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Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil-borne plant pathogenic fungi

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Abstract

Fungi isolated from the cortical tissue of surface sterilized tomato roots collected from field plots produced secondary metabolites in nutrition broth that were highly toxic to *Meloidogyne incognita*. Especially strains of *Fusarium oxysporum* were highly active with 13 of 15 strains producing culture filtrates toxic to nematodes. The mechanism of action of the toxic metabolites produced by the non-pathogenic *F. oxysporum* strain 162 with proven biological control of *M. incognita* in pot experiments was investigated. These metabolites reduced *M. incognita* mobility within 10 min of exposure. After 60 min, 98% of juveniles were inactivated. Juveniles were initially inactivated within a few minutes of exposure, but with exposure of 5 h 50% of the juveniles were dead and 24 h exposure resulted in 100% mortality. In a bioassay with lettuce seedlings metabolite concentrations >100 mg/l reduced the number of *M. incognita* juveniles on the roots comparing to the water control. The *F. oxysporum* toxins were highly effective towards sedentary parasites and less effective towards migratory endoparasites. Non-parasitic nematodes were not influenced at all. Metabolites of strain 162 also reduced significantly the growth of *Phytophthora cactorum*, *Pythium ultimum* and *Rhizoctonia solani in vitro*. Secondary metabolites of endophytic fungi on plant-parasitic nematodes and soil-borne fungi should be considered for control of plant parasitic nematodes and plant pathogenic fungi. The results also show the need for proper selection of target nematodes in *in vitro* bioassays.

Introduction

Fusarium oxysporum Schlecht emend. Sny. & Hans. has been shown repeatedly to be antagonistic to plant parasitic nematodes [Crump, 1987; Quadri and Saleh, 1990] as well as to fungi causing vascular diseases [Postma and Rattink, 1992; Alabouvette *et al.*, 1993]. In addition, metabolites of this species can cause nematode mortality [Mani, 1983; Hallmann and Sikora, 1994b] and death of fungal pathogens [Walz-Borgmeier, 1991]. Our experiments with a non-pathogenic endophytic *F. oxysporum* isolated from the cortical tissue of tomato roots demonstrated his antagonistic potential when actively colonizing plant tissue [Hallmann and Sikora, 1994a]. Tomato roots colonized endophytically by *F. oxysporum* showed a 60% reduced *Meloidogyne incognita* infection. The mode-of-action of this mutualistic interrelationship between *F. oxysporum* and host plant is not fully understood. In the investigations presented in this paper we attempted to determine the presence of secondary metabolites produced *in vitro* by endophytic fungi and their effects on *M. incognita* activity. Furthermore, a non-pathogenic endophytic strain of *F. oxysporum* which effectively controls *M. incognita* in pot experiments [Hallmann and Sikora, 1994a] was selected. The properties of the culture filtrates of this strain, from now on called strain 162, on *M. incognita* activity and inhibition of soil-borne fungi was determined *in vitro*.

Materials and methods

General techniques

The fungal isolates tested for toxic metabolites were isolated from the cortical tissue of surface sterilized tomato roots growing in fields in Kenya [Hallmann and Sikora, 1994c]. The non-pathogenic *Fusarium oxysporum* strain 162 used in experiments II–V was isolated at Jomo Kenyatta Centre for Agriculture and Technology, Kenya and identified by Dr. Nirenberg (BBA Berlin). In experiment V metabolites were tested against following soil-borne fungi: *F. oxysporum* Schlechtend.: Fr. f. sp. *lycopersici* (Sacc.) Sny. & Hans., *Phytophthora cactorum* (Leb. & Cohn) Schröt, *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn. These fungi were taken from the collection of the Institut für Pflanzenkrankheiten (Bonn) and cultured on potato dextrose agar (PDA).

