

final : 1988

FRG

EFFECT OF DIFFERENT WEST AFRICAN SPECIES
AND STRAINS OF *ARTHROBOTRYS* NEMATOPHAGOUS FUNGI
ON *MELOIDOGYNE* SPECIES

R. DUPONNOIS⁽¹⁾, T. MATEILLE⁽¹⁾, V. SENE⁽²⁾, A. SAWADOGO⁽³⁾ & M. FARGETTE⁽⁴⁾

⁽¹⁾ ORSTOM, B.P. 1386, Dakar (Sénégal). Fax: (221) 32.16.75;
E-mail: duponnoi@belair.orstom.sn and mateille@belair.orstom.sn

⁽²⁾ ISTOM, Institut d'Agro-Développement International,
32 Boulevard du Port, 95094 Cergy-Pontoise cedex (France).

⁽³⁾ INERA, Protection des végétaux, BP 403, Bobo Dioulasso (Burkina Faso). Fax: (226) 97.09.60;

⁽⁴⁾ ORSTOM/CIRAD, B.P. 5035, 34032 Montpellier cedex (France). Fax: (33) 467.61.55.81;
E-mail: fargette@orstom.orstom.fr

Different species of *Arthrobotrys* nematophagous fungi and several strains of *A. oligospora* have been studied for their antagonistic effects against nematodes of the genus *Meloidogyne*, important pests of vegetables. All fungi trapped *M. mayaguensis* and *M. incognita* juveniles *in vitro* but had no effect on the juveniles of *M. javanica*. In pot experiments with *M. mayaguensis*, all fungi reduced the nematode populations and stimulated the growth of tomato seedlings. In a field trial, a strain of *A. oligospora*, isolated in Senegal and incorporated into compost blocks, was efficient in increasing the tomato seedling growth. The introduction of nematophagous fungi in compost blocks as a biological biocontrol technique against phytophagous nematodes adapted for developing countries is discussed.

KEY-WORDS: nematodes, biological control, fungi, trapping activity, vegetables.

INTRODUCTION

Plant parasitic nematodes and in particular root-knot nematodes (*Meloidogyne* spp.) are cosmopolitan and important pest problems affecting the production of both subtropical and tropical crops (Johnson & Fassuliotis, 1984). During the last decades, nematode control methods have been based on the use of chemical products applied to the soil or the cultivated plants. However the toxicity of some pesticide compounds used and the intensification of agriculture have stimulated research into new methods for managing pest nematodes. Methods of biological control in particular were promoted. To control plant parasitic nematodes, investigations have focused on the application of nematode-trapping fungi. Several species of the hyphomycete *Arthrobotrys* have been tested against *Meloidogyne* spp.. *A. irregularis* has provided significant reduction of the development of *M. incognita* and *M. arenaria* (Cayrol, 1983; Pelagatti *et al.*, 1986; Vouyoukalou, 1993). In Senegal, root-knot nematodes are the most widespread pest nematodes in vegetable crops. In that country, however, studies on nematophagous fungi and on their potential in terms of nematode control are very limited.

The aims of this study by ORSTOM were to (i) test the potential of nematophagous fungal strains isolated in Burkina Faso and Senegal for trapping juveniles of *Meloidogyne*

Fonds Documentaire IRD



010022454

Fonds Documentaire IRD

Cote : B* 22454 Ex : 1

spp., (ii) develop a control method using the nematophagous fungi incorporated into compost blocks to control *Meloidogyne* populations in both pots and mixed populations of *M. javanica*, *M. incognita* and *M. mayaguensis* which are common in field vegetables in Sahelian countries.

MATERIALS AND METHODS

ISOLATION AND CULTURE OF *ARTHROBOTRYS* SP. STRAINS (Table 1)

Petri dishes were filled with diluted brewery wort (1.8-2.0 g.l⁻¹ total sugars after dilution, pH 5.5) and solidified with 20 g.l⁻¹ of agar. The plates were sprinkled with 1 g of soil sampled from several vegetable-producing areas in Senegal and Burkina Faso. The plates were sealed and incubated for one week at 25°C in the dark. Single conidia were picked off under the dissecting microscope and transferred to other Petri dishes also filled with diluted brewery wort solidified with agar (20 g.l⁻¹). These single-spore cultures were maintained aseptically in the dark at 25°C on the nutrient broth (8 g.l⁻¹) agar (20 g.l⁻¹) medium.

