Danish Research Service for Plant and Soil Science
Research Centre for Plant Protection
Institute of Plant Pathology
Zoological Department
DK-2800 Lyngby

Report no. 1817

The effect of *Hirsutella heteroderae* on the multiplication of the beet cyst nematode on sugar beet

Hirsutella heteroderae's indflydelse på opformeringen af roecystenematoden på sukkerroe

Mogens Juhl †

Summary

The hyphomycet *Hirsutella heteroderae*, a soil inhabiting fungus with sticky spores, was found to be highly effective as parasite on larvae of the beet cyst nematode *Heterodera schachtii* in a pot experiment carried out in a greenhouse.

In autoclaved soil with added larvae of *H. schachtii* a reduction in cyst formation of up to 58% was obtained. In naturally infested soil, a reduction of up to 92% was obtained – both by adding up to 730.6 $\times 10^6$ spores of *H. heteroderae* to 1 l soil.

Key words: *Hirsutella heteroderae, Heterodera schachtii,* biological control.

Resumé

Hyphomyceten *Hirsutella heteroderae*, en jordboende svamp med klæbrige sporer, er ved potteforsøg i drivhus fundet at kunne være særdeles effektiv som parasit på larver af *Heterodera schachtii.*

I autoklaveret jord tilsat larver af *H. schachtii* opnåedes en reduktion af cystedannelsen på op til 58% og i naturligt inficeret jord på op til 92% ved indblanding af op til 730,6 $\times 10^6$ sporer af *H. heteroderae* pr. l jord.

Nøgleord: *Hirsutella heteroderae, Heterodera schachtii,* biologisk bekæmpelse.

Introduction

Hirsutella heteroderae was described for the first time when it was found in Bavarian hop gardens in 1974. It was found in the root zone of hop plants attacked by *Heterodera humuli.* Free-living larvae of *H. humuli* were found parasitized by the fungus, which was isolated from such larvae in 1976. The fungus turned out to be a hyphomycet belonging to the *Hirsutella* family, a cosmopolitan family comprising about 35 species, which – with a few exceptions – are only known from insects and mites (4, 5).

Hirsutella heteroderae is the first *Hirsutella* species known to parasitize nematodes. The name of the species may probably be ascribed to the fact that out of nematodes from a number of families it seems to have a special affinity to larvae of the *Heterodera* family. *H. heteroderae* is widespread in sugar beet areas in Western Germany (3). *H. heteroderae* may be synonymous with *H. rhossiliensis* (2).

At present it is known that at least 19 families of nematodes may be parasitized by this fungus. These include *Aphelenchoides, Criconema, Ditylenchus, Globodera, Heterodera, Pratylenchus, Rotylenchus* and *Tylenchorrhynchus* (5 and *Nuñez* pers. com.).

The conidiophores of *H. heteroderae* are arising more or less at right angles from the hyphae and are swollen towards the base, i.e. they are bottleshaped, and consequently the conidiae are called phialospores. These are about 5 μ in diameter and are lemonshaped. The surface is glutinous and stick to the cuticula of nematodes which happen to pass by. Thus spore attachment mostly takes place at the head end of the nematode. The mobility of a nematode is not immediately reduced, but it usually dies within about 24 hours after attachment of a *Hirsutella* spore. The interior parts of the nematode are gradually replaced by a compact mycelium growth arising from a haustorium which has grown through the cuticula from the attached spore.

About 4–10 days after spore contact – depending on the nematode species – mycelium grows out of the nematode body at various places. Phialids with phialospores are formed from the mycelium. Phialids may also grow out direct through the cuticula.

H. heteroderae may be grown on agar, for instance PDA (potato dextrose agar) or CMA (corn meal agar). The growth is slow. After one month on 1% of CMA at approx. 22°C the diameter had grown to 3 cm. The optimum temperature is 20–25°C. At 5°C the growth is extremely slow. The optimum pH is found to be 5.7–6.8.

As *H. heteroderae* parasitize by attachment of sticky spores to the nematode cuticula, the only stages at which cystforming nematodes may be attacked is as free-living larvae in the soil on their way from cyst to root and as males free from the root on their way to fertilizing a female. Spores or mycelium of *H. heteroderae* has never been found in cysts.

