**Effect of environment on the abundance and activity of the nematophagous fungus *Hirsutella minnesotensis* in soil**

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Received 23 January 2009; revised 23 October 2009; accepted 23 October 2009.

Final version published online 17 December 2009.

DOI: 10.1111/j.1574-6941.2009.00810.x

**Introduction**

The fungus *Hirsutella minnesotensis* Chen, Liu & Chen was first isolated from second-stage juveniles (J2) of the soybean cyst nematode (*SCN, Heterodera glycines* Ichinohe) in Waseca, MN, in 1996 (Chen *et al.*, 2000), and was subsequently detected in China, Germany, and Poland (Ma *et al.*, 2005; Balazy *et al.*, 2008). Like the related species, *Hirsutella rhossiliensis, H. minnesotensis* is an endoparasite of nematode and produces conidia that adhere to, penetrate, and eventually kill nematodes (Sturhan & Schneider, 1980; Jaffee *et al.*, 1992). *Hirsutella minnesotensis* is the most prominent fungal parasite of SCN J2 in northeastern China and was detected in 27 of 53 soil samples collected in Heilongjiang Province, China, whereas *H. rhossiliensis* was detected in only one sample (Ma *et al.*, 2005). *Hirsutella minnesotensis* has shown potential for the biocontrol of SCN in greenhouse and field plot trials and may be responsible for natural suppression of SCN in some field soils (Xiang *et al.*, 2010; S.Y. Chen, unpublished data).

Soil environmental factors, such as soil temperature, moisture, and texture, greatly affect the colonization, multiplication, activity, and spread of microorganisms that have been added to soil (Keller & Zimmermann, 1989). Some nematophagous fungi are among the most important enemies of nematodes and can substantially reduce nematode numbers in soils. The influence of environmental factors on various nematophagous fungi, including the nematode-trapping fungi, has been studied (Gray, 1985). Those nematode-trapping fungi that only produce traps in the presence of nematodes were more frequently isolated from soils with low organic matter and low moisture levels, whereas those nematode-trapping fungi that produce traps whether or not nematodes are present were more abundant in soils with high organic matter and high moisture levels (Gray, 1985). Petri dish studies suggest that the trapping fungi may produce more spores than traps when soil is dry (Jaffee *et al.*, 1992).

The effect of environment on the abundance and activity of *H. rhossiliensis* has been extensively investigated. The...
optimal temperature for infection of the sugar beet cyst nematode Heterodera schachtii by H. rhosiliensis lies between 20 and 25 °C (Tedford et al., 1995a); high soil moisture reduced infection (Timper & Brodie, 1993). Transmission of H. rhosiliensis to either H. schachtii or the root-knot nematode Meloidogyne javanica in loam was not affected by soil moisture content between 8% and 14% but transmission to H. schachtii was suppressed outside that range. In loamy sand and sand, transmission of H. rhosiliensis spores to H. schachtii and M. javanica was greatest at the lowest moisture levels tested and decreased linearly with increasing soil moisture (Tedford et al., 1992).

Organic amendments to soil are thought to stimulate the activity of some nematophagous fungi, but they did not enhance parasitism of nematodes by H. rhosiliensis (Jaffee et al., 1994). However, addition of organic matter to soil may indirectly enhance the endoparasitic fungus Drechmeria coniospora by increasing the numbers of bacterivorous nematodes available for infection (van den Boogert et al., 1994). Hirsutella rhosiliensis, Monacrosporium ellipsosporum, and Arthrobotrys dactyloides parasitized significant proportions of H. schachtii when introduced into untreated loamy sand in the form of parasitized nematodes, and they infected fewer nematodes when the soil was physically disturbed (Jaffee et al., 1992).

