# The Effect of Arthrobotrys conoides on Meloidogyne incognita Population Densities in Corn as Influenced by Temperature, Fungus Inoculum Density, and Time of Fungus Introduction in the Soil[1]

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*Abstract:* In greenhouse experiments, the effect of *Arthrobotrys conoides* on *Meloidogyne incognita* population densities as affected by soil temperature, inoculum density, and green alfalfa was determined. The effect on *M. incognita* population densities was greater at a soil temperature of 25 C than at 18 or 32 C. Nematode control by *A. conoides* was most effective when the fungus was introduced into the soil 2 wk prior to nematode inoculation and planting of corn. Inoculum density of *A. conoides* was positively correlated with plant shoot weight (r = 0.81) and negatively correlated with numbers of *Meloidogyne* juveniles (r = –0.96), eggs (–0.89) and galls per gram of root (–0.91). *A. conoides* was not isolated from green alfalfa, but was isolated from alfalfa-amended soil to which no fungus had been added. *Key words:* biological control, root-knot nematode, predaceous fungus, nematophagus fungi.

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Biological control of nematodes involves complex biological and biochemical interactions. Linford (9) and Linford et al. (10) controlled *Meloidogyne* spp. by amending soil with chopped pineapple shoots. Various organic amendments have reduced population densities of *Meloidogyne* and root-knot disease severity (5,11,14,17,19). Organic compounds have direct effects on nematodes (4,19); however, bacteria and fungi may be involved in the effect of organic amendments on the disease expression caused by root-knot nematodes (12). Nematode-trapping fungi were stimulated by organic amendments (10), and have been implicated in biological control (13).

The fungus *Arthrobotrys conoides* Drech. captures nematodes by means of a sticky hyphal network (6). Nematodes are killed by the intrusion of globose structures and then the consumption of body contents by assimilative hyphae arising from the globose bodies (6).

The interaction of nematode-trapping fungi and nematodes is complex (13). Activities of nematophagous fungi may be influenced by soil pH, moisture, and temperature (8,12,20). Since *A. conoides* is common in North Carolina soils (16) and there are many questions about the ecological requirements of nematode-trapping fungi, this study was undertaken to determine 1) the effect of inoculum density and time of introduction of *A. conoides* on biological control of *Meloidogyne incognita* (Kofoid & White) Chitwood on corn, 2) the optimal soil temperature for the growth of *A. conoides,* and 3) the presence of *A. conoides* in green alfalfa.

## MATERIALS AND METHODS

All experiments were conducted in the greenhouse. *Meloidogyne incognita* (isolate NC 83–1) was cultured on tomato (*Lycopersicon esculentum* Mill. 'Rutgers'). Infected tomato roots were placed in 0.5% NaOCl to dissolve the gelatinous matrix containing the eggs (2). Aliquots containing ca. 10,000 eggs were mixed thoroughly with 1,500 cm$^3$ of a steam-sterilized soil mixture of equal parts of sandy loam soil and sand and placed into 15-cm-d pots, unless otherwise specified.

A North Carolina isolate of *A. conoides* was cultured on cornmeal agar for 3–5 days at 24 C, then transferred to another medium (1) and grown for 5 wk at room temperature so the medium would be permeated with the fungus. The medium consisted of a 600-cm$^3$ nutrient suspension containing 35 g Czapek dox broth and 20 g cornmeal per liter of distilled water mixed with 1,500 cm$^3$ grade 2 vermiculite. The mixture was autoclaved for 50 min at 121 C, cooled for 48 h, then autoclaved again for 50 min.

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The medium was soaked with water, squeezed to remove the excess water and nutrients, then air dried until it was friable. All other pots received an equal amount of medium without fungus.

Two seeds of corn (*Zea mays* L. 'Pioneer 3368A') were planted in each pot, and then thinned to one plant/pot 7 days later. The pots were arranged in a randomized complete block design. The plants were fertilized with a complete fertilizer as needed.

Shoot and root fresh weights, number of nematode galls per gram of root, and population density of *M. incognita* and *A. conoides* from soil were determined at the termination of each experiment. Juveniles and roots from 200 cm$^3$ soil were extracted with a semi-automatic elutriator (2). Final separation of juveniles from soil was done by centrifugal flotation (2). Egg masses attached to roots or in soil were extracted with 0.5% NaOCl (2). *Arthrobotrys conoides* was recovered from soil by the baited plates method (3). Regression and analysis of variance were the principal statistics used to evaluate the data.

