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Influence of *Glomus fasciculatum* on *Meloidogyne hapla* Infecting *Allium cepa*[1]

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Abstract: The impact of *Glomus fasciculatum* on *Meloidogyne hapla* associated with *Allium cepa* was evaluated in two experiments. Nematode density was not different in mycorrhizal and nonmycorrhizal plants 10 weeks after the joint inoculation of *M. hapla* and *G. fasciculatum.* Differences in the age structure of *M. hapla* populations reared on mycorrhizal and nonmycorrhizal plants were noted. *G. fasciculatum* enhanced leaf and bulb growth of *A. cepa* in the absence of *M. hapla,* but did not affect plant weight when nematodes were present. Survival and reproduction of *M. hapla* were not affected by *G. fasciculatum* or phosphorus (P). The estimated time required for inoculated second-stage juveniles (J2) to mature to the adult stage was 1,000 degree hours (base = 9 C) greater in mycorrhizal than in nonmycorrhizal plants supplemented with P. Although the infectivity of J2 was not measured directly, colonization of *A. cepa* by *G. fasciculatum* appeared to alter the ability of *M. hapla* to penetrate roots.

Key words: mycorrhizae, onion, nematode development, interaction.

Vesicular-arbuscular mycorrhizae (VAM) and phytoparasitic nematodes are commonly associated with the roots of plant species. The stimulation and inhibition of plant growth by VAM and nematodes, respectively, have been described in detail (10,11). Although the concomitant influence of VAM and nematodes on host plants is not yet thoroughly understood, nematode parasitism is generally not as deleterious to plants with an established VAM association compared with plants grown in the absence of these fungal symbionts (5). It is not clear if the increased ability of plants to withstand nematode infection when colonized by VAM is caused by the reduction of nematode population levels, and hence damage, or to the increased tolerance of mycorrhizal plants to nematode parasitism.

VAM colonization resulted in decreased population levels of *Globodera solanacearum, Meloidogyne hapla,* and *M. incognita* infecting tobacco, carrot, and tomato, respectively, indicating that VAM adversely affects the ontogeny of these nematodes in some hosts (4,12). Conversely, mycorrhizal grape plants supported greater numbers of *M. arenaria* and were larger than nonmycorrhizal plants, suggesting that the enhanced nutrition associated with VAM colonization increased the tolerance of grape to nematode infection (1). The duration and sampling schedule of past experiments seem to be the most variable and, possibly, the most important factors determining the outcome of concomitant nematode–VAM colonization research (2,7).

The *M. hapla–G. fasciculatum–A. cepa* system was selected for our research because it was well suited for determining the stage-specific development of nematodes and because little information was available on the relationship between these organisms. The objectives were to 1) assess the individual and joint influence of *M. hapla* and *G. fasciculatum* on the growth of *A. cepa,* 2) compare the growth of a *M. hapla* population in mycorrhizal and nonmycorrhizal *A. cepa,* and 3) establish the influence of *G. fasciculatum* colonization on a single *M. hapla* generation reared on *A. cepa.*

Materials and Methods

Influence of Glomus fasciculatum on M. hapla and A. cepa: Approximately 1,500 cm^3 of a pasteurized Houghton muck soil (100% aggregate of organic matter) with a pH of 6.6 and a phosphorus (P) level of 60 ppm was thoroughly mixed with *M. hapla* eggs or *G. fasciculatum* spores and placed in each of 120 20-cm-d clay pots. Inocula for the following four treatments were added in 5 ml water: 1) 10,000 *M. hapla* eggs, 2) 2,000 *G. fasciculatum* spores, 3) 10,000 eggs and

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2,000 spores, 4) water passed over eggs and spores. One *A. cepa* cv. Krummery Special seed, pregerminated for 48 hours, was planted in each pot. The plants were maintained under greenhouse conditions and watered daily for 10 weeks.