Eight nematode species representing different trophic groups were tested: 1. sedentary plant parasites: *Heterodera schachtii* (Schmidt), *Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, 2. *migratory endoparasites: Pratylenchus zeae* Graham and *Radopholus similis* (Cobb) Thorne, 3. mycophagous: *Aphelenchoides composticola* Franklin and 4. bacteriophagous: *Panagrellus redivivus* Linnaeus. Juveniles of *Meloidogyne* spp. were extracted from galled tomato roots and juveniles of *H. schachtii* from cysts of sugar beets using standard extraction procedures, respectively [Hussey and Barker, 1973; Ayoub, 1980]. *P. zeae* and *R. similis* originated from monoxenic cultures whereas *A. composticola* was maintained on PDA inoculated with *F. oxysporum* and *P. redivivus* on Miluvit-agar inoculated with *Escherichia coli*. In tests with sedentary endoparasites second-stage juveniles were used as inoculum, whereas, migratory endoparasites and non-parasitic species were applied as a mixed population of juveniles and adults.

Culture filtrates with secondary metabolites were obtained by growing fungi in 100 ml flasks on 50 ml gliotoxin-fermentation-medium (25 g dextrin, 2 g KH_2PO_4, 2 g NH_4-tartrate, 1 g $MgSO_4 \times 7\ H_2O$, 0,01 g $FeSO_4$) for 7 days under continuous agitation in an incubator at 25 °C. Initial fungal inoculum per flask consisted of 5 PDA discs of 0.5 cm in diameter taken from 7 day old culture dishes. After fermentation, fungal mycelia was seperated from the broth by passage through a Sartorius filter combination of C/N-filters with 8 μm, 0,45 μm and 0,2 μm pore size and the filtrate was tested immediately. Sterile 100 ml-flasks were filled each with 18 ml of the culture filtrate, 1 ml antibiotic solution and 1 ml of tap water containing 2000 *M. incognita* juveniles. The final antibiotic concentration in the solution was 150 ppm penicillin and 150 ppm streptomycin and was needed to prevent microbial growth. Previous tests demonstrated that the antibiotic mixture used efficiently controlled bacterial growth without affecting nematode activity. After increasing duration of incubation, or after 24 h in experiments I and II respectively, 1 ml subsamples were examined under a microscope for effects on nematode activity. Inactive nematodes appeared to be rigid and elongated with head and tail sometimes slightly bent. Specimens which were still inactivated after transferring them into tap water and incubating them for 24 h were classified as dead.

Experimental design

I: Screening of endophytic growing fungal isolates for toxic metabolite activity to Meloidogyne incognita
Thirty-four endophytic growing isolates were randomly selected from our endophyte collection and screened for their ability to produce toxic metabolites which inactivate or cause death of *M. incognita* juveniles. The juveniles were exposed for 24 h to the fungal culture filtrate containing secondary metabolites and nematode activity was recorded. Endophytic colonization of the selected strains was previously proven by inoculating tomato seedlings with the endophyte and reisolation of the endophyte from surface sterilized tomato roots four weeks after inoculation.

II: Effect of secondary metabolites of Fusarium oxysporum *strain 162 on nematode trophic groups*
Nematodes of each species were exposed to the culture filtrate of *F. oxysporum* strain 162 or gliotoxin-fermentation-medium in the control respectively for 24 h and then nematode activity was recorded.

III: Exposure time and nematode inactivation and/or mortality
Sterile 100 ml-flasks were filled with twice the amount of culture filtrate and nematode inoculum as that described previously. At each pre-determined exposure time a 2 ml subsample was taken and adverse effects on nematode activity measured. Samples were taken at 0, 10, 30 and 60 min and after 2, 5, 9, 24 and 48 h. Each subsample was divided into two 1 ml portions. One ml was used for recording percentage

of juvenile inactivation and the other for percentage mortality. Nematode mortality was determined after the nematodes were washed on a 20 μm aperture sieve with tap water until the culture filtrate was completely removed and juvenile incubation in 10 ml tap water on a rotary shaker for 24 h. Juveniles still inactive after treatment procedure were classified as dead.