TABLE 1
List of the strains of trapping fungi used

Identification	Code	Origin	Author
<i>Arthrobotrys oligospora</i>	S 30	Burkina Faso	Sawadogo A.
<i>Arthrobotrys oligospora</i>	S 31	Burkina Faso	Sawadogo A.
<i>Arthrobotrys conoides</i>	S 42	Burkina Faso	Sawadogo A.
<i>Arthrobotrys</i> sp.	BF 10	Burkina Faso	Sawadogo A.
<i>Arthrobotrys</i> sp.	BF 74	Burkina Faso	Sawadogo A.
<i>Arthrobotrys</i> sp.	BF 80	Burkina Faso	Sawadogo A.
<i>Arthrobotrys</i> sp.	SOSU 2	Burkina Faso	Sawadogo A.
<i>Arthrobotrys</i> sp.	ORS 18690 S2	Sénégal	Duponnois R.
<i>Arthrobotrys oligospora</i>	ORS 18692 S5	Sénégal	Duponnois R.
<i>Arthrobotrys oligospora</i>	ORS 18692 S7	Sénégal	Duponnois R.

TRAPPING ACTIVITY

Populations of *M. mayaguensis*, *M. javanica* and *M. incognita* were reared on tomato (*Lycopersicon esculentum* Mill.), cv Roma roots. Two months after inoculation, the roots were harvested, cut into short pieces and placed in a mist chamber for one week for egg hatching (Seinhorst, 1950).

Each fungus was cultured on the nutrient broth agar medium for 2-3 weeks. Agar plugs (6 mm diam.) were taken from the margin of the fungal colonies and transferred into Petri dishes (90 mm diam.) filled with distilled water agar (20 g.l⁻¹).

One week later, 100 second stage juveniles were introduced in a water droplet into one week old fungal cultures growing in Petri dishes on distilled water agar medium. After two days, the number of juveniles trapped by the fungus were counted using a dissecting microscope. Each combination *Meloidogyne* species — fungal isolate was replicated five

Every week, the height of the plants, the mortality and number of inflorescences per plot in each treatment were determined. Every month from transplanting to the end of the experiment, one tomato plant per plot was uprooted. The nematodes were extracted from the roots and counted as described above. A 250 g sample of the soil surrounding the plant was taken from each plot and the nematodes were extracted using an elutriator (Seinhorst, 1962).

The data were treated with a one-way analysis of variance and the mean values were compared with the Student's "t" test ($P < 0.05$). For nematodes, data were previously transformed by $\log(x + 1)$.

RESULTS

TRAPPING ACTIVITY

All fungal isolates trapped juveniles of *M. mayaguensis* but in different proportions (Table 2). The optimal rate was observed with fungal strains ORS 18692 S7, S30, S31 and S42. Juveniles of *M. incognita* were mainly trapped by strains S30, S31 and S42, but strain S31 only had a trapping activity against juveniles of *M. javanica*.

TABLE 2
In vitro efficacy (% trapped *Meloidogyne* spp. juveniles) of various fungal isolates

Fungal strains	<i>M. mayaguensis</i> *	<i>M. incognita</i> *	<i>M. javanica</i> *
ORS 18690 S2	11 c	0	0
ORS 18692 S5	26 b	3 b	0
ORS 18692 S7	74 a	0	0
S 30	78 a	65 a	0
S 31	82 a	70 a	20
S 42	82 a	60 a	0
BF 10	10 c	4 b	0
BF 74	9 c	2 b	0
BF 80	14 c	2 b	0
SOSU 2	8 c	16 b	0

(*) Data followed by the same letter did not significantly differ according to the one way analysis of variance ($P > 0.05$).

GLASSHOUSE EXPERIMENT

Average height of tomato plants was significantly higher than the control when fungal isolates ORS 18690 S2, ORS 18692 S7 and S42 have been inoculated regardless of concentration (Table 3): A similar effect was also recorded in terms of shoot biomass. For the root biomass, all fungi inoculated at the rate 1:10, except strain SOSU 2, allowed a greater plant development than the control. At the rate 1:100, no effect was observed with strains BF 10 and SOSU 2, root biomass being significantly greater than the control for the other isolates.

