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Population Dynamics of *Meloidogyne incognita* on Corn Grown in Soil Infested with *Arthrobotrys conoides*[1]

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Abstract: Microplot and greenhouse experiments were conducted to evaluate the effects of soil incorporation of the nematophagous fungus *Arthrobotrys conoides* and green alfalfa mulch on the population dynamics of *Meloidogyne incognita* on corn. Reproduction of *M. incognita* and the incidence of root galling were reduced by the addition of *A. conoides* and/or green alfalfa in all tests. Numbers of juveniles were reduced by as much as 84%, and eggs were fewest in early to mid-season soil samples from microplots. Yields increased in treatments with *A. conoides* and/or green alfalfa in greenhouse tests and in the microplot tests in 1979. No interaction was found between the fungus and green alfalfa in the reduction of the nematode population. *Key words:* biological control, population dynamics, ecology, green alfalfa, root-knot nematode, soil amendment, nematophagous fungus, nematode trapping fungus, organic mulch.

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The role of nematophagous fungi as biological deterrents toward plant-parasitic nematodes is not clear (6,17). For instance, *Meloidogyne* population reductions in soil amended with pineapple shoots were attributed to the stimulation of nematophagous fungi (9,11). Of five such fungi tested, only *Dactylella ellipsospora* Grove protected potted pineapple plants from root-knot nematode injury (10). *Heterodera* spp. were controlled in greenhouse tests but not in the field (7). Damage from *M. incognita* (Kofoid & White) Chitwood in other tests were not suppressed by the addition of several nematophagous fungi (16,17).

The introduction of organic amendments to soil has also been correlated with reduced population densities of *Meloidogyne* species (5,8,9,11,12,13,14,15,19,21, 22,24,25,26). This reduction may be due to increased organic compounds or activity of other micro-organisms. For example, amendment of soil with margosa oil cake increased the concentration of phenolic substances (24) and fatty acids (25). More suppression of root-knot disease on tomato was obtained with 10 tons of oil cake per acre than with 5 tons and when the amendment was added 8 months before assaying than for shorter times (12,15).

The objective of this study was to determine the effect of *Arthrobotrys conoides*

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Drechsl. alone and in soil amended with green alfalfa on *M. incognita* population dynamics on corn. These organisms were selected because both are common in North Carolina.

MATERIALS AND METHODS

Inoculum: Meloidogyne incognita was cultured on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in the greenhouse. A North Carolina isolate of *A. conoides* was cultured on cornmeal agar and allowed to grow for 3–5 days at 24 C, then transferred into a modified vermiculite medium (18). For the modified medium, a 600-cm³ nutrient suspension consisting of 35 g Czapek dox broth and 20 g cornmeal per liter of distilled water was mixed into 1500-cm³ grade 2 vermiculite. The mixture was autoclaved for 50 min at 121 C, cooled for 48 h, then autoclaved again for 50 min. When the medium had cooled, several 8-mm-d disks from the cornmeal agar culture were introduced. After 5-wk growth the contents of each container were wrapped in two layers of cheesecloth, soaked with tap water for approximately 10 sec, and squeezed by hand to remove excess water and nutrient solution. This inoculum then was spread thinly on a paper-covered bench and air dried until it was friable. The dried inoculum was placed in polyethylene bags and agitated to obtain a uniform mixture.

Microplot experiments: Microplots (78 cm d) (2) were established in a Norfolk loamy sand soil at the Central Crops Research Station near Clayton, North Carolina. All plots were fumigated with methyl bromide at the rate of 100 g/m² and aerated for 2 wk.

A factorial experiment with eight treatments was used in 1979. The treatments were *A. conoides*, green alfalfa, and *M. incognita*, alone and all possible combinations, and an untreated control. Green alfalfa (900 g/plot = 5,572 kg/ha) was chopped and then incorporated 15 cm deep into designated plots. Thirty days later the appropriate plots were infested with *M. incognita* (500 cm³ of sand containing ca. 150,000 eggs and juveniles in chopped tomato roots) and *A. conoides* (500 cm³ infested vermiculite medium). Plots were limed and fertilized according to soil test recommendations. Several corn seeds (*Zea mays* L., 'Pioneer 3368A') were planted in the plots, then thinned to three plants/plot 8 days later. A randomized complete block design with seven replicates per treatment was used.