*Effect of soil temperature:* The soil in each experiment was infested with either *A. conoides*, *M. incognita*, both organisms, or neither organism. Plastic pots (15-cm-d) filled with *A. conoides*-infested soil or uninfested soil were placed in Wisconsin-type temperature tanks at either 18, 25, or 32 C with ambient temperatures ranging from 20 to 28 C. One week later, eggs of *M. incognita* were mixed into soil from selected pots and corn was planted in all pots. Treatments were replicated six times (one pot/replication). The experiment was terminated after 56 days.

In order to determine growth rate of *A. conoides*, a piece of mycelium was placed in the center of a 100-mm-d petri plate containing cornmeal agar. Six plates were placed in each incubator at 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34 C. The diameter of the cultures was measured at 48, 72, 96, 120, and 144 h.

*Time of fungus introduction: A. conoides* was mixed into the soil at 0, 2, 4, or 6 wk prior to nematode inoculation and planting to corn. The treatments were placed on a greenhouse bench, arranged in a randomized complete block design, and replicated six times. The experiment was concluded 70 days later.

*Inoculum density of A. conoides:* Five inoculum levels (0, 50, 100, 150, or 200 cm$^3$) of the nutrient-enriched verminculite medium infested with mycelium of *A. conoides* were mixed thoroughly into 1,500 cm$^3$ steam sterilized soil. Seven days later, *M. incognita* eggs were mixed into the soil and corn planted. Treatments were arranged on a greenhouse bench in a randomized complete block design with five replications (one pot/replication). The experiment was terminated 70 days later.

*Root penetration by M. incognita:* A 3 × 3 factorial experiment was used with the factors being three inoculum densities of *M. incognita* and three of *A. conoides*. Aliquants of 0, 30, or 60 cm$^3$ of the fungus-infested, nutrient enriched vermiculite medium were mixed thoroughly into the potting soil and kept moist. One week later, 0, 3,000, or 6,000 eggs of *M. incognita* were mixed into the soil of selected pots and corn planted. The treatments were replicated 16 times (one pot/replication). Four replicates of each treatment were evaluated at four times: 4, 8, 16, and 32 days after planting. Soil was gently washed from the roots with running tap water. The roots were chopped and 1-g subsamples were stained with lactophenol-acid fuchsin. After clearing overnight in lactophenol, the root samples were pressed between two glass slides and nematodes inside the roots were observed and counted using a stereoscopic microscope.

*Determining presence of A. conoides in green alfalfa:* Green alfalfa was collected from the North Carolina State University Research Farm Unit 2 at Raleigh. The alfalfa was chopped, half was steamed for 30 min (121 C) to kill any associated organisms, and the other half was untreated. A sample from the steamed and nonsteamed alfalfa was plated on water agar medium to determine presence of *A. conoides*.

A 3 × 2 factorial experiment was also conducted. Treatments were 1) no alfalfa, 2) 20 g steamed alfalfa, and 3) 20 g unsteamed alfalfa. The alfalfa was incorporated into the soil. Two weeks later half of the pots were infested with *M. incognita* eggs and planted with corn. The experi-

ment was terminated 60 days later. *A. conoides* was not artificially added to the soil.

## RESULTS

*Effect of soil temperature:* Numbers of *Meloidogyne incognita* juveniles were reduced in soils infested with *A. conoides* at all soil temperatures but significantly ($P = 0.01$) only at 25 C (Table 1). Similar results occurred with root galling. Nematode populations were greatest at 32 C and least at 18 C.

Plant biomass was similar at 18 C among treatments (Table 2). Compared to the control, shoot weight was suppressed ($P = 0.05$) by *M. incognita* alone at 25 and 32 C, but not in the *M. incognita* + *A. conoides* treatments. Root weights were not significantly affected by any treatment. *A. conoides* was recovered from all pots to which it was added at 18 and 25 C, but from only 33% of the pots at 32 C.

The optimum temperature for radial growth in vitro was in the 24–28 C range (Table 3). Little mycelial growth occurred at 32 C and none at 34 C. The rate of mycelial growth was reduced as the temperature was lowered from 26 C.

