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Effectiveness of *Hirsutella minnesotensis* and *H. rhossiliensis* in control of the soybean cyst nematode in four soils with various pH, texture, and organic matter

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RESEARCH ARTICLE

Effectiveness of *Hirsutella minnesotensis* and *H. rhossiliensis* in control of the soybean cyst nematode in four soils with various pH, texture, and organic matter

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The parasitism of soybean cyst nematode, *Heterodera glycines*, by the fungi *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* and their biocontrol effectiveness against the nematode were investigated in four soils with various pH, texture, and organic matter. Fungal parasitism was assayed in the soils in 25 mL vials. As expected, percentage of *H. glycines* second-stage juveniles (J2) parasitized by either fungus increased with increasing number of fungus-colonized J2 initially added into the soils. Parasitism of J2 by the fungi was negatively related with soil pH. Both positive and negative relationships with fungal parasitism were observed for soil sandiness and organic matter. In greenhouse study, both fungi at 0.2–0.8 g fresh mycelium of liquid culture per 0.3 L pot and 1% corn-grits culture effectively reduced nematode population density. The relationship between biocontrol effectiveness and the soil factors depended on fungal species and inoculation levels. In general, percentage reduction of egg population density in the soil was negatively correlated with soil pH and positively correlated with sandiness. There was no or weak correlation between egg reduction and organic matter. The percentage of J2 parasitized by the fungi 2 months after planting did not correlate with the soil factors. Plant growth was better in the two soils with intermediate pH and sand than the soil with high pH and low sand or with low pH and high sand. It appeared that soil pH and/or texture are important in influencing biocontrol effectiveness, but further studies are needed to determine the effect of individual factors because they are correlated.

Keywords: biological control; *Heterodera glycines*; *Hirsutella minnesotensis*; *Hirsutella rhossiliensis*; nematophagous fungi; organic matter; parasitism; soil pH; soil texture; soybean cyst nematode

Introduction

Hirsutella rhossiliensis Minter & Brady and *Hirsutella minnesotensis* Chen, Liu & Chen (2000) are two fungal endoparasites of nematodes (Sturhan and Schneider 1980; Chen, Liu, and Chen 2000). The fungi produce adhesive conidia that may attach to cuticle of vermiform nematodes and initiate infection. Only the conidia that remain on the conidiogenous cells have infectivity; once detached, they will loss ability of attaching to the nematode cuticle (McInnis and Jaffee 1989). Studies have

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shown that a single conidium attached to cuticle is sufficient for infecting a nematode (Cayrol and Frankowski 1986). Once the fungus penetrates the nematode, it proliferates in the nematode's body, converts the nematode's contents into mycelium, and produces new conidia within a few days (Jaffee 1992).

Hirsutella rhossiliensis has been reported from numerous nematodes throughout the world (Kerry and Jaffee 1997; Chen and Dickson 2004). In some fields, the fungus parasitized a high percentage of nematodes (Muller 1982; Jaffee, Gaspard, and Ferris 1989; Liu and Chen 2000) and may be partially responsible for suppression of nematode populations. For example, in some orchards in the southeastern USA, populations of the ring nematode *Mesocriconema xenoplax* have been reported to be suppressed by *H. rhossiliensis* (Zehr 1985). Muller (1982, 1985) reported that *H. rhossiliensis* parasitized as high as 90% of the sugar beet cyst nematode *Heterodera schachtii* second-stage juveniles (J2) in a field in Germany, and suppressed *H. schachtii* populations on oil radish. Parasitism of the cyst nematode *Heterodera daverti* by *H. rhossiliensis* was detected in 90% of soil samples collected from carnation fields in Naples, Italy (del Sorbo, Marziano, and D'Errico 2003). The fungus was presumably responsible for decrease in severity of the nematode attacks over years. In a field in Minnesota, USA, the population density of the soybean cyst nematode, *Heterodera glycines* Ichinohe, remained at a relatively low level in monoculture of soybean over 27 years. *Hirsutella rhossiliensis* parasitized 11–53% of J2 during the soybean growing seasons (Chen 1997). The fungus was at least partially responsible for suppressing infection of soybean by *H. glycines* in the field (Chen 2007).

Hirsutella minnesotensis has been observed on *H. glycines* J2 in North Central USA (Chen et al. 2000) and Northern China (Ma, Liu, Jian, and Li 2005). Recently *H. minnesotensis* was isolated from mites in Germany and Poland (Balazy, Wrzosek, Sosnowska, Tkaczuk, and Muszewska 2008). In laboratory assays, *H. minnesotensis* also can parasitize a wide range of nematodes including plant-parasitic, entomopathogenic, fungal-feeding, and bacterial-feeding nematodes (Liu and Chen 2001a).

In a survey in 237 fields in 27 counties across southern Minnesota in the USA, *H. rhossiliensis* and *H. minnesotensis* were commonly found on the J2 of *H. glycines* (Liu and Chen 2000). Parasitism of J2 by *H. rhossiliensis* was observed in soils from 43% of the fields and by *H. minnesotensis* in soils from 14% of the fields. However, there was large variation in percentage of J2 parasitized among fields. In some fields, as high as 60% of J2 were parasitized by one or both species (Chen and Reese 1999; Liu and Chen 2000).

