Journal of Nematology 21(2):179–183. 1989.

Biological Control of *Meloidogyne hapla* on Alfalfa and Tomato with the Fungus *Meria coniospora*

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Abstract: This study was to determine whether *Arthrobotrys flagrans, A. oligospora,* and *Meria coniospora* would control the root-knot nematode *Meloidogyne hapla* on alfalfa and tomato. Alfalfa seeds were coated with a fungus-rye powder in 2% cellulose and were planted in infested soil. Three-week-old seedlings from seed treated with *M. coniospora* had 60% and 58% fewer galls in two experiments than did seedlings from untreated seeds. Numbers of J2 in the soil were not reduced. Plant growth did not improve. When seed of tomato were coated with *M. coniospora* and planted in *M. hapla*-infested soil, roots had 34% fewer galls and 47% fewer J2 in the soil at 28 days. After 56 days there was no reduction in J2 numbers. Plant growth did not improve. When roots of tomato transplants were dusted with *M. coniospora* fungus-rye powder or sprayed with a spore suspension before planting in *M. hapla*-infested soil, 42% and 35%, respectively, fewer galls developed in 28 days on treated roots than on roots not treated with fungus. The numbers of J2 extracted from roots or recovered from soil were not reduced, however, and plant growth did not improve.

Key words: alfalfa, *Arthrobotrys flagrans, Arthrobotrys oligospora,* biological control, *Lycopersicon esculentum, Medicago sativa, Meloidogyne hapla, Meria coniospora,* nematode-destroying fungi, northern root-knot nematode, tomato.

The northern root-knot nematode, *Meloidogyne hapla* Chitwood, is a common plant parasite in agricultural soils in Ontario (7,9). Microplot studies have shown that *M. hapla* can reduce yields of alfalfa and tomato (6,10). Fumigation of soils for field crops such as alfalfa is economically impractical. Precision application of systemic nematicides to seed could reduce the cost of chemical control. The application of oxamyl to alfalfa seed resulted in a significant reduction in the number of root galls caused by *M. hapla* on the roots and an increase in top growth (11). Similarly, applying nematode-destroying fungi to seed or to roots of transplants may be alternative methods for more precise application. Galling on tomato by *Meloidogyne incognita* and *M. javanica* was reduced 76–87% by the nematode-destroying fungus *Meria coniospora* in greenhouse pot trials (4). The objective of our study was to determine the effects of *M. coniospora* Drechsler, *Arthrobotrys flagrans* Fres., and *A. flagrans* (Dudd.) Mekhtieva applied to seed or roots of alfalfa and tomato on the damage caused by *M. hapla.*

MATERIALS AND METHODS

The isolate of *M. hapla* used in this study was recovered from soil collected in the Niagara Peninsula of southern Ontario. The nematode was reared on celery (*Apium graveolens* L. cv. Utah 15) growing in large plastic tubs (46 × 46 × 27 cm) of Vineland silt loam (61% sand, 28% silt, 11% clay). Infested soil was diluted to the desired inoculum density with steam-sterilized Vineland silt loam that had been exposed to the air for several weeks to develop a microflora.

Alfalfa experiments: The three fungi used in this study were *A. oligospora, A. flagrans,* and *M. coniospora,* which were recovered from agricultural soils using techniques described previously (1). They were maintained on potato dextrose agar (PDA). Inoculum for seed treatments was prepared as follows: Rye grain (75 g) was placed in distilled water (75 ml) in a 500-ml flask, autoclaved for 30 minutes, cooled overnight, and autoclaved again. When cool, each flask was inoculated with four 4-mm discs from a culture of the fungus to be tested. The flasks, maintained at 24 C were shaken daily for 3 weeks at which time the rye kernels were heavily overgrown with

Received for publication 25 March 1988.

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the fungus. The contents of each flask were spread thinly in a tray and allowed to air dry for 48 hours. The dried kernels were ground for 60 seconds in a coffee grinder, and the resulting powder was passed through a 0.85-mm-pore screen to remove the larger particles. Alfalfa (*Medicago sativa* L. cv. Saranac) seeds were coated three times with a nematode-trapping fungus. First a fungus-rye suspension was prepared by adding 1 g macerated fungus-rye powder to 10 ml of a 2% aqueous carboxy methyl cellulose solution. Then in a mechanical tumbler (11), 0.5 ml of the fungus-rye-cellulose suspension was added with a pipette to 3 g of alfalfa seed as the tumbler rotated for 2 minutes. The seed were dried on a screened trough in which a hair dryer was inserted (11). A second and third coat were applied to the seed as described, using 1 ml of the fungus-rye-cellulose suspension each time. Coated alfalfa seed were prepared with each of the three fungi.

