chitwood, is associated with dormant alfalfa in cool temperate areas. However, it is not unusual to find both *D. dipsaci* and *M. hapla* in the same field. Both nematode species are pathogenic on alfalfa and are involved in disease complexes (12,13). Re-

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sistant varieties appear to be the only practical way to control these nematodes. Since both susceptible and resistant alfalfas are used by growers, a study was made to determine 1) the relationship between *M. hapla* and *D. dipsaci* in dual infections on resistant and susceptible alfalfas and 2) whether the presence of *D. dipsaci* affects the resistance or susceptibility of alfalfa to *M. hapla.*

MATERIALS AND METHODS

Alfalfa cultivars and genotypes used in this study were 'Nev Syn XX'—a selection containing resistance to both *D. dipsaci* and *M. hapla,* 'Vernal 298'—a selection resistant to *M. hapla* and susceptible to *D. dipsaci,* 'Lahontan'—a cultivar resistant to *D. dipsaci* and susceptible to *M. hapla,* and 'Ranger'—a cultivar susceptible to both *D. dipsaci* and *M. hapla* (8,9,16).

For each experiment, seeds of the four alfalfas were scarified, surface sterilzed in 0.05% sodium hypochlorite, rinsed in distilled water, and germinated on filter pads saturated with captan (CIS-N-[trichloromethyl]thio-4-cyclohexene-1,2,-dicarboximide). The *D. dipsaci* inoculum was extracted from alfalfa tissue cultures with a Baermann funnel. Nematodes from the funnel were rinsed six times in distilled water. The *M. hapla* inoculum was from a Utah tomato plant culture. The egg masses were surface sterilized in a 0.5% sodium hypochlorite solution, rinsed in distilled water, and hatched in an oxygenator at 25 C.

To study the interaction between *M. hapla* and *D. dipsaci* in the penetration of resistant and susceptible alfalfas, germinated seeds (with 5–10-mm radicles) of the four alfalfas were planted singly in 10-cm containers of methyl bromide fumigated Provo sand (17% MHC) and inoculated with 50 *D. dipsaci,* 100 *M. hapla* larvae, both of these, or neither. The seedlings were grown in growth chambers with a 16-h light period at 16, 20, 24, and 28 C. The experiment, including uninoculated controls, was replicated 20 times and grown in paired series. Plants were harvested after 14 d and stained in a lactophenol-acid fuschin solution and the number of nematodes per plant determined under a stereomicroscope.

To study the pathogenicity of *M. hapla* and *D. dipsaci* in sequential infections on alfalfa, germinated seeds of all four alfalfas in 10-cm containers were inoculated with 100 *D. dipsaci* and 0, 2, 4, 6, and 8 wk later with 1,000 *M. hapla* larvae. The growth-chamber conditions and replication were as described above. After 7 wk of growth, plants were harvested and their percentages of galling and root-indices (1 = no galling; 2 = 1–10% galled; 3 = 11–30% galled; 4 = 31–70% galled; 5 = 71–90% galled, and 6 = 91–100% galled root system) were determined (1).

Differences in penetration between the two species was sought in a study of the attractiveness of the alfalfas to the nematodes. Inoculum containing both *D. dipsaci* and *M. hapla* larvae was placed 12.5 mm from a 7-d-old alfalfa seedling in 25 ml of 0.20% water agar in 150- × 20- × 15-mm plastic sampling boxes. The experiment was replicated 20 times. After 4 d at 20 C, the nematodes' migration and orientation to the seedlings were recorded. In a similar experiment, 100 larvae of each species were placed midway between 'Ranger' and 'Nev Syn XX' (12.5 mm from each) germinated seed in the 5–15 radicle stage. The experiment was replicated 20 times. After 4 d at 20 C, the movement of the nematodes was determined (11).

RESULTS AND DISCUSSION

Root penetration: More ($P = 0.05$) *D. dipsaci* penetrated 'Ranger' than penetrated 'Nev Syn XX' (Fig. 1). Confirming a previous study (9), no differences in invasion were observed between 'Ranger' and 'Lahontan.'