Because the two fungi effectively parasitize nematodes in natural soils, they are attractive candidates as potential biological control agents. In greenhouse assays, *H. rhossiliensis* suppressed *Globodera pallida* on potato (Velvis and Kamp 1996), *H. schachtii* on cabbage (Jaffee and Muldoon 1989), *Pratylenchus penetrans* on potato (Timper and Brodie 1994), *H. glycines* on soybean (Liu and Chen 2001b, 2005; Chen and Liu 2005), and *Meloidogyne incognita* on tomato (Amin 2000). *Hirsutella minnesotensis* was effective in control of *H. glycines* on soybean (Liu and Chen 2001b, 2005; Chen and Liu 2005), and *Meloidogyne hapla* on tomato (Mennan, Chen, and Melakeberhan 2006, 2007) in the greenhouse studies. However, field and microplot studies showed inconsistent results (Jaffee, Muldoon, and Westerdahl 1996; Jaffee and Muldoon 1997; Jaffee and Zasoski 2001; Chen

2003). The reasons for the variability in field occurrences and the biocontrol effectiveness are unclear.

Nematophagous fungi are influenced by numerous biotic and abiotic soil factors. Soil pH is an important factor that directly or indirectly affects the activities of nematophagous fungi, and different fungi may have different requirements of soil environments (Chen and Dickson 2004). The presence of predatory fungi was more influenced by soil pH than other soil factors (Gray 1985). Conidium-forming endoparasites were isolated from samples with relatively low soil pH (Gray 1987). Jaffee and Zasoski (2001) reported that the activity of pelletized *H. rhossiliensis* was negatively correlated with soil pH above 4.5. Maximum activity occurred at pH 4.5, and activity gradually declined to near zero as the pH increased to 6.5 and rapidly declined to near zero as the pH dropped below 4.0. It is concluded that low soil pH suppresses soil organisms that otherwise interfere with growth of *H. rhossiliensis* from alginate pellets (Jaffee and Zasoski 2001). Soil texture is another important factor that affects both activity of nematophagous fungi and nematode movement. Transmission of nematodes by *H. rhossiliensis* was higher in loam than sandy soils (Tedford, Jaffee, and Muldoon 1992). Organic matter may enhance activities of some nematophagous fungi, especially trap-forming fungi, in natural soil (Gray 1987). But the parasitism of *Mesocriconema xenoplax* by *H. rhossiliensis* was inhibited by addition of organic matter to a natural soil (Jaffee, Ferris, Stapleton, Norton, and Muldoon 1994).

The objective of this study was to determine fungal parasitism and biocontrol effectiveness of *H. minnesotensis* and *H. rhossiliensis* against the soybean cyst nematode in four soils with various pH, texture and organic matter.

Materials and methods

Soil

Four soils were collected from a soybean field in Waseca County, Minnesota. Soil textures, pH and organic matter were analyzed in the Agvise Laboratory in Benson, Minnesota, and are summarized in Table 1. Each of the soils was passed through a 5 mm aperture sieve, autoclaved at 121°C for 1 h, air dried, and water content was adjusted to 10% (w/w).

Table 1. Characteristics of soils used in experiments in vials and greenhouse.

Characteristics	Soil 1	Soil 2	Soil 3	Soil 4
Soil type	Clay loam	Silty clay	Clay loam	Silty clay loam
% sand	31.6	18.3	22.4	15.1
% silt	35.0	40.6	38.4	45.8
% clay	33.4	41.1	39.2	39.1
Bulk density (kg L^{-1})	1.32	1.26	1.27	1.26
pH before autoclaving	5.28	6.25	7.17	7.94
pH after autoclaving	4.85	5.88	6.88	7.79
% organic matter	6.1	7.7	6.6	9.0

Nematode origin and preparation

Soybean cyst nematode race 3 (HG Type 0-) collected from a field in Waseca, Minnesota, was cultured on soybean cv. Sturdy in sterilized soil in the greenhouse. Newly formed females and cysts were washed with a vigorously applied water stream through an 850 μm aperture sieve onto a 250 μm aperture sieve and extracted by centrifugation in 63% (w/v) sucrose solution. Eggs were released from the cysts by crushing the cysts on a 150 μm aperture sieve with a rubber stopper mounted on a motor (Faghihi and Ferris 2000). The eggs were separated from debris by centrifugation in a 35% (w/v) sucrose solution for 5 min at $1500 \times g$, transferred to an antibiotic solution (streptomycin sulfate, chlortetracycline and 8-quinolinol at 100, 50, and 20 mg L^{-1}, respectively), and maintained at 4°C before being used within 2 days. Prior to use, the antibiotic solution was replaced with water. For preparation of J2, the eggs were rinsed with deionized water and transferred to a 4 mM $ZnCl_2$ solution at 22–25°C to hatch. The J2 that hatched within 2 days were collected, rinsed with sterile deionized water and washed into a sterile beaker with 4.5 mM KCl before being used in the assays in vial soil.