Six replicates of each treatment were harvested 2, 4, 6, 8, and 10 weeks after planting. Root, bulb, and leaf fresh weights and bulb and leaf dry weights were determined at each harvest. Soil samples from each pot (100 cm^3) were assayed for nematodes and fungal spores by a centrifugal-flotation technique (6). The entire root system of each plant, stained in a solution of lactophenol containing 0.01% acid fuchsin, was observed for mycorrhizae using a stereoscopic microscope. Nematodes in soil and roots were counted by life stage. Data from plants that died during the experiment from no apparent cause other than nematode damage were included in the analysis of plant growth but were not considered in the analysis of nematode population growth, since *M. hapla* is an obligate parasite that cannot develop in the absence of a host plant.

Influence of G. fasciculatum on the development of M. hapla: Onions (cv. Krummery Special) were sown in compartments of plastic nursery trays containing 217 g (dry wt) of a 7:1 mix of pasteurized sand and a Houghton muck soil with a pH of 6.5 and a soil P level of 1.3 ppm. One-third of the trays were inoculated ca. 3.0 cm below the surface with 1,600 *G. fasciculatum* spores in 5 ml water. Another third of the trays were fertilized with 43 mg KH_2PO_4 per compartment, with 48 ppm P added to the soil to stimulate onion growth comparable to mycorrhizal colonization. The remaining trays were not fertilized or inoculated. Uniformity between treatments in all regards except P level was accomplished by adding water passed over spores to the trays not receiving mycorrhizae inoculum and by adjusting soil nutrient levels with KNO_3 and NH_4NO_3 for trays unamended and amended with KH_2PO_4, respectively. The plants were grown in an environmentally controlled chamber at 20–23 C with 12 hours of light. After 1 month, and confirmation of mycorrhizal colonization, each plant was inoculated with 650 *M. hapla* second-stage juveniles (J2) in 2 ml water. Four days after inoculation, the plants were gently removed from the trays, washed free of soil and nematodes, and transplanted into 10.5-cm-d pots containing ca. 500 g muck soil with a pH of 6.5 and a soil P level of 16 ppm. The three soil treatments were repeated at transplanting, except that the spore and P levels added were 1,230/pot and 14.8 mg/pot, respectively. Levels of N and K were adjusted as described previously. The pots were maintained in the greenhouse and watered daily. Soil temperature was recorded hourly using a Campbell CR-21 micrologger for degree-hour computation, commencing at the time of transplanting.

Seven plants from each soil treatment were harvested every 3 days (ca. 1,000 degree hours [base 9 C]) for 33 days. Fresh root and shoot weights were determined and entire root systems stained in lactophenol containing 0.01% acid fuchsin. Nematodes were teased from roots, observed through a stereoscopic microscope, and enumerated according to the following scheme: 1) Early J2 (EJ2) had a slender body shape. 2) Intermediate J2 (IJ2) had a broadened body shape and pointed terminus. 3) Late J2 (LJ2) had a broadened body shape with rounded terminus and mucro. 4) Third- and fourth-stage juveniles (J3–J4) had multiple unshed cuticles and absence of stylet. 5) Adult females had mature gonads, rounded body shape, and absence of eggs or gelatinous matrix. 6) Ovipositing females were as previous stage except egg mass was present.

Nematode development was computed by a technique described by Manley (9). Frequency counts of each stage were plotted against time. It was assumed that nematodes assigned to each stage had completed 50% of the development necessary to complete that stage (i.e., each stage designation represented the midpoint between the previous and subsequent stage). To obtain the estimated median stage value for each sample date, the frequency of each stage was multiplied by a stage designation number (1, 2, . . . , 6), and the resulting product was divided by the total number of nematodes present on that date. The median stage estimates were then plotted against a physiological time scale (degree hours), and a cubic function was fitted to the points to determine the median rate of nematode development.