Although the effect of soil environmental factors on nematophagous fungi has been studied, the efficacy of these fungi as biological control agents of nematodes depends not only on their abundance but also on their activity. To quantify both the biomass and activity of H. minnesotensis, Xiang et al. (2010) have combined a real-time quantitative PCR method with a parasitism bioassay. The purpose of the current study was to determine how the biomass and activity of H. minnesotensis are affected by soil environmental factors (temperature, water content, and texture) and to determine the optimal conditions for the multiplication and parasitism of this fungus. The information is needed to better understand the ecology of the fungus, evaluate the potential of H. minnesotensis as a biological control agent, and to improve application strategies for nematode control.

Materials and methods

Fungus, nematode, and soil

Fungal cultures of H. minnesotensis CBS115627 were applied to soil in this study. The fungal inoculum consisted of mycelial slurry, which was prepared from fungal colonies harvested from liquid culture (Zhang et al., 2006) on an orbital shaker at 150 r.p.m. for 6 days at 25 °C. The fungal colonies were collected and blended following the method of Liu & Chen (2005). The mycelial slurry was used immediately after preparation.

SCN race 4 originally from a soybean field in a suburb of Beijing, China, was cultured on soybean cultivar Zhonghuang 13 in autoclaved soil in the greenhouse. SCN J2 was obtained by hatching eggs from newly formed cysts (Liu & Chen, 2001a).

Soil was collected from a cornfield in Gaomi County, Shandong Province, China, and consisted of 52% sand, 33% silt, 15% clay, and 1.03% organic matter with a pH of 6.7. The soil was passed through a 2-mm aperture sieve and stored at room temperature (22–25 °C) for 2 months before being used. SCN was not detected in this soil by wet sieving and sucrose centrifugation (Jenkins, 1964), and Hirsutella species were not observed on native nematodes or on SCN J2 added to the soil. The soil at 8% water content (g water 100 g⁻¹ dry soil) was treated by microwave heating (1 kg lot of soil in a plastic bag at 800 W for 1.5 min) to eliminate nematodes and other small animals but to allow most fungi and bacteria to survive (Chen et al., 1995).

General procedures in tube soil assay

The fresh mycelial slurry was added to the microwaved field soil at 1.0 g mycelia (fresh weight) into 100 g soil (dry weight). The soil was mixed thoroughly, amended with silicon dioxide sand as described in the following sections, and placed in 50-mL centrifuge tubes (50 g of soil per tube) with a 7-mm pore at the bottom of each tube. Three replicate tubes were used for each treatment. Silicon dioxide sand was added to prevent the soil from compacting while it was being experimentally handled. Soil without fungal inoculum was used as the control. The tubes were placed in plastic boxes containing wet cheesecloth to maintain moisture and the water content of the soil was measured at the end of the experiment to confirm the moisture constant. Both the boxes and the cheesecloth were sterile at the start of the experiment. After the tubes had been incubated for 3 weeks under different environmental conditions, 500 SCN J2 in 0.4 mL of 4.5 mM KCl were added to the soil surface. Three days later, the soil in each tube was mixed thoroughly and 40 g was used for extraction of J2 (Liu & Chen, 2001b) and 10 g soil was stored at −80 °C for extraction of DNA.

Effect of soil temperature, water content, and texture

To determine the effect of soil temperature on the fungal abundance and activity, soil was amended with 10% silicon dioxide sand (wet weight) and adjusted to 14% water content before it was placed in tubes. The soil tubes were incubated for 3 weeks at 5, 10, 15, 20, 25, and 30 °C before the soil was assayed. To test the effect of soil water content, soil was amended with 10% silicon dioxide sand and adjusted to 6%, 10%, 14%, 18%, or 22% water content before it was placed in tubes. The tubes were incubated at...
15 °C for 3 weeks before the soil was assayed. The effect of soil texture was tested by addition of silicon dioxide sand or fine soil particles (diameter < 74 μm, mostly silt and clay). The fine soil particles were obtained by passing the soil through a 74-μm sieve. The sand or fine particles were added at the following rates (wet weight): 10%, 30%, 50%, and 70%. The tubes were incubated at 15 °C for 3 weeks before the soil was assayed. The experiments of soil temperature and water content effects were carried out three times each, and the experiment on soil texture was repeated once.