IV: Dose-response

100 ml of the culture filtrate of *F. oxysporum* strain 162 was concentrated by rotary evaporation in a water bath at 50 °C. The concentrate was absorbed in 15 ml distilled water and concentrations of 100, 1.000 and 10.000 mg/l were made by dilution in additional distilled water. Additionally, unconcentrated culture filtrate at full strength and at 10% and 1% concentration in distilled water were tested. Control treatments consisted of equivalent concentrations of non-inoculated gliotoxin-fermentation-medium and tap water respectively. A 5 ml sample of each treatment was added to 0.5 g Sephadex G150 in 6 cm petri dishes. The suspension was thoroughly mixed by slight agitation of the petri dishes. After 10 min to allow polymerization of the Sephadex, 500 μl of tap water with 500 *M. incognita* juveniles were inoculated at one concentrated point close to the side of the petri dish. After 48 h, 3 lettuce seedlings (*Lactuca sativa*) with approximately 1.5 cm long roots were pushed into the Sephadex opposite to the nematodes. After another 24 h the number of nematodes within 1 mm from the roots were counted. Each treatment was replicated 3 times and data were analyzed according to standard procedures for analysis of variance and least significant difference (LSD). Comparisions to the water control were determined after Abbott [1925] and named control efficacy.

V: Activity toward soil-borne fungal pathogens

Gliotoxin-fermentation-broth from *F. oxysproum* strain 162 inoculated and non-inoculated shaked cultures was mixed with sterile distilled water to obtain filtrate dilutions of 3%, 25% and 75% respectively and added to petri dishes. Actual agar concentration was maintained at 1%. For the control water agar was used. The dishes were then inoculated with the soil-borne pathogenic fungi *F. oxysporum* f. sp. *lycopersici*, *P. ultimum*, *P. cactorum*, *R. solani* and the non-pathogenic *F. oxysporum* strain 162 being used for toxic metabolite production. Fungal inoculation was made by placing PDA-discs of 0.5 cm in diameter taken from 7 day old culture dishes in the center of the petri dish with the culture filtrates. Fungal radial growth in mm was measured after 3 days incubation at 20 °C in the dark. Data were subjected to ANOVA and differences between means were evaluated following Duncan's multiple range test.

Table 1. Effect of fungal secondary metabolites obtained from endophytic fungi isolated from surface sterilized tomato roots on *Meloidogyne incognita* mobility after 3 h exposure

Strain	Inactive L_2 %	*Fusarium oxysporum*[3]	Strain	Inactive L_2 %	*Fusarium oxysporum*
Control[1]	1		158	37	X
Control[2]	0		159	100	
10	3		160	100	
22	100	X	161	80	
33	31		162	96	X
34	100	X	163	97	X
53	6		165	10	
143	89	X	166	82	
144	81	X	170	0	
145	100	X	171	90	
146	94	X	172	14	
147	64	X	179	82	
148	73	X	183	18	
150	83	X	184	8	
152	59		185	18	
153	96	X	186	5	
154	67		188	33	
157	100	X	189	0	X

[1] Tap water
[2] Gliotoxin-fermentation-medium
[3] Fungi determined as *Fusarium oxysporum*

Results

I: Screening of endophytic growing fungal isolates for toxic metabolite activity to Meloidogyne incognita

The secondary metabolites in the culture filtrates of 20 out of 34 endophytic growing fungi isolated from tomato roots inactivated *M. incognita* juveniles over 60 percent (Table 1). Culture filtrates of 7 isolates caused <10% inactivation. Tap water and gliotoxin-fermentation-broth controls did not alter juvenile activity. The most effective isolates were identified as *Fusarium oxysporum*. In contrast, only 2 of 14 isolates that were considered less active were *F. oxysporum*. The *F. oxysporum* strain 162 was used for more detailed studies.