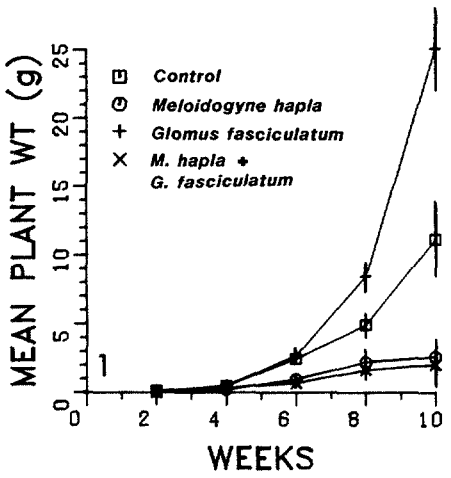


FIG. 1. Fresh weight (± standard error) of *Allium cepa* inoculated with *Meloidogyne hapla, Glomus fasciculatum, Meloidogyne hapla* plus *Glomus fasciculatum,* and noninoculated control.

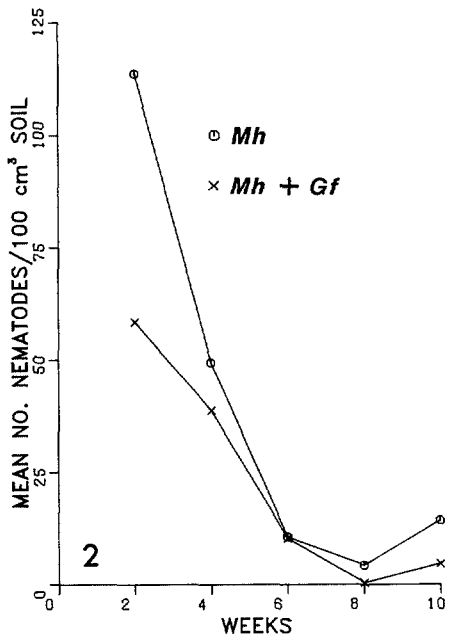


FIG. 2. Number of *Meloidogyne hapla* second-stage juveniles in 100 cm^3 soil surrounding *Allium cepa* inoculated with *Meloidogyne hapla* alone or with *Meloidogyne hapla* plus *Glomus fasciculatum.* Mean values within a treatment followed by the same letter are not significantly ($P = 0.05$) different according to Duncan's multiple-range test.

Egg production by adult female nematodes was monitored from the first appearance of eggs, 24 days following inoculation, until 39 days after the experiment was initiated. Egg masses were removed from the roots as the nematodes were counted, agitated in an aqueous solution of 1.05% NaOCl, and counted under a stereoscopic microscope. The total number of eggs and empty egg shells for each treatment and sample date was divided by the mean number of ovipositing females to obtain an estimate of the mean number of eggs produced per female.

RESULTS

Influence of Glomus fasciculatum on M. hapla and A. cepa: Plants inoculated with *G. fasciculatum* alone were significantly ($P = 0.05$) larger after 10 weeks than were control plants or plants inoculated with *M. hapla* (Fig. 1). Plants infected with *M. hapla* were consistently smaller than noninfected plants throughout the experiment, regardless of the presence of VAM. These trends were also noted in separate comparisons of fresh and dry leaf weights and fresh bulb weights. The fresh root weight of VAM-treated plants after 10 weeks was significantly ($P = 0.05$) greater than that of plants inoculated with nematodes but not different from that of noninoculated control plants.

The development of *M. hapla* associated with mycorrhizal or nonmycorrhizal *A. cepa* was similar. Densities of J2 in the soil were greatest 2 weeks after planting and then declined. Nematode density in the soil increased significantly ($P = 0.05$) between 8 and 10 weeks after planting for mycorrhizal but not for nonmycorrhizal plants (Fig. 2). Juveniles were observed within roots on all harvest dates. Adult pre-ovipositing and ovipositing females were first observed 4 and 6 weeks after planting, respectively. Between 2 and 10 weeks after planting, the total number of nematodes per plant increased 1.8-fold in mycorrhizal plants and 3.1-fold in nonmycorrhizal plants.