At the rate 1:10, the number of galls per plant was higher with strain S31 than the control (Table 4), whereas strain SOSU 2 decreased the number of galls per plant. No effect

times. The trapping rate (trapped juveniles/total juveniles) was transformed by arcsin(sqrt) and treated with a one way analysis of variance, the mean values being compared with the Student's *t* - test at 0.05 probability level.

GLASSHOUSE EXPERIMENT

Solid fungus inocula were prepared in 0.5 dm³ glass flasks containing 0.3 dm³ compost made from a mixture of cattle and sheep offal (SERAS company in Thies, Senegal). Its chemical characteristics were as follows: pH H₂O 7.5; carbon 36.9%; nitrogen 20%; total cations 3.6 meq%; cation exchange 0.9 meq% and total P₂O₅ 24 000 ppm. The jars, closed with cotton wool, were autoclaved at 120°C for 20 min. Then the substrate was moistened to field capacity with liquid nutrient broth medium (8 g.l⁻¹) and autoclaved a second time (120°C for 20 min). After cooling, 8 mycelial plugs from an agar culture of each fungal isolate, were laid on top of the substrate. The cultures were incubated for 5 weeks at 25°C.

The 3 components of the system (juveniles of *M. mayaguensis*, fungal strain and tomato plants) were placed in 60 cm³ polythene cells filled with sandy soil (pH H₂O 7.1; fine silt 0.6%; coarse silt 1.4%, fine sand 61.6%, coarse sand). The soil has been previously autoclaved (140°C, 40 min) and inoculated with the fungal inoculum at 2 concentrations: 1:10 or 1:100 (v:v). Soil mixed with sterilised compost at the same concentrations but without fungus served as control. A one week old tomato seedling cv Roma was transplanted into each cell. After 1 week, the tomato plant was inoculated with a 5 ml suspension of 100 7-day-old second stage juveniles of *M. mayaguensis* (water alone for the control). The experiment was conducted in a greenhouse under natural climatic conditions (temperatures from 20°C to 35°C; about 15 hours light per 24 h). The cells were watered daily and arranged in a randomized, complete block design with 14 replicates.

One month after nematode inoculation, the height of the seedlings was measured, plants

TABLE 3

Effect of various fungal isolates inoculated at two concentrations 1:10 and 1:100 (v:v) on the growth of the tomato seedlings inoculated with 100 juveniles of M. mayaguensis per plant

Fungal strains	Height* (cm)		Shoot biomass* (mg dry weight)		Root biomass* (mg dry weight)	
	1/10	1/100	1/10	1/100	1/10	1/100
Control	24.9 b	23.1 b	327 b	406.0 b	111 b	101 b
ORS 18690 S2	34.0 a	31.3 a	609 a	573 a	217 a	194 a
ORS 18692 S7	33.4 a	31.3 a	656 a	580 a	229 a	184 a
S 31	25.3 b	22.3 b	436 b	403 b	209 a	174 a
S 42	30.9 a	31.9 a	677 a	536 ab	219 a	163 a
BF 10	20.9 b	21.7 b	409 b	313 b	246 a	109 b
SOSU 2	21.1 b	22.9 b	503 ab	344 b	150 b	140 ab

(*) Data in the same column followed by the same letter did not significantly differ according to the one way analysis of variance ($P > 0.05$).

was observed with the other treatments. At the lower level of inoculation (1:100), most nematophagous fungi, except ORS 18692 S7, decreased the number of galls per plant compared to the control treatment. The number of juveniles detected per plant was significantly reduced with all fungi inoculated at the rate 1:100 whereas the majority of the fungi had no effect on the development of *M. mayaguensis* at the rate 1:10, except strain SOSU 2 which significantly reduced the multiplication of juveniles (Table 4).

FIELD EXPERIMENT

Thirteen days after planting, the height of the tomato plants transplanted with the compost blocks was significantly greater than in the control (without blocks), the effect

TABLE 4

Effect of various fungal isolates inoculated at two concentrations 1:10 and 1:100 (v:v) on the development of M. mayaguensis juveniles in the glasshouse.