*Time of fungus introduction: Meloidogyne incognita* juveniles and gall numbers were less ($P = 0.01$) in all *A. conoides*-infested pots (Table 4). The ranges of suppression for the various fungus treatments were 54–88%, 45–55%, and 67–84% for juveniles, eggs, and galls per gram root, respectively. Fewest juveniles and galls occurred when the fungus was added to the

Table 1. Numbers of *Meloidogyne incognita* juveniles, eggs, and galls on corn in response to temperature and presence of *Arthrobotrys conoides*.*

| | Soil temperature | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 18 C | | | 25 C | | | 32 C | | |
| Treatment | Juveniles | Eggs | Galls/g root | Juveniles | Eggs | Galls/g root | Juveniles | Eggs | Galls/g root |
| *M. incognita* | 40 | 208 | 3.0 | 348 | 6648 | 6.5 | 1368 | 23064 | 13.9 |
| *M. incognita* + *A. conoides* | 24 | 208 | 2.6 | 96 | 4448 | 3.8 | 900 | 12688 | 11.1 |
| LSD (0.05) | NS | NS | NS | 114 | NS | 1.0 | NS | NS | NS |
| (0.01) | | | | 166 | | 1.5 | | | |
| % Reduction | 40 | 0.0 | 14 | 72 | 33 | 41.0 | 34 | 45 | 20 |

*Initial population density of *M. incognita* was 10,000 eggs/pot; that of *A. conoides* was 100 $cm^3$ of a nutrient-enriched vermiculite permeated with mycelium.

Table 2. Fresh root and shoot weight of corn grown in soil at 18, 25, 32 C and infested with *Meloidogyne incognita* and/or *Arthrobotrys conoides*.

| | Soil temperature | | | | | |
|---|---|---|---|---|---|---|
| | 18 C | | 25 C | | 32 C | |
| Treatment | Shoot wt (g) | Root wt (g) | Shoot wt (g) | Root wt (g) | Shoot wt (g) | Root wt (g) |
| Control | 94 | 41 | 131 | 55 | 143 | 59 |
| *A. conoides* | 90 | 39 | 129 | 52 | 140 | 45 |
| *M. incognita* | 93 | 37 | 109 | 41 | 129 | 35 |
| *M. incognita* + *A. conoides* | 92 | 41 | 127 | 53 | 133 | 40 |
| LSD (0.05) | NS | NS | 6.57 | NS | 11 | NS |
| (0.01) | | | 9.24 | | | |

Table 3. Colony diameter (cm) of *Arthrobotrys conoides* grown on cornmeal agar at 10 temperatures ranging from 16 to 34 C.

| Time (h) | Temperature (C) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 32 | 34 |
| 48 | 1.6 | 2.5 | 3.0 | 3.3 | 4.1 | 4.6 | 4.4 | 2.0 | 0.8 | 0.0 |
| 72 | 2.3 | 3.4 | 3.5 | 4.5 | 6.4 | 6.6 | 6.7 | 3.0 | 0.8 | 0.0 |
| 96 | 3.0 | 4.0 | 4.1 | 6.5 | 7.6 | 8.7 | 9.0 | 4.8 | 0.9 | 0.0 |
| 120 | 3.8 | 5.0 | 4.9 | 7.3 | >9.0 | >9.0 | >9.0 | 7.2 | 0.9 | 0.0 |
| 144 | 4.4 | 6.4 | 6.5 | >9.0 | >9.0 | >9.0 | >9.0 | >9.0 | 0.9 | 0.0 |

soil 2 wk prior to nematode infestation and planting, but the difference was not significant among other fungus treatments.

Shoot and root weight were suppressed ($P = 0.01$) by *M. incognita* (Table 4). Fresh plant weights were not significantly different among the control and *A. conoides* treatments, except root weights were less ($P = 0.05$) than in the control when the fungus was added 4 or 6 wk before the nematode.

*Inoculum density of A. conoides:* Fungus inoculum density was negatively correlated with numbers of juveniles, eggs, and galls ($r = -0.96$, $-0.89$, and $-0.91$, respectively). Inoculum density was positively correlated with root and shoot wt ($r = 0.81$ and 0.92, respectively) (Table 5).