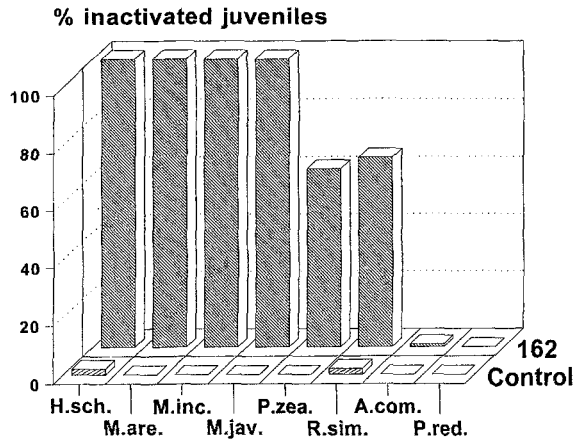

Fig. 1. Inactivation of different nematode trophic groups following 24-h exposure to secondary metabolites of endophytic *Fusarium oxysporum* strain 162 (162) and Gliotoxin-fermentation-medium (Control); phytophagous: H.sch. = *Heterodera schachtii*, M.are. = *Meloidogyne arenaria*, M.inc. = *M. incognita*, M.jav. = *M. javanica*, P.zea. = *Pratylenchus zeae*, R.sim. = *Radopholus similis*; mycophagous: A.com. = *Aphelenchoides composticola*; bacteriophagous: P.red. = *Panagrellus redivivus*.

II: Effect of secondary metabolites of Fusarium oxysporum *strain 162 on nematode trophic groups*

The secondary metabolites in culture filtrates of the fungal endophyte *F. oxysporum* strain 162 selectively inactivated plant parasitic nematode species and had no affect on the non-parasitic mycophagous and bacteriophagous species (Fig. 1). Infective juveniles of the sedentary endoparasitic nematodes *H. schachtii*, *M. arenaria*, *M. incognita* and *M. javanica* were completely inactivated after 24 h exposure. Inactivation of approximately 65 percent of juveniles and adults of the migratory endoparasites *P. zeae* and *R. similis* were recorded. The activity of the mycophagous nematode *A. composticola* and the bacteriophagous nematode *P. redivivus* were not altered. The control treatment with gliotoxin-fermentation-broth had no effect on nematode activity irrespective of nematode trophic group.

III: Exposure time and nematode inactivation and/or mortality

Exposure of *M. incognita* juveniles for 30 min to the culture filtrate of *F. oxysporum* strain 162 caused 80% inactivation and exposure >60 min resulted in 100% inactivation, respectively (Fig. 2). Juveniles treated 30 min and transferred to tap water for 24 h completely recovered and showed normal activity (Fig. 3). Exposure for more than 30 min however, resulted in

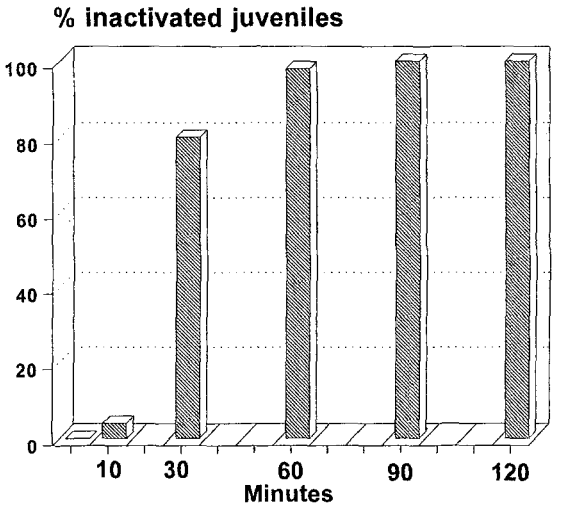

Fig. 2. Effect of exposure time to secondary metabolites of endophytic *Fusarium oxysporum* strain 162 on inactivation of *Meloidogyne incognita* juveniles.

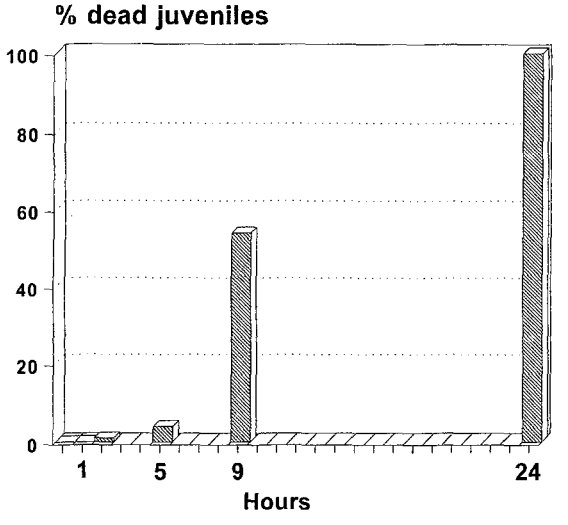

Fig. 3. Effect of exposure time to secondary metabolites of endophytic *Fusarium oxysporum* strain 162 on mortality of *Meloidogyne incognita* juveniles.

non-recovery of the juveniles in tap water, which was considered equivalent to death. After 9 h exposure 50% of the juvenile were dead and 100% death was obtained when juveniles were treated for >24 h. Exposure for 48 h caused visual disintegration of internal tissue structures. The degradation of tissue caused the juvenile to float to the surface of the solutions.

