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Parasitism of the Nematode *Heterodera glycines* by the Fungus *Hirsutella rhossiliensis* as Influenced by Crop Sequence[1]

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Abstract: The effect of crop sequence on parasitism of second-stage juveniles (J2) of *Heterodera glycines* by *Hirsutella rhossiliensis* was investigated. Data were collected from plots of a long-term crop rotation experiment established in 1982. Crop sequences included (i) continuous monoculture of corn and soybean; (ii) annual rotation of the two crops; and (iii) 1, 2, 3, 4, or 5 years of each crop following 5 years of the other crop. The nematode J2 density and percentage of J2 parasitized by the fungus were determined at planting, midseason, and end of season in 1997 and 1998. A significant effect of the crop sequence on parasitism of J2 was observed at midseason in both years and at end of season in 1998. In plots of first-year soybean following 5 years of corn, fungal parasitism increased from an undetectable level at planting to 2% and 4% of J2 parasitized by ends of season in 1997 and 1998, respectively. Fungal parasitism was similar in plots of second-through-fifth-year soybean after 5 years of corn and in plots of soybean monoculture. Parasitism of J2 in the soybean plots in annual rotation with corn increased from undetectable and 2% at planting to 6% and 23% at midseason in 1997 and 1998, respectively. The effect of crop sequence on the fungal parasitism of J2 may be attributed to a density-dependent relationship between the parasite and its host. Season also affected the fungal parasitism; percentage of J2 parasitized by the fungus was the highest at midseason and the lowest at planting.

Key words: biological control, corn, crop rotation, crop sequence, *Glycine max, Heterodera glycines, Hirsutella rhossiliensis,* nematode, nematophagous fungus, soybean, soybean cyst nematode, *Zea mays.*

The nematophagous fungus *Hirsutella rhossiliensis* Minter & Brady produces conidia enveloped in an adhesive mucus sheath. The conidia adhere to, penetrate, and infect motile nematodes that contact them. Assimilative hyphae grow through the still-living host, and within several days the nematode is dead and filled with hyphae. Fungal hyphae then emerge from the cadaver and produce conidia that infect live nematodes (Jaffee, 1992). Pathogenicity of the fungus may vary depending on fungal isolate and nematode species (Tedford et al., 1994). Cayrol and Prankowski (1986) reported that a single conidium of *H. rhossiliensis* attached to *Ditylenchus dipsaci* Pilipjev was sufficient to kill the nematode.

Hirsutella rhossiliensis (Hr) has been investigated as a biological control agent of several plant-parasitic nematodes. Muller (1985) reported that the fungus may have suppressed cyst nematodes in some sugar beet fields in Germany. High numbers and percentages of *Mesocriconema xenoplax* Raski parasitized by Hr were found in some California peach orchards (Jaffee et al., 1989). In a laboratory test, Jaffee and Muldoon (1989) demonstrated that Hr suppressed penetration of cabbage roots by *Heterodera schachtii* Schmidt. Timper and Brodie (1994) reported that Hr reduced root penetration by *Pratylenchus penetrans* Cobb and suppressed nematode populations 60 days after nematode inoculation. However, results obtained by Tedford et al. (1993) indicated that long-term interactions between Hr populations and cyst or root-knot nematodes did not result in biological control in microplots.

Parasitism of nematodes by Hr is density-dependent (Jaffee, 1992). The percentage of *M. xenoplax* parasitized by the fungus was dependent on nematode density; however, the correlation was weak and the variation in percentage parasitism was large (Jaffee et al., 1989). *Hirsutella rhossiliensis* is a poor soil competitor. Local populations of the fungus

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may go extinct unless supplied with some minimum number of nematodes (the host threshold density); thus, natural epidemics of this fungus among populations of nematodes may develop slowly and only after long periods of high host densities (Jaffee, 1992). It appeared that Hr was a weak regulator of *M. xenoplax* population densities (Jaffee et al., 1989).

