# Effect of Plant Age on Resistance of Alfalfa to Meloidogyne hapla[1]

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*Abstract: Meloidogyne hapla*-resistant plants grown from cuttings and inoculated with *M. hapla* larvae were free of galls. However, 35 to 48% of the seedling intercross progeny of resistant genotypes that were inoculated in the germinated seed stage were galled. There was an inverse relationship between the age of plants grown from seed and the percentage of plants galled by *M. hapla*; the older the plants at inoculation, the greater the percentage of gall-free plants. The per cent of galled plants was significantly reduced when galled roots were removed and plants reinoculated. Reproduction of *M. hapla* on galled progeny of resistant plants was significantly less than that on susceptible plants. There were no differences in nematode reproduction on galled progeny of resistant plants, regardless of age at time of inoculation. An increase in inoculum levels from 100 to 10,000 *M. hapla* larvae did not affect resistance or susceptibility. There was a direct correlation between galling of inoculated seedlings of resistant progeny and temperature; inoculated 8-week-old cuttings of resistant plants were galled only at 32 C. *Key words:* selections, temperature.

In 1958, Stanford *et al.* (9) found resistance to the northern root-knot nematode, *Meloidogyne hapla* Chitwood, in Vernal alfalfa. Goplen and Stanford (3) later determined that resistance in Vernal selections M-7 and M-9 was governed by a single dominant gene. Hunt *et al.* (5) incorporated *M. hapla* resistance into strains of alfalfa having desirable agronomic characteristics and resistance to other pests, but found inconsistencies in resistance of plants inoculated with *M. hapla* in the germinated seed and seedling stage.

The effect of age on the tolerance of plants to plant pathogens has been reported from diversified fields. Linford (8) observed that only young alfalfa roots, before secondary thickening has disturbed the primary cortex,

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appear to be susceptible to *Aphanomyces euteiches* Drechs. Keitt and Jones (6) showed that young, growing apple leaves pass through a stage of maximal susceptibility into a period of increasing resistance to *Venturia inaequalis* (Cke.) Wint. Giddings (2) stated that young sugarbeet plants showed greater symptoms and injury to curly top virus than did older plants, and Benda and Bennett (1) found that recovery of tomato from infection by curly top virus, was associated with an increase in plant age.

In an alfalfa breeding program, seed, seedlings, and cuttings of different ages are often used; this study was initiated to determine whether plant age has an effect on the resistance of alfalfa plants to *M. hapla*.

## MATERIALS AND METHODS

Alfalfa (*Medicago sativa* L.) seeds used in this study were triplex-quadruplex (three-four alleles dominant for resistance), duplex (two alleles dominant for resistance), and nulliplex (four recessive or susceptible alleles) *M. hapla*-resistant alfalfa genotypes.

Germinated seed (4-10 mm radicle) and 6-week-old cuttings (100 progeny of each of the three genotypes) were inoculated with 200 *M. hapla* larvae/germinated seed or plant, and grown in Provo sand in 15.2 cm clay pots at 22 ± 4 C. Six weeks after inoculation, plant roots were checked for galling. A similar experiment involved inoculating germinated seed, and 2-, 4-, 6-, and 8-week-old seedling progeny of resistant and intercross progeny of susceptible alfalfa selections with 200 *M. hapla* larvae/germinated seed or plant. Plants (200 of each selection) were grown for 6 weeks at 22 ± 4 C, and the numbers of galled plants and galls per plant were recorded.

To further evaluate the host-parasite relationship between *M. hapla* and resistant alfalfa, 2-, 4-, and 8-week-old seedlings of triplex-quadruplex and nulliplex intercross progeny were inoculated with 1000 *M. hapla* larvae. Plants were replicated 100 times and grown in Provo sand. After 6 weeks, soil was carefully washed from the roots, and the galling and nematode reproduction were determined (11). Plants were root-pruned to remove galls, and planted into 15.2-cm clay pots of Provo sand. Two weeks after transplanting, when the initiation of new root growth had begun, plants were reinoculated with 1000 *M. hapla* larvae/plant. Six weeks after the second inoculation, roots were re-examined for root-knot galls.