Two experiments were conducted, each with four treatments: 1) untreated seed, 2) *A. flagrans*-coated seed, 3) *A. oligospora*-coated seed, and 4) *M. coniospora*-coated seed. The *M. hapla*-infested soil contained 3 J2/g in the first experiment and 10 J2/g in the second. Styrofoam pots (11 cm d × 7.5 cm high) were filled with 425 cm^3 of *M. hapla*-infested soil. Twenty seed sites were impressed into the soil with a multipoint dibble, and a single seed was placed in each site. Treatments were replicated 10 times in a randomized block design. The pots were placed in a growth room with a 17-C light period of 11,000 lux for 16 hours and a 14-C dark period. Seedlings were counted daily from day 3 to day 12. At 21 days shoots and roots were weighed and *M. hapla* galls counted. *Meloidogyne hapla* J2 were extracted from 50-g soil samples by the pan method (8) over a 2-week period.

Tomato experiments: Two experiments were conducted with *M. coniospora* on tomato (*Lycopersicon esculentum* Mill cv. Rutgers). In the first experiment, a fungus-rye suspension for coating seed was prepared by adding 2 g of the macerated fungus-rye powder to 10 ml of 2% carboxy methyl cellulose solution. One milliliter of the prepared fungus-rye-cellulose suspension was applied to 1 g of seed in each of the three coats applied. A second lot of seed was coated with rye-cellulose suspension without the fungus. Seventy-four styrofoam pots (11 cm d × 13.5 cm high) were filled with uniformly mixed *M. hapla*-infested soil and three tomato seeds were planted in each pot. The experiment had three treatments: 1) untreated seed, 2) rye-powder coated seed, and 3) fungus-rye coated seed. Each treatment had 14 replicates. The pots were arranged in a randomized block design in a growth room with a 20-C light period of 11,000 lux for 16 hours and an 18-C dark period. After germination, the seedlings were thinned to one per pot. Tomatoes in 14 pots from each treatment were harvested 28 days and 56 days after planting. At each harvest, top and root weights were determined, *M. hapla* galls were counted, and J2 were extracted from 50-g soil samples by the pan method (8) for 2 weeks. At the 56-day harvest, J2 were extracted also from the roots in a mistifier for 2 weeks. Data were subjected to analyses of variance.

In a second experiment with tomato, root systems of transplants were treated rather than the seed. Tomato plants were grown in sterile compost soil in seedling trays (each cylinder 33 mm d × 90 mm high) for 4 weeks. Sufficient *M. hapla*-infested soil to fill 70 styrofoam pots (11 cm d × 13.5 cm high) was mixed thoroughly in a shaker. The equivalent of 14 pots of infested soil was placed in a 20-liter plastic container. The soil was injected with Telone (180 liters/ha) and the container was sealed for 1 week. The fumigated soil was then aerated for 3 weeks. During fumigation and aeration the remainder of the infested soil was stored at 5 C. The experiment consisted of five treatments: 1) transplants with roots untreated, 2) untreated transplants in fumigated soil, 3) roots of transplants rolled in rye powder, 4) roots of transplants rolled in fungus-rye powder, and 5) roots of transplants sprayed with spores (155 mil-

TABLE 1. Growth of Saranac alfalfa and galling and reproduction of *Meloidogyne hapla* when alfalfa seed were coated with nematophagus fungi.

Parameters	Control	*Arthrobotrys flagrans*	*Arthrobotrys oligospora*	*Meria coniospora*	$LSD_{5\%}$
		Experiment 1			
Seedlings/pot	16	16	16	16	ns
Shoot wt (g/pot)	1.6	1.6	1.6	1.6	ns
Root wt (g/pot)	1.6	1.4	1.1	1.2	ns
Nodules/pot	46	45	37	41	ns
Nodules/g root	29	32	36	36	ns
Galls/pot	5	4	5	2	2
Galls/g root	3	3	4	2	ns
		Experiment 2			
Seedlings/pot	16	16	17	15	ns
Shoot wt (g/pot)	1.5	1.5	1.5	1.6	ns
Root wt (g/pot)	1.0	1.0	1.0	1.1	ns
Nodules/pot	36	34	45	46	8
Nodules/g root	36	34	43	43	ns
Galls/pot	12	10	7	5	5
Galls/g root	12	9	7	5	3
J2/50 g soil	500	400	570	450	ns

Each number is the average of 10 replicates.

lion spores per root system). Each treatment was replicated 14 times. The pots were arranged in a randomized block design in a growth room with a 20-C light period of 11,000 lux for 16 hours and an 18-C dark period. The experiment was terminated at 6 weeks. Growth parameters were measured, galls on the roots were counted, and J2 in the roots and soil were extracted by the mistifier and pan methods (9), respectively, and counted. Data were subjected to analyses of variance.

RESULTS

Alfalfa experiments: Plants treated with *M. coniospora* had fewer *M. hapla* galls than plants without the fungus (Table 1). Galls on the roots of 3-week-old seedlings were reduced by 60% and 58% in two experiments. Numbers of J2 in the soil, however, were not reduced. The weight of shoot and root growth was not affected. The fungi did not affect the number of nodules per gram of root; however, in experiment 2 the number of nodules per pot was greater when seed were coated with *A. oligospora* or *M. coniospora* (Table 1). Generally *A. oligospora* and *A. flagrans* had no effect.

