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FUNGI ASSOCIATED WITH *HETERODERA SCHACHTII* IN JORDAN. II. IDENTITY AND INCIDENCE

by
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Summary. The mycoflora associated with *Heterodera schachtii* in Jordan has been investigated. Eight fungal species were isolated from white and new brown cysts at harvest time. *Verticillium chlamydosporium*, *Aspergillus flavus*, *Fusarium oxysporum* and *F. solani* were the most common fungi. Fourteen more fungi were isolated from cysts or cyst contents two months after harvest. *A. erythrocephalus*, *A. fumigatus*, *A. versicolor* and *Penicillium aurantiogriseum* were most common among those fungi. Only 29.8% of cysts were healthy at this time. Direct microscopic observation of cysts at the same time showed thad 72.4% of cysts are associated with fungal structures in the field.

The sugar beet cyst nematode *Heterodera schachtii* Schmidt has recently been found on cabbage in Jordan (Saleh, 1987). Sugarbeet is not cultivated in the country and crucifers are considered to be the main host. Investigations on the nematode distribution has shown that it may be restricted to a small area near the old Roman city of Jerash (unpublished). A large proportion of the cysts were observed to have a variety of fungal structures associated with them. Although the fungal parasitism of *H. schachtii* has been intensively studied in temperate regions (Bursnall and Tribe, 1974; Nigh *et al.*, 1980; Heijbroek, 1983; Crump, 1987), there is only limited knowledge of it in subtropical regions. The objective of the present work was to isolate and identify fungi associated with *H. schachtii* in Jordan and to determine the level of infection of the nematode in the field.

Materials and methods

A bulk soil sample was collected from a field infested with *H. schachtii* in the Jearsh area in August 1987, two months after a cabbage crop was harvested. At the next harvesting time, June 1988, further samples were taken from the same field and another sample from a second field in the same area. Cysts were extracted by suspending soil in water and sieving through a 250 μm sieve. Cysts from the first sample were selected at random with the aid of a stereomicroscope, fractured on a glass slide in a drop of methylene blue-tinted lactophenol and examined with a microscope. The cysts were classified as either full (with < 50% of eggs unhatched) or empty (with > 50% of eggs hatched). Each category was further classified as: 1) healthy cysts with no or few diseased eggs); 2) partially diseased (few to 50% of eggs parasitized); 3) fully diseased cysts (with > 50% of eggs parasited).

Fungi were isolated from cysts by collecting the cysts in a small vial, washing them vigorously in sterile water and surface sterilizing them with 70% alcohol for one minute. They were washed again in sterile water followed by immersion in an antibiotic solution (100 ppm). The cysts were then transferred to petri dishes containing potato dextrose agar medium (PDA) subsidized with streptomycin (100 μm/ml) and incubated at 25° C for five days (Morgan-Jones *et al.*, 1981).

To isolate fungi associated with cyst contents, 100 surface sterilized cysts were divided into 10 batches, each batch was homogenized in 10 ml sterile water in a tissue homogenizer. To separate the cyst walls, the homogenized suspension was passed through a 100 μm sieve set over a 25 μm sieve. One ml of the cyst suspension collected on the lower sieve was plated on PDA in each of two petri dishes and incubated (modified from Nigh *et al.*, 1980). The procedure was repeated whereby the suspension was diluted to 100 ml before plating. The resulting colonies

TABLE I - *The pathology of* Heterodera schachtii *cysts in the field two months after harvest.*

Cyst category	% healthy cysts	% partially diseased	% fully diseased	Total
Full cysts (new)	13.4	22.5	13.0	48.9
Empty cysts (old)	14.2	23.1	13.8	51.1
Total (487 cysts)	27.6	45.6	26.8	100

were counted and pure cultures of almost all fungi were established. Cultures were sent to Prof. W. Gams (Centralbureau voor Schimmelcultures, Baarn, Netherlands) for identification, or identified in our laboratory.

Percent egg parasitism was determined according to the method described by Kerry and Crump (1977).

Results and discussion

Direct microscopic observation of cysts (total 487) collected two months after the cabbage crop was harvested in 1987 showed that 72.4% of them were associated with fungal structures. This is higher than observed on *H. schachtii* in Germany (Rademacher and Schmidt, cited by Tribe, 1979) and in Britain (Tribe, 1979). Percentages of partially or fully diseased cysts found among empty (old) cysts were almost similar to those found among full (new) ones.

Eight fungal species were isolated from white cysts and new brown ones from both fields at the end of the growing season, 1988 (Table II). *Verticillium chlamydosporium*, the most common egg and female parasite of *H. schachtii* (Rodriguez-Kabana and Morgan-Jones, 1988), was the fungus most frequently found. This emphasises the role of this fungus as a cosmopolitan biocontrol agent of the nematode. *Fusarium* spp., mainly *F. oxysporum* and *F. solani* were also frequently isolated. These two species have been recovered from cyst nematode populations in several parts of the world and have been shown to be capable of parasitizing eggs of both *Heterodera* and *Meloidogyne* species (Nigh *et al.*,1980; Godoy *et al.*,1983 and Morgan-Jones *et al.*, 1984). Except for *A. flavus*, the other seven species are known colonizers of *H. schachtii* in other parts of the world. Also, except for this species, microscopic observation made on cysts from which fungi grew showed that a direct colonization of eggs was common for these fungi. Whether this colonization occurred as a result of the saprophytic activity of these fungi on eggs killed by other agents, or it is a more specific parasitism of eggs has to be studied. However, 16% natural egg parasitism existed in the field at harvest time.

