Attraction of *Ditylenchus dipsaci* and *Meloidogyne hapla* by Resistant and Susceptible Alfalfa Seedlings[1]

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Abstract: Ditylenchus dipsaci Kühn were equally attracted to and equally invaded resistant ('Lahontan') and susceptible ('Ranger') germinating alfalfa (*Medicago sativa* L.) seedlings exposed singly in moist sand except at a distance of 12.5 mm at 20 C when the susceptibles proved more attractive than the resistants. Larvae hatching from egg-masses of *Meloidogyne hapla* Chitwood were also attracted equally to germinating seedlings of resistant ('M-9') and susceptible ('Lahontan') alfalfa offered singly. When hatched midway between resistant and susceptible, however, more larvae were attracted to the susceptibles. *M. hapla* larvae were attracted equally to the root and stem apices, region of elongation, and upper hypocotyl of resistant and susceptible plants. *Key Words:* Tropism, Cultivars.

The positive tropism of plant-parasitic nematodes toward infection courts in susceptible hosts vastly improves their pathogenic and reproductive potentials.

Linford (7) first demonstrated the attraction by showing that *Meloidogyne* sp. [*Heterodera marioni* (Cornu)], *Pratylenchus pratensis* (deMan), and *Helicotylenchus multicinctus* (Cobb) were attracted to roots and tissues of several host plant species. Lownsbery and Viglierchio (9) found that *Meloidogyne hapla* Chitwood larvae were attracted to germinating tomato seeds. Wieser (13, 14) stated that *M. hapla* larvae were attracted to seedlings and excised root tissue of tomato, bean, egg plant, and soybean. He also found fast-growing tomato plants attracted *M. hapla* more strongly than slow-growing plants. Widdowson, Doncaster, and Fenwick (12) demonstrated that larvae of *H. rostochiensis* Wollenweber concentrated at root tips and at lateral root primordia of tomato. Loewenberg, Sullivan and Schuster (8) likewise found that *M. incognita incognita* (Kofoid & White) larvae were attracted to the terminal centimeter of tomato seedlings. Wieser (14) reported that the apical 2-mm of excised tomato tips had a repelling effect on *M. hapla* larvae.

The following study was made to determine whether *M. hapla* and *Ditylenchus dipsaci* (Kühn) were differently attracted to resistant and susceptible alfalfa cultivars.

MATERIALS AND METHODS

To study the attractiveness of alfalfa to *D. dipsaci,* 200 nematodes/cc water, taken from susceptible alfalfa plants, were introduced into one-liter polyethylene containers (110 × 110 mm) of Provo sand (M.H.C. = 13%) at distances of 12.5, 25, and 50 mm from germinating 'Lahontan' (resistant to *D. dipsaci*) and 'Ranger' (susceptible) alfalfa seed (Fig. 1). Soil was at or near field capacity after nematodes were added. The seed were scarified, and swollen in water 24 hr prior to planting. Plants were grown at 10, 15, and 20 C; 20 replicates/temperature; and the experiment repeated twice. After development of the unifoliate leaf (7, 12, and 20 days at 20, 15, and 10 C, respectively), whole seedlings were harvested and then stained and examined for nematode infection.

To determine whether *M. hapla* larvae were differently attracted to resistant and susceptible alfalfa, germinating seed of 'M-9' (a resistant selection from 'Vernal') (11, 3)

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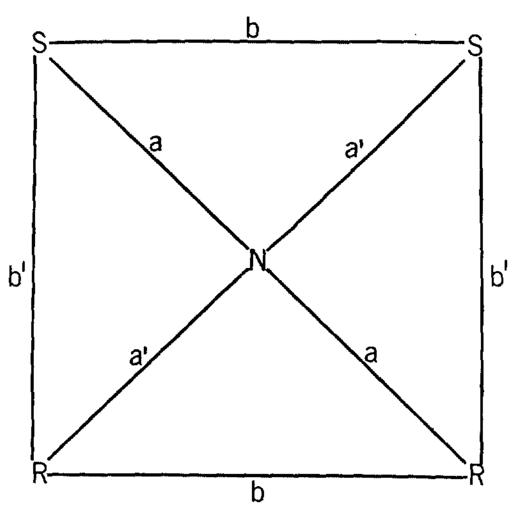

