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Influence of *Glomus fasciculatum* and *Meloidogyne hapla* on *Allium cepa* in Organic Soils[1]

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Abstract: The influence of *Meloidogyne hapla* and *Glomus fasciculatum* on *Allium cepa* (onion) grown in organic soil was evaluated under greenhouse conditions. In the absence of *G. fasciculatum, M. hapla* significantly retarded the growth of *A. cepa* cv. Krummery Special and MSU 8155 × 826, but had no detrimental influence on Downing Yellow Globe, Spartan Banner, or Spartan Sleeper. All five cultivars maintained populations of *M. hapla.* Final root population densities of *M. hapla* associated with Spartan Banner, Krummery Special, MSU 8155 × 826, and Spartan Sleeper were significantly greater than those recovered from Downing Yellow Globe. Final root population densities of *M. hapla* were directly proportional to the initial population densities. Root colonization of onion by *G. fasciculatum* significantly enhanced the growth and development of Downing Yellow Globe. The rate of increase of *A. cepa* growth and the final spore density were directly proportional to the initial spore density of *G. fasciculatum.* Final population densities of *M. hapla* in the presence of *G. fasciculatum* were generally greater than in the absence of the fungus. After 15 weeks, *A. cepa* plants grown in the presence of both *M. hapla* and *G. fasciculatum* were significantly larger than those grown in the presence of only *M. hapla.*

Key words: vesicular-arbuscular mycorrhizae, northern root-knot nematode, onion.

Endomycorrhizal fungi alter the susceptibility of plants to disease (2,9,14,16). Most studies of nematode–mycorrhizae interactions have been with cotton, soybean, or tobacco. *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 population densities associated with mycorrhizal tobacco were less than those on nonmycorrhizal plants (1), but some soybean–symbiont combinations resulted in increased root diseases (14). Lower yields of both nematode susceptible and resistant tobacco cultivars occurred in the presence of both *Globodera solanacearum* (Miller & Gay, 1972) Mulvey & Stone, 1976 and mycorrhizal fungi than with either organism alone. Reduced reproductive capacity of the nematode or the fungus was also reported (4,15). *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans-Stekhoven, 1941 had no influence on mycorrhizal fungi, sporulation, or plant growth in cotton, although the symbiont alone resulted in significant growth increases in mycorrhizal plants (8). There is, however, a growing literature indicating that under a number of environmental conditions, the detrimental influence of plant-parasitic nematodes is less on mycorrhizal than on nonmycorrhizal plants (9,18–20).

Olthof and Potter (12) established the relationships between initial population densities of *Meloidogyne hapla* Chitwood, 1949 and marketable yields of *Allium cepa* L. (onion) grown in mineral soil. Similar information for this association is not available for onion grown in organic soils. This host–parasite relationship has not been studied in relation to mycorrhizal fungi. The objective of this study was to evaluate

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the concomitant influence of *M. hapla* and *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe on the growth and development of *A. cepa* in relation to the population dynamics of both microorganisms.

Materials and Methods

Cultures of *M. hapla* were maintained on tomato (*Lycopersicon lycopersici* L. cv. Rutgers) under greenhouse conditions. Eggs were extracted from roots in 1% sodium hypochlorite as described by Hussey and Barker (7). Spores of *G. fasciculatum* were obtained from *Sorghum vulgare* Pers. greenhouse cultures (provided by Dr. G. R. Safir, Michigan State University) and extracted from soil by a centrifugal-flotation method (10). In all experiments, water suspensions of the desired inoculum densities of *M. hapla* or *G. fasciculatum* were added to plastic pots containing 1,000 cm^3 steamed organic soil.

