Fundam. appl. Nematol., 1993, **16** (4), 321-324

Effect of *Arthrobotrys irregularis* on *Meloidogyne arenaria* on tomato plants

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Accepted for publication 4 November 1992.

Summary – The effectiveness of *Arthrobotrys irregularis* against *Meloidogyne arenaria* on tomato plants was tested in experiments carried out in a growth chamber. The application of the fungus in the soil reduced root galling at both rates tested (1 % and 10 %) and forms of inoculum. Galling was reduced at all population densities and the plant weight was influenced by the presence of the fungus in the soil.

Résumé – *Action d'*Arthrobotrys irregularis *envers* Meloidogyne arenaria *sur tomate* – L'action d'*Arthrobotrys irregularis* envers *Meloidogyne arenaria* sur tomate a été étudiée lors d'expériences en chambre de culture. L'incorporation du champignon au sol réduit le nombre des galles, indépendamment du type et des taux d'inoculum (1 et 10 %) utilisés. Le nombre de galles diminue quelle que soit la densité des populations, et le poids du végétal est influencé par la présence du champignon dans le sol.

Key-words : Nematode trapping fungi, *Arthrobotrys, Meloidogyne arenaria,* biological control.

Arthrobotrys (Hyphomycetes) has been tested against *Meloidogyne* since 1975, by Uladova. Zopf (1888) first demonstrated the capture of living eelworms by *Arthrobotrys oligospora*. The fungus attracts the nematodes by a mechanism which is not fully understood. Nematode excretion products and direct contact with hyphae play a role in the initiation of trap formation which is also dependent on environmental conditions (Nordbring-Hertz, 1977). Once traps are present the nematodes are captured within one hour (Nordbring-Hertz & Stalhammar-Carlemaln, 1978). Trap formation induced by nematodes has been correlated with motility of nematodes (Jansson & Nordbring-Hertz, 1981).

Arthrobotrys irregularis has been tested for the control of *M. incognita* on tomato. A commercial product, Royal 350, based on *A. irregularis* was used in pot and field experiments (Cayrol, 1983; Pelagatti *et al.*, 1986) and in the laboratory (Cayrol, 1983). In the pot experiment, sterilized soil was used and *Arthrobotrys* was applied 40 days before planting and inoculation with *Meloidogyne* juveniles. In field experiments the fungus was applied in *Meloidogyne* infested soil. Both investigators used the same dose (140 g/m²) and they obtained significant control.

Arthrobotrys oligospora did not provide effective control of *Meloidogyne* (Kunkum & Gopal, 1985).

The recent awareness of the possible danger of toxic pesticides, and the continued intensification of agriculture, and the need for better methods for managing pests, is likely to promote research on biological control methods and their integration in control programmes.

This work was the first attempt to seek better methods for minimizing the number of nematodes in the vegetable crops in Crete where large amount of chemicals are used every year for soil fumigation. Nematodes of the genus *Meloidogyne* are the most widespread in the Island of Crete.

Materials and methods

CULTURE OF NEMATODES FOR EXPERIMENTS

Egg masses of *Meloidogyne* species were collected from heavily infested roots of tomato plants and transferred to a 80-100 μm aperture sieve (ten to fifteen per dish) without filter paper. The sieve was placed in a small dish filled with water such that the mesh touched the water. It was left overnight at 10-15 °C and it was transferred into an incubator at 23 °C for 5-10 days. Nematodes were collected every other day and kept at 10-15 °C. When enough juveniles (J2) had hatched the suspension was poured on the funnel of the micropore filter connected to the water pump. A filter paper of 8 μm aperture was placed in the micropore. When the water had nearly passed through, 100 ml of sterile water was added, slowly, and allowed to pass so the cuticles of nematodes were washed to remove bacteria and spores of fungi that may have been attached. When the water had passed through, the filter paper was removed and placed in a beaker with sterile water. These nematodes were inoculated onto tomato plants cv. Dombo and used throughout the experiments.

CULTURE OR *ARTHROBOTRYS* FOR APPLICATION IN THE SOIL

The fungus was cultured on potato dextrose or corn meal agar (PDA or CMA) for 3 to 5 days at 25 °C and then transferred into a modified vermiculite medium

and allowed to grow for 5 weeks at room temperature (25 °C).