The effect of inoculum levels on plant resistance was also considered. Germinated seed, and 4- and 8-week-old progeny of resistant and susceptible alfalfa were inoculated with 100, 1000, and 10,000 *M. hapla* larvae/germinated seed or plant and grown in 15.2-cm clay pots of Provo sand. Each treatment was replicated 20 times, and plants were grown in the greenhouse at 22 ± 4 C. After 6 weeks, roots were washed free of soil, and the number of galls was recorded.

A final experiment involved the relationship between plant age and temperature on plant resistance. Germinated seed and 8-week-old cuttings of plants that were nulliplex, duplex, and triplex-quadruplex for resistance were planted into 1-liter polyethylene containers of Provo sand and inoculated with 500 *M. hapla* larvae/plant. Plants (20 replicates/treatment) were grown in water baths at 20, 24, 28, and 32 C. Nematode pathogenicity was determined after 6 weeks.

## RESULTS AND DISCUSSION

Cuttings of all ages of resistant plants grown at 22 ± 4 C were free of galls. However, 35 and 48% of the intercross progeny of triplex-quadruplex and duplex resistant genotypes, respectively, inoculated as germinated seed, were galled. All nulliplex plants inoculated as germinated seed and 6-week-old cuttings were galled. Significant differences occurred in the number of galls per plant between the progeny of resistant and susceptible plants, but not among the progeny of resistant genotypes. There were 9.4 and 8.6 galls/plant on inoculated germinated seed and 6-week-old cuttings of the nulliplex genotype. This compared with 2.4 and 1.9 galls/plant on inoculated germinated seed of triplex-quadruplex, and duplex, resistant genotypes. We did not detect genetic dosage effects in the inheritance of resistance to *M. hapla*.

An inverse relationship occurred between the age of the progeny of resistant plants grown from seed and the percentage of galled plants. When inoculated at 0, 2, 4, 6, and 8 weeks of age, 40, 23, 15, 7, and 3%, respectively, of the progeny of resistant plants were galled. All nulliplex plants were galled regardless of age. Some of the variation in the percentage of galled progeny of resistant plants may result

TABLE 1. Effect of plant age on galling of resistant and susceptible alfalfa by *Meloidogyne hapla.*[a]

| Genotype | Age of plants at inoculation (wk) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Resistant | 2.9[b] | 2.7 | 2.9 | 2.8 | 2.9 |
| Susceptible | 8.4 | 7.6 | 8.6 | 8.8 | 8.4 |
| LSD .01 | 1.9 | | | | |

[a]Plants inoculated with 200 *M. hapla* larvae and grown at 22 ± 4 C for 6 weeks.
[b]Galls per galled plant.

TABLE 2. Effect of plant age on root galling of resistant and suceptible alfalfa by *Meloidogyne hapla.*

| Genotype for resistance | Plant age at 1st inoculation[a] | % Galled | Plant age at 2nd inoculation[b] | % Galled |
|---|---|---|---|---|
| Triplex-quadruplex | 2 | 48 | 10 | 12 |
| | 4 | 33 | 12 | 8 |
| | 8 | 12 | 16 | 2 |
| Nulliplex | 2 | 100 | 10 | 100 |
| | 4 | 100 | 12 | 100 |
| | 8 | 100 | 16 | 100 |

[a]Plants inoculated with 1000 *M. hapla* larvae and grown at 22 ± 4 C; susceptibility determined after 6 weeks.
[b]Root galls of first inoculation removed, plants transplanted, plants inoculated 2 weeks later, and galling determined.

from a differential susceptibility related to age; factors affecting resistance may be produced in some plants sooner than in others. Talboys (10) suggested the probability of low resistant zones in roots of highly resistant plants. This may explain why the roots of some progeny of resistant alfalfa plants were slightly galled. A significantly greater number of galls were found on progeny of susceptible plants than on progeny of resistant plants, but age did not affect the severity of galling (Table 1).