As part of a cooperation within the IOBC (International Organization for Biological and integrated Control of noxious animals and plants) Integrated Control of Soil Pests working group, sub-group: pathogens of nematodes, pot tests with *H. heteroderae* were carried out in 1984 in a greenhouse at Lyngby. The purpose of the experiment was to examine the influence of the fungus on the propagation of the beet cyst nematode *Heterodera schachtii.*

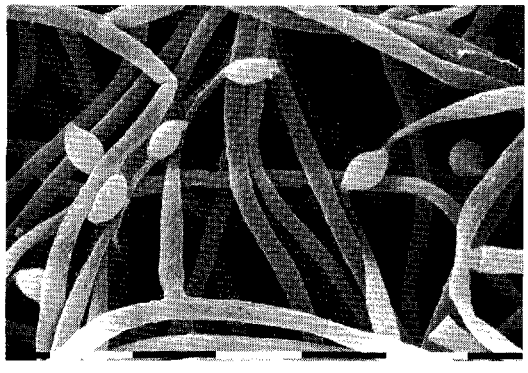

Fig. 1. *Hirsutella rhossiliensis (heteroderae)* mycelium with phialides and phialospores. Bar = 10 μm. SEM. (Photo: *Carmen Nuñez)*

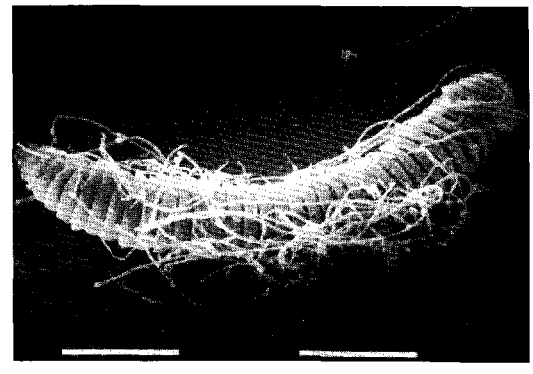

Fig. 2. *Criconema* with attached spores and growth of mycelium, phialides and phialospores of *Hirsutella rhossiliensis (heterodera).* Bar = 0.1 mm. SEM. (Photo: *Carmen Nuñez)*

Method and materials

The experiment was carried out in 1 l pots with one sugar beet plant – Magna mono – per pot. 2 types of soil were used for the experiment – A: Autoclaved sand-mixed, cyst-free garden soil and B: sand-mixed nematode-infested field soil.

In type B an average of 673 healthy cysts with a total of 134.4×10^3 eggs and larvae per l soil were found before start of the experiment. The fungal parasitism of eggs and larvae was in the autumn of 1983 21%; so the actual number of eggs and larvae was about 106×10^3 or less per l soil (pot).

6000 hatched, living larvae of the beet cyst nematode were introduced into each pot of A-soil at the 2-leaf stage of the beet plants. The larvae were introduced as a 1 ml suspension into 3 2.5–3.0 cm deep holes at a distance of about 2 cm from the root. As it turned out that 8.7% of the hatched larvae had died (did not move) at the time of introduction of the larval suspension, 6000 + 8.7% = 6522 larvae were added to each pot.

The cultures of *H. heteroderae* originating from West Germany were grown on corn meal agar (5 g CMA in 500 ml distilled water for 30 9 cm petri dishes).

As the fungus grows very slowly, it had to be spread by plating. The number of spores per petri dish was determined as 121.77×10^6 by means of a hemacytometer after about one month's growth.

Either type of soil received 5 treatments where the agar was chopped up and mixed with soil in the following quantities before being potted:

Contents of 6 petri dishes with *H. heteroderae* per l soil											(2aH)
–	–	3	–	–	–		–		–	–	– (1aH)
–	–	6	–	–	without		–		–	–	– (2a)
–	–	3	–	–	–		–		–	–	– (1a)
No agar or fungus added											(0a)

With 6 replicates per treatment the experiment comprised 60 pots in all. The experiment was scheduled to run for a period with a temperature sum of 500 day-degrees above 10°C (the time for 1 generation of *Heterodera schachtii)*. At the end of the experiment, the top of the plants was cut off and weighed, while the pots with soil and roots were left unwatered until extraction for counting of cysts.