Soil temperature affected ($P = 0.05$) the penetration of all four alfalfas by *D. dipsaci.* Penetration by *D. dipsaci* was highest at 20, 24, and 28 C with 'Nev Syn XX' and at 20 and 24 C with 'Ranger,' 'Lahontan,' and 'Vernal 298.' Over the range of temperatures tested, penetration of all alfalfas by *M. hapla* increased with temperature. 'Ranger' and 'Lahontan' were penetrated by significantly more ($P = 0.01$) *M. hapla* larvae than were 'Vernal 298' and 'Nev Syn XX.' Inoculating with both *D. dipsaci* and *M. hapla* did not alter individual penetration patterns.

Plant weight depression: Except for

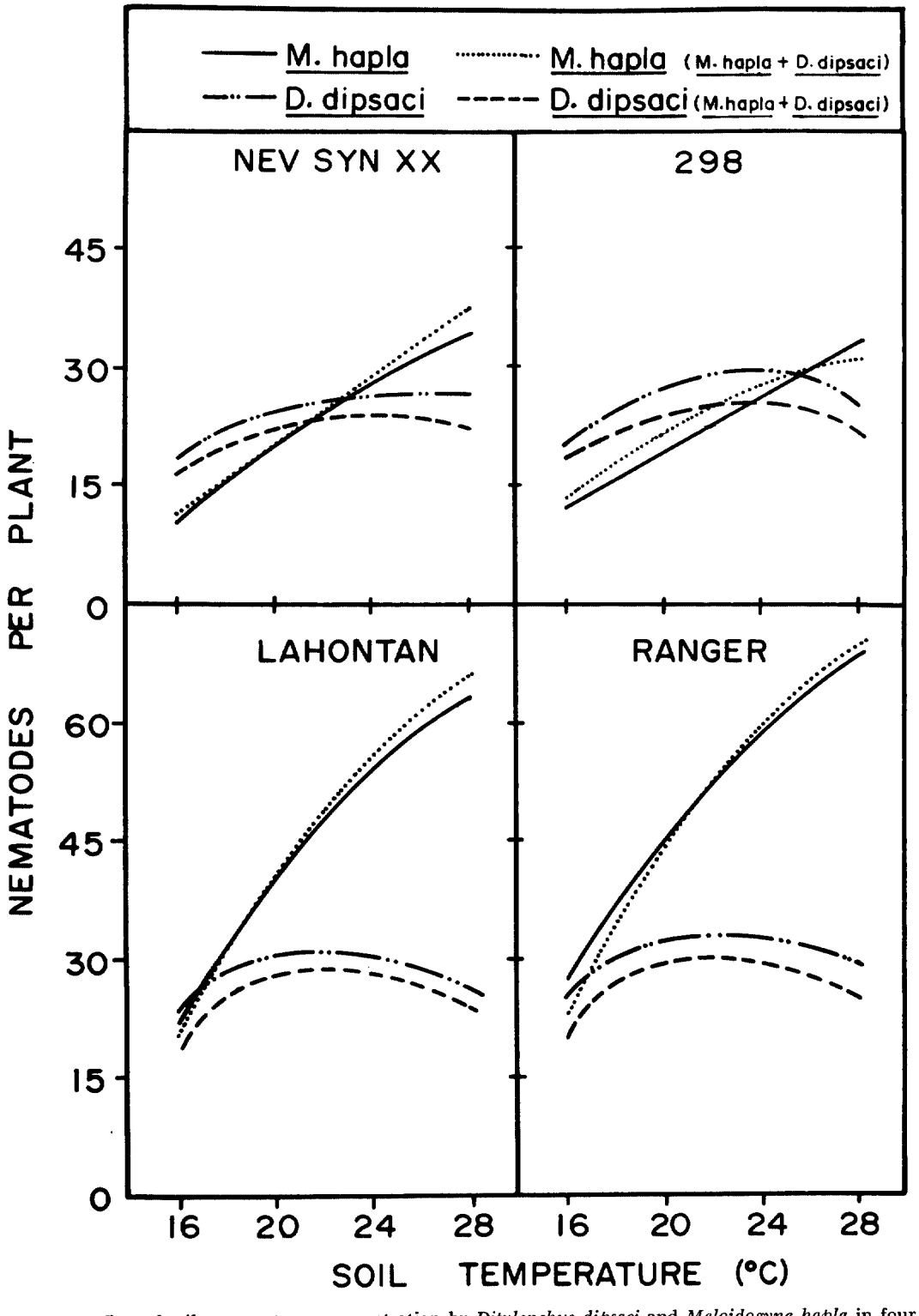

Fig. 1. Effect of soil temperature on penetration by *Ditylenchus dipsaci* and *Meloidogyne hapla* in four alfalfas: 'Nev Syn XX'—a selection resistant to *D. dipsaci* and *M. hapla,* 'Vernal 298'—a selection resistant to *M. hapla* and susceptible to *D. dipsaci,* 'Lahontan'—a cultivar resistant to *D. dipsaci* and susceptible to *M. hapla,* and 'Ranger'—a cultivar susceptible to both *D. dipsaci* and *M. hapla.*