Fungal parasitism of J2 in vial soil

The fungi *H. minnesotensis* isolate SD3-2 and *H. rhossiliensis* isolate OWVT-1 were cultured on corn grits at room temperature (22–25°C) for 4–5 weeks. Five thousand healthy J2 in 1 mL of 4.5 mM KCl were added to 50 g of corn-grits fungal culture in a 10 cm diameter Petri dish. After incubation at room temperature for 3 days, J2 were recovered from the corn grits by a sucrose-flotation and centrifugation technique (Jenkins 1964). The nematode suspensions in 0.3 mL 4.5 mM KCl solution containing 100, 500, and 2500 fungus-colonized J2 were individually mixed with 25 g of each soil. The soil was then placed in a 25 mL vial, in which a drain hole (7 mm diameter) was made at the bottom and a circle of polyester fabric was placed in the bottom to retain soil (Liu and Chen 2001b). At 0, 10 and 30 days after adding the fungus-colonized J2, 300 healthy J2 (hatched within 2 days) in 0.3 mL of 4.5 mM KCl were added to the surface of the soil in each vial. After 3 days, J2 were recovered from the soil with the sucrose-flotation and centrifugation method (Jenkins 1964). The percentage of the assay J2 colonized by each of the fungi or with attached fungal spores was determined. Five replicates (5 vials) were used for each treatment. The experiment was performed twice.

Biocontrol effectiveness in the greenhouse

Fungal inoculum preparation: Both liquid and solid cultures of *H. minnesotensis* isolate SD3-2 and *H. rhossiliensis* isolate OWVT-1 were prepared following the procedures in previous reports (Liu and Chen 2001b, 2005). The colony-forming units of the fungal cultures are summarized in Table 2.

General procedures

The experiment was a factorial design including four soils, two fungi, and six fungal inoculation levels. Nematode eggs were added to 300 mL soil at a rate of 3000 eggs

Table 2. Fungal isolates, their types of culture, and colony-forming units (CFU) of the cultures used in this study.

		Origin			CFU per g of corn grits or mL of suspension	
Fungal species	Fungal isolates	Host	Locality	Type of culture	Run 1	Run 2
H. rhossiliensis	OWVT-1	*H. glycines*	Minnesota	Corn grits	$(5.52 \pm 0.22) \times 10^6$	$(4.53 \pm 0.54) \times 10^6$
				Potato dextrose broth	$(1.57 \pm 0.34) \times 10^6$	$(1.56 \pm 0.26) \times 10^6$
H. minnesotensis	SD3-2	*H. glycines*	South Dakota	Corn grits	$(4.58 \pm 0.34) \times 10^6$	$(4.36 \pm 0.32) \times 10^6$
				Potato dextrose broth	$(1.65 \pm 0.21) \times 10^6$	$(1.67 \pm 0.24) \times 10^6$

$0.1\ L^{-1}$ soil. For the liquid culture inoculum, 10 mL of fungal suspension at application rates of 0.2, 0.4 and 0.8 g fresh mycelium per pot were added to the soil. The corn-grits culture of each fungus was mixed with the soil at a rate of 1% (corn grits:soil). Controls included the soil amended with 1% autoclaved corn grits, and the soil without addition of corn grits and fungal culture. The soils with the eggs and with/without fungal inoculum were mixed thoroughly, and placed in a 10 cm diameter pot. Seeds of 'Sturdy' soybean were surfaced disinfected with 0.1% of NaOCl for 3 min and three seeds were sown per pot. Five replicates were used for each treatment. The pots were maintained in the greenhouse at approximately 26°C (range 20–30°C) with 16 h of artificial lights and 8 h of dark each day. After 1 week, the soybean plants were thinned to two plants per pot. Two months after inoculation, egg population densities, percentage of J2 colonized by the fungi or with attached fungal conidia, and plant fresh weights were measured. This experiment was performed twice.

Statistical analysis

For the fungal parasitism in the vials, the data of percentage of J2 parasitized by the fungi were degree-arcsine ($x^{0.5}$)-transformed before being subjected to analysis of variance (ANOVA) to determine the interactive effects of four soils, fungal species, fungal density, and incubation time. Because the interactions among the four factors were significant at all levels, further analyses were focused on variables of soil factors and inoculation level. For this purpose, linear regression was performed for data of individual fungal species and incubation time using the model $Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2$, where Y is degree-arcsine ($x^{0.5}$)-transformed percentage of J2 parasitized by fungus, X_1 is inoculation level, and X_2 is either pH, percentage of sand, or organic matter.

For the greenhouse study, the $\log_{10}$ (x)-transformed egg population densities, degree-arcsine ($x^{0.5}$)-transformed percentages of J2 parasitized by fungi and non-transformed plant fresh shoot weights were subjected to ANOVA. The means were compared with the least significant difference test (LSD) at $\alpha=0.05$. The Pearson simple linear correlation analyses were performed to determine the relationship between the percentage of J2 parasitized by the fungi and soil characters, and between the egg reduction and soil characters.

Results

Parasitism of J2 in vial soil

There was no difference between the two repeated runs of the experiment, and thus the data of the two runs are combined. Interactions among soil pH, fungal species, fungal inoculation density, and incubation time were significant at all levels. The ANOVA of individual fungal species are presented separately in Table 3. The effect of soil factors on fungal parasitism of J2 depended on fungal species, fungal inoculation level, and length of incubation time (Table 4). Overall, the percentage of assay J2 with attached conidia increased with increasing number of colonized J2 initially added into the soil. Soil pH negatively related with percentage of J2 parasitized by *H. rhossiliensis* at days 0 and 10 and by *H. minnesotensis* at days 10 and 30. Interaction of soil pH and inoculation level was observed at day 10 for

Table 3. Analysis of variance of percentage of second-stage juveniles of *Heterodera glycine*s parasitized by *Hirsutella rhossiliensis* (Hr) and *H. minnesotensis* (Hm) in vial soil.

	df	Hr	Hm
Soils (A)	3	***	***
Fungal density (B)	2	***	***
Incubation time (C)	2	***	NS
A × B	6	***	*
A × C	6	***	***
B × C	4	**	**
A × B × C	12	***	NS

*,**,***Significant at $P < 0.05$, 0.01 and 0.001, respectively.
NS, not significant at $P \geq 0.05$.