FIG. 1. Schematic drawing of procedure used to determine attraction of *Ditylenchus dipsaci* to 'Lahontan' (resistant) and 'Ranger' (susceptible) alfalfa.

and 'Lahontan' (susceptible to *M. hapla*) were placed on 25 cc of 0.20% water agar in 150 × 20 × 15 mm plastic sampling boxes. An egg mass harvested from tomato roots 6 weeks after inoculation, was placed (i) 12.5 mm from a germinating resistant seed; (ii) 12.5 mm from a germinating susceptible seed; and (iii) between germinating resistant and germinating susceptible seeds (12.5 mm from each). After 4 days in the laboratory at 22 ± 4 C, seedlings were examined for presence of larvae. Each treatment group consisted of 40 replicates. A similar study, using the same technique, determined the effect of temperature on the attraction of *M. hapla* larvae to alfalfa. Plastic sampling boxes, 150 × 20 × 15 mm, were maintained at 15, 20, 25, and 30 C for 4 days and nematode migration recorded. Treatment groups each contained 40 replicates, and the experiment was repeated once.

Differences in the growth rate were determined for the three alfalfa cultivars, 'M-9', 'Lahontan', and 'Ranger'. Scarified, preswollen seeds were placed singly in test tubes (25 × 150 mm) containing 50 ml 0.20% water agar. Growth, root tip to base of cotyledons, was recorded daily for 4 days at 22 ± 4 C.

RESULTS

ATTRACTIVENESS OF ALFALFA TO *DITYLENCHUS DIPSACI*: When nematodes were placed 12.5 mm from 'Ranger' and 'Lahontan' seed at 20 C, a significantly greater number of 'Ranger' plants were infected, and a significantly higher incidence of infection occurred (Table 1). There were, however, no significant differences in infection by nematodes placed 12.5, 25, or 50 mm from germinating seed at 10 and 15 C, and at 25 and 50 mm at 20 C. Differences in infection of plants at different nematode-placement distances within temperature treatment groups were significant. The closer the nematodes were to the seed, the greater the infection.

ATTRACTIVENESS OF ALFALFA TO *MELOIDOGYNE HAPLA*: There was little difference in the attractiveness of resistant and susceptible alfalfa to *M. hapla* when an egg mass was placed 12.5 mm from a single germinating 'M-9' (resistant) or 'Lahontan' (susceptible) alfalfa seed at 22 ± 4 C. 'M-9' and 'Lahontan' attracted 77 and 90% of the larvae, respectively. However, significantly greater numbers of larvae from an egg mass placed midway between germinating seeds of 'M-9' and 'Lahontan' (12.5 mm from each) were attracted to 'Lahontan'; 72% were attracted to 'Lahontan' and 28% to 'M-9'.

The percent of larvae attracted to both 'M-9' and 'Lahontan' increased slightly with a temperature change from 15 to 30 C when an egg mass was placed 12.5 mm from a germinating resistant or susceptible seed singly. 'Lahontan' attracted 76, 75, 84, and 89% of larvae while 'M-9' attracted 68, 74, 73, and 84% of the larvae at 15, 20, 25, and 30 C, respectively. When an egg mass was placed between germinating 'M-9' and 'Lahontan' seeds (12.5 mm from each), a significantly greater percentage of larvae were also attracted to 'Lahontan'. However, there was a decrease in the attraction to 'Lahontan' and an increase in the attraction to 'M-9' at the higher temperatures (81, 76, 73, and 64% of the larvae were attracted to 'Lahontan', and 19, 24, 27, and 36% were attracted to 'M-9' at 15, 20, 25, and 30 C, respectively).

M. hapla larvae were strongly attracted to the root apical meristem and the region of elongation and, to a lesser extent, to the upper hypocotyl and the stem apical meristem of both resistant and susceptible seedlings (Fig. 2). Few or none were found along the transition area. We found no repulsion of *M. hapla* larvae from the root terminus of alfalfa seedlings.