Tomato experiments: Growth parameters and root galling were affected when seed or roots were treated with *M. coniospora* (Tables 2, 3). There were 49% fewer galls per gram of root 28 days after *M. coniospora*-coated seed were planted in *M. hapla*-infested soil, and at 56 days there were 41% fewer galls per gram of root compared with the control without the fungus (Table 2). At 28 days, 47% fewer J2 were obtained from the infested soil planted with *M. coniospora*-coated seed than in the infested control soil planted with untreated seed. After 56 days the number of J2 in the infested soil was not different from the control, even though there were fewer galls on the roots of plants growing from *M. coniospora*-coated seed.

In the *M. hapla*-infested soil, seed treated with fungus-rye powder or rye powder alone germinated better than untreated seed (Table 2). Shoot and root growth of plants growing from fungus-rye powder-coated seed did not weigh more than the control plants at day 28 even though there were fewer galls on the roots. At 56 days the roots of plants from fungus-rye powder-coated seed weighed more than those of control plants.

When the roots of tomato transplants

TABLE 2. Germination of *Meria coniospora*-coated Rutgers tomato seed, tomato growth, and *Meloidogyne hapla* galling and reproduction.

Parameters	Control	Control (rye)	Fungus (rye)	$LSD_{5\%}$
		Day 16		
Seedlings/pot	1.2†	2.1	1.9	0.9
		Day 28		
Shoot wt (g/pot)	3.9	3.7	4.5	ns
Roto wt (g/pot)	0.9	0.9	1.3	ns
Galls/root system	44	38	29	13
Galls/g root	47	48	24	15
J2/50 g soil	19	16	10	5
		Day 56		
Shoot wt (g/pot)	58.1	57.0	58.3	ns
Roto wt (g/pot)	10.8	11.9	13.5	1.6
Galls/root system	485	597	375	98
Galls/g root	46	50	27	7
J2/50 g soil	730	470	550	ns
J2/root system	28,000	23,390	22,350	ns
J2/g root	2,920	2,040	1,710	ns

Each number is the average of 14 replicates.
† Tomato seedlings were reduced to one per pot for balance of the experiment.

were treated with *M. coniospora*-rye powder or a *M. coniospora* spore suspension, *M. hapla* galls were 46% fewer than on the control at day 28 (Table 3). Roots of tomatoes grown in the fumigated soil had no galls. At day 28, J2 densities were the same in the soil in all treatments except in the fumigated soil (Table 3). The numbers of J2 extracted from the roots of transplants treated with a spore suspension of *M. coniospora* were about 3.5 times greater than the numbers extracted from the roots of the control. There was no improvement in growth of shoots or roots at day 28 on those transplants that had been treated with fungus-rye powder or with a spore suspension of *M. coniospora*. In the fumigated soil, shoot and root growth at day 28 was greater than that of the control.

DISCUSSION

Galls of *M. hapla* on tomato treated with *M. coniospora* were relatively more numerous than galls of *M. incognita* and *M. javanica* on plants treated with the isolate of Jannson et al. (4). These differences may be due to differences in treatment techniques. Our fungus was applied as a seed treatment, whereas Jannson et al. applied theirs as a spore suspension to the soil or used infected *Panagrellus redivivus* to introduce and spread the fungus (4). Control of nematode diseases using seed treatments may be more economical, but seed treat-

TABLE 3. Growth of Rutgers tomato plants after applying *Meria coniospora* spore suspension or mycelium powder to the tomato roots and *Meloidogyne hapla* galling and reproduction.

Parameters	Control	Control (rye)	Fumigation	Fungus (rye)	Fungus (spores)	$LSD_{5\%}$
Shoot wt (g/pot)	70.6	68.9	79.6	72.6	72.3	3.4
Root wt (g/pot)	20.5	20.5	29.1	21.8	24.7	3.8
Galls/root system	1,214	1,270	0	704	788	210
Galls/g root	61	60	0	33	33	8
J2/50 g soil	590	330	1	350	690	280
J2/root system	25,630	17,710	2	19,590	87,710	13,910
J2/g root	1,330	870	1	970	3,870	640

Each number is the average of 14 replicates.

ments are more demanding biologically than soil supplements with conidia or infected hosts. Differences may also be explained by the use of different tomato varieties. *M. hapla* may be less susceptible than *M. incognita* or *M. javanica* to *M. coniospora*, or our isolate of *M. coniospora* may be less aggressive. Fungistasis may have suppressed greater activity of our isolate of *M. coniospora* (2,3,5). Although our tub cultures contained only one plant-parasitic nematode, *M. hapla*, it contained a number of saprophytic nematodes and may have contained other fungi and bacteria. *M. hapla* galling on alfalfa was reduced more than on tomato. There was no growth response in our studies or in Jansson's (4).

The lack of J2 reduction cannot be explained readily. *Meria coniospora* may not grow rapidly enough to destroy the J2 or perhaps the more vigorous J2 escaped or were resistant to parasitism. Thus the use of a highly aggressive strain of *M. coniospora* in the biological control of plant-parasitic nematodes is very important.

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