H. rhossiliensis, and pH had stronger effect on the percentage of J2 parasitized at high than low fungal population densities. Effect of soil texture and organic matter was inconsistent and appeared to be less important. The relationship between sandiness and parasitism of J2 by fungus was positive for *H. rhossiliensis* at days 0 and 10, and negative for *H. rhossiliensis* at day 30 and for *H. minnesotensis* at days 10 and 30 (Table 4). There was a positive interaction between inoculation level and organic matter on parasitism of J2 by *H. rhossiliensis* at day 10. The relationship between organic matter and fungal parasitism of J2 was negative for *H. rhossiliensis* at days 0 and 10 and for *H. minnesotensis* at day 30, but positive relationship was also observed for *H. rhossiliensis* at day 30 (Table 4).

Biocontrol effectiveness in the greenhouse

The ANOVA of nematode population densities, percentage of J2 parasitized, and plant weights are summarized in Table 5. The effects of soil (pH, texture, and organic matter), fungal species, and fungal inoculation level on the biocontrol effectiveness differed in the two runs of the experiment, thus the data of both runs are separately presented. The effect of soil on biocontrol effectiveness differed at different fungal inoculation levels and with different fungi.

Egg population density

Overall *H. minnesotensis* and *H. rhossiliensis* effectively reduced the number of eggs mL^{-1} soil as compared with the soil-only control in most cases in the two runs (Table 6). However, the control effectiveness was more consistent across different soil pH and texture levels in Run 1 than Run 2 of the experiment. In Run 1, lower number of eggs mL^{-1} soil as compared with soil-only control was observed for both fungi in all four soils. In Run 2, lower eggs mL^{-1} soil were consistently observed for all inoculation levels of both fungal species only in Soil 1. In Soil 2 and Soil 3, most fungal treatments did not result in significant lower egg population densities. In Soil 4, *H. rhossiliensis* at 1% corn-grits culture and *H. minnesotensis* at 0.4 and 0.8 g mycelium and 1% corn-grits culture also resulted in significant lower eggs mL^{-1} soil. In comparison with the soil-only control, the treatment of 1% corn grits generally

Table 4. Linear relationship of inoculation level (Inoc), soil pH, texture (% sand), and organic matter (OM) with percentage of second-stage juveniles of *Heterodera glycine*s parasitized by *Hirsutella rhossiliensis* (Hr) and *H. minnesotensis* (Hm) in vial soils.[1]

		pH and Inco		Sand and Inoc		OM and Inoc	
Fungus	Day	Model	R^2	Model	R^2	Model	R^2
Hr	0	$Y=35.2+0.0037X_1-2.7X_2$	0.54	$Y=10.3+0.0037X_1+0.354X_2$	0.45	$Y=36.9+0.0037X_1-2.57X_2$	0.52
	10	$Y=29.1+0.019X_1-2.16X_2-0.0022X_1X_2$	0.82	$Y=7.8+0.34X_2+0.00024X_1X_2$	0.64	$Y=15.4+0.021X_1-0.0022X_1X_2$	0.59
	30	$Y=13.3+0.0041X_1$	0.32	$Y=22.1+0.0041X_1-0.402X_2$	0.42	$Y=-1.8+0.0041X_1+2.06X_2$	0.41
Hm	0	$Y=19+0.0038X_1$	0.54	$Y=19+0.0038X_1$	0.54	$Y=19+0.0038X_1$	0.54
	10	$Y=27.6+0.0042X_1-1.26X_2$	0.47	$Y=14.8+0.0042X_1-1.217X_2$	0.47	$Y=19.6+0.0042X_1$	0.43
	30	$Y=49.1+0.0027X_1-4.48X_2$	0.66	$Y=6.7+0.0027X_1-0.637X_2$	0.48	$Y=44+0.0027X_1-3.18X_2$	0.42

[1]Linear regression was performed using the model $Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2$, where Y is degree-arcsine ($x^{0.5}$)-transformed percentage of J2 parasitized by fungus, X_1 is inoculation level, and X_2 is either pH, sand, or organic matter. The coefficients of the remaining terms in the model are significant at $P<0.05$.

Table 5. Analysis of variance of nematode population densities, percentage of second-stage juveniles of *Heterodera glycines* parasitized, and plant weights treated with *Hirsutella rhossiliensis* and *H. minnesotensis* in four soils.

	df	Eggs 0.1 L^{-1} soil	% J2 parasitized	Plant weight
Experiment (A)	1	***	***	**
Soil (B)	3	***	***	***
Fungi (C)	1	NS	***	*
Inoc. level (D)	5	***	***	***
A × B	3	***	**	NS
A × C	1	NS	***	NS
A × D	5	***	***	NS
B × C	3	NS	**	NS
B × D	15	***	***	***
C × D	5	NS	***	*
A × B × C	3	***	***	NS
A × B × D	15	***	***	NS
B × C × D	15	NS	***	NS
A × C × D	5	NS	***	NS
A × B × C × D	30	NS	NS	NS

*,**,***Significant at $P < 0.05$, 0.01 and 0.001, respectively.
NS, not significant at $P \geq 0.05$.

resulted in similar population density, but reduced egg population densities in Soil 1 in the Run 2 (Table 6).

