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Fungal parasites of the root-knot nematodes *Meloidogyne* spp. in southern Bulgaria

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Abstract. Results of mycological surveys of root-knot nematodes from the southern region of Bulgaria indicated that three species were associated with *Meloidogyne*: *Fusarium oxysporum*, *Verticillium chlamydosporium*, and *Gliocladium roseum*. The fungi infected up to 8.7% of the eggs in the females of *Meloidogyne* spp. In the field the egg parasitism by fungi was observed during September. In the pot experiments the eggs developed infection with the fungi in the first generation at the end of July. The fungi destroyed from 7.6% to 23.5% of the eggs in the next generations.

Key words: nematophagous fungi, egg parasites, root-knot nematodes.

INTRODUCTION

Root-knot nematodes are serious pests of many cultivated crops around the word. The *Meloidogyne* spp. complex is one of the economically most important pests in Bulgaria Damage to tomato by *Meloidogyne* spp. was first observed in Bulgaria by Malkov (1903). Later research showed that these nematodes are widespread in the country (Stoyanov, 1980). To control the root-knot nematodes resistant varieties and chemicals are used (Stoyanov, 1989; Choleva et al., 2006; Salkova et al., 2006). The root-knot nematodes can be infected by fungi at different stages of their life cycles. Soils are a reservoir for microorganisms that are highly varied in activity and composition. Natural regulation of the soil densities of the root-knot nematodes occurs in monocropping areas. Our review of the literature on fungi associated with cyst and root-knot nematodes revealed that about 160 fungal species belonging to 70 genera had been reported on these nematodes (Qadri, 1989; Qadri & Saleh, 1990; De Leij & Kerry, 1991; Khan & Akram, 2000). Casual observations of populations of *Meloidogyne* spp. in southern Bulgaria revealed the presence of black eggs in some females.

The purpose of the present investigation was to describe and isolate fungi from eggs of *Meloidogyne* spp. collected from a greenhouse in southern Bulgaria. These studies were conducted to assess the frequency of occurrence of the causative fungus in the nematodo-endemic areas.

MATERIALS AND METHODS

The experiment was undertaken at the Plant Protection Institute in Kostinbrod in 2002–2004. Monthly root samples were collected from a greenhouse previously identified as infested with *Meloidogyne* spp. The samples were taken on a zig-zag pattern over the field. Roots were washed free from soil with a fine jet of water. The females were picked off the roots and recorded whether infected or healthy. The egg numbers were determined per 1 g of roots by crushing the females. The healthy and diseased eggs were counted with the aid of a microscope (×400) in three replicates for each sampling time.

The effects of parasites upon eggs in females were determined using the following two categories: (1) infested eggs – those developing a fungal colony and eggs that were not identifiable as juveniles or embryos, and (2) normal eggs – those containing embryos or second-stage juveniles (Lopez-Llorca & Boag, 1993).

Part of the soil collected from the field was used in a pot experiment. Surface sterilized seeds (0.1% mercuric chloride for 2 min) of tomato were sown in sterilized soil. Fifteen days after germination the seedlings were thinned to one per pot. Pots were filled with infested soil and planted with tomato seedlings (cv. Ideal) at the end of May. The plants were inoculated with freshly hatched juveniles of *Meloidogyne* spp. at a rate of 1500/pot. Three pots were removed at monthly intervals and 10 females were randomly chosen from each pot, rinsed several times in sterile water, and preserved at room temperature in sterile sand in glass vials. During January the females were separated from sand, crushed in water, and the numbers of the diseased and healthy eggs were recorded.

Part of the soil collected from the infested field was steam-sterilized and put into 24 pots. A second set of 24 pots were filled with infested field soil. Tomato plants were placed in the pots and inoculated with 1500 juveniles/pot in a water suspension pipetted around the roots. From July onward the pots were examined more frequently and when the females emerged they were examined at ×400 magnification to ascertain if there was any fungal infection. Weekly counts of healthy and diseased eggs were recorded in both of the sets in three replications.

