Suppression of *Criconemella xenoplax* by the Nematophagous Fungus *Hirsutella rhossiliensis*

C. G. EAYRE, Former Graduate Assistant, Department of Plant Pathology and Physiology, Clemson University; B. A. JAFFEE, Assistant Professor, Division of Nematology, University of California-Davis 95616; and E. I. ZEHR, Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377

ABSTRACT

Eayre, C. G., Jaffee, B. A., and Zehr, E. I. 1987. Suppression of *Criconemella xenoplax* by the nematophagous fungus *Hirsutella rhossiliensis*. Plant Disease 71:832-834.

The ability of the fungus *Hirsutella rhossiliensis* to suppress *Criconemella xenoplax* on peach seedlings in steamed soil in the greenhouse was studied. Suppression was observed in five of nine experiments. Addition of KCl to the soil did not affect the level of suppression but appeared to stimulate parasitism in one experiment.

Criconemella xenoplax (Raski) Lúc & Raski contributes to the peach-tree-short-life syndrome in the southeastern United States (11). In the 1970s, the nematicide dibromochloropropane was used to control this ectoparasitic nematode and increase tree longevity (13), but this chemical is now unavailable to growers. Consequently, new methods for controlling *C. xenoplax* are needed.

The hyphomycete *Hirsutella rhossiliensis* Minter & Brady was isolated from *C. xenoplax* and was able to parasitize this nematode in laboratory experiments (4–6). In these tests, nematodes were inoculated with conidia of *H. rhossiliensis* and incubated in soil extracts or salt solutions. Parasitism was positively correlated with electrical conductivity of soil extracts and was enhanced by K^+ and certain other ions (5). The effects of the fungus on the nematode in soil are unknown, although populations containing a high proportion of infected individuals have been reported (4).

The purposes of this study were to determine 1) the effect of *H. rhossiliensis* on population densities of *C. xenoplax* in soil and 2) if potassium amendments increased the impact of the fungus on nematode populations in soil. A preliminary report was published earlier (3).

MATERIALS AND METHODS

H. rhossiliensis (ATCC 46487) was grown in 10-cm-diameter petri plates containing 40 cm^3 of fine vermiculite moistened with 30 ml of V-8 broth (200 ml of V-8 juice, 3 g of $CaCO_3$, and 800 ml of H_2O). Plates were autoclaved, inoculated with a suspension of hyphae and conidia in 1 ml of H_2O, and incubated at 25 C until the fungus colonized the vermiculite (3–4 wk).

Troup sand (>90% sand), collected from a peach-tree-short-life site in Edgefield County, SC, was screened (0.6 cm) and then autoclaved or treated with aerated steam for 30 min at 60 C (1). Six-week-old Nemaguard or Red Globe peach seedlings were transplanted into 15-cm-diameter plastic pots containing 1,500 cm^3 of screened, steamed soil into which 75 cm^3 of fungal inoculum (moist, colonized vermiculite) was thoroughly mixed (5% v/v). Control pots received 75 cm^3 of autoclaved fungal inoculum. Pots were randomly distributed on a greenhouse bench (24 ± 5 C).

One week after the seedlings were transplanted into soil infested with *H. rhossiliensis*, 500 recently extracted *C. xenoplax* (juveniles and adults obtained from peach seedlings grown in autoclaved soil) were added to each pot. Pots were watered whenever the soil surface became dry. Unless noted otherwise, pots were fertilized every 2 wk with 100 ml of solution A (45 g of KNO_3, 12 g of triple superphosphate (0-46-0), and 2 ml of Stoller's Crop Mix in 19 L of H_2O) alternating with solution B (45 g of $Ca(NO_3)_2$ and 15 g of $MgSO_4$ in 19 L of H_2O). Mites were controlled with regular applications of cyhexatin or dicofol.

The objective of the first four experiments was to determine the potential for suppression of *C. xenoplax* during a specified period (8 or 12 wk). In two additional tests lasting 16 wk, samples were taken at 12 and 16 wk in the first instance (experiment 5) and at 2-wk intervals beginning 4 wk after inoculation in the second (experiment 6) to study the pattern of nematode increase in the presence or absence of *H. rhossiliensis*. No salts were added other than fertilizer in these experiments.

The influence of potassium (added as KCl) was studied in three other experiments (experiments 7–9). In experiments 7 and 8, each pot was watered with tap water or 1, 2, or 3 μM KCl; plants were watered so that the entire soil volume was moistened whenever the top of the soil appeared dry. Fertilizer was applied to soil in experiments 7 and 8 as previously described. For experiment 9, each pot received, in addition to standard watering, 100 ml of 0, 4.25, or 8.5 μM KCl each week; no fertilizer was applied to the soil, but the foliage was dipped in a nutrient solution ($Ca(NO_3)_2$ + spreader-sticker) once a week.