A 5 × 3 factorial experiment with 15 treatments, five inoculum densities of *M. incognita*, and three of *A. conoides* was used in 1980. Either 0, 500 cm³, or 2,000 cm³ of *A. conoides*-infested vermiculite were introduced into each plot. Ten days later, 0, 7,100, 71,000, 355,000, or 710,000 *M. incognita* juveniles and eggs were added to the appropriate plots. The plots were hand weeded and sprayed with carbaryl to reduce insect populations as needed. A randomized complete block design with four replicates was used.

Greenhouse experiments: The treatments were the same as in the 1979 microplot experiment. A steam-sterilized mixture of equal parts sand and sandy loam soil were placed in 15-cm-d clay pots. Twenty grams of chopped alfalfa and 100 cm³ of *A. conoides*-infested vermiculite were added to the appropriate pots 2 wk before planting. Just before planting, 10,000 *M. incognita* eggs were mixed with the potting medium in selected pots. Corn was sown and pots watered regularly and fertilized as needed with a complete fertilizer.

At 50 days after planting, plants were harvested, root and shoot weights recorded, root gall index (0 = 0, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = 101 + galls) determined, and 200-cm³ soil samples taken from each pot for nematode and fungus assay. The remaining soil was returned to the pot and 300 cm³ of fresh potting soil was added. The pots were then replanted with corn and returned to the greenhouse. Fifty days later plants were harvested and data were taken as before. A randomized complete block design with five replicates was used. Results in both experiments were similar, so the data were combined for analysis.

Sampling and statistical analyses: Soil samples for nematode and fungus assays were collected from the microplots three times during the growing season (at 50, 80, and 120 days in 1979; 40, 70, and 130 days in 1980). Each sample consisted of 6–8 cores/plot, taken with a 2.5-cm-d soil

sampling tube to a depth of 15–20 cm. A 200-cm³ subsample was processed from each composite sample. In the greenhouse, soil samples were collected when the tests were terminated and a 200-cm³ aliquant processed. Juveniles and roots were extracted from each sample by a combination of elutriation and centrifugation (1). Egg masses attached to roots or in soil were dispersed with 0.5% NaOCl (1). *Arthrobotrys conoides* was reisolated from microplot and greenhouse soils by a baited plates method (3). Nematode population data were transformed to $\log_{10}$ (X + 1) or $\log_{10}$ (X), for statistical analysis.

RESULTS

Microplot experiments: In 1979 the population density of *Meloidogyne incognita* juveniles and eggs was suppressed in plots containing *A. conoides* and green alfalfa after 50 days, but at harvest (120 days after planting) only juveniles were suppressed (Table 1). Similarly, *M. incognita* juveniles and egg populations at 40 days, and juveniles at 70 days in 1980 were suppressed in plots containing *A. conoides* (Table 2).

Arthrobotrys conoides was recovered from plots infested with the fungus or amended with green alfalfa (Tables 1 and 2). Greatest recovery was at 40 or 50 days after planting and then decreased with each sampling time to harvest. *M. incognita* suppressed yield in 1979 in plots without *A. conoides*. Both the fungus and the alfalfa mulch enhanced yields, but the effects were independent of each other. There were growth differences early in 1980, but yields were not different (Table 2).

Greenhouse experiments: Results of the two greenhouse experiments were similar, so only the first experiment is discussed. The addition of *A. conoides* and/or alfalfa mulch, both alone and in combination, reduced the numbers of *M. incognita* by 43–64% and the amount of root galling by 31–33% (Table 3).