Infection of the soybean cyst nematode, *Heterodera glycines* Ichinohe, by Hr in natural soil was first observed in a Minnesota soybean field in 1995 (Chen, 1997). A survey was then conducted to determine the infestation level and distribution of the fungus in southern Minnesota. The results indicated that the fungus was common in soybean fields in the region, but the fungal population density varied among fields. High percentages of parasitized second-stage juveniles (J2) were observed in some fields (unpubl.) but not in others. There is no information on factors affecting fungal epidemics in *H. glycines* populations. The objective of this study was to investigate the effect of crop sequence on parasitism of *H. glycines* by Hr.

Materials and Methods

A field site was established at the Southern Experiment Station at Waseca, Minnesota, in 1982 to study the effect of long-term corn-soybean crop sequences on crop yields. The field soil was a Nicollet clay loam (fine loamy, mixed, mesic Aquic Hapludoll) with 6.1% organic matter measured in 1998.

The experiment included 14 treatments arranged in a randomized complete block with four replicates. Cropping sequences included (i) continuous monoculture of corn and soybean; (ii) annual rotation of the two crops; and (iii) 1, 2, 3, 4, or 5 years of each crop following 5 years of the other crop. The experiment was designed so that all sequences of either crop were planted in each year of the study. Each plot consisted of 6 rows, each 18 m long and with a row spacing of 76 cm (Crookston et al., 1991).

In 1996, the soybean cyst nematode and Hr were discovered at the site. Subsequently, soil samples were taken at planting, midseason, and end of season in 1997 and 1998 to determine the effect of crop sequence on nematode population density and level of parasitism by Hr.

Crops were planted on 10 May 1997 and 30 April 1998 and harvested on 30 September 1997 and 15 September 1998. Corn cultivars Pioneer 3730 and Dekalb 493rr were used in 1997 and 1998, respectively. Soybean cultivars Parker and Asgrow 2101 were used in 1997 and 1998, respectively. Dekalb 493rr and Asgrow 2101 are resistant to the herbicide glyphosate (Roundup, Monsanto).

A conventional tillage regime of fall moldboard plowing and spring field cultivation prior to planting was used during 1997–1998 and previous years. Fertilizer was applied according to University of Minnesota Soil Testing Service recommendations. On 30 April 1997, 450 kg/ha N, a high level due to incorrect calibration, was applied to all corn plots. On 22 April 1998, 168 kg/ha N was applied to corn plots following soybean and 196 kg/ha N was applied to corn plots following corn. No fertilizer was used in soybean plots. In 1997, as in previous years, preemergence herbicides alachlor (Lasso, Monsanto) at 3.4 kg a.i./ha and linuron (Lorox, du Pont) at 0.275 kg a.i./ha were applied on 22 May. In 1998, alachlor at 3.4 kg a.i./ha was applied on 6 May and glyphosate at 0.92 kg a.i./ha was applied on 23 June.

A soil sample composed of 30 cores was taken from the root zone in the two central rows of each plot. Cores were collected to a depth of 20 cm with a 2.5-cm-diam. soil probe. Sampling dates were 3 June, 5 August, and 20 October 1997, and 4 May, 24 July, and 18 September 1998. The soil samples were stored at 4 °C for up to 1 week. Each soil sample was thoroughly mixed, a subsample of 100 cm^3 was taken, and J2 were extracted from the soil with sucrose-flotation and centrifugation (Jenkins, 1964). Numbers of J2 were recorded. The J2 parasitized by Hr were counted from the first 50 J2 examined. When the number of J2 was less than 50, all J2 were examined. Percent J2 was computed. Any J2 with one or more

attached Hr conidia or J2 colonized with fungal mycelium were considered as being parasitized by the fungus. This assumption was based on the morphology and size of conidia attached to the cuticle and on the observation that only Hr was isolated when colonized J2 were plated onto potato dextrose agar. It was assumed that even if other nematophagous fungi with adhesive conidia existed in the field, the population density of such fungi would be low and would not significantly affect quantification of Hr populations. When the J2 density was less than 10/100 cm^3 of soil, fungal parasitism level was not determined.