Starting date for soil A: 19 June. Temperature sum: 556.6°C
Starting date for soil B: 15 June. Temperature sum: 613.3°C
(4 readings per 24 hours)
Test period for soil A: 56 days
Test period for soil B: 60 days

Results

The results from the experiment will be seen from Table 1 and 2 and from Fig. 3. Because of the large number of cysts, which makes counting very time-consuming, treatment (1a) was omitted.

A preliminary examination of cyst content of the A-soil revealed a parasitism which did not exceed 1%, and it was not by *H. heteroderae*.

Discussion

There was no correlation between treatment and top fresh weight. Due to the gradual drying out of roots from the end of the experiment to the last extraction, root weight is of no vital significance in this connection. However, all the roots were very bearded.

The number of newly formed cysts in the auto-

Table 1. Greenhouse pot experiment with *Hirsutella heteroderae* 1984. Top fresh weight, numbers of cysts, eggs and larvae of *Heterodera schachtii* after soil treatment with different numbers of spores from *H. heteroderae*. *Potteforsøg i drivhus med* Hirsutella heteroderae *1984. Friskvægt af top samt antal af cyster, æg og larver af* H. schachtii *efter jordbehandling med forskelligt antal sporer fra* H. heteroderae.

Treatment	Top fresh weight g	Number cysts	Number eggs & larvae × 1000	Treatment	Top fresh weight g	Number cysts	Number* eggs & larvae × 1000
A,2a	54.4	3078	517	B,2a	72.6	4408	898
	55.8	2753	475		75.8	1557	359
	62.4	11490	1299		40.0	4250	715
	53.2	12558	1344		52.8	2358	539
	43.8	8643	864		67.9	5758	1381
	83.5	10414	925		60.6	10196	2102
Sum	353.1	48936	5414		369.7	28527	5995
Mean	58.9	8156.0	902		61.6	4754.5	999
A,0a	45.8	2677	720	B,0a	32.2	4274	570
	62.5	7967	948		61.9	3600	333
	83.2	9467	1465		68.0	3258	358
	68.2	6262	1107		59.6	1727	232
	98.7	5300	573		45.1	8326	1805
	49.1	5105	669		60.8	3534	306
Sum	407.5	36778	5482		327.6	24719	3604
Mean	67.9	6129.7	914		54.6	4119.8	601
A,1aH	48.9	3853	542	B,1aH	43.8	5288	1000
	54.2	6771	850		61.3	836	126
	63.7	6502	845		70.4	2666	548
	42.2	2700	537		52.9	1950	566
	56.1	4354	771		51.3	2692	595
	47.7	2793	439		20.1	1541	281
Sum	312.8	26973	3983		299.8	14973	3115
Mean	52.1	4495.5	664		50.0	2495.5	519
A,2aH	51.2	3737	644	B,2aH	45.3	581	109
	55.1	4497	625		42.0	3195	810
	70.9	3911	651		18.8	691	150
	61.3	2800	533		30.0	497	101
	51.1	4384	684		22.9	646	149
	77.5	1457	273		25.9	521	57
Sum	367.1	20786	3408		184.9	6113	1376
Mean	61.2	3464.3	568.0		30.8	1028.8	229

*): The number of cysts in the infested soil: 673, has not been deducted.

Table 2. Greenhouse pot experiment with *Hirsutella heteroderae* 1984. Mean number and proportional of newly formed cysts of *Heterodera schachtii* on sugar beet roots after soil treatment with different amounts of spores from *Hirsutella heteroderae.*

Drivhusforsøg med Hirsutella heteroderae *1984. Gennemsnitligt antal nydannede cyster af* Heterodera schachtii *på rødder af sukkerroer og forholdstal for antal cyster efter jordbehandling med forskelligt antal sporer fra* Hirsutella heteroderae.

Treatment	Mean no. of cysts found per plant	Cysts found less original no. of cysts	Proportional	Dispersion on mean of cysts found
A,2a	8156		100	±1740
A,0a	6130		75	± 969
A,1aH	4496		55	± 725
A,2aH	3464		42	± 469
B,2a	4755	4082	100	±1251
B,0a	4120	3447	84	± 910
B,1aH	2496	1823	45	± 628
B,2aH	1029	346	8	± 436