Fungal strains	Number of galls per plant*		Number of juveniles per plant*	
	1/10	1/100	1/10	1/100
Control	14.3 b	21.3 a	2 624 a	7 046 a
ORS 18690 S2	15.3 b	15.4 b	2 266 a	4 008 b
ORS 18692 S7	12.4 b	18.3 ab	1 803 a	2 112 b
S 31	19.4 a	13.6 b	3 832 a	3 611 b
S 42	12.4 b	14.4 b	1 634 a	2 470 b
BF 10	15.6 b	12.7 b	1 489 a	2 527 b
SOSU 2	8 c	12.4 b	699 b	2 132 b

(*) Data in the same column followed by the same letter did not significantly differ according to the one way analysis of variance ($P > 0.05$).

being even greater when the fungus was inoculated at the rates 1:100 and 1:50 (Table 5). At the end of the experiment (62 days), treatments which combined the use of the compost and the fungus determined a significant increase in the height of tomato plants. This cultural technique also decreased the mortality of the seedlings which was very high during the first 3 weeks of the experiment in the control. This peat block effect was strengthened when the fungal isolate was mixed with the compost at the lowest rate (1:100).

TABLE 5
Effect of Arthrobotrys oligospora ORS 18692 S7 on height and mortality of tomato plants and on the number of inflorescences per plot in the field experiment

Parameter	Treatment	Days after planting							
		13	20	27	34	41	48	55	62
Height* (cm)	Control**	4.5 c	7.5 c	9.9 c	10.4 c	12.1 c	14.4 c	16.5 c	18.3 c
	No fungus	8.6 b	12.2 b	15.6 b	16.7 b	18.2 b	20.0 b	22.0 bc	23.2 bc
	1:100	10.9 a	15.1 a	18.8 a	20.5 a	23.6 a	26.6 a	29.9 a	31.8 a
	1:50	10.5 a	14.5 a	17.5 ab	18.7 ab	21.3 ab	22.6 ab	25.3 ab	27.0 ab
	1:20	9.1 b	12.5 b	16.5 ab	17.8 ab	21.3 ab	24.2 ab	27.2 ab	29.0 ab
	1:10	8.7 b	12.8 b	16.5 ab	17.6 ab	20.1 ab	22.8 ab	25.7 ab	27.4 ab
Number of inflorescences per plot*	Control**	0	0	0	1.9 c	3.1 c	6.9 b	11.4 b	16.6 b
	No fungus	0	0	0	3.7 bc	4.9 bc	7.7 b	10.5 b	14.6 b
	1:100	0	0	0.7 a	11.2 a	16.2 a	25.4 a	30.5 a	40.7 a
	1:50	0	0	0.4 ab	8.9 ab	12.3 ab	18.4 a	24.6 a	29.6 ab
	1:20	0	0	0.1 b	9.1 ab	13.8 ab	23 a	29.9 a	36.1 ab
	1:10	0	0	0.2 ab	10.5 a	16.5 a	25.7 a	30.8 a	40.7 a
Mortality* (%)	Control**	26.0 a	43.6 a	44.4 a	44.4 a	46.8 a	51.6 a	52.4 a	52.8 a
	No fungus	8.8 b	16.4 b	20.4 b	20.8 b	22.8 b	34.0 b	36.0 b	36.8 b
	1:100	1.2 b	4.8 c	8.0 c	8.0 c	8.8 c	14.8 c	15.6 c	16.4 c
	1:50	6.0 b	11.6 bc	16.0 bc	16.4 bc	18.0 bc	24.4 bc	26.4 bc	27.6 bc
	1:20	7.6 b	16.8 b	22.4 b	22.8 b	24.0 b	29.2 b	31.2 b	33.2 b
	1:10	2.8 b	10.0 bc	12.0 bc	12.0 bc	13.6 bc	18.8 c	21.2 bc	21.6 bc

(*) For each parameter, data in the same column followed by the same letter did not significantly differ according to the one way analysis of variance ($P > 0.05$).

(**) Treatment without compost and nematophagous fungus.

Inflorescences were observed earlier in the fungal treatments than in the control (Table 5). From day 34, the number of inflorescences per plot was significantly greater in the treatments 1:100 and 1:10 than in the treatment without fungus.

Compared to the control (without compost and fungus), the use of the blocks increased the shoot biomass only towards the end of the experiment. In terms of root biomass, a stimulating effect was only observed after 3 weeks. Also the shoot and root biomass of tomato plants in the 1:100 treatment were significantly greater than in the treatment without fungus (Table 6).

No significant differences was observed on the development of the community of *M. javanica* and *M. mayaguensis* except that at the end of the experiment, the number of juveniles per plant and per dm^3 of soil were significantly greater in the 1:10 treatment than in the treatment without fungus and in the control (Table 7).