Detection of *H. minnesotensis* in soil by a parasitism bioassay and real-time PCR

SCN J2 were extracted from the 40 g subsample of soil by sucrose flotation and centrifugation (Chen & Liu, 2005). All extracted nematodes were observed at × 100 magnification with an inverted microscope. J2 filled with hyphae or with conidia characteristic of *H. minnesotensis* on their cuticle were counted as parasitized. The percentage of J2 that were parasitized was calculated.

Fungal DNA was extracted from 0.5 g of the 10 g subsample of soil from each tube. The soil sample was treated with a mini-bead beater (Biospec Products, Bartlesville, OK) at 4200 r.p.m. for 90 s to break open cells. The DNA was extracted with the MoBio UltraClean soil DNA isolation kit (MoBio Laboratories, Solana Beach, CA) following the manufacturer’s protocols. Soil DNA was diluted 10-fold and subjected to real-time PCR as described previously (Xiang *et al.*, 2010). The DNA was stored at −20 °C if it could not be assayed immediately.

Statistical analysis

Data were subjected to ANOVA, and means were compared with Fisher’s protected least significant difference at α = 0.05. Data were subjected to regression analysis. SPSS software (version 15.0) was used. An arcsine transformation was applied to the percentage parasitism data before statistical analysis.

Results

Effect of soil temperature

The quantity of *H. minnesotensis* DNA in soil as determined by real-time PCR was highest at 5 and 10 °C, sharply declined between 10 and 15 °C, gradually declined between 15 and 20 °C, and did not change from 20 to 30 °C (Fig. 1). *Hirsutella minnesotensis* was not detected by real-time PCR in soil tubes that were not inoculated with the fungus (data not shown).

Soil temperature also significantly affected the activity of *H. minnesotensis*, as indicated by the percentage of SCN J2 that were parasitized. Although the greatest quantity of DNA was detected at 5 and 10 °C, the highest percentage of J2 parasitized by *H. minnesotensis* was at 10 and 15 °C; parasitism levels were very low at higher or lower temperatures (Fig. 1). In this test and in all other tests in this paper, all parasitized J2 appeared to be parasitized by *H. minnesotensis*, based on the morphology of the spores adhering to the J2 cuticle.

Effect of soil moisture

The quantity of *H. minnesotensis* DNA was highest at 6% soil water content and declined with increasing soil water content (Fig. 2). The percentage of SCN J2 parasitized was highest at 6% and 10% soil water content and then dramatically decreased with increasing soil water content (Fig. 2).
Infecting SCN J2 was 15–25
nematode, the optimum temperature for
1995b). Although soil temperature affects both fungus and
that a nematode will encounter a conidium (Tedford
nematode mobility, factors that determine the probability
depended on the numbers of conidia per unit soil and on
perature was outside of this range, the percentage decreased.

quantity of DNA but not the parasitic activity (Fig. 3).

Fig. 3. Effect of soil texture on the quantity of Hirsutella minnesotensis DNA and the activity of H. minnesotensis in soil. Values are the means (+SE) of three replications (244 × 167 mm).

Influence of soil texture

There were no significant differences in the percentage of parasitized J2 in the native soil, in soil amended with 10–70% fine soil particles, and in soil amended with 10% sand (P = 0.083), whereas the percentage of parasitized J2 decreased with the increase of sand in the soils (30%, 50%, 70% sand added) (P < 0.001). In contrast, the quantity of H. minnesotensis DNA was highest in the native soil, and in soil amended with 30%, 50%, and 70% fine soil particles (P = 0.359). Increasing the ratio of silicon dioxide sand to native soil resulted in the dramatic decline in the quantity of H. minnesotensis DNA and H. minnesotensis parasitic activity. However, addition of 10% fine soil particles to the soil decreased the quantity of DNA but not the activity of the fungus relative to the native soil. Decreasing the ratio of fine soil particles to native soil from 70% to 10% reduced the quantity of DNA but not the parasitic activity (Fig. 3).