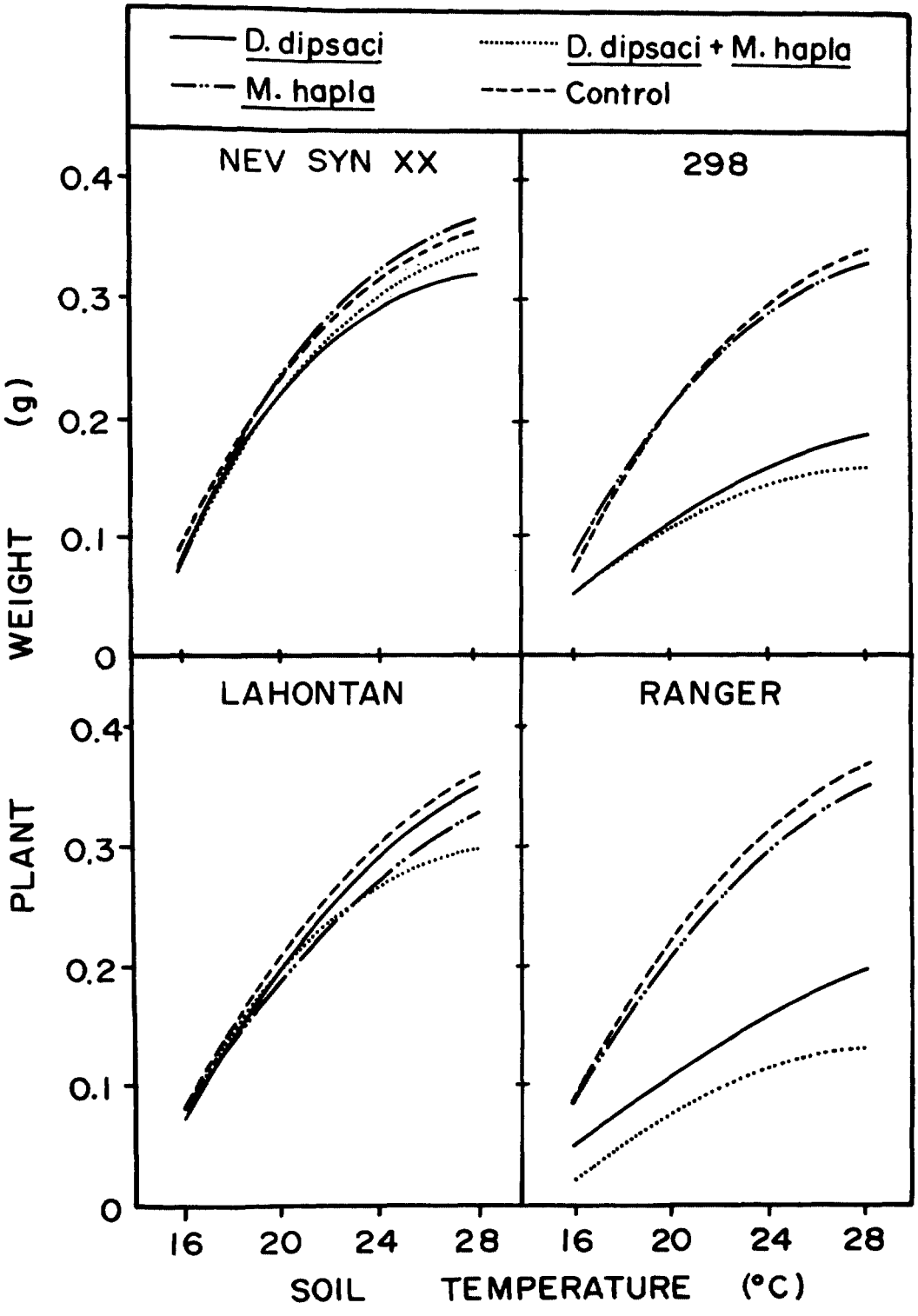

Fig. 2. Effect of soil temperature on weight of 'Nev Syn XX,' 'Vernal 298,' 'Lahontan,' and 'Ranger' alfalfa inoculated as germinated seeds alone and in combination with *Ditylenchus dipsaci* and *Meloidogyne hapla* larvae.

'Vernal 298' at 16 C, weights of alfalfas susceptible to *D. dipsaci* ('Ranger' and 'Vernal 298') were significantly ($P = 0.05$) depressed when inoculated with *D. dipsaci* alone or in combination with *M. hapla* (Fig. 2). Plant weights for alfalfas resistant to the stem nematode ('Lahontan' and 'Nev Syn XX') were not depressed under the same conditions. Infection with *M. hapla* alone caused no significant depression in plant weight of any of the alfalfas, but the combination of *M. hapla* and *D. dipsaci* resulted in a synergistic weight depression in 'Ranger' at all temperatures.

Root galling: Inoculation of seedlings with *D. dipsaci* 0, 2, 4, 6, and 8 wk before their inoculation with *M. hapla* did not influence galling by *M. hapla* in *M. hapla*-susceptible 'Lahontan' or 'Ranger.' Roots of 'Ranger' and 'Lahontan' plants were galled in all soil temperatures after inoculation with *M. hapla* alone or with a combination