The relationship between percentage of egg reduction and soil characters depended on different inoculation levels and different fungi (Table 7). In general, the egg reduction by *H. rhossiliensis* was negatively correlated with soil pH and positively correlated with sand; there was no significant correlation between the egg reduction and soil organic matter for *H. rhossiliensis*.

In *H. minnesotensis* treatments, significantly negative correlation between percentage egg reduction and soil pH was observed at 0.4 and 0.8 g mycelium and 1% corn-grits culture in the Run 1 and 0.2 g mycelium in Run 2 (Table 7). The percentage of egg reduction was positively correlated with sand at 0.4 g mycelium and 1% corn-grits culture in Run 1 and all fungal inoculation levels in Run 2. Significant correlation between egg reduction and organic matter was observed in 0.2 and 0.4 g mycelium and 1% corn-grits culture in Run 1 and 0.2 g mycelium in Run 2 (Table 7).

Fungal parasitism

All factors, i.e., soil, fungal species, and fungal inoculation levels, affected percentage of J2 parasitized at the end of experiment (2 months after planting). The interactions among the three factors were significant at all combinations (Table 5). No parasitism of J2 by *H. minnesotensis* or *H. rhossiliensis* in the soil of either control was observed, indicating that there was no detectable cross contamination among the pots. There was no difference in percentage of J2 parasitized by fungi among the inoculation levels in most cases, but in a few cases corn-grits culture and/or 0.2 g mycelium resulted in lower percentage of J2 parasitized (Table 8).

Table 6. Effect of *Hirsutella rhossiliensis* (Hr) and *H. minnesotensis* (Hm) on *Heterodera glycines* egg population density (eggs mL^{-1}) in four soils in greenhouse.[1]

Fungi	Experiment	Inoculation level	Soil 1			Soil 2			Soil 3			Soil 4		
Hr	Run 1	0.8 g mycelium/pot	19	b	B	50	bc	AB	109	b	A	99	b	A
		0.4 g mycelium/pot	29	b	B	51	bc	B	126	b	A	107	b	A
		0.2 g mycelium/pot	19	b	B	100	b	A	123	b	A	107	b	A
		1% corn-grits culture	20	b	B	28	c	B	104	b	A	103	b	A
		1% corn grits	384	a	B	305	a	B	522	a	A	361	a	B
		soil only	359	a	A	446	a	A	448	a	A	474	a	A
	Run 2	0.8 g mycelium/pot	140	b	A	118	bc	A	135	a	A	106	ab	A
		0.4 g mycelium/pot	142	b	A	143	abc	A	193	a	A	132	a	A
		0.2 g mycelium/pot	221	b	A	242	ab	A	200	a	A	168	a	A
		1% corn-grits culture	47	c	B	106	c	AB	191	a	A	68	b	AB
		1% corn grits	72	c	C	287	a	A	282	a	AB	112	ab	BC
		soil only	561	a	A	320	a	B	280	a	B	209	a	B
Hm	Run 1	0.8 g mycelium/pot	41	b	A	37	b	A	73	bc	A	88	c	A
		0.4 g mycelium/pot	43	b	C	58	b	BC	95	b	AB	144	b	A
		0.2 g mycelium/pot	59	b	B	67	b	B	49	c	B	168	b	A
		1% corn-grits culture	17	c	C	50	b	B	47	c	B	118	bc	A
		1% corn grits	384	a	B	305	a	B	522	a	A	361	a	B
		soil only	359	a	A	446	a	A	448	a	A	474	a	A
	Run 2	0.8 g mycelium/pot	63	bc	A	182	a	A	149	ab	A	98	b	A
		0.4 g mycelium/pot	80	b	A	207	a	A	170	ab	A	102	b	A
		0.2 g mycelium/pot	100	b	B	257	a	A	184	ab	AB	146	ab	AB
		1% corn-grits culture	35	c	C	247	a	A	85	b	B	60	c	BC
		1% corn grits	72	bc	C	287	a	A	282	a	AB	112	ab	BC
		soil only	561	a	A	320	a	B	280	a	B	209	a	B

[1]The values are means of five replicates. The data were transformed to $\log_{10}(x)$ before being subjected to statistical analysis. The values followed by the same letter in the column within the same fungal species and run of experiment or the values followed by the same uppercase letter in the row were not significantly different according to the LSD test at $P \geq 0.05$.

Table 7. Correlation coefficients (r) between reduction of *Heterodera glycines* egg population density by *Hirsutella rhossiliensis* (Hr) and *H. minnesotensis* (Hm) and soil characters.