At harvest, root, leaf, bulb, and total plant fresh weights were determined. Root samples (0.25 gram) were stained with acid fuchsin in lactophenol and observed with a light microscope to estimate root infection by *G. fasciculatum* and *M. hapla.* Soil samples (100 cm^3) were processed by the centrifugal-flotation method to recover spores of *G. fasciculatum* and second-stage juveniles of *M. hapla.*

Because of the loss of nematodes during extraction (17), the following calibration procedure was used to standardize *M. hapla* population densities recovered using the centrifugal-flotation technique. Second-stage juveniles of *M. hapla* were extracted from soil by a Baermann pan procedure, and aliquants were counted and added as water suspensions to 100-cm^3 samples of autoclaved soil. The soil samples were then processed by a centrifugal-flotation method and the nematodes counted. The extraction efficiency (E) was calculated.

$$E = \frac{\text{nematodes recovered}}{\text{nematodes added}}$$

The actual population density (Pa) was estimated from the population density recovered (Pr) for each sample,

$$Pa = Pr/E = KPr$$

where the extraction coefficient was $K = 1/E$. K was assumed to be constant for a given set of procedural conditions. All soil population density data for *M. hapla* reported were adjusted using an extraction coefficient of K = 3.2.

Influence of M. hapla on A. cepa growth: Three seeds of Downing Yellow Globe, Krummery Special, Spartan Banner, Spartan Sleeper, or MSU 8155 × 826 hybrid were planted in 1,000 cm^3 steamed organic soil in pots and inoculated with 0 or 15,000 *M. hapla* eggs/pot. After emergence, six replicates of each of the 10 treatments were thinned to one plant per pot and grown in soil temperature control benches in a greenhouse (soil temperature 15 ± 2 C and air temperature = 21 ± 3 C). After 6 weeks, the pots were moved to a greenhouse bench (21 ± 3 C) with 14 hours a day of supplemental lighting. Plants were harvested after 15 weeks and analyzed as previously described.

In a second greenhouse experiment, four initial population densities of *M. hapla* were evaluated in relation to their influence on Downing Yellow Globe. Five replicate pots of 1,000 cm^3 steamed organic soil were infested with 0, 10, 100, 1,000, or 10,000 *M. hapla* eggs/pot and seeded with three seeds of Downing Yellow Globe. The plants were maintained and evaluated as described for the first experiment.

Influence of Glomus fasciculatum on A. cepa growth: Three seeds of Downing Yellow Globe were planted in each of 25 plastic pots of 1,000 cm^3 steamed organic soil. Each pot was inoculated with 0, 5, 50, 500, or 5,000 spores of *G. fasciculatum* to provide five replicates of each treatment. The plants were grown as previously described in soil temperature control benches for 9 weeks and then moved to greenhouse benches. All plants were harvested after 16 weeks and processed as described above.

Interactions of M. hapla, G. fasciculatum, and A. cepa: Interactions among *M. hapla, G. fasciculatum,* and Downing Yellow Globe were assessed in two greenhouse tests. The first experiment consisted of four treatments and was terminated after 6 weeks. The treatments included noninoculated controls, 5,000 *M. hapla* eggs/plant, 1,500 *G. fasciculatum* spores/plant, and both *M. hapla* and *G. fasciculatum* at these inoculum densities. Twenty-four replicate pots of 1,000 cm^3 steamed organic soil were used for each treatment. Four replicates of each treatment were harvested at weekly inter-

TABLE 1. Influence of *Meloidogyne hapla* on the fresh weight in grams of five *Allium cepa* cultivars grown in steamed organic soil and maintained in the greenhouse for 15 weeks.

Cultivar	*M. hapla*	Bulb	Root	Leaf	Total
Downing Yellow Globe	−	2.20 (0.40)	5.1 (0.75)	9.4 (0.72)	16.7 (0.80)
	+	2.78	4.7	10.3	17.8
Krummery Special	−	5.25 (0.04)	11.0 (0.04)	15.3 (0.03)	31.5 (0.02)
	+	1.32	2.5	5.9	9.6
MSU 8155 × 826	−	3.62 (0.02)	6.7 (0.01)	13.1 (0.02)	23.4 (0.01)
	+	1.52	2.9	7.0	11.4
Spartan Banner	−	2.18 (0.70)	3.3 (0.26)	4.6 (0.48)	10.0 (0.43)
	+	1.77	2.2	3.6	7.6
Spartan Sleeper	−	1.63 (0.64)	3.1 (0.32)	4.7 (0.61)	9.4 (0.50)
	+	1.42	2.2	3.9	7.5

Statistics in parentheses represent level of significant difference between comparable treatment means (control and *M. hapla*) according to the 2-tail Student's *t*-test.

vals for 6 weeks, beginning 2 weeks after planting. The plants were grown in soil temperature control benches at 21 ± 3 C for the duration of the experiment.