of *M. hapla* and *D. dipsaci,* and, as in previous studies (8), the severity of galling was influenced by soil temperature. Root-knot galling indices, for 8-wk-old plants inoculated with *M. hapla* alone were 4.9, 6.0, 6.0, and 6.0 on 'Lahontan,' and 5.1, 6.0, 6.0, and 6.0 on 'Ranger' at 16, 20, 24, and 28 C, respectively.

'Nev Syn XX' was not susceptible to *M. hapla* or *D. dipsaci* in any of the experimental treatments.

Galling of 'Vernal 298' by *M. hapla* was affected by soil temperature, plant age, and single vs. combined inocula. Increases in plant age and soil temperature had opposite effects on galling (Fig. 3). Greatest galling by *M. hapla* alone was at 28 C in plants inoculated as germinated seed (5–10-mm radicle). Except for treatments resulting in no galling, the least galling from *M. hapla* alone was at 20 C in plants inoculated at 4 wk of age. No galling occurred with *M. hapla* alone at 16 C in plants inoculated at 4, 6, and 8 wk of age, at 20 C in plants inoculated at 6 and 8 wk of age, or at 24 C in plants inoculated at 8 wk of age. This agrees with previous findings (10). The resistance in alfalfa to galling by *M. hapla* increases with the age of the plant.