Meloidogyne incognita alone suppressed shoot and root fresh weights compared to the untreated control (Table 3). The addition of *A. conoides* and/or green alfalfa nematode-infested soil increased corn fresh weight compared to the nematode alone treatment. Plant weights in alfalfa-amended soils were greater than in the untreated control, but were not different among the alfalfa-amended treatments (Table 3).

DISCUSSION

Suppression of *Meloidogyne incognita* populations and subsequent retardation of root-knot disease of corn was obtained with the use of *A. conoides* and/or green alfalfa in both microplot and greenhouse tests. Similar suppressions of *Meloidogyne* populations and disease severity with organic amendments have been reported on pineapple (9,11), tomato (19,25,26), okra (8), and tobacco (21). Two mechanisms (4,23) might be involved: i) the decomposition products released from soil amendments into soil by soil micro-organisms are directly toxic to plant nematodes; ii) the addition of soil amendments initiates a succession of events favoring the build-up of bacteria, microbivorous nematodes, nematode-trapping fungi, and other soil organisms that are antagonistic to nematodes.

The consistent recovery of *A. conoides* from alfalfa-amended soil, even though it was not added, is suggestive of contamination or a favorable environment for its establishment. The latter is the most probable explanation because *A. conoides* was also recovered in tests utilizing autoclaved alfalfa (Al-Hazmi, unpublished data).

The successful establishment of *A. conoides* and subsequent control of *M. incognita* in these experiments may be related to several factors. The soil was fumigated which reduced the populations of competing organisms. A vigorous culture of the fungus was incorporated into the fumigated soil to which a large population of *M. incognita* was subsequently added. Edaphic and other ecological factors not determined may also have been favorable during this experiment. The ecological relationships of nematode-trapping fungi are variable and complex, and very little is known about how effective the fungi are in suppressing specific nematode populations (17).

Although corn supports high populations of *M. incognita* in the field, chemical control of this nematode has rarely resulted

Table 1. Numbers† of *Meloidogyne incognita* juveniles and eggs/200 cm³ soil, and the percentage recovery of *Arthrobotrys conoids* at 50, 80, and 120 days after planting corn (*Zea mays* 'Pioneer 3368A') in microplots amended with alfalfa and/or infested with *A. conoides* and/or *M. incognita*. Grain yield at 120 days after planting is also shown. 1979.

Treatment	Days after planting									Yield‡ (g/microplot)§
	50			80			120			
	Juveniles	Eggs	% Ac recovery	Juveniles	Eggs	% Ac recovery	Juveniles	Eggs	% Ac recovery	
Control	—	—	0	—	—	0	—	—	0	650
M. incognita (N)	384	27058	0	652	3908	0	2314	2252	0	484
A. conoides (Ac)	—	—	100	—	—	86	—	—	71	640
Alfalfa mulch (OM)	—	—	100	—	—	100	—	—	71	757
N + Ac	148	965	100	497	2092	100	1586	2525	43	609
N + OM	154	925	57	591	2097	57	1538	3188	43	467
Ac + OM	—	—	86	—	—	86	—	—	57	679
N + Ac + OM	58	280	100	480	1857	86	732	3363	57	815
F Values:										
A. conoides	7.28*	6.27*		2.24	0.67		28.07**	0.81		4.60*
M. incognita	—	—		—	—		—	—		5.93*
Organic matter	1.38	7.80*		0.24	3.37		34.80**	0.68		10.98**
Ac × OM	1.38	0.41		0.00	0.00		1.93	0.33		1.20

*Significant at $P = 0.05$. **Significant at $P = 0.01$.
†Original data were transformed to $\log_{10}$ (X + 1) in samples collected early season and to $\log_{10}$ (X) in samples collected mid-season and at harvest.
‡Weight adjusted to 15.5% moisture.
§Microplots were 78 cm d.

Table 2. Numbers† of *Meloidogyne incognita* juveniles and eggs/200 cm³ soil, and the percentage recovery of *Arthrobotrys conoides* at 40, 70, and 130 days after planting corn (*Zea mays* 'Pioneer 3368A') in microplots amended with alfalfa and/or infested with *A. conoides* and/or *M. incognita*. Grain yield at 130 days after planting is also shown. 1980.