IV: Dose-response

The dose-response of unconcentrated culture filtrates from *F. oxysporum* strain 162 on *M. incognita* infection was tested in petri dishes with lettuce seedlings. In the water control an average of 68 juveniles were detected on the roots. At full strength metabolite in the medium no juveniles were observed around the roots resulting in a control efficacy of 100 (Table 2). At this high metabolite concentration the juveniles were completely inactivated and remained at the inoculation spot. Comparable concentrations of gliotoxin-fermentation-broth alone resulted in an efficacy of 80. With decreasing metabolite concentrations in the medium the number of juveniles close to the roots increased associated with a significant decrease in control efficacy. At dilutions of 10% control efficacy was 66 whereas at 1% dilution efficacy reached only 39. With gliotoxin-fermentation-broth alone in the medium the control efficacy was 34 at 10% and 19 at 1% dilution respectively.

Increasing levels of concentrated culture filtrate caused a similar dose response reaction (Table 2). At culture filtrate concentrations of 10.000 mg/l control efficacy reached 82, followed by 38 and 15 at 1.000 mg/l and 100 mg/l respectively. With fermentation broth alone control efficacy was 36 at 10.000 mg/l, 62 at 1.000 mg/l and 11 at 100 mg/l. At culture filtrate concentrations of 1% or 100 mg/l no inactivated juveniles were detectable. Plant growth was negatively affected only at highest concentrations of gliotoxin fermentation broth with and without the fungal metabolites. In these cases seedling growth was reduced and the roots developed a necrotic brown color.

V: Activity towards soil-borne fungal pathogens

With the exception of *F. oxysporum* strain 162 and *F. oxysporum* f. sp. *lycopersici*, radial growth of the fungal pathogens tested decreased with increasing metabolite concentrations in the medium (Table 3). At 75% fungal metabolite in the medium the radial growth of *P. ultimum*, *P. cactorum* and *R. solani* was significantly reduced to 0.5 cm, 0.7 cm and 2.9 cm respectively. At 3% fungal metabolite only the growth of *R. solani* was significantly reduced, whereas *P. ultimum* and *P. cactorum* showed similar growth like on water agar. In contrast, the growth of the endophytic *F. oxysporum* strain 162, which was the source of the tested metabolite, and *F. oxysporum* f. sp. *lycopersici* decreased significantly with reduced metabolite concentrations. At 75% fungal metabolite radial growth of *F. oxysporum* strain 162 was 2.6 cm and for *F. oxysporum* f. sp *lycopersici* 2.7 cm whereas at 3% fungal metabolite radial growth was 2.0 cm and 2.2 cm respectively. A corresponding growth reduction was also observed in the controls with gliotoxin-fermentation-broth alone. Maximum growth of both *F. oxysporum* isolates were similar. Nevertheless gliotoxin-fermentation-broth alone positively affected fungal growth of all tested fungi.