The second experiment with Downing Yellow Globe consisted of four treatments, and was terminated after 15 weeks. The treatments included noninoculated controls, 30,000 *M. hapla* eggs/plant, 600 *G. fasciculatum* spores/plant, and both *M. hapla* and *G. fasciculatum* at the above inoculum densities. Twenty-four replicate pots of 1,000 cm^3 steamed organic soil were used for each treatment. Four replicates of each treatment were harvested at 2, 4, 6, 8, 11, and 15 weeks after planting. All plants were maintained in soil temperature control benches (21 ± 3 C) for the first 6 weeks of the experiment. Controls for each treatment were inoculated with a spore-free filtrate of the inoculum of *G. fasciculatum* to serve as a control. Plants were grown for 4 weeks in soil temperature control benches (21 ± 3 C) and then moved to greenhouse benches. The entire root system of each plant was stained to determine nematode and mycorrhizal infection. Growth data and soil samples were processed as described previously.

RESULTS

Influence of M. hapla on A. cepa: The total fresh weight of Krummery Special and MSU 8155 × 826 was significantly ($P \leq 0.02$) less when exposed to an initial population density of 15 *M. hapla* eggs/cm^3 soil than when grown in the absence of this nematode (Table 1). The fresh weight of bulb, roots, and leaves of these cultivars were significantly ($P \leq 0.04$) less in the presence of *M. hapla* than in its absence.

All five cultivars of *A. cepa* maintained populations of *M. hapla* (Table 2). Soil population densities of *M. hapla* associated with Krummery Special, Spartan Banner, and Spartan Sleeper were significantly ($P = 0.05$) greater than those associated with MSU 8155 × 826. Population densities of *M. hapla* recovered from roots of Downing Yellow Globe were significantly less than those recovered from the other five cultivars. These differences in *M. hapla* population densities were not observed among the total final population densities per experimental unit. The rate of reproduction (R = final population density [Pf]/initial population density [Pi]) was low, ranging from 0.03 to 0.10.

TABLE 2. Population densities of *Meloidogyne hapla* associated with five *Allium cepa* cultivars grown in organic soil in the greenhouse for 15 weeks.

	M. hapla		
Cultivar	No./100 cm^3 soil	No./g root tissue	Pf*
Downing Yellow Globe	42 bc	37 b	581 b
Krummery Special	60 c	256 c	1,173 b
MSU 8155 × 826	12 ab	119 c	477 b
Spartan Banner	65 c	121 c	917 b
Spartan Sleeper	95 c	181 c	1,464 b

* Pf = final soil and root population density per pot.

Column means followed by the same letter are not statistically different ($P = 0.05$) according to the Student-Newman-Keuls multiple-range test of $\log_{10}(x + 1)$ of the transformed data. Means followed by the letter "a" are not significantly different from means of the noninoculated controls. All means of noninoculated controls equal zero and are not listed in the table.

TABLE 3. Influence of *Meloidogyne hapla* on the growth of *Allium cepa* cv. Downing Yellow Globe after 16 weeks of growth under greenhouse conditions, with reference to the initial and final population densities of *M. hapla*.

*M. hapla** (Pi)	*A. cepa* fresh weight (g)				*M. hapla*	
	Bulb	Leaf	Root	Total	Pf†	Pf/Pi
0	7.2 a	11.8 a	9.5 a	28.5 a	0 a	0.00
1	4.2 a	7.9 a	7.1 a	19.2 a	0 a	0.00
10	5.4 a	7.7 a	6.9 a	20.0 a	214 b	2.14
100	4.3 a	10.1 a	7.4 a	21.8 a	710 c	0.71
1,000	4.4 a	9.4 a	9.7 a	23.5 a	4,033 c	0.40

Column means followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls multiple-range test.

* Pi = initial density of *M. hapla* eggs per 100 cm³ soil.

† Pf = final soil and root population density per pot.