Galling resulting from sequential inoculation of 'Vernal 298' with *D. dipsaci* and *M. hapla* (Fig. 4) was higher than that from

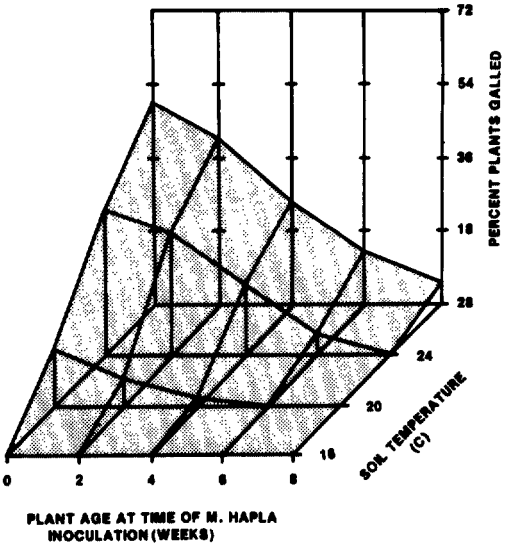

Fig. 3. Effect of plant age (at time of exposure to *Meloidogyne hapla*) and soil temperature on percentage of 'Vernal 298' alfalfa plants galled.

M. hapla alone (Fig. 3), particularly in the older plants at higher soil temperatures. At 24 and 28 C the combination of *M. hapla* and *D. dipsaci* increased galling over that of *M. hapla* alone by 25 and 31%, respectively, in plants inoculated with *M. hapla* 8 wk after inoculation with *D. dipsaci*. Galling was also increased (15 and 16%, 12 and 18%, 15 and 16%, and 22 and 30%, respectively) at the same temperatures when inoculated under similar conditions with

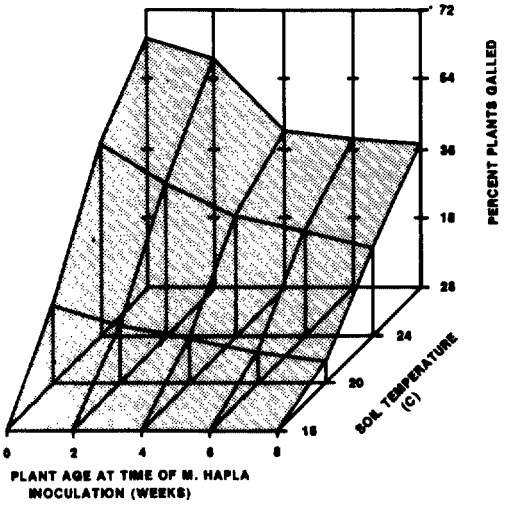

Fig. 4. Effect of plant age (at time of exposure to *Meloidogyne hapla*) and soil temperature on galling of 'Vernal 298' alfalfa plants also inoculated with *D. dipsaci*.

M. hapla 0, 2, 4, and 6 wk after inoculation with *D. dipsaci.*

Plant age, soil temperature, and use of single-species vs. combined inocula had affected the galling (root-knot indices) on 'Vernal 298' alfalfa (Figs. 5, 6). The highest root-knot index was observed at 28 C in plants inoculated as seeds with a combination of *M. hapla* and *D. dipsaci.*

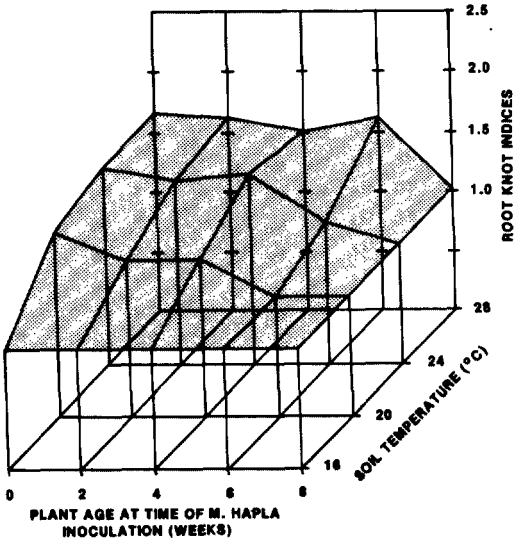

Fig. 5. Effect of plant age (at time of exposure to *Meloidogyne hapla*) and soil temperature on severity of galling (root-knot indices) in 'Vernal 298' alfalfa (1 = no galling, 2 = 1–10% of root system galled).