Individual pots were sampled once per experiment. Roots were rated for necrosis, and roots and shoots were weighed. The soil from each pot was

Table 1. Suppression of *Criconemella xenoplax* on peach seedlings by *Hirsutella rhossiliensis* in the greenhouse[a]

Exp.	Replicate	Length of exp. (wk)	P_i[b]	P_f −/+[c]	Percent reduction (or increase)	*P* value[d]	Percent colonized[e]
1	3	12	33	1,228/771	37	0.25	1.9
2	6	12	33	892/302	66	0.01	4.6
3	5	8	750	2,486/1,472	41	0.01	2.8
4	4	12	33	1,618/675	58	0.26	0
7	20	12	33	1,344/1,489	+11	0.61	...[f]
8	20	12	33	416/473	+14	0.58	...[f]

[a] Six-week-old peach seedlings were transplanted into steamed soil containing living or dead inoculum of *H. rhossiliensis*. *C. xenoplax* were added 1 wk later.
[b] Initial nematode population density, *C. xenoplax* per 100 cm^3 of soil.
[c] Final nematode population density in soil containing living fungal inoculum (+) or dead inoculum (−).
[d] Determined by the *t* test.
[e] Percentage of nematodes exposed to live *H. rhossiliensis* that were colonized at termination of the experiment.
[f] Percentage colonization was not determined.

Present address of first author: Department of Plant Pathology, North Carolina State University, Raleigh 27695.

Technical Contribution 2529 of the South Carolina Agricultural Experiment Station, Clemson University.

Accepted for publication 26 December 1986.

mixed thoroughly. Nematodes were extracted from a 100-cm^3 sample by elutriation (2) followed by centrifugation (9). Experiments 1–6 were analyzed by the *t* test for equal or unequal variances as appropriate (12). Experiments 7–9 were analyzed by factorial analysis of variance (12); data were subjected to a power transformation if necessary.

RESULTS AND DISCUSSION

H. rhossiliensis significantly ($P = 0.01$) suppressed *C. xenoplax* population levels in two of the first four experiments, although nematode population levels increased whether or not living fungal inoculum was added (Table 1). In the two experiments that lasted 16 wk, suppression continued for the duration in experiment 5 but was not detected in the last sampling of experiment 6 (Table 2). The reason(s) for these inconsistent results is unclear but might be related to the behavior and population dynamics of the fungus. The percentage of nematodes colonized by *H. rhossiliensis* was low in each of the first four experiments (Table 1), but many nematodes were infested with ungerminated spores. Many infested but uninfected nematodes have been seen also in samples collected from the field (*unpublished*). The percentage of parasitism might have been underestimated because parasitism of J2 and J3 larvae is easily overlooked. Although potassium and certain other cations have increased parasitism in laboratory tests (5), factors that influence germination and viability of *H. rhossiliensis* spores are not well understood.

The purpose of the last three experiments was to determine if parasitism in soil was enhanced by the addition of potassium ions, which had increased parasitism in laboratory tests (5). In experiments 7 and 8, *H. rhossiliensis* did not suppress *C. xenoplax* regardless of the level of potassium added (Table 1). Parasitism occurred, but the percentage of infection was not determined. In experiment 9, populations were reduced by *H. rhossiliensis* whether or not potassium was added, but nematodes increased in all pots (Table 3). The percentage of nematodes colonized after 13 wk increased with potassium addition (0.5, 5.6, and 7% parasitized at 0, 4.25, and 8.5 μM KCl, respectively). Root weight, shoot weight, and root necrosis were not affected by the treatments.

The failure of KCl amendments to affect nematode suppression is not easily explained. Stimulation of infection by KCl seemed to occur in experiment 9, but the extent of parasitism may have been limited by other factors such as fungal survival, sporulation, or inoculation of nematodes. Other epidemiological factors that affect virulence of the fungus or time for an epidemic to become established might have limited parasitism. The results reported here, however, are consistent with an 18-mo field study in which addition of potassium did not influence nematode suppression by *H. rhossiliensis* (E. I. Zehr and B. A. Jaffee, *unpublished*).

Inocula of nematophagous fungi may be added to soil in different ways. In this and other studies (e.g., 10), dead organic substrates were used to carry the biocontrol agent and to provide the biocontrol agent with an initial food base. Recent research suggests that *H. rhossiliensis* is not an aggressive saprophyte and may not be able to protect and utilize certain dead organic substrates in the presence of other soil microorganisms (6). Although pasteurized or autoclaved soil was used in the present study, it was always aged 3–4 wk before use and was probably colonized by fast-growing saprophytic fungi. Such fungi may have limited the ability of *H. rhossiliensis* to sporulate from the vermiculite/V-8 juice carrier.

Another endoparasitic fungus, *Meria coniospora*, was successfully introduced into soil as a conidial suspension or on infected "carrier" nematodes (8). Unlike *M. coniospora*, which adheres specifically to the cuticle of certain nematodes (7), *H. rhossiliensis* spores adhere to a variety of substrates. Consequently, it is difficult to obtain an aqueous spore suspension of *H. rhossiliensis* by flooding agar plates; once dislodged from phialides, spores adhere to hyphae, agar, or petri plates. Even if a spore suspension were obtained and mixed into the soil, it is likely that the spores would adhere to soil particles and would be unable to adhere to and infect passing nematodes. We believe that successful inoculation and infection of nematodes in soil probably occurs only if the spore is produced and presented on the tip of the phialide; in this position, the spore readily adheres to passing nematodes.