TABLE 6

Effect of Arthrobotrys oligospora ORS 18692 S7 on average shoot and root biomass of tomato plants in the field experiment

Parameters	Treatment	Days after planting		
		20	42	62
Shoot biomass* (g dry weight per plant)	Control**	0.3 c	1.28 b	4.76 c
	No fungus	0.6 bc	2.45 ab	7.07 b
	1:100	1.02 a	3.78 a	12.62 a
	1:50	0.84 ab	2.63 ab	10.34 ab
	1:20	0.51 bc	3.49 ab	10.97 ab
	1:10	0.51 bc	4.11 a	10.95 ab
Root biomass* (g dry weight per plant)	Control**	0.03 b	0.20 b	0.95 b
	No fungus	0.13 a	0.58 ab	0.65 b
	1:100	0.11 a	0.42 ab	1.76 a
	1:50	0.07 ab	0.32 ab	1.32 ab
	1:20	0.05 ab	0.43 ab	1.03 ab
	1:10	0.08 ab	0.65 a	1.40 ab

(*) For each parameter, data in the same column followed by the same letter did not significantly differ according to the one way analysis of variance ($P > 0.05$).

(**) Treatment without blocks and nematophagous fungus.

TABLE 7

Effect of Arthrobotrys oligospora ORS 18692 S7 on *Meloidogyne* spp. juvenile numbers per tomato

DISCUSSION

The various fungal isolates of the genus *Arthrobotrys* were able to trap the juveniles of *M. mayaguensis* and *M. incognita* but one strain only (S31) had some activity against *M. javanica*. The mechanisms involved during the fungal predation of the juveniles are well known (Imerglík, 1981; Gaspard & Mankau, 1987), recognition and attachment of the mycelium to the cuticle of the juvenile being mainly due to the existence of compatible glycoproteins such as lectins. The best target for these Sahelian fungi was *M. mayaguensis*.

In the glasshouse experiment, fungi had a great efficiency on the plant growth at the both inoculum rates. They however had a higher activity against *M. mayaguensis* when inoculated at the lowest rate (1:100). Soil colonization by the fungus depends on a number of environmental factors such as nutrients. These saprophytic fungi can grow on organic matter and they can also use phytoparasitic nematodes as an energy source. Moreover, the incorporation of the fungal inoculum in the soil may be accompanied with some residual simple sugars such as glucose from the culture medium. As a consequence, the fungi are offered several nutritional options and may reduce their predatory activity.

In the control treatments, nematode development was lowest when organic matter was added at the rate 1:10. It is well known that organic matter may have several direct effects on nematode populations (Akhtar & Alam, 1993). The production of some secondary breakdown products could be toxic for juveniles of *M. mayaguensis*.

In the field experiment, the association of compost blocks and the nematophagous fungus *A. oligospora* ORS 18692 S7 inoculated at the lowest rate induced a significant decrease of

RÉSUMÉ

Effet de différentes espèces et souches ouest-africaines du champignon nématophage *Arthrobotrys* sur des nématodes du genre *Meloidogyne*

Différentes espèces d'*Arthrobotrys*, champignon nématophage, et plusieurs souches d'*A. oligospora*, isolées au Sénégal et au Burkina Faso, ont été étudiées pour leur effets antagonistes envers les nématodes du genre *Meloidogyne*, parasites importants des cultures maraîchères en Afrique de l'Ouest. Toutes les souches utilisées ont piégé les juvéniles de *M. mayaguensis* et *M. incognita in vitro* mais n'ont eu aucun effet sur *M. javanica*. Testées sur culture de tomate en pots, toutes les souches ont diminué le développement des populations de *M. mayaguensis* et ont permis un meilleur développement des plantes. Testée sur culture de tomate au champ, une souche d'*A. oligospora* isolée au Sénégal et incorporée à des mottes de compost a permis une meilleure croissance des plantes. La production de plants maraîchers en mottes de compost combinée à l'incorporation de champignons

nématophages aux mottes est proposée comme une technique de lutte biologique contre les nématodes phytoparasites adaptée aux pays en voie de développement.

REFERENCES

- Akhtar, M. & Alam, M. M. — 1993. Utilization of waste materials in nematode control: a review. — *Bioresource Technology*, 3: 116-118.