Discussion

Real-time PCR indicated that the quantity of H. minnesotensis DNA was highest in relatively cool soils and decreased as temperature increased. We suspect that the quantity of DNA was greater at low temperatures because low temperatures slowed the decomposition of the fungus, as reported for H. rhossiliensis growing on agar plates (Zhang, 2005).

The highest percentage of SCN J2 parasitized by H. minnesotensis occurred at 10–15 °C. When the soil temperature was outside of this range, the percentage decreased.

In previous studies, the infection of SCN J2 by H. rhossiliensis depended on the numbers of conidia per unit soil and on nematode mobility, factors that determine the probability that a nematode will encounter a conidium (Tedford et al., 1999b). Although soil temperature affects both fungus and nematode, the optimum temperature for H. rhossiliensis infecting SCN J2 was 15–25 °C (Zhang, 2005), which was higher than for H. minnesotensis in the current study. The differences in the effects of temperature on nematode infection between those two studies are probably explained by effects of temperature on the fungi, because the nematode was the same in both studies. We infer that temperature is a major factor affecting the colonization and activity of both Hirsutella species. Although there is no evidence that H. minnesotensis prefers a cooler climate than H. rhossiliensis, H. minnesotensis is a prominent SCN J2 parasite in soils of northeastern China, whereas H. rhossiliensis is prominent in the soils of Minnesota (Liu & Chen, 2000; Ma et al., 2005). Hirsutella minnesotensis could be a better candidate for biological control of SCN in northeastern China.

Soil moisture influences not only fungal growth, sporation, and degradation but also nematode mobility and survival. Mycelia persist better in dry soil than in wet soil (Studdert & Kaya, 1990a,b), which probably explains why the quantity of H. minnesotensis DNA declined as water content increased in the present study. For H. rhossiliensis, high soil moisture reduces growth and sporulation and can inhibit fungal spore dissemination (Timper et al., 1991). Hirsutella minnesotensis parasitized a higher percentage of nematodes at 6–10% soil water content than at higher soil water contents. Although low soil water content generally favors parasitism of nematodes by H. minnesotensis, nematodes are aquatic animals and require a film of water to move through soil pores. Soil water contents < 6% probably reduced nematode mobility and therefore reduced the chances that nematodes would encounter spores and become infected.

Parasitism of nematodes by H. rhossiliensis is affected by the size of soil particles (Jaffee et al., 1990; Tedford et al., 1992; Liu & Chen, 2009). Soil texture affects nematode movement and survival. Generally, soils with a higher content of fine particles restrict nematode movement and may reduce aeration, which can reduce nematode survival. The present study also found that more J2 were recovered from the sand-amended soils than from the fine particle-amended soil (data not show). However, in our study, parasitism of SCN J2 by H. minnesotensis was greatest in the fine particle-amended soil, intermediate in the native soil, and lowest in sand-amended soil. Similar results were reported for H. rhossiliensis and H. minnesotensis by Timper et al. (1991) and Liu & Chen (2009). The quantity of H. minnesotensis DNA was also higher in fine particle-amended than in the sand-amended soils. In addition to having physical effects on nematode movement and fungal growth, the fine soil particles might also have provided nutrients that enhanced the growth of D. coniospora (van den Boogert et al., 1994).

In summary, this research indicates that H. minnesotensis may be suited for control of SCN in northeastern China because H. minnesotensis prefers cooler soils. The results also

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suggest that the fungus will provide the most control in relatively dry soils with a high content of fine particles.

**Acknowledgements**

This research was jointly supported by the National Natural Science Foundations of China (nos 30800732 and 30770072) and the 863 Program (2006AA10A211).

**References**