Fungus	Experiment	Inoculation level	pH	Sand	Organic matter
Hr	Run 1	0.8 g mycelium/pot	−0.57**	0.44 NS	−0.24 NS
		0.4 g mycelium/pot	−0.65**	0.45*	−0.19 NS
		0.2 g mycelium/pot	−0.48*	0.51*	−0.28 NS
		1% corn-grits culture	−0.65**	0.44 NS	−0.25 NS
	Run 2	0.8 g mycelium/pot	−0.62**	0.55*	−0.39 NS
		0.4 g mycelium/pot	−0.69***	0.59**	−0.34 NS
		0.2 g mycelium/pot	−0.44 NS	0.51*	−0.40 NS
		1% corn-grits culture	−0.44 NS	0.35 NS	0.00 NS
Hm	Run 1	0.8 g mycelium/pot	−0.40*	0.23 NS	−0.19 NS
		0.4 g mycelium/pot	−0.68***	0.54*	−0.51*
		0.2 g mycelium/pot	−0.43NS	0.40 NS	−0.60**
		1% corn-grits culture	−0.75***	0.75***	−0.80***
	Run 2	0.8 g mycelium/pot	−0.38NS	0.48*	−0.29NS
		0.4 g mycelium/pot	−0.39NS	0.52*	−0.28NS
		0.2 g mycelium/pot	−0.50*	0.66**	−0.48*
		1% corn-grits culture	−0.08 NS	0.43*	−0.30NS

*,**,***Significant at $P < 0.05$, 0.01 and 0.001, respectively.
NS, not significant at $P \geq 0.05$.

In Run 1, no significant difference among the four soils in percentage of J2 parasitized at the end of experiment was observed for all of the three inoculation levels of the liquid culture of both fungi (Table 8). In the treatments with 1% corn-grits culture, however, *H. rhossiliensis* parasitized more J2 at the end of the experiment in Soil 1 and Soil 3 than Soil 2 and Soil 4, and *H. minnesotensis* parasitized more J2 in Soil 2 than Soil 1.

In Run 2, parasitism of J2 by *H. rhossiliensis* did not differ among the soils except that 0.4 g mycelium resulted in lower parasitism in Soil 4 than Soil 3 (Table 8). *Hirsutella minnesotensis* at 0.4 and 0.8 g mycelium parasitized more J2 in Soil 1 and Soil 3 than Soil 2 and Soil 4. When 0.2 g mycelium was used, the percentage of J2 parasitized by *H. minnesotensis* was greater in Soil 3 than Soil 1 and Soil 4. There was no significant difference in percentage of J2 parasitized by *H. minnesotensis* when corn-grits culture was used in Run 2 (Table 8).

No significant correlation between the percentage of J2 parasitized and soil pH was observed for the two fungi in the two runs (data not shown). Significant positive correlation between the fungal parasitism and sandiness was observed only in treatments of 1% corn-grits culture of *H. rhossiliensis* in Run 1 and 0.8 g mycelium of *H. minnesotensis* in Run 2. Significant correlation between percentage of J2 parasitized and organic matter was observed in treatment of 1% corn-grits culture of *H. rhossiliensis* in Run 1 and 0.4 and 0.8 g mycelium of *H. minnesotensis* in Run 2 (data not shown).

Plant growth

ANOVA indicated that the plant fresh shoot weight differed between the two runs of experiment, among different soils, between the two fungal species and among the

fungal inoculation levels, and that the soil effects on plant growth differed among different fungal inoculation levels (Tables 5 and 9). In the cases where differences are significant, the fresh shoot weight was generally greater in Soil 2 and Soil 3 with intermediate soil pH and percent sand than Soil 1 with the lowest pH and highest percent sand and Soil 4 with the highest pH and lowest percent sand (Table 9).

Hirsutella rhossiliensis at 0.4 g mycelium resulted in greater weight than any other inoculation levels in Soil 1 in both Runs (Table 9). In Soil 2, no significant difference in shoot weight was observed among the inoculation levels of the fungus. In Soil 3, *H. rhossiliensis* at 0.4 and 0.8 g mycelium produced greater shoot weights than the soil-only control in Run 2 but not in Run 1. In Soil 4, the three levels of liquid culture resulted in greater plant weights than the soil-only control in both Runs, and the 1% corn-grits culture produced greater plant weight than the 1% corn-grits control in Run 2 (Table 9).

Hirsutella minnesotensis produced greater shoot weights in all of the three liquid inoculation levels than the soil-only control in Soil 1 in both Runs, but solid culture did not increase plant weights in the Soil 1 (Table 9). In soil 2, only 0.8 g mycelium increased shoot weight as compared with the control in Run 2. In Soil 3, significant increase of plant weights with the fungal treatment as compared with its control was observed only at 0.4 and 0.8 g mycelium in Run 2. In Soil 4, the liquid culture at 0.2 and 0.4 g in Run 1 and all three inoculation levels in Run 2 resulted in greater plant weights than soil-only control, but the solid culture did not increase plant growth as compared with corn-grits control (Table 9).

Discussion

This study demonstrated that *H. rhossiliensis* and *H. minnesotensis* activities and biocontrol effectiveness against the soybean cyst nematode differed in the four soils with different soil pH, texture and organic matter. The original objective of this study was focused on the soil pH effect on the fungal parasitism and biocontrol effectiveness. The soils were collected from the same field and we expected little difference in soil texture and organic matter. However, the pH in the four soils was positively correlated with organic matter and negatively correlated with soil sand, making it difficult to determine the effect of soil pH on the fungal activities and biocontrol effectiveness. Further studies are needed to determine whether the differences in the fungal activities and biocontrol of *H. glycines* in the four soils were due to the soil pH, texture, organic matter, or their combinations.