The medium consisted of nutrient suspension (1000 ml) containing 24 g potato dextrose and 20 g oat meal per 100 ml distilled water, mixed with 2500 ml vermiculite. The mixture was autoclaved for 50 min at 121 °C, cooled for 48 h and autoclaved again. After the medium was cooled, four discs from the edges of *Arthrobotrys* cultures were introduced to each flask with the vermiculite medium. After one week the flasks were shaken, and after five weeks the material was washed between two layers of cheese cloth, squeezed by hand to remove the excess water and nutrient solution. The medium was then spread on a paper covered bench and allowed to dry at room temperature. A part of the modified medium was not inoculated with the fungus but treated in the same way, to be used as a control.

Effect of the form of inoculum and application rate

The nematode population used in this experiment originated from Crete (Greece) and was cultured as described. The predominant species in this population was *Meloidogyne arenaria*.

Arthrobotrys irregularis was cultured and grown as described and half of the medium (colonized or not) was not washed. The colonized medium was added to steam sterile sandy soil at the rates 1 and 10 % (v/v) soil. Uncolonized medium was mixed at the same rates and added to the pots and unamended soil added to others to act as control treatments.

The inoculum was thoroughly mixed with the soil before dispensing 1500 ml of the mixture to plastic pots.

The pots were planted after 15 days with one-month-old tomato plants and 500 J2 (age 2-7 days) were added to the pots two days before planting.

Each treatment was replicated five times and the pots were arranged in randomized blocks in a growth chamber with 14 h photoperiod. The soil mean temperature was 25 °C and the soil moisture was maintained at 80 % field capacity.

The number of galls in the roots of the tomato plants were counted 55 days after planting and it was expressed as number of galls per gram of root system.

Influence of the nematode population density

Meloidogyne arenaria was derived from single egg masses from previously identified females extrated from cucumber roots and was cultured as described.

Nutrient enriched medium without washing was added to the steam sterile soil at the rate 10 % (v/v). Uncolonized medium was mixed at the same rate to act as control treatments.

The nematodes were applied at the levels of 0, 250, 750, and 1000 J2 per plant (N0, N1, N2, N3). Application of fungus and nematodes was carried out in the same way as the previous experiment.

The experiment was terminated 40 days after planting and the following measurements were taken :

- galls per gram root,
- fresh top weights,
- root weights,
- leaf water content,
- leaf nutrient analysis was performed in the atomic absorption analyzer. For this purpose, two leaves were collected from each plant, washed four times in distilled water and dried at 60 °C. Nematode data were transformed in the $\log_{10}$ scale for statistical analysis.

Results

Effect of the form of inoculum and application rate of *A. irregularis* on *Meloidogyne* on tomato plants

The fungus application, either washed of nutrients or not, gave significant control ($P < 0.001$) of root galling of tomato plants. No differences were observed between the fungus treatments (Table 1).

Table 1. Effect of *Arthrobotrys irregularis* on gall production of *Meloidogyne arenaria* on tomato plants 55 days after planting. Number of galls per g roots.

Treatment		Washed	Unwashed
No amendment			69.49 *b*
(Vermiculite only)	1	107.62 *b*	90.40 *b*
	10	47.28 *b*	170.05 *b*
Fungus	1	3.11 *a*	4.67 *a*
	10	6.54 *a*	5.21 *a*

* Means within columns followed by the same letter are not significantly different ($P < 0.001$).

There was a significant difference ($P < 0.05$) between the washed and the unwashed vermiculite medium (not inoculated) at 10 % inoculum level [LSD = 77.09 (0.53 log scale)].

The reduction in galling in percentages fluctuated between 86 to 97 % between the various fungus treatments compared to their control and 91-95 % when the fungus treatments were compared to non-amended soil.

Nematode population density

There was strong evidence for differences between the control treatments and the fungus inoculated treatments and the fungus inoculated ($P = 0.001$) concerning the root galling of tomato plants (Table 2). Galling was reduced to 48, 41 and 43 % respectively for the 250, 750 and 1000 J2 treatments.

Table 2. Tomato top weights, leaf water content and number of galls per g root as affected by different levels of *Meloidogyne arenaria* and *Arthrobotrys irregularis*.