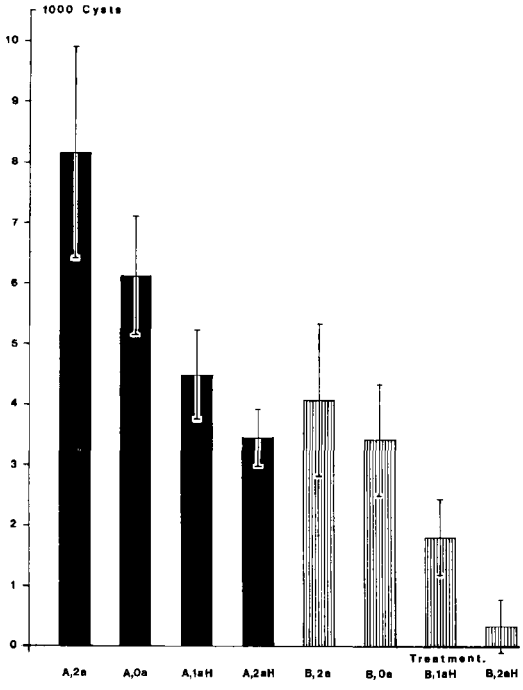

Fig. 3. Number of new cysts of *Heterodera schachtii* on sugar-beet roots after soil treatment with different amounts of spores from *Hirsutella heteroderae,* with dispersion of mean given.

claved soil (soil A) was very great and exceeded what might be caused by the number of larvae added. This shows that cysts and females had developed from 2nd generation larvae. The temperature sum of 556.6 day-degrees days above 10°C, which was obtained after 56 days, was under the given conditions too high for obtaining only 1 generation of *Heterodera schachtii.* The average temperature in the greenhouse during the test period was 19.9°C (13.6–26.5). In the open, a temperature sum of about 500 day-degrees above 10°C has been found suitable for 1 nematode generation to develop during the growth period. In general, the nematode development takes place more quickly with higher temperatures.

If the treatments 2a and 0a are compared, it is seen that both with A- and B-soil the admixture of agar to the soil stimulates the cyst formation, possibly by giving the larvae better possibilities of moving.

The effect obtained by adding *Hirsutella* spores was significant in both types of soil and increased with increased introduction of spores, especially in soil B, where a reduction of 92% in the number of newly formed females and cysts was obtained (Table 2).

The smaller number of cysts formed in soil B than in soil A in spite of the initial larger number of larvae, and the greater effect obtained when introducing spores (92% against 48%) is probably due to the fact that the larvae of *H. schachtii* were more evenly distributed in soil B than in soil A, where the larvae were introduced concentrated in 3 2.5–3.0 cm deep holes at a distance of only 2 cm from the root. Thus most of the larvae in soil B had to move over considerably greater distances with a greater risk of encountering a spore.

The mean number of eggs per cyst in all treatments was 152.0 and 187.4 for soil A and B, respectively, which might indicate a large number of newly formed females of the 2nd generation, especially in soil A, but it might also indicate that autoclaving of the soil reduced the fertility of the individual females.

Unfortunately, we do not know anything about the spore density of the fungus *H. heteroderae* in naturally infected soil. Fungi with sticky spores seem to prefer heavy soil to light soil (1), but the test demonstrated that with the present spore densities *H. heteroderae* may be used as an efficient biological control of the beet cyst nematode *Heterodera schachtii*.

Acknowledgement

I wish to express my gratitude to *Carmen Nuñez*, Laboratory of Zoology, University of Gent, Belgium, for valuable information.

Literature

1. *Groppe, B.* 1984. Untersuchungen zum Vorkommen räuberischer und endoparasitärer nematophager Pilze. Mitt. aus der Biol. Bundesanst. Land- und Forstwirts., Berlin-Dahlem 223, 187.
2. *Jaffee, B. A. & Zehr, E. J.* 1985. Parasitic and saprophytic abilities of the nematode-attacking fungus *Hirsutella rhossiliensis*. J. Nematol. 17, 341–345.
3. *Kerry, B. R.* 1984. Report of a meeting of the IOBC/WPRS Integrated Control of soil pest working group (Cambridge, September 19th–22nd 1983). Pedobiologia 27, 151–157.
4. *Minter, D. W. & Brady, B. L.* 1980. Mononematous species of *Hirsutella*. Trans. Br. Mycol. Soc. 74, 271–282.
5. *Sturhan, D. & Schneider, R.* 1980. *Hirsutella heteroderae*, ein neuer nematodenparasitärer Pilz. Phytopath. Z. 99, 99–115.

Manuscript received 19 November, 1985.