The linear regression or simple correlation analysis indicated that there was stronger correlation of fungal parasitism in vial soil or nematode egg reduction in greenhouse pots with soil pH than with soil sandiness and organic matter. This may suggest that pH was the most important factor affecting fungal parasitism and biocontrol effectiveness in these soils. If soil pH was the major factor responsible for the treatment effect, our data suggest that the percentage of parasitism declined with increasing soil pH ranged from 4.85 to 7.79 for both fungi. This result is similar to a previous study in which the activities of *H. rhossiliensis* decreased when soil pH increased from 4.5 to 6.5 (Jaffee and Zasoski 2001). In the previous study, natural soil was used and it was concluded that low soil pH inhibited activities of other microorganisms that could be antagonistic to *H. rhossiliensis*. In our study, however, the soil was autoclaved, and there should be limited activities of other microbial antagonists. Thus, it is unlikely that the effect of soil pH on the fungal activities in

Table 8. Effect of *Hirsutella rhossiliensis* (Hr) and *H. minnesotensis* (Hm) on parasitism of *Heterodera glycines* second-stage juveniles (J2) in four soils in greenhouse.[1]

			Percentage of J2 parasitized											
Fungus	Experiment	Inoculation level	Soil 1			Soil 2			Soil 3			Soil 4		
Hr	Run 1	0.8 g mycelium/pot	18.6	a	A	14.2	a	A	13.4	a	A	17.2	a	A
		0.4 g mycelium/pot	14.4	a	A	13.0	a	A	13.7	a	A	15.3	a	A
		0.2 g mycelium/pot	14.1	a	A	13.5	a	A	13.8	a	A	9.8	b	A
		1% corngrits culture	16.4	a	A	5.2	b	B	14.1	a	A	6.6	b	B
	Run 2	0.8 g mycelium/pot	14.3	a	A	22.0	a	A	20.6	a	A	10.7	a	A
		0.4 g mycelium/pot	9.2	ab	AB	14.5	ab	AB	15.5	ab	A	5.5	b	B
		0.2 g mycelium/pot	6.5	b	A	9.4	bc	A	7.7	bc	A	2.7	bc	A
		1% corngrits culture	5.2	bc	A	13.9	ab	A	11.2	abc	A	13.3	a	A
Hm	Run 1	0.8 g mycelium/pot	14.9	a	A	17.2	a	A	19.5	a	A	12.5	a	A
		0.4 g mycelium/pot	14.4	a	A	11.5	a	A	18.4	a	A	10.1	a	A
		0.2 g mycelium/pot	13.0	a	A	10.1	a	A	15.4	a	A	10.3	a	A
		1% corngrits culture	8.3	a	B	20.2	a	A	9.5	a	AB	9.9	a	AB
	Run 2	0.8 g mycelium/pot	52.7	a	A	32.8	a	B	59.3	a	A	24.9	a	B
		0.4 g mycelium/pot	39.8	a	AB	27.2	a	B	56.5	a	A	18.0	a	B
		0.2 g mycelium/pot	17.2	b	B	21.2	ab	AB	35.0	b	A	11.7	ab	B
		1% corngrits culture	8.4	bc	A	12.6	bc	A	7.9	c	A	20.7	a	A

[1]The values are means of five replicates. The data were transformed to degree asin ($x^{0.5}$) before being subjected to statistical analysis. The values followed by the same letter in the column within the same fungal species and run of experiment or the values followed by the same uppercase letter in the row were not significantly different according to the LSD test at $P \geq 0.05$.

Table 9. Effect of *Hirsutella rhossiliensis* (Hr) and *H. minnesotensis* (Hm) on growth of soybean infected by *Heterodera glycines* in four soils in greenhouse.[1]

			Plant fresh weight (g)											
Fungi	Experiment	Inoculation level	Soil 1			Soil 2			Soil 3			Soil 4		
Hr	Run 1	0.8 g mycelium/pot	3.1	b	B	7.1	a	A	8.0	a	A	4.2	abc	B
		0.4 g mycelium/pot	6.7	a	A	4.7	a	A	7.4	a	A	6.1	a	A
		0.2 g mycelium/pot	3.3	b	A	6.2	a	A	4.9	a	A	5.7	ab	A
		1% corngrit culture	2.6	b	B	6.4	a	A	6.4	a	A	3.6	bc	B
		1% corn grits	3.5	b	B	8.1	a	A	6.3	a	A	3.3	c	B
		soil only	2.0	b	B	6.5	a	A	6.5	a	A	3.3	c	B
	Run 2	0.8 g mycelium/pot	3.5	b	C	7.9	a	AB	8.6	a	A	5.9	a	B
		0.4 g mycelium/pot	7.6	a	A	5.6	a	A	8.4	ab	A	7.4	a	A
		0.2 g mycelium/pot	3.9	b	A	7.1	a	A	7.0	abc	A	6.1	a	A
		1% corngrit culture	3.6	b	B	6.7	a	A	7.2	abc	A	5.8	a	A
		1% corn grits	3.7	b	BC	7.0	a	A	5.7	bc	AB	3.1	b	C
		soil only	2.3	b	B	5.6	a	A	5.3	c	A	3.4	b	AB
Hm	Run 1	0.8 g mycelium/pot	6.6	a	AB	9.2	a	A	7.5	a	A	4.6	ab	B
		0.4 g mycelium/pot	4.6	ab	A	7.7	a	A	7.1	a	A	6.0	a	A
		0.2 g mycelium/pot	6.0	a	A	7.1	a	A	7.2	a	A	6.6	a	A
		1% corngrit culture	3.2	bc	B	7.7	a	A	4.1	a	AB	1.9	c	B
		1% corn grits	3.5	bc	B	8.1	a	A	6.3	a	A	3.3	bc	B
		soil only	2.0	c	A	6.5	a	A	6.5	a	A	3.7	bc	A
	Run 2	0.8 g mycelium/pot	6.6	a	B	9.4	a	A	9.3	a	A	6.1	ab	B
		0.4 g mycelium/pot	5.4	ab	B	8.1	ab	A	8.4	ab	A	6.8	a	AB
		0.2 g mycelium/pot	6.5	a	A	7.6	ab	A	7.7	abc	A	7.0	a	A
		1% corngrit culture	4.1	bc	B	8.3	ab	A	5.7	bc	AB	4.6	bc	B
		1% corn grits	3.7	cd	BC	7.0	ab	A	5.7	bc	AB	3.1	c	C
		soil only	2.3	d	B	5.6	b	A	5.3	c	A	3.4	c	AB