Discussion

Our results demonstrated that endophytic growing fungi isolated from internal root tissue of surface-sterilized tomato roots produce *in vitro* secondary metabolites inhibitory as well as toxic to *M. incognita* juveniles. Based on this results endophytic strains can be selected to prove fungal metabolite production in the plant itself. Culture filtrates of 20 fungi out of 34 tested, inactivated *M. incognita* juveniles >60%, thus demonstrating the broad occurrence of toxic substances in endophytic fungi. The most effective isolates included 15 isolates identified as *F. oxysporum*. This species was also the most frequently isolated endophyte from tomato plants in earlier studies [Hallmann and Sikora, 1995]. Schuster [1992], who also tested culture filtrates of different fungi reported that metabolites in culture filtrates of all egg pathogenic fungi tested, inactivated *Panagrellus redivivus*, whereas, culture filtrates of predacious fungi had no effect on nematode mobility. This author mentioned differences in nematode take up of the filtrate related to fungal trophic group as a possible reason for selective activity. At least nine toxins have been isolated worldwide from *F. oxysporum* [Marasas *et al.*, 1984], not including common metabolites like acetic acid which may also kill nematodes. Few investigations of the effect of Fusarium toxins on plant parasitic nematodes have been reported: Mani and Sethi [1984] working with culture filtrates of *F. solani* reported reductions in hatch and mobility of *M. incognita*. Fattah and Webster [1983] observed inhibited development of *M. javanica* in roots colonized by *F. oxysporum* f. sp. *lycopersici*. A decrease in number of *M. incognita* females and increase in the proportion of males was described by Moussa and Hague [1988] for soybeans invaded by *F. oxysporum* f. sp. *glycines*. The same authors mentioned toxins produced by the fungus as possible mode of action.

Table 2. Dose response of untreated and concentrated gliotoxin-fermentation-medium (GFM) without (−Endophyte) and with culture filtrates of non-pathogenic *Fusarium oxysporum* strain 162 (+Endophyte) on control efficacy of *Meloidogyne incognita* in a bioassay with lettuce plants

GFM	Control efficacy according to Abbott					
	Concentration of GFM without and with culture filtrates					
	100%	10%	1%	10.000 mg/l	1.000 mg/l	100 mg/l
−Endophyte	81ab[1]	34d	19 d	36 bc[1]	62a	11d
+Endophyte	100a[1]	66 bc	39 cd	82 a[1]	38 b	15 cd
LSD		27.61			22.73	

Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Number of replicates:3.
[1] Phytotoxicity: root growth reduction of about 50%.

Table 3. Effect of concentration of gliotoxin-fermentation-medium without (−Endophyte) and with culture filtrates of non-pathogenic *Fusarium oxysporum* strain 162 (+Endophyte) on the radial growth of 5 soil-borne fungi after 3 days

Concentration Gliotoxin-Fermentation-Medium		Radial growth (cm)				
	pH[1]	*Fusarium oxysporum* strain 162	*Fusarium oxysporum lycopersici*	*Pythium ultimum*	*Phytophthora cactorum*	*Rhizoctonia solani*
Control[2]	5.9	2.6 ab	2.6 bc	8.5 a	1.3 c	5.2 a
−Endophyte						
75%	4.8	2.8 a	2.9 a	8.5 a	1.7 a	5.0 a
25%	5.0	2.8 a	2.5 c	8.5 a	1.5 b	4.0 b
3%	5.3	2.2 c	2.5 c	8.5 a	1.3 c	3.3 c
+Endophyte						
75%	3.9	2.6 b	2.7 b	0.5 c	0.7 e	2.9 d
25%	4.0	2.2 c	2.2 d	4.9 b	1.1 d	2.6 e
3%	4.4	2.0 c	2.2 d	8.5 a	1.3 c	3.4 c
LSD		0.2	0.2	0.5	0.1	0.3

Means followed by the same letter in the column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Number of replicates: 4.
[1] pH of the medium
[2] Fungal growth on water agar

The *F. oxysporum* strain 162 used in our tests selectively inactivated nematode species related to similar trophic groups. After 24 h exposure plant parasitic nematode species were inactivated between 60–100%, whereas the mobility of the mycophagous and bacteriophagous species was not altered. Within the plant parasitic trophic group, sedentary endoparasites were inactivated 100% by the metabolites and migratory endoparasites approximately 65%. Similar results were achieved by Cayrol *et al.* [1989] with culture filtrates of *Paecilomyces lilacinus*. In their studies plant parasitic nematodes were inactivated up to 100%, in contrast to an average of 25% for mycophagous, bacteriophagous and entomopathogenic species. The results indicate that the more intimate the relationship of the nematode species is to the plant and away from the soil environment the more sensitive are the species to fungal metabolites. Differentiations in surface coat or detoxification systems of the nematode species that may have developed evolutionarily due to constant contact of mycophagous and bacteriophagous species with saprophytic fungal toxins may be responsible. The different magnitude of inhibition observed between sedentary and migratory endoparasites may also be related to nematode stage tested. Only second-stage juveniles of sedentary species were tested whereas

juvenile and adult nematodes of migratory endoparasites were involved. Amin and Sikora [pers. communication] showed that second-stage juveniles of *Pratylenchus zeae* are more sensitive to fungal metabolites in culture filtrates than more advanced juvenile stages or adults.