Initial population densities of 1, 10, 100, and 1,000 *M. hapla* eggs/100 cm³ soil had no significant ($P = 0.05$) influence on the final fresh weights of Downing Yellow Globe bulbs, roots, or leaves (Table 3). Initial egg densities of 10 *M. hapla* eggs/100 cm³ soil were required for the detection of final populations of *M. hapla*. The final population densities of *M. hapla* were directly proportional to the initial population densities. The rate of reproduction, however, was greatest (Pf/Pi = 2.14) for an initial population density of 10 *M. hapla* eggs/100 cm³ soil and declined with increasing initial nematode population densities.

Influence of Glomus fasciculatum on A. cepa: Initial inoculum levels of 500 *G. fasciculatum* spores/plant resulted in a significant ($P = 0.05$) increase in the bulb, leaf, and root weights of Downing Yellow Globe (Table 4). Onion bulb weight was significantly ($P = 0.05$) greater with an initial density of 5,000 compared with 500 spores/plant. Initial spore densities as low as five per plant significantly ($P = 0.05$) increased total plant weight. Root tissue colonization by *G. fasciculatum* increased with increasing initial spore densities through 500 spores/pot. Final spore density increased with increasing initial spore densities, but the rate of spore production decreased with increasing initial spore densities.

Interaction among M. hapla, G. fasciculatum, and A. cepa: Root colonization of *G. fasciculatum* was detected 4 weeks after inoculation. It was not influenced by the presence of *M. hapla* in either of the interaction studies, and it reached a maximum of 15% 15 weeks after inoculation. Spore density, however, was significantly ($P = 0.05$) lower in the presence of *M. hapla* through week 6 of both experiments than

TABLE 4. Influence of *Glomus fasciculatum* on the growth of *Allium cepa* cv. Downing Yellow Globe after 16 weeks of growth under greenhouse conditions, with reference to the final spore density of *G. fasciculatum* and root colonization.

*G. fasciculatum** (Pi)	*A. cepa* fresh weight (g)				*G. fasciculatum*		
	Bulb	Leaf	Root	Total	Root colonization (%)	Spores/ 100 cm³	Pf/Pi
0	5.4 a	12.1 a	8.8 a	26.2 a	4 a	0 a	0.0 b
5	8.2 a	27.0 ab	14.8 a	50.1 b	14 a	174 a	348.0 a
50	8.7 a	29.5 ab	14.3 a	52.5 b	22 a	273 a	54.6 b
500	18.7 b	51.9 c	26.7 b	97.2 c	48 b	1,655 b	33.1 b
5,000	35.4 c	42.9 bc	27.8 b	106.1 c	42 b	3,932 c	7.9 b

Column means followed by the same letter are not statistically different ($P = 0.05$) according to the Student-Newman-Keuls multiple-range test.

* Initial density of *G. fasciculatum* spores per pot.

TABLE 5. Influence of *Meloidogyne hapla* on *Glomus fasciculatum* spore production, and of *G fasciculatum*-colonized *Allium cepa* cv. Downing Yellow Globe on population densities of *M. hapla*.

Harvest time and treatment	*G. fasciculatum* spores/100 cm³		*M. hapla* (Pf)	
	Expt. 1*	Expt. 2†	Expt. 1*	Expt. 2†
Week 4				
G. fasciculatum	12 a		48 a	
M. hapla and *G. fasciculatum*	2 b		100 b	
Week 6				
G. fasciculatum	16 a	29 a	45 a	306 b
M. hapla and *G. fasciculatum*	1 b	17 b	250 c	433 c
Week 11				
G. fasciculatum		11 b		143 a
M. hapla and *G. fasciculatum*		13 b		127 a
Week 15				
G. fasciculatum		39 a		295 b
M. hapla and *G. fasciculatum*		45 a		510 c

Column means for each week followed by the same letter are not statistically different ($P = 0.05$) according to the Student-Newman-Keuls multiple-range test.

* *M. hapla* initial population density = 5,000 eggs/pot. *G. fasiculatum* initial population density = 1,500 spores/pot.