The age structure of the nematode populations associated with mycorrhizal and nonmycorrhizal *A. cepa* differed. Six weeks after planting more than 50% of the nematodes in nonmycorrhizal plants had developed to the adult stage, as compared to only 25% in mycorrhizal plants ($P = 0.08$). Over the course of the experiment, there was no significant ($P = 0.05$) change in the number of juvenile *M. hapla,* and a significant increase ($P = 0.05$) in the number of

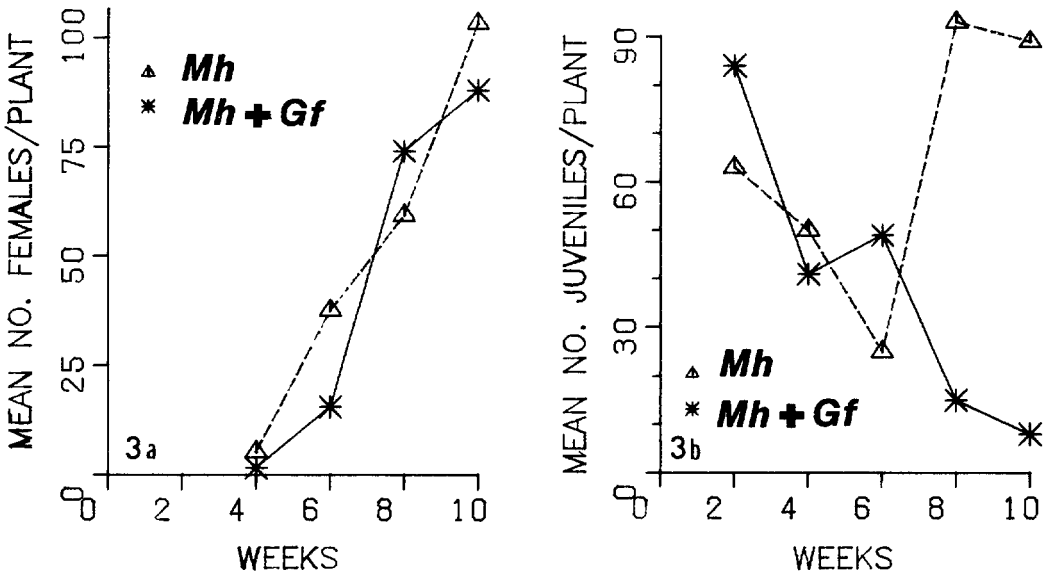


FIG. 3. Number of *Meloidogyne hapla* females (a) and juveniles (b) associated with *Allium cepa* inoculated with the nematode alone or with the nematode plus *Glomus fasciculatum*.

adult females associated with nonmycorrhizal plants (Fig. 3). The number of adult females associated with mycorrhizal plants also increased ($P = 0.05$) between 4 and 10 weeks after planting, while the number of juvenile nematodes decreased ($P = 0.05$) (Fig. 3).

There was no difference in the total number of nematodes or the number of any nematode stage inhabiting root tissue between mycorrhizal and nonmycorrhizal plants. The density of J2 and eggs in soil of *M. hapla* were also similar between treatments, except for the first sampling date when more nematodes ($P = 0.05$) were observed in the soil surrounding nonmycorrhizal plants (Fig. 2).

The staining technique used in this experiment was not adequate for quantifying *G. fasciculatum* development. Colonization by *G. fasciculatum* was first observed 3 weeks after planting in all inoculated plants. The levels of *G. fasciculatum* spores in the soil were not different on any sampling date between plants inoculated with the fungus alone and plants inoculated with both the fungus and nematodes.

Influence of Glomus fasciculatum on the development of M. hapla: All stages of *M. hapla* occurred over the course of the experiment. The inoculum consisted of early EJ2. The first appearance of IJ2, signifying the commencement of endoparasitic feeding, was observed after 1,075 degree hours (DH) for all treatments (Fig. 4). Late LJ2 were first observed after 2,145 DH for the P-fertilized plants and after 3,314 DH for the control and VAM-treated plants. The combined J3–J4 and the adult stage were observed after 4,360 and 5,240 DH, respectively, for all three treatments. Egg production commenced after 8,545 DH for the P-fertilized and VAM-treated plants and after 9,662 DH for the control plants.