*Root penetration by M. incognita:* In comparison to the control, fewer ($P = 0.01$) *M. incognita* juveniles penetrated roots after the addition of *A. conoides* at all sampling times (Table 6). The maximum reduction in number of nematodes per gram of root was 95%. All inoculum densities of *A. conoides* tested inhibited penetration by *M. incognita*. When *A. conoides* was present, most nematodes inside roots were juveniles at 32 days; none of the adults had produced eggs. However, when *A. conoides* was not present, almost all nematodes were female adults with eggs at 32 days.

*Determining presence of A. conoides in organic matter: Arthrobotrys conoides* was not isolated from steamed or nonsteamed fresh alfalfa. Numbers of juveniles, eggs, and galls were fewer ($P = 0.01$) and plant growth was greater ($P = 0.05$) in alfalfa-amended soil, whether steamed or nonsteamed, than in nonamended soil (Table 7). Numbers of galls, juveniles, and eggs were 86, 55, and 87% less in pots amended with nonsteamed alfalfa, respectively. At harvest, *A. conoides* was isolated in 60% of alfalfa amended pots (Table 7).

## DISCUSSION

The addition of *A. conoides* to *M. incognita*-infested soil suppressed juvenile

Table 4. The effect of *Arthrobotrys conoides* and/or *Meloidogyne incognita* on corn shoot and root fresh weight, number of nematode juveniles and eggs/200 $cm^3$ soil, number of galls/gram root, and the percentage of pots from which *A. conoides* was recovered.

| Treatment | Shoot wt (g) | Root wt (g) | Juveniles | Eggs | Galls | Fungus recovery (%) |
|---|---|---|---|---|---|---|
| Control | 172 | 84 | 0 | 0 | 0 | 0 |
| *A. conoides* (F) | 170 | 78 | 0 | 0 | 0 | |
| *M. incognita* (N) | 108 | 52 | 738 | 13193 | 15.9 | 0 |
| N + F simultaneously | 168 | 75 | 343 | 7240 | 5.2 | 100 |
| F + N 2 wk later | 171 | 81 | 166 | 7240 | 2.6 | 100 |
| F + N 4 wk later | 155 | 67 | 255 | 6020 | 4.9 | 83 |
| F + N 6 wk later | 153 | 66 | 261 | 5920 | 4.3 | 83 |
| LSD (0.05) | 21 | 16 | 217 | NS | 2.4 | |
| (0.01) | 28 | 21 | 293 | NS | 3.3 | |

Table 5. Corn shoot and root fresh weight, numbers of *Meloidogyne incognita* juveniles and eggs/200 cm³ soil, number of galls/gram root, and the percentage of pots with *Arthrobotrys conoides* as affected by inoculum densities of *A. conoides*.

| Treatment | Shoot* wt (g) | Root* wt (g) | Juveniles | Eggs | Galls | Fungus recovery (%) |
|---|---|---|---|---|---|---|
| Control | 260a | 146b | | | | 20 |
| *M. incognita* (N) | 202c | 120c | 212a | 3112a | 7.8a | 20 |
| *A. conoides* ($F_1$)† | 252a | 170a | | | | 100 |
| N + $F_1$ | 230abc | 124c | 128ab | 3376ab | 4.3b | 80 |
| N + $F_2$ | 208bc | 123c | 104b | 1296ab | 2.3c | 100 |
| N + $F_3$ | 233ab | 138b | 88b | 1328ab | 2.1cd | 100 |
| N + $F_4$ | 257a | 148b | 22c | 904b | 1.4d | 100 |

*Means in columns followed by the same letter are not significantly different at $P = 0.05$. Data were transformed to $\log_{10}$ (X) and multiple range comparisons determined using the least significant difference.

†$F_1, \ldots F_4$ = *A. conoides* inoculum densities (50, 100, 150, and 200 cm³ of a nutrient-enriched vermiculite medium permeated with *A. conoides* mycelium per 15-cm-d pots, respectively).

numbers and gall development and increased corn root and shoot weight. Predation, the most likely explanation for this suppression, requires mycelial growth and trap formation (4). The optimal temperature for mycelial growth in culture (24–28 C) corresponded well with the temperature at which greatest nematode reduction occurred (25 C). Infected nematode cadavers were not observed, but the suppressive effects on the nematode populations and disease severity are circumstantial evidence that considerable biological predation was occurring.