Fungi were isolated from the eggs. The surface of the egg-masses was sterilized by agitation for 3 min in 5 mL of 0.1% NaOCl, then removed and washed twice with sterile distilled water. They were carefully crushed in distilled water and the suspension was placed in Petri dishes containing oat agar (Kerry & Crump, 1977). The plates were incubated at room temperature for 14 days to allow fungi to grow. Fungal colonies were identified to species using Barnett (1960) and Barron (1968).

RESULTS AND DISCUSSION

In the field the tomato plants were sown at the end of July. Most of the eggs of *Meloidogyne* spp. had hatched from August to October and the population of healthy eggs increased from 81.6 to 139.6 eggs/1 g roots. The first diseased eggs

(5.0%) in the field were observed in September (Table 1). Their number increased during October (8.7%). Between 5.0% and 8.7% of the eggs were destroyed by fungi in the naturally infested field soil. The fungi became active from August onward. The availability of soil moisture and conductive temperature during this period may be responsible for their activity. It is interesting to note that up to 9.0% of the eggs of the first generation nematodes were infested. The survey clearly showed the spread of diseased eggs in the *Meloidogyne* infested area.

In the pot experiment the plants were sown at the end of May. The first diseased eggs were observed in the naturally infested soil during August. The eggs removed from pots containing naturally infested soil in July and preserved in sterile sand had not developed diseased eggs by January. The eggs removed from August onward however contained from 7.6% to 23.5% diseased eggs by January. The pot experiment confirmed findings from the field experiment (Table 2). When the juveniles from healthy eggs emerged and started penetrating into the roots the percentage of diseased eggs increased to 23.0%. Eggs developed fungal infection at the end of the first generation and the numbers of infected eggs increased in the following generations.

Table 1. Surveillance on diseased eggs of *Meloidogyne* spp. in infested field soil

Month	Healthy eggs*	Infected eggs*	Total*	% diseased eggs
July	0.0	0.0	0.0	0.0
August	81.66 ± 5.0	0.0	81.66 ± 5.0	0.0
September	157.0 ± 13.5	10.0 ± 1.6	167.0 ± 8.0	5.02 ± 2.0
October	139.6 ± 7.0	13.3 ± 1.8	152.0 ± 7.0	8.69 ± 3.2

* Mean number of eggs per 1 g of roots ± SD.

Table 2. Development of diseased eggs of *Meloidogyne* spp. in the pot experiment

Time of egg collection	Mean number of eggs ± SD per pot during January		
	Healthy eggs	Diseased eggs	% diseased eggs
June	0.0	0.0	0.0
July	33.0 ± 3.0	0.0	0.0
August	54.0 ± 2.3	7.6 ± 1.5	12.21
September	123.6 ± 8.9	10.3 ± 1.6	7.69
October	161.6 ± 12.0	27.3 ± 3.2	14.45
November	109.3 ± 11.0	27.6 ± 3.2	20.16
December	110.3 ± 10.3	34.0 ± 3.0	23.56

In the second experiment females emerging on plants grown in naturally infested soil were attacked by fungal parasites at the end of July. The fungi destroyed from 7.3% to 14.0% of the eggs in naturally infested soil during July and August. In the sterilized soil infested artificially there were no infected eggs (Table 3).

Table 3. Fungal parasitism of nematode eggs in the soil infested artificially and naturally with *Meloidogyne* spp.

Observation month and week		Mean number of eggs per plant ±SD				
		Artificially infested soil		Naturally infested soil		
		Healthy	Diseased	Healthy	Diseased	% diseased
July	1	0.0	0.0	0.0	0.0	0.0
	2	8.3±1.0	0.0	0.0	0.0	0.0
	3	12.6±1.5	0.0	10.3±1.7	0.0	0.0
	4	31.3±5.1	0.0	25.6±1.5	3.2±0.4	10.4
August	1	54.3±2.0	0.0	26.0±3.1	3.0±1.6	10.3
	2	98.0±5.4	0.0	50.3±4.2	4.0±1.4	7.3
	3	66.3±4.1	0.0	55.0±4.0	9.0±3.1	14.0
	4	168.6±9.0	0.0	78.3±5.5	12.6±2.3	13.8

The present investigation shows that suppression of the mycoflora in soil from a field heavily infested with the root-knot nematode can result in increases of the number of healthy eggs of *Meloidogyne* spp. Similar results were reported earlier by several workers (Crump & Kerry, 1984, 1987; Kerry, 1984).