[1]The values are means of five replicates. The values followed by the same letter in the column within the same fungal species and run of experiment or the values followed by the same uppercase letter in the row were not significantly different according to the LSD test at $P \geq 0.05$.

our study was due to the indirect effect of microbial antagonists of the *Hirsutella* species as reported in the previous study (Jaffee and Zasoski 2001). It is possible, though, that the interaction between soil pH and *H. rhossiliensis* in the autoclaved soil may differ from that in natural fields.

The trend of decrease of fungal activities with increasing soil pH was most evident at day 10 for *H. rhossiliensis* and day 30 for *H. minnesotensis*. The fungal activities and nematode population may be dynamic temporally, and the soil effects on the variables applied in this study may depend on the time of taking measurements. The difference in fungal parasitism of J2 between the two fungi in response to pH, texture, or organic matter may be due to the difference in time for the fungi to establish in the soil. A previous study indicated that *H. rhossiliensis* established its population in the soil faster than *H. minnesotensis* (Chen and Liu 2005). This is probably the reason why *H. rhossiliensis* responded to the soil factors faster than *H. minnesotensis* in their parasitism of *H. glycines* J2.

Soil texture has been shown to influence the activities of *H. rhossiliensis* in previous studies. Transmission of *H. rhossiliensis* to *H. schachtii* was greatest in loamy sand, intermediate in loam, and lowest in coarse sand (Tedford et al. 1992). In our study, however, the fungal activities increased with increasing soil sandiness suggesting it is an important factor. In the present study, the four soils were clay loam, silty clay, clay loam, and silty clay loam, respectively, which contained less sand than the soil in the previous study (Tedford et al. 1992). Based on these two studies, if the soil texture is an important factor, the relationship between biocontrol effectiveness may be reduced in either coarse sandy or heavy clay soil.

In our study, the relationship between parasitism of J2 by *H. rhossiliensis* and organic matter changed from negative at days 0 and 10 to positive at day 30. Based on our observations and previous reports (Gray 1987; Jaffee et al. 1994), effects of organic matter may differ in different soils and time. Further study is needed to determine any organic matter effect in soils with different pH and texture.

The corn grits may have affected the nematode population. The number of eggs was lower in the 1% corn-grits control than in the soil-only control. The mechanism of the inhibitory effect of corn grits on the nematode population density was unclear. Possible reasons included toxic effect on the nematode and/or positive effect on any contaminating antagonists of the nematode.

There was no significant relationship between nematode density and the percentage of J2 parasitized in soil in pots at the end of the experiments. The estimates of percentage J2 parasitized at the end of the experiment could not quantitatively explain the biocontrol of the nematode by the fungi (Liu and Chen 2001b). Percentage parasitism might change over time. A highly pathogenic isolate could parasitize a high percentage of the J2 at some point of time during the experiment. These parasitized J2 may rapidly degrade and become non-detectable (Jaffee 1992). Furthermore, the highly pathogenic isolate might suppress the nematode population density; in return, the fungal population also decreased because of its density-dependent parasitism (Jaffee et al. 1989). This is probably the reason why there was no or weak correlation between the percentage of J2 parasitized and the soil pH, texture, and/or organic matter

Hirsutella rhossiliensis and *H. minnesotensis* were highly effective in suppressing *H. glycines* population densities in greenhouse studies (Chen and Liu 2005). However, results from fields and microplots have been controversial (Jaffee et al.

1996; Jaffee and Muldoon 1997; Jaffee and Zasoski 2001; Chen 2003). Jaffee and Zasoski (2001) determined that the activity of *H. rhossiliensis* was good in acidic soil and poor in neutral soil (pH 6.5) and that the pH effect may explain performance of pelletized *H. rhossiliensis* in two vineyards with distinct soil pH (Jaffee 1999). Further greenhouse and field studies are needed to determine the individual soil factors affecting the activities of the two fungi and their biocontrol effectiveness on various nematodes and under different conditions.

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