The median developmental stage values were similar for *M. hapla* reared on control, P-fertilized, and VAM-inoculated plants (Fig. 5), 5.26, 5.37, and 5.47, respectively, on the final sample date (33 days). The experiment was terminated before 50% of the population in any treatment reached the ovipositing female or sixth designated stage.

Initial densities of *M. hapla* per plant were greatest ($P = 0.05$) in the control plants, both before and after adjustment by an analysis of covariance using root weight as a covariate. Three days after transplanting, control, mycorrhizal, and VAM-inoculated plants supported 251, 219, and 190 nematodes, respectively. The total number of nematodes did not differ between treatments on any other sample date, according to analysis of variance and covariance. Of

those nematodes which entered roots, 8.7, 18.5, and 21% survived for the duration of the experiment in control, VAM-treated, and P-fertilized plants, respectively.

Egg production per *M. hapla* female was not different between treatments on any sampling date. Covariance analysis of the egg density counts, according to the weight of the root system each nematode inhabited, also did not reveal any difference in nematode reproduction between treatments. On the final sampling date (13,715 DH), females produced an average of 134.8, 150.4, and 209.7 eggs, respectively, in control, P-fertilized, and VAM-treated plants.

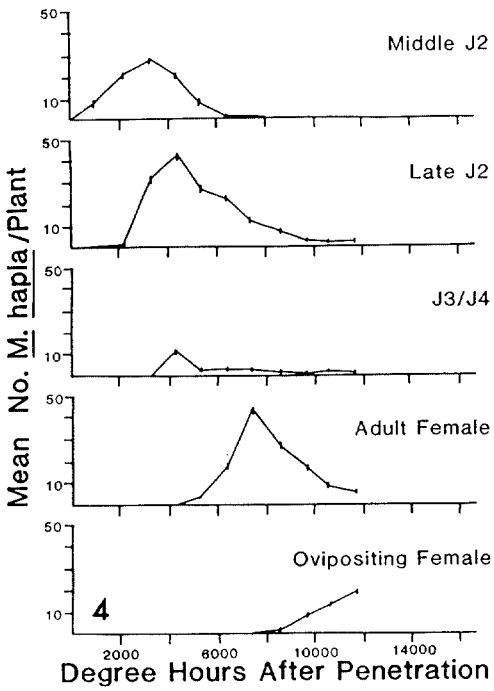
The fresh root weight of nonamended control *A. cepa* was less ($P = 0.05$) than that of P-fertilized plants until 30 days (10,631 DH) following transplanting. The fresh root weight of VAM-treated plants was equal to or greater than that of P-fertilized plants except for the first, seventh, and eighth sampling dates on days 3, 18, and 21 following transplanting, respectively.

Discussion

G. fasciculatum did not increase the tolerance of *A. cepa* to *M. hapla* parasitism. The fungus enhanced the growth of *A. cepa* when *M. hapla* was not present but had no effect on the growth of nematode-infected plants. Nor was there a difference in the number of *M. hapla* supported by mycorrhizal and nonmycorrhizal plants.

The data indicate that the population density of *M. hapla* may have differed in mycorrhizal and nonmycorrhizal plants had the experiment continued beyond 10 weeks. The nematodes comprising the initial inoculum infected *A. cepa* before, or concurrent to, *G. fasciculatum* and completed one generation. An influx of second-generation nematodes occurred in nonmycorrhizal plants, as evidenced by an increasing number of adult nematodes without an accompanying decrease in the level of juvenile nematodes and by a comparison of final to initial root densities (Pf/Pi = 3.1). In contrast, levels of juvenile nematodes within mycorrhizal plants indicate that few second-generation nematodes entered these plants (Pf/Pi = 1.8).