Treatment	Days after planting: 40 Juveniles	40 Eggs	40 % Ac recovery	70 Juveniles	70 Eggs	70 % Ac recovery	130 Juveniles	130 Eggs	130 % Ac recovery	Yield‡ (g/microplot)§
Control	0	0	0	0	0	0	0	0	0	505
A. conoides (Ac_1)§	0	0	100	0	0	100	0	0	75	515
A. conoides (Ac_2)	0	0	100	0	0	100	0	0	100	512
M. incognita (N_1)	12	70	0	35	27	0	1185	3560	0	502
$Ac_1 + N_1$	10	30	100	15	180	75	792	3150	100	509
$Ac_2 + N_1$	5	40	100	15	140	100	642	3420	75	497
M. incognita (N_2)	22	80	0	290	990	0	1440	3250	0	480
$Ac_1 + N_2$	12	50	100	145	740	75	1030	2720	50	516
$Ac_2 + N_2$	12	40	100	92	560	100	857	2850	75	457
M. incognita (N_3)	40	330	0	597	2810	0	1370	3440	0	415
$Ac_1 + N_3$	35	210	100	320	910	75	1040	2490	75	475
$Ac_2 + N_3$	22	90	100	275	790	75	487	3440	75	529
M. incognita (N_4)	47	400	0	795	1340	0	1475	3380	0	418
$Ac_1 + N_4$	30	380	100	352	1050	75	920	1960	50	452
$Ac_2 + N_4$	5	150	100	305	880	100	810	3000	100	455
F-Values:										
A. conoides (Ac)	6.83**	5.88**		12.46**	5.40**		13.88**	0.88		NS
M. incognita (N)	6.19**	14.24**		21.26**	7.45**		1.02	0.24		NS
Ac × N	1.26	1.43		1.95	1.98		0.38	0.05		NS

**Significant at $P = 0.01$.

†Original data were transformed to $\log_{10}$ (X + 1) when zeroes occurred in counts and $\log_{10}$ (X) otherwise.

‡Seed weight adjusted to 15.5% moisture.

§Ac_1 and Ac_2 = 500 cm² and 2,000 cm² media containing *A. conoides*, respectively. N_1, N_2, N_3 and N_4 = 0.1, 1.0, 5.0 and 10.0 eggs and juveniles/cm³ soil, respectively.

Table 3. Numbers of *Meloidogyne incognita* juveniles and eggs/200-cm³ soil, fresh corn shoot and root weight, galling index, and the percentage recovery of *Arthrobotrys conoides* in greenhouse experiments in which the soil was amended with green alfalfa and/or infested with *A. conoides* and/or *M. incognita*.

Treatment	Juveniles	Eggs	Fresh weight (g)		Gall index†	% Ac recovery
			Shoot	Root		
Control	—	—	101	44	—	20
M. incognita (N)	633	4344	73	27	3.6	20
A. conoides (Ac)	—	—	121	51	—	100
Alfalfa mulch (OM)	—	—	175	72	—	60
N + Ac	362	2496	107	40	2.5	100
N+ OM	284	1912	159	66	2.5	60
Ac + OM	—	—	164	67	—	100
N + Ac + OM	230	1672	162	70	2.4	100
LSD (0.05)	155	1676	21	11	0.7	
(0.01)	213	—	28	15	—	

†Gall index represents degree of infection based on number of galls on roots: 0 = none, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = greater than 100 galls per root system.

in a yield increase of corn (H. E. Duncan, personal communication). Corn growth and yield in our tests were reduced by *M. incognita* if *A. conoides* was not present. Since *A. conoides* is widespread in corn fields in North Carolina (20), *A. conoides* might be effective in limiting the damage caused by *M. incognita*. However, a better understanding of the ecological requirements of the fungus is needed before *A. conoides* can be used in the suppression of *M. incognita* in the field.

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