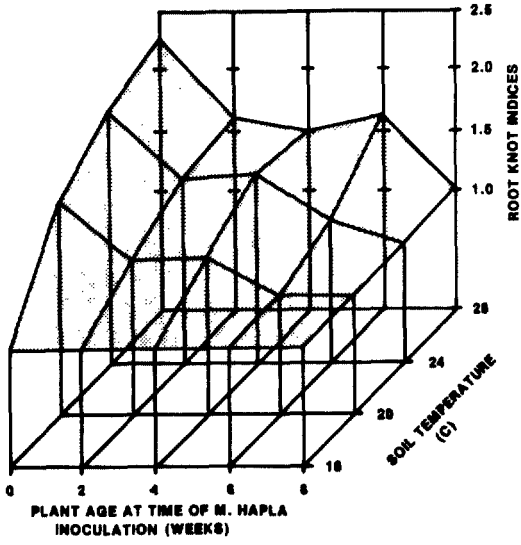

Fig. 6. Effect of plant age (at time of exposure to *Meloidogyne hapla*) and soil temperature on severity of galling (root-knot indices) of 'Vernal 298' alfalfa plants also inoculated with *Ditylenchus dipsaci* (1 = no galling, 2 = 1–10% of root system galled, and 3 = 11–30% root system galled).

The concomitant infection with another species of nematode apparently slowed the development of a factor(s) or physiological reaction(s) of the plant that leads to total resistance. The negative effect of the interaction of *M. hapla* and *D. dipsaci* on the resistance of 'Vernal 298' was not sufficient to consistently increase the severity of galling (root-knot indices) over that with *M. hapla* alone, and only a slight to moderate degree of galling occurred.

Seedling attractiveness: The attractiveness of the four alfalfas to *D. dipsaci* or *M. hapla* epicotyl or hypocotyl plant tissue did not differ when nematodes were placed 12.5 mm from single seedlings. *D. dipsaci* attracted to hypocotyl tissue were 48, 47, 46, and 44 nematodes for 'Ranger,' 'Vernal 298,' 'Lahontan,' and 'Nev Syn XX,' respectively; 52, 53, 54, and 56 nematodes were attracted to epicotyl tissue of the same alfalfas. The numbers of *M. hapla* larvae attracted to hypotocyl tissue were 54, 53, 52, and 52, respectively, and the number attracted to epicotyl tissue were 46, 47, 48, and 48, respectively. Although there was always more *M. hapla* larvae attracted to hypocotyl and *D. dipsaci* attracted to epicotyl tissue, the differences were not significant. Penetration of the hypocotyl tissue of any of the alfalfas by *D. dipsaci* was never observed, and penetration of the epicotyl tissue by *M. hapla* larvae was observed only once when two larvae were found in a Ranger seedling.

When 100 *D. dipsaci* and 100 *M. hapla* larvae were placed midway between germinating seeds of 'Nev Syn XX' and 'Ranger' (12.5 mm from each seed), significantly ($P = 0.01$) more nematodes of both species were attracted to Ranger; 72 *D. dipsaci* and 76 *M. hapla* larvae were attracted to 'Ranger' and 28 and 24, respectively, to 'Nev Syn XX.'

It is apparent the *M. hapla* and *D. dipsaci* can interact in producing root-knot galls on alfalfas. Since *M. hapla* is usually only mildly parasitic on alfalfa, its major importance in alfalfa is when susceptible alfalfa is used in rotation with less tolerant plant cultivars. However, the influence of

M. hapla on the growth of alfalfa should be even more important in areas of *D. dipsaci* infestations when *D. dipsaci* susceptible alfalfa cultivars are grown. The problem is further compounded when alfalfa susceptible to both nematode species is grown; poor alfalfa stands have been observed in alfalfa fields where newly planted alfalfa susceptible to both *M. hapla* and *D. dipsaci* was invaded by both species.

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