Values of J2 density were transformed to $\log_{10}(x + 1)$, and percentages of J2 parasitized by Hr were arcsine-transformed before being subjected to ANOVA. Means of each treatment were compared with LSD at $P = 0.05$. Regressions were performed to determine whether year, sampling date, and J2 density were related to fungal parasitism, with the percentage of J2 parasitized as the dependent variable and year, season, and J2 density as independent variables.

Results

Nematode J2 density varied among sampling dates and crop sequence treatments (Fig. 1). In general, J2 density was higher in 1998 than in 1997 ($P < 0.001$). There was an interaction between year and sampling date. In 1997, average J2 density was lowest at planting and highest at end of season. In 1998, average J2 density was not different among the three sampling dates.

Crop sequence affected J2 density. In general, J2 density was higher in soybean plots than in corn plots ($P < 0.001$). There was an interaction between sampling date and crop sequence on J2 density ($P < 0.001$). In soybean plots, J2 density increased or remained similar throughout the growing season, except for the fifth-year soybean (S5) in 1998, in which J2 density decreased. In corn plots, J2 density decreased during the growing seasons within each year (Table 1). In plots with the first-year soybean following 5 years of corn (S1), J2 were nearly undetectable at planting. The J2 density in these plots increased rapidly with a Pf/Pi (final population density/initial population density) of 120 in 1998 (Table 1). At the ends of season, J2 densities reached 136 and 235 J2/100 cm^3 of soil in 1997 and 1998, respectively (Fig. 1). Pf/Pi decreased with an increasing number of years of soybean (Table 1, S1 to S5). However, no significant difference in J2 densities was observed at midseason and at end of season among the third-, fourth-, and fifth-year soybean plots (S3, S4, and S5) in 1997 and 1998 (Fig. 1). A significant difference in Pf/Pi was observed between S3 and S5 in 1998, but not among S3, S4, and S5 in 1997, between S3 and S4, or between S4 and S5 in 1998 (Table 1). Regardless of differences among initial J2 densities, final J2 densities in soybean plots were similar among the treatments (Fig. 1). In plots of corn planted after 5 years of soybean (C1 to C5), J2 density decreased with an increasing number of years of corn (Fig. 1). In the fourth (C4) and fifth (C5) years of corn, J2 decreased to almost undetectable levels (Fig. 1). Density of J2 in plots with monoculture of soybean (SS) was similar to the J2 density in plots of fourth-(S4) and fifth-(S5) year soybean, except that J2 density in SS plots was lower than in S4 plots at end of season in 1997 (Fig. 1). Initial J2 population density in soybean plots in annual rotations was low, but increased through the season so that the density at the end of season did not differ from the densities in plots with 3–5 years of soybean monoculture (S3–S5) (Fig. 1).

The overall percentage of J2 parasitized by Hr was similar in 1997 and 1998 (Fig. 2). There was a significant effect of crop sequence on the percentage of J2 parasitized at midseason in both years and at end of season in 1998 (Fig. 2). In plots of first-year soybean following 5 years of corn (S1), J2 were undetectable and fungal parasitism was not determined at planting in 1997 and at planting and midseason in 1998. At midseason in 1997, no parasitism of J2 was observed. By end of season, the fungus parasitized 2% and 4% of J2 in 1997 and 1998, respectively. The percentage of J2 parasitized was less in plots of first-year soybean