† *M. hapla* initial population density = 30,000 eggs/pot. *G. fasciculatum* initial population density = 600 spores/pot.

in the absence of this nematode (Table 5). After 4, 6, and 15 weeks, final root and soil population densities of *M. hapla* were significantly ($P = 0.05$) greater in the presence than in the absence of *G. fasciculatum*. Approximately 10% of the original *M. hapla* inoculum was recovered as second-stage juveniles after 2 weeks. Second-stage juveniles of *M. hapla* were observed entering root tissue during weeks 2 and 4, and again during weeks 11 and 15. Egg production was first observed 8 weeks after initiation of the experiment.

No consistent differences were observed in *A. cepa* growth during the first 11 weeks of the second interaction experiment. *M. hapla*-inoculated plants were smaller than those of other treatments, but were not significantly different from the nontreated controls (Fig. 1). After 15 weeks, however, *A. cepa* plants grown in the presence of both *M. hapla* and *G. fasciculatum* were significantly larger ($P = 0.05$) than plants

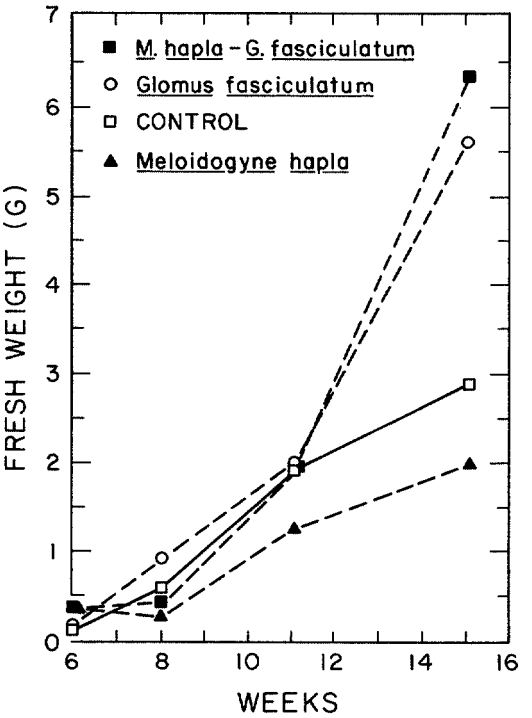

FIG. 1. Influence of *Meloidogyne hapla* and *Glomus fasciculatum* on fresh weight in grams of *Allium cepa* cv. Downing Yellow Globe.

grown in the presence of *M. hapla* alone (Fig. 1).

DISCUSSION

The onion cultivars examined varied in both suitability for nematode reproduction and tolerance to damage from infection by *M. hapla*. Both parameters must be considered in cultivar evaluation and development (13). Downing Yellow Globe is resistant to *M. hapla*, resulting in low nematode reproduction and tolerance to nematode infection.

In the presence of *G. fasciculatum*, increased growth of *A. cepa* was observed compared to plants lacking the fungal symbiont. This is the first report of this *G. fasciculatum*/*A. cepa* symbiotic association (3,5,6,9,11). *G. fasciculatum* occurs in onion fields in Michigan. Although the rate of reproduction of *M. hapla* was increased in the presence of *G. fasciculatum*, the growth of *A. cepa* was also enhanced by the fungus, resulting in greater growth of nematode-infected mycorrhizal onions compared to nematode-infected plants without mycorrhizae. This indicates that *G. fasciculatum*

may enhance tolerance of Downing Yellow Globe to *M. hapla* (9,18–20).

No direct interaction was observed between fungal hyphae and *M. hapla*. Reduced early *G. fasciculatum* spore production in the presence of *M. hapla* may indicate competition for host nutrients. Since the responses of both endomycorrhizal fungi and root-knot nematodes can be altered by genetic variation of the host, further research into the mycorrhizal responses of onion cultivars susceptible to *M. hapla* is necessary to provide additional insight into this host–symbiont–parasite interrelationship.

Michigan onion growers frequently rotate onions with carrots. In many cases, fields are maintained 1 year out of every 5 in a covercrop such as *Sorghum vulgare* × *S. sudanense* Hitchc. (sudax), an excellent host for the increase of *G. fasciculatum*. These cultural procedures provides ways to manipulate population densities of both *M. hapla* and mycorrhizae spores. The results of these studies indicate that, whenever possible, onion production systems should be designed to stimulate activity of appropriate endomycorrhizal fungi.

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