We isolated three species of nematophagous fungi from eggs of *Meloidogyne* spp. This is the first report of isolation of *Fusarium oxysporum* Schlecht, *Verticillium chlamydosporum* Goddard, and *Gliocladium roseum* Bainier from infected females of *Meloidogyne* spp. in Bulgaria.

The synergistic interaction of the root-knot nematode and *Fusarium* spp. is known in many crops (Khan & Husain, 1991; Fazal et al., 1994; Dababat & Sikora, 2006). Many *Fusarium* spp. are reported to be toxin producers and can attack nematodes by toxins or by enzymes (Morgan-Jones et al., 1983; Marasas et al., 1984; Khan & Akram, 2000).

Verticillium ssp. are known to be capable of colonizing the rhizosphere of many crops and this genus has been described as the most important egg parasite of *Heterodera avenae* (Woll.) and *H. schachtii* (Schmidt) (Kerry et al., 1984; De Leij & Kerry, 1991).

The significance of *Gliocladium roseum* as a destructive parasite was studied by Barnett & Lilly (1962) and Rodrigues-Kabana et al. (1984).

The nematodes are a very important part in root decay complexes involving the nematodes, the plant, and the fungi or bacteria. The metabolic activities of any one part of the complex influence those of the other components. Root-knot nematode infections on certain hosts effectively predispose these roots to subsequent invasion by a range of other organisms present in the rhizosphere. Although some aspects of the biology of fungi infecting the eggs of root-knot nematodes are known, their mode of action is not yet fully understood (Fattan & Webster, 1983). The composition of the associated mycoflora of individual fields probably is determined by such factors as plant host, cropping history, soil fertility, and climatic conditions. There is need to understand the effects of cropping sequences, cultural practices, and fungicides on the population of fungi associated with nematodes. The mycoflora associated with phytonematodes shows a wide

capacity for adaptation to seasonal changes in temperature (Rodrigues-Kabana et al., 1984). Presumably some fungal species can be expected to be active on nematodes in the southern soil at all times of the year. According to Sosnowska & Banaszak (2000) and Choleva et al. (2006), we may be able to manipulate the antagonistic mycoflora of the soil to increase destruction of nematode eggs by the use of organic soil amendments.

This is the first report of identification of fungi infecting *Meloidogyne* spp. in Bulgaria. In order to establish the aggressiveness of these fungi as egg parasites and their suitability as biocontrol agents pathogenicity tests are required.

CONCLUSIONS

The results obtained from the research allow us to draw the following conclusions:

- In southern Bulgaria eggs of *Meloidogyne* spp. were infected by three species of nematophagous fungi: *Fusarium oxysporum*, *Verticillium chlamydosporium*, and *Gliocladium roseum*.
- Infection of the first generation of the root-knot nematodes occurred at the end of July. The fungi destroyed from 7.6% to 23.5% of the eggs in the following generations.
- Egg parasitism by fungi (8.7%) was observed in the field during September.

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Juurnematoodi *Meloidogyne* spp. seenparasiitidest Lõuna-Bulgaarias

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Lõuna-Bulgaaria kasvuhoonetes leviva juurnematoodi *Meloidogyne* spp. Munadest on leitud kolme liiki seenparasiite: *Fusarium oxysporum* Schlecht, *Verticillium chlamydosporum* Goddard ja *Gliocladium roseum* Bainier. Läbiviidud laboratoorsete katsete tulemusel selgus, et 7,6…23,5% seente poolt nakatatud juurnematoodide järgmise põlvkonna munadest hävines. Edaspidistes uuringutes on vaja suuremat tähelepanu pöörata seente kui juurnematoodi parasiitide patogeensele agressiivsusele ja võimalikule kasutamisele bioloogiliseks kontrolliks.