With the advancement in cyst age (two months after harvest), cysts are subjected to attacks from soprophytes like *Aspergillus* spp. generally common in the soils of warm regions (Domsch *et al.*, 1982). In the fields that were investigated, *A. fumigatus*, *A. erythrocephalus*, *A. versicolor* (isolated from cyst contents only) and *Penicillium aurantiogriseum* were the dominant ones in addition to *A. flavus*. these saprophytes have a degree of association or parasitism with insects (Domsch *et al.*, 1982), and have been found associated with cyst nematodes from different parts of the world (Gintis *et al.*, 1982, 1983 and Morgan-Jones *et al.*, 1984). The high incidence of these fungi in our field indicate their active role in the reduction of *H. schachtii* multiplication. In addition to these species, a diverse

TABLE II - *Occurrence of fungi on* Heterodera schachtii *white (W), new brown (NB) and brown cysts (B) at two dates (D) in percents (total 120-140 cysts/column).*

	Field I		Field II	
	DI[1]		DII	DI
Fungus	W	NB	B	W + NB
Acremonium sclerotigenum Gams	0.0	0.0	4.2	0.0
Aspergillus erythrocephalus Berk.	0.0	0.0	8.5	0.0
A. flavus Link	7.0	5.2	–	12.1
A. fumigatus Fres.	0.0	0.0	12.7	0.0
Cylindrocarpon olidium Woll.	1.4	0.8	0.0	2.2
Fusarium equiseti Sacc.	0.7	0.8	5.9	2.2
F. oxysporum Schlecht	1.4	10.4	2.5	1.1
F. solani Sacc.	1.4	5.2	9.3	1.1
F. solani Sacc. (macroconidial isolate)	0.7	1.6	1.7	0.0
Sterile mycelium	0.0	7.2	3.4	4.2
Verticillium chlamydosporium Gold.	4.9	25.9	11.0	14.3
Miscellaneous fungi[2]	0.0	0.0	11.0	0.0
Healthy cysts	82.5	43.1	29.8	62.8
% parasitized eggs	16.0	–	–	–

[1] DI: At harvesting time; DII: Two months later; [2] Their names are listed in Table III.

group of fungi referred to here as «miscellaneous fungi» were found on and in cysts at low incidence (<1% for each). Even though the majority of fungi isolated from *H. schachtii* at this stage have not been reported previously on this nematode, the majority e. g. *A. flavus*, *A. fumigatus*, *Mortierella* spp., *F. culmorum*, *F. flocciferum*, *F. equiseti*, *Ulocladium consortiale*, *Preussia sp.* and others were reported on other cyst nematodes elsewhere (see citation, Table III). This might indicate that cyst colonization with such fungi is not accidental, in spite of the low incidence.

A large number of fungal propagules (= colonies) was found in cysts two months after harvest (87 propagules/cyst), so that a dilution of the suspension of cyst content was necessary to give a small number of fungal colonies per petri dish. This allowed the isolation and identification in pure cultures. Since a similar mycoflora was isolated by plating intact cysts (Table II, field I-DII), or by plating cyst content after the exclusion of cyst wall (Table III), we conclude that both methods can be used to determine fungi associated with *H. schachtii*. This also confirms that these fungi are not just external cyst contaminants.

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TABLE III - *Fungi isolated from* Heterodera schachtii *cyst content two months after harvest given as number of propagules (colonies)/cyst and their percents.*

Fungus	Propagules number/cyst	in percent	Reference (reported on *H. schachtii*)
Aspergillus erythrocephalus	3.5	4.0	
A. flavus	7.0	8.1	
A. fumigatus	8.0	9.2	
A. versicolor Tirab.	3.5	4.0	Vinduska, 1982
Fusarium oxysporum	14.0	16.1	Crump, 1987
F. solani	17.5	20.1	Bursnall & Tribe, 1974
F. solani (macroconidial) isolate)	3.0	3.4	
Penicillium aurantiogriseum Dierckx	6.0	6.9	
Preussia sp. Fuckel	2.0	2.3	
Sterile mycelium	2.0	2.3	
Verticillium chlamydosporium	13.5	15.5	Bursnall & Tribe, 1974
Miscellaneous fungi (< 1 % each)	7.0	8.1	
Cladosporium sphaerospermum Penz.			
Fusarium culmorum Sacc.			Crump, 1987
F. equiseti			Crump, 1987
F. flocciferum Corda			Crump, 1987
Gliocladium roseum Bain			
Mortierella hyalina Gams			
M. polycephala Coemans			
Pleospora sp.			
Trichoderma sp.			
Ulocladium consortiale Simmons			
Total	87.0	100	

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