Treatment	Shoot weight (g)	Water content (%)	Galls (n)
No fungus			
N0 (J2 = 0)	59.51 *b*	88.21	0 *a*
N1 (J2 = 250)	58.64 *b*	87.12	5.42 *a*
N2 (J2 = 750)	59.41 *b*	87.40	15.39 *b*
N3 (J2 = 1000)	54.60 *ab*	87.68	12.60 *b*
Fungus			
N0 (J2 = 0)	50.34 *a*	87.15	0 *a*
N1 (J2 = 250)	57.88 *b*	87.66	2 61 *a*
N2 (J2 = 750)	54.52 *a*	87.24	6.23 *a*
N3 (J2 = 1000)	50.03 *a*	87.51	5.35 *a*

* Each figure is mean of five replicates.
* Means followed by the same letter are not significantly different ($P < 0.001$).

The leaf water content was similar in all treatments. Fresh plant shoot weight seemed to be reduced by the fungus (Table 2). From the micronutrient concentration in leaves (Table 3). Boron approaches the deficiency level (30 ppm) and Mn is increased in the fungus treatments significantly ($P = 0.05$).

Table 3. Leaf concentration of macro- and micronutrients in tomatoes 45 days after planting.

	% dry matter					ppm dry matter			
	N	P	K	Ca	Mg	B	Fe	Mn	Zn
V0	3.72	0.30	5.03	1.40	0.99	26.10	85.81	251.60	22.65
V1	3.60	0.25	5.07	1.38	0.96	22.70	93.22	255.27	18.30
V2	3.85	0.31	5.56	1.72	1.02	26.90	111.03	299.46	26.97
V3	3.70	0.25	4.32	1.15	0.77	24.60	93.22	257.59	23.19
A0	3.37	0.34	4.64	1.08	0.76	28.30	85.89	302.22	23.52
A1	4.39	0.36	5.24	1.42	1.04	28.35	88.12	314.00	30.25
A2	3.57	0.29	5.90	2.05	1.13	26.20	99.92	326.89	29.61
A3	3.39	0.23	5.69	1.83	1.23	26.25	100.95	362.77	32.35

(All figures on the table are means of two samples.)
V = vermiculite only; A = vermiculite inoculated with fungus.
0..3 = 0, 250, 750, 1000 J2 per plant.

Discussion

The effect of *A. irregularis* on *Meloidogyne arenaria* was quite good concerning the reduction of root galling which was significantly lower in plants treated with the vermiculite inoculated with the fungus. There was no effect of the increased inoculum level in either experiments.

The form of inoculum (washed of unwashed) did not influence the gall development on the roots and the control level of nematodes was similar (Table 1).

In Table 2 we can see a distinct difference concerning the plant growth which is not so clear on the pictures because 40 days are not enough to show such differences. The retardation of plant growth is clearly evident in the treatments where no nematodes were added to the plants and this is attributed to the fungus only. This type of fungi excretes, during its metabolism, products like phenols which can be toxic to the plants (Domsch *et al.*, 1980). By washing the inoculum medium most of these products, produced during the five-week incubation, are possibly removed. From our experience (unpublished data) the vermiculite itself improves the plant growth.

After adding the fungus inoculum to the soil it is possible that the soil microfauna changes and other microorganisms make the Mn in the soil more available to the plants, so its concentration in the leaves of the plants which received the fungus treatments was higher than in the plants which received the vermiculite only.

Despite the adverse effect of the fungus it was effective against the nematodes. Galling was reduced at all nematode population densities. This reduction was more spectacular in the first experiment because there the plants were maintained for 55 days and part of the galls were due to a partial second generation.

Pelaggatti *et al.* (1986) reported good results by adding nematodes in tomato plants two days after the fungus incorporation into the soil.

It is notable that in our experiments the low fungus inoculum level suppressed the nematode development as well as the high inoculum level, because it minimizes the amount of medium to be prepared and applied to 20 m³/ha. Doubling the amount of inoculum did not influence the predacity of the fungus (Jansson, 1982). Cayrol (1984) suggested as an effective dose 1.4 t/ha. Al-Hazmi *et al.* (1982), although he used 300 m³/ha to reduce the number of nematodes in the soil, observed that there were many left in the soil at the end of the experiment.

For the correct evaluation of the activity of the nematode-trapping fungi it is essential that, when they are added into the soil, the effect of the amendment added in the soil is taken into account. It is important to have the appropriate control to compare with, otherwise the results might be misleading because these changes in the soil environment influence plant growth, directly or indirectly.

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