The culture filtrates of *F. oxysporum* strain 162 reduced *M. incognita* juvenile activity within minutes whereas considerable levels of mortality first occured after >60 min exposure. Cayrol *et al.* [1989] obtained 100% inactivation of different plant parasitic nematodes in culture filtrates of *P. lilacinus* within 30 min. The nematodes were paralysed and recovered in tap water. Exposure for more than 72 h caused 100% mortality. The authors assumed that fungal metabolites affecting the nerve system caused the rapid inactivation of the nematodes. In contrast, culture filtrates of *F. solani* investigated by Mani and Sethi [1984] showed delayed effects on *M. incognita* with 100% inactivated juveniles only after 48 h exposure to the culture filtrate. Studies of juvenile mortality were not discussed.

In our experiments, nematode exposure to culture filtrates of *F. oxysporum* strain 162 for more than 48 h caused disintegration of juvenile body tissues resulting in floatation of the juveniles to the surface of the test solution. Schuster [1992] obtained similar results with nematodes exposed for 48 h to culture filtrates of egg pathogens, but in his case the effect was restricted to *P. redivivus* and did not appear in plant parasitic nematodes.

Besides the nematicidal effect the tested culture filtrate of *F. oxysporum* strain 162 also negatively affected fungal growth of various soil-borne plant pathogens. A 75% mixture of the culture filtrate to water agar completely inhibited growth of *P. ultimum* and significantly reduced the growth of *P. cactorum* and *R. solani*. Nevertheless, *F. oxysporum* f. sp. *lycopersici* did not react to the culture filtrate of the endophyte produced metabolites. Fungal growth inhibition was also reported by Walz-Borgmeier [1991] for culture filtrates of *Trichoderma viride* and *Gliocladium roseum*. In contrast to our results Walz-Borgmeier achieved growth inhibition of pathogenic *Fusarium* species with metabolites in culture filtrates of non-pathogenic *F. oxysporum*, which may indicate race-specific metabolite production.

An increased control efficacy, i.e. reductions in total number of *M. incognita* juveniles on lettuce roots grown in culture filtrates of *F. oxysporum* strain 162 was observed at dilutions of 1% or 100 mg/l respectively. No inactivated juveniles were detectable at this concentration in the Sephadex dishes bioassay, but did occur in full strength filtrate. Nevertheless we do not know if the culture filtrate unables the juveniles to move to the root or if the attraction to the root is disturbed due to the filtrates. In general, nematode control efficacy increased rapidly with increasing metabolite concentrations. Cayrol and Djian [1990] reported 100% inactivation of *M. arenaria* juveniles in non-diluted culture filtrate of *F. roseum* var. *arthrosporoides* whereas a 10% dilution caused no alteration in nematode activity when compared to the water control. Similar results were obtained by Cayrol *et al.* [1989] with culture filtrates of *P. lilacinus* where 100% inactivation of *M. arenaria* was detected in non diluted filtrates and <2% inactivation of juveniles in a 10% dilution.

Full strength culture filtrate containing *F. oxysporum* strain 162 metabolites affected plant growth in our experiments. Growth retardation in the Sephadex dish bioassay was probably due to the ingredients of the fermentation medium, most likely by unused residues of sugars and salts still present in the culture filtrate. The fact that such organic compounds can negatively affect plant growth is well known and has to be taken into consideration for practical aspects. If gliotoxin fermentation medium affects plant growth, we would expect less juveniles being attracted to the plant roots correlated with an increased control efficacy. This would explain the high effect of the gliotoxin fermentation medium on *M. incognita* in the Sephadex bioassay comparing with the *in vitro* experiments I–III where only the direct effect of the medium on juvenile bahaviour was measured.

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