We first hypothesized that the development of *M. hapla* was inhibited in the



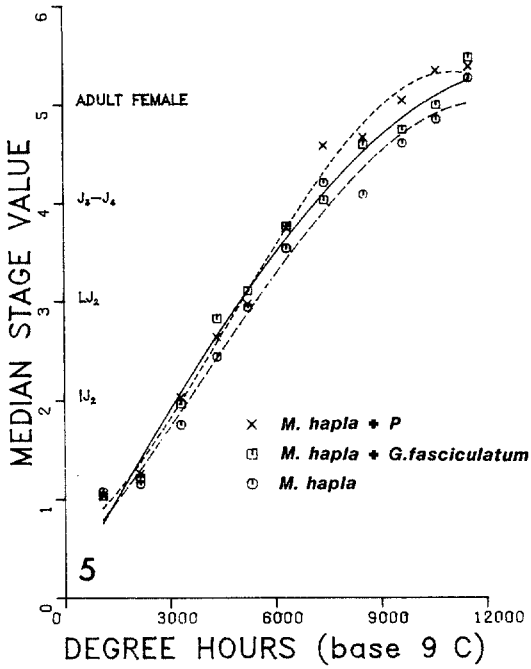


FIG. 4. Incidence of five stage-classes of *Meloidogyne hapla* inhabiting *Allium cepa* supplemented with phosphorus.

FIG. 5. Stage-specific incidence of the median individual (or 50% of the population) of *Meloidogyne hapla* inhabiting *Allium cepa* amended with phosphorus, inoculated with *Glomus fasciculatum*, or unamended control.

presence of *G. fasciculatum* because of the two-fold difference ($P = 0.08$) in the proportion of nematodes which had reached the adult stage at the time oviposition was first noted. Delayed development of nematodes in these, as compared with nonmycorrhizal plants, would result in the production of fewer progeny after 10 weeks and explain why the numbers of juveniles did not remain steady or increase in mycorrhizal plants. In the more detailed experiment, however, there was no significant difference in the calculated median developmental stage of nematodes reared on nonmycorrhizal vs. mycorrhizal *A. cepa* on 11 sampling dates. These results may have been confounded by differences in the initial number of nematodes penetrating roots for the three treatments. It is possible that the high nematode density per root system in control plants had an equal or greater effect than VAM on the development of *M. hapla.* In fact, more nematodes exited the unamended nonmycorrhizal *A. cepa* than the nonmycorrhizal-P and mycorrhizal plants.

Nematode density and survival were similar in nonmycorrhizal plants receiving P and mycorrhizal plants. Although there was no significant difference in the median developmental stage values of nematodes reared on these plants for any sampling date, the estimated time required for 50% of the population to attain the adult stage was more than 1,000 DH_9 greater for nematodes inhabiting mycorrhizal plants. Even if the difference in nematode development, ca. 3 days in this experiment, was responsible for the results observed in this research, it is probably not sufficient to affect nematode population growth in the field.

Nematodes maintained in roots with well-established VAM in our second experiment did not exhibit impaired reproduction or survival. The most apparent effect of *G. fasciculatum* on *M. hapla* was to reduce the number of nematodes penetrating roots in the first 96 hours after inoculation. This phenomenon, reported by us (8) and others (3,13), seemed to be related to root P levels, since nonmycorrhizal plants supplemented with P also supported less root penetration by *M. hapla.*

Reduced penetration by *M. hapla* in VAM-colonized roots could account for the different trends in population growth noted in our first experiment. Nematodes were observed in roots before VAM colonization. The fungus was established, however, by the time progeny of the inoculated nematodes were observed. The apparent absence of a second nematode generation in mycorrhizal plants could have been caused by the inability of juveniles to enter root tissue. This interpretation is supported by the increase in nematode levels surrounding, but not within, mycorrhizal plants.

Our results indicate that there is no direct competition or antagonism between *M. hapla* and *G. fasciculatum* inhabiting *A. cepa* as reported for other nematode–VAM–host systems (12). Nematodes which entered mycorrhizal roots survived and matured. It is more likely that VAM colonization alters the physiology of the host plant which, in turn, mediates the quantity and quality of food reserves and living environment for nematodes. A host-mediated influence of VAM would be subject to edaphic conditions. In addition, our data show that the duration, inoculation sequence, and sampling schedule are very important experimental variables that should be reviewed in detail before designing and conducting VAM–nematode interaction research.

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