Species of nematophagous fungi have different optimum temperatures for activity. The optimum temperature for growth of *Dactylella oviparasitica* Stirling and Mankau was in the 24–27 C range in vitro, but active parasitism of *M. incognita* eggs occurred at lower temperatures (20). Parasitism of *Heterodera schachtii* eggs by *Acremonium strictum* was greater at 24 C than at 28 C, whereas parasitism by *Fusarium oxysporum* was similar at both temperatures (15). Based on the growth rate of *A. conoides* in vitro, predation would be most active between 24 and 28 C.

Table 6. Effect of *Arthrobotrys conoides* on the penetration of corn roots by *Meloidogyne incognita* juveniles.

| Treatment† | Nematodes/gram of roots | | | |
|---|---|---|---|---|
| | 4 days | 8 days | 16 days | 32 days |
| *M. incognita* ($N_1$) | 31 | 40 | 68 | 40 |
| $F_1$ + $N_1$ | 2 | 14 | 16 | 4 |
| $F_2$ + $N_1$ | 2 | 14 | 5 | 2 |
| *M. incognita* ($N_2$) | 105 | 88 | 100 | 56 |
| $F_1$ + $N_2$ | 20 | 5 | 35 | 6 |
| $F_2$ + $N_2$ | 38 | 2 | 15 | 3 |
| | Analysis of Variance F-values‡ | | | |
| *A. conoides* | 9.87** | 121.71** | 133.52** | 45.41** |
| *M. incognita* | 14.08** | 7.36* | 37.88** | 2.06 |
| *A. conoides* × *M. incognita* | 2.02 | 32.57** | 2.85 | 1.20 |

†$F_1$ and $F_2$ represent 30 and 60 cm³ of a nutrient-enriched medium permeated with *A. conoides* mycelium/10-cm-d pot, respectively. $N_1$ and $N_2$ represent 3,000 and 6,000 eggs of *M. incognita*/10-cm-d pot, respectively.

‡Asterisks * and ** indicate significance at $P = 0.05$ and $P = 0.01$, respectively.

Table 7. Numbers of *Meloidogyne incognita* juveniles and eggs/200 $cm^3$ soil, number of galls/gram root, corn shoot and root weight, and percentage recovery of *Arthrobotrys conoides* from soil infested with *M. incognita* and/or *A. conoides* and amended with steamed and nonsteamed green alfalfa.

| Treatment | Shoot wt (g) | Root wt (g) | Juveniles | Eggs | Galls | Fungus recovery (%) |
|---|---|---|---|---|---|---|
| Control | 190 | 80 | | | | 20 |
| *M. incognita* (N) | 125 | 55 | 380 | 9008 | 9.9 | 20 |
| Non-steamed green alfalfa (OM) | 208 | 91 | | | | 20 |
| Steamed green alfalfa (SOM) | 208 | 93 | | | | 60 |
| OM + N | 177 | 72 | 172 | 1152 | 1.2 | 60 |
| SOM + N | 186 | 81 | 168 | 1384 | 1.8 | 60 |
| LSD (0.05) | 25 | 15 | 78 | 3101 | 2.0 | |
| (0.01) | 34 | 21 | 110 | 4348 | 2.8 | |

Nematode development and activity are slower at the temperature optimum for the fungus than they are in warmer soils (7).

The presence of *A. conoides* was more important than time of introduction, although it might be advantageous to have an established fungal population. Organic matter such as green alfalfa apparently provides good conditions for the establishment of the fungus. Earlier experiments (1,5,9,10, 11,12,14) have shown that addition of organic matter enhances nematode control and plant growth. The benefit of adding organic matter to soil may result from increased soil fertility as well as the establishment of *A. conoides*. Growth of additional micro-organisms antagonistic to nematodes may also be stimulated simultaneously by the addition of organic soil amendments (18).

Although there was a trend toward fewer *M. incognita* eggs produced in treatments with *A. conoides* than in those without the fungus, egg numbers generally were not significantly different among treatments with or without the fungus. The decrease in nematode penetration may allow nematodes infecting the root to produce more eggs. Even though plant weights were significantly greater in *M. incognita*-infested pots with *A. conoides* than in those without the fungus, the inoculum potential of the nematode remains high and could be a hazard to the subsequent crop.

It is difficult to demonstrate yield losses of corn due to *M. incognita* in North Carolina, even though corn is a good host. *A. conoides* is widespread in agricultural soils in North Carolina (16) and may negate some damage on corn caused by *M. incognita*.

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