A significant difference (chi-square test) occurred in the percentage of galled progeny of resistant plants between plants inoculated at 2 or 4 weeks of age and plants inoculated at 8, 10, 12, or 16 weeks of age ($X^2$ = 100.4; Table 2). A significant decrease in the percentage of galled plants also occurred between the first and second inoculation of progeny of resistant plants ($X^2$ = 53.9). Age did not affect the severity of galling of either resistant or susceptible plants, and results were similar to those shown in Table 1.

Reproduction of *M. hapla* was greater on progeny of susceptible than on progeny of resistant plants, which agrees with previous findings (4). There were no differences in the production of viable larvae on progeny of resistant plants regardless of age at inoculation. Numbers of viable larvae produced per gram of galled root on progeny of resistant plants inoculated at 2, 4, and 8 weeks of age were 648, 594, and 539, respectively, while numbers on progeny of susceptible plants inoculated at 2, 4, and 8 weeks of age were 6268, 6432, and 6980, respectively.

An increase in inoculum levels did not cause a corresponding increase in the number of galled plants. The percentage of galled plants in progeny of resistant clones subjected to inoculum densities of 100, 1000, and 10,000 *M. hapla* larvae/plant were, respectively, 40, 50, and 45% on plants inoculated in the germinated seed stage; 25, 20, and 20% on plants inoculated at 4 weeks of age; and 0, 5, and 5% on plants inoculated at 8 weeks of age. An increase in inoculum density did not increase the severity of galling on resistant plants (Table 3).

All susceptible plants (inoculated in the germinated seed and 6-week-old cutting stage) were galled at 20, 24, 28, and 32 C (Table 4). Inoculated seeds of resistant plants were galled at all temperatures, but corresponding inoculated cuttings were galled only at 32 C. These differences were significant ($X^2$ = 24.4), and apparently were due to a shift in plant metabolism governing resistance (7). There was also a significant increase in the percentage of

TABLE 3. Effect of inoculum densities on resistance of alfalfa to *Meloidogyne hapla.*[a]

| Genotype and inoculum densities | Age of plant at inoculation (wk) | | |
|---|---|---|---|
| | 0[b] | 4 | 8 |
| Resistant | | | |
| 100 | 2.00 ± .19[c] | 1.00 ± .07 | 0.00 ± .00 |
| 1000 | 2.00 ± .21 | 1.00 ± .09 | 1.00 ± .09 |
| 10,000 | 2.30 ± .26 | 1.50 ± .13 | 1.00 ± .04 |
| Susceptible | | | |
| 100 | 3.95 ± .27 | 3.27 ± .18 | 2.98 ± .09 |
| 1000 | 5.00 ± .00 | 5.00 ± .00 | 4.85 ± .06 |
| 10,000 | 5.00 ± .00 | 5.00 ± .00 | 5.00 ± .00 |

[a]Plants grown at 22 ± 4 C for 6 wk.
[b]Germinated seed (4- to 10-mm radicle).
[c]Root-knot indices.

TABLE 4. Effect of temperature and plant age on the pathogenicity of *Meloidogyne hapla* to resistant and susceptible alfalfa.[a]

| | Percent of plants galled | | | | | |
|---|---|---|---|---|---|---|
| | Nulliplex | | Duplex | | Triplex-quadruplex | |
| Temp (C) | Seed[b] | Cuttings[c] | Seed | Cuttings | Seed | Cuttings |
| 20 | 100 | 100 | 35 | 0 | 35 | 0 |
| 24 | 100 | 100 | 30 | 0 | 30 | 0 |
| 28 | 100 | 100 | 35 | 0 | 40 | 0 |
| 32 | 100 | 100 | 90 | 55 | 70 | 40 |

[a]Plants inoculated with 500 *M. hapla* larvae/plant and grown for 6 weeks (20 plants/treatment).
[b]Germinated seed (4- to 10-mm radicle) at inoculation.
[c]Eight weeks old at inoculation.

seed galled at 32 C ($X^2$ = 35.1). Differences in resistant genotypes did not affect galling.

In this study, we found a direct correlation between plant age and resistance and susceptibility of alfalfa to *M. hapla*. A similar correlation may exist with other plants and nematodes. Therefore, the importance of plant age should be considered whenever it becomes a factor in a host-parasite relationship.

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