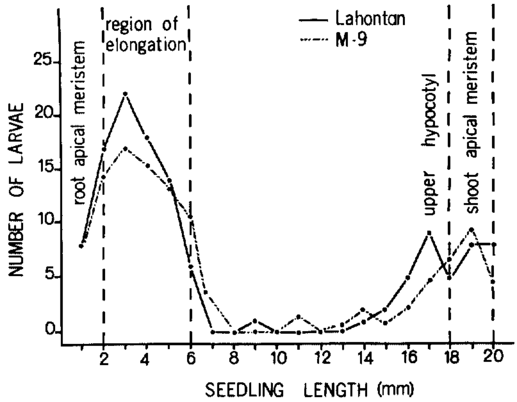

FIG. 2. Orientation of *M. hapla* larvae to different parts of 'M-9' (a resistant selection from 'Vernal') and 'Lahontan' (susceptible) alfalfa plant at 25 C.

No differences in the growth of 'Lahontan' and 'M-9' seedlings were observed. 'M-9' growth was 4, 9, 14, and 19 mm after 1, 2, 3, and 4 days, respectively at 22 ± 4 C. This compared with 7, 10, 16, and 19 mm for the growth of 'Lahontan' over the same length of time at the same temperature. 'Ranger' made the greatest growth: 8, 16, 22, and 30 mm over the same corresponding period.

TABLE 1. Infection of 'Lahontan' (resistant) and 'Ranger' (susceptible) alfalfa seedlings by *Ditylenchus dipsaci*.

Temperature (C)	Distance[a] (mm)	Percent plants infected[b]		Nematodes per infected plant[b]	
		Lahontan	Ranger	Lahontan	Ranger
10	12.5	100	100	21	22
	25	72	76	16	17
	50	32	35	6	8
15	12.5	84	92	15	19
	25	43	50	2	4
	50	4	4	1	2
20	12.5	72	88	4	17
	25	60	55	4	5
	50	6	12	2	2
LSD 0.05			12		8
0.01			16		11

[a] Distances at which 200 *D. dipsaci* were placed from four quadrilaterally placed germinating seed.
[b] Plants harvested at 20, 15, and 10 C for 7, 12, and 20 days, respectively.

DISCUSSION

D. dipsaci infects resistant as readily as susceptible alfalfa plants when inoculum is placed directly over the swollen seed (5). It is assumed that any differences in infection of resistant and susceptible alfalfa is due to differences in numbers of nematodes attracted to infection courts. Although susceptible alfalfa seedlings attracted *D. dipsaci* more strongly than resistant seedlings at 20 C, factor(s) regulating the attraction may be limited in range as the diffusion gradient of the attractant lessens with increased distances from the root. We have found greatest nematode infection occurs during the spring when nematodes migrate to and infect crown buds over a long period of time because of a slow plant growth at cool temperatures and high soil moisture. This is why a greater percentage of plants were infected and by more nematodes at 10 C, than at the higher temperatures. It also explains why we found no differences in infection of resistant or susceptible alfalfa at 10 and 15 C; *D. dipsaci* was able to overcome any differences in attraction by random migration, and reach and infect the plants before emergence from the soil.

Susceptible alfalfa plants have a stronger attraction to *M. hapla* larvae than do resistant plants. This may be responsible for a higher infection occurring in susceptible than in resistant plants (4). However, differences in attraction decreased as temperature increased from 15 to 30 C, which may reflect reduced production of attractant by susceptible plants, or an increase in attractant produced by resistant plants or both. Temperature effects upon nematode activity may also be operative.

M. hapla larvae were not repelled by the alfalfa seedling root terminus which agrees with the findings of Loewenberg *et al.* (8) and Widdowson *et al.* (12).

Carbon dioxide has been shown to be an important attractant to nematodes (6). Since we found no differences in the growth of root-knot susceptible and resistant plants, there would have to be a difference in the respiratory ratio of the two alfalfa cultivars, 'M-9' and 'Lahontan', for CO_2 to be the only or major factor in the attraction complex.

Besides CO_2 several other factors including amino acids (10), electrical potential (1), and temperature (2), have been implicated in the attraction process between plants and nematodes. There are other explanations that may be considered. These are (i) removal of some agent(s) from the environment by the growing plant roots or soil microflora, (ii) both attractive and repulsive exudates being produced by the same plant (some in greater quantities than others); (iii) different root attractant(s) being produced by different plant species; and (iv) species of nematodes being affected differently by a root attractant.

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