Although *H. rhossiliensis* does not compete well for certain substrates, only a limited number of substrates were considered (6), and it is possible that a more suitable substrate could be found and used for introducing high levels of the fungus into soil. The possibility of using infected nematodes as inoculum should also be considered.

Table 2. Effects of *Hirsutella rhossiliensis* on population density of *Criconemella xenoplax* on peach seedlings through time[a]

	C. xenoplax per 100 cm^3 of soil (wk after addition of nematodes)						
Fungal inoculum	**4**	**6**	**8**	**10**	**12**	**14**	**16**
Experiment 5							
Dead	...	...	...	...	1,965[b]	...	7,520
Alive	...	...	...	...	946	...	1,457
$P =$	...	...	...	...	0.30	...	0.04
Experiment 6							
Dead	10[c]	80	150	171	765	1,668	1,453
Alive	5	25	33	130	392	233	1,510
$P =$	0.47	0.02	0.01	0.60	0.12	0.01	0.89

[a] Six-week-old peach seedlings were transplanted into steamed soil containing living or heat-killed *H. rhossiliensis*. *C. xenoplax* (33/100 cm^3 of soil) were added 1 wk later.
[b] In experiment 5, each value is the mean of six replicates; each replicate was destructively sampled once.
[c] In experiment 6, each value is the mean of five replicates; each replicate was destructively sampled once.

Table 3. Effects of KCl soil amendments on suppression of *Criconemella xenoplax* by *Hirsutella rhossiliensis* in the greenhouse[a]

	C. xenoplax per 100 cm^3 of soil (μM KCl per pot per week[b])			
Fungal inoculum	**0**	**4.25**	**8.5**	$\bar{x}$
Heat-killed	1,346[c]	1,295	1,410	1,350
Alive	656	525	480	520

[a] Six-week-old peach seedlings were transplanted into steamed soil containing living or heat-killed *H. rhossiliensis*. *C. xenoplax* (33/100 cm^3 soil) were added 1 wk later. The experiment was terminated 13 wk after addition of nematodes.
[b] Each pot received 100 ml of tap water and 4.25 or 8.5 M KCl per week in addition to regular watering.
[c] Each value is the mean of six replicates. The interaction between fungal inoculum and KCl levels was not significant ($P = 0.88$). The main effect of inoculum was significant ($P = 0.01$), but the main effect of KCl level was not significant ($P = 0.86$).

LITERATURE CITED

1. Aldrich, R. A., and Nelson, P. E. 1969. Equipment for aerated steam treatment of small quantities of soil and soil mixes. Plant Dis. Rep. 53:784-788.
2. Byrd, D. W., Jr., Barker, K. R., Ferris, H., Nusbaum, C. J., Griffin, W. E., Small, R. H., and Stone, C. A. 1976. Two semiautomatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.
3. Eayre, C. G., Jaffee, B. A., and Zehr, E. I. 1983. Suppression of *Criconemella xenoplax* by the fungus *Hirsutella rhossiliensis*. (Abstr.) Phytopathology 73:500.
4. Jaffee, B. A., and Zehr, E. I. 1982. Parasitism of the nematode *Criconemella xenoplax* by the fungus *Hirsutella rhossiliensis*. Phytopathology 72:1378-1381.

5. Jaffee, B. A., and Zehr, E. I. 1983. Effects of certain ions, osmotic potential, and soil solutions on parasitism of *Criconemella xenoplax* by *Hirsutella rhossiliensis*. Phytopathology 73:544-546.
6. Jaffee, B. A., and Zehr, E. I. 1985. Parasitic and saprophytic abilities of the nematode-attacking fungus *Hirsutella rhossiliensis*. J. Nematol. 17:341-345.
7. Jansson, H.-B., Jeyaprakash, J., and Zuckerman, B. M. 1984. Differential adhesion and infection of nematodes by the endoparasitic fungus *Meria coniospora*. Appl. Environ. Microbiol. 49:552-555.
8. Jansson, H.-B., Jeyaprakash, J., and Zuckerman, B. M. 1985. Control of root-knot nematodes on tomato by the endoparasitic fungus *Meria coniospora*. J. Nematol. 17:327-329.
9. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
10. Mankau, R., and Wu, X. 1985. Effects of the nematode-trapping fungus, *Monacrosporium ellipsosporium* on *Meloidogyne incognita* populations in field soil. Rev. Nematol. 8:147-153.
11. Nyczepir, A. P., Zehr, E. I., Lewis, S. A., and Harshman, D. C. 1983. Short life of peach trees induced by *Criconemella xenoplax*. Plant Dis. 67:507-508.
12. SAS Institute. 1982. SAS User's guide: Statistics. The Institute: Cary, NC. 584 pp.
13. Zehr, E. I., Miller, R. W., and Smith, F. H. 1976. Soil fumigation and peach rootstocks for protection against peach tree short life. Phytopathology 66:689-694.