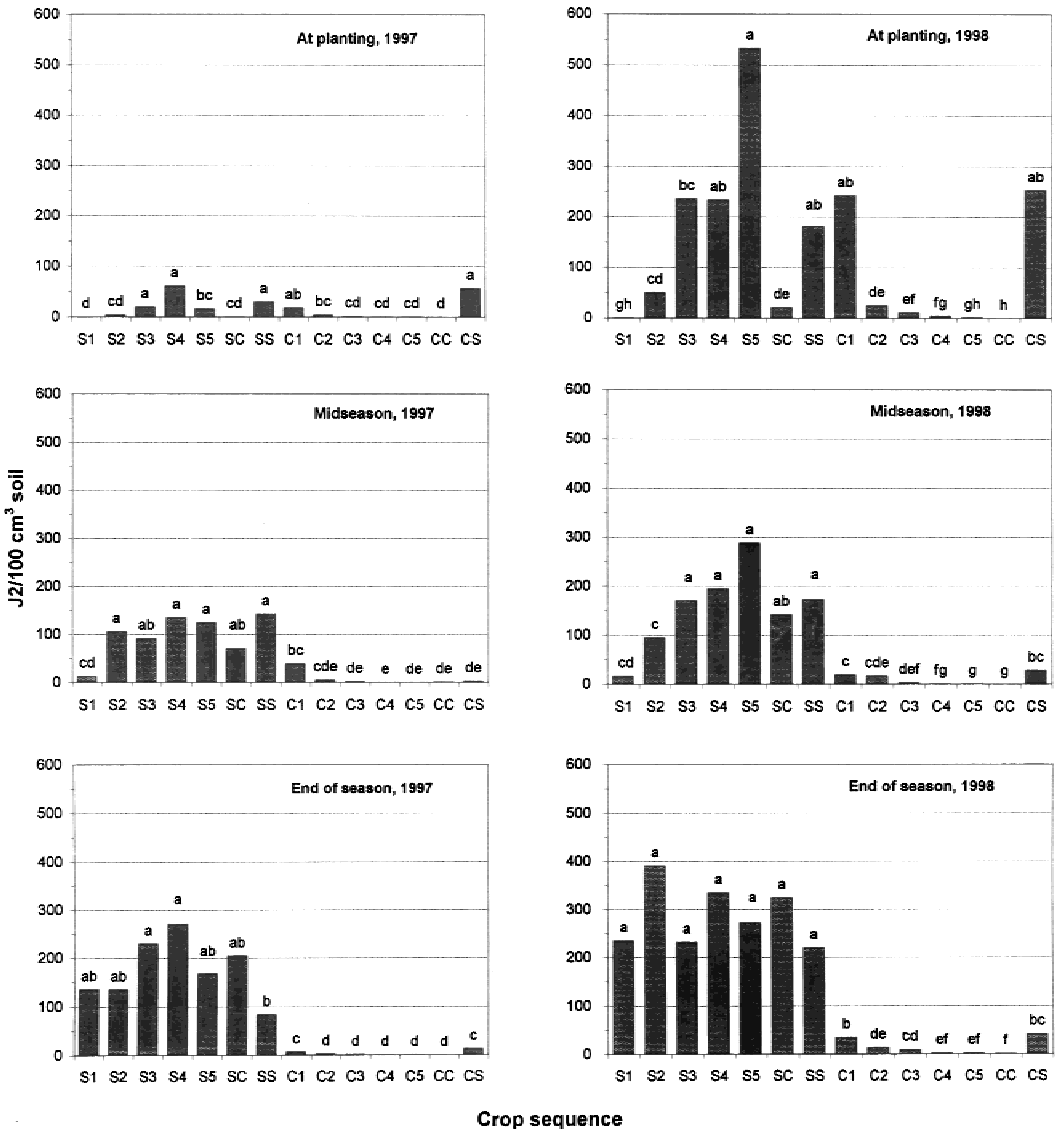

FIG. 1. Effect of various crop sequences on densities of second-stage juveniles (J2) *Heterodera glycines*. S1, S2, S3, S4, and S5 = first-, second-, third-, fourth-, and fifth-year soybean, respectively, following 5 years of corn. SC = soybean in annual rotation with corn. SS = soybean in monoculture. C1, C2, C3, C4, and C5 = first-, second-, third-, fourth-, and fifth-year corn, respectively, following 5 years of soybean. CS = corn in annual rotation with soybean. CC = corn in monoculture. The values were transformed to $\log_{10}(x + 1)$ for ANOVA. Presented numbers are non-transformed data with four replicates. Bars with a common letter within a chart are not significantly different ($P = 0.05$) according to an LSD test.

(S1) than in plots of the second- to fifth-year soybean (S2 to S5) at midseason in 1997, and at end of season in 1998. Parasitism was similar in the plots of second- to fifth-year soybean. Fungal parasitism in plots of soybean monoculture (SS) was similar to that in plots of second- to fifth-year soybean. In 1997, fungal parasitism at planting in soybean plots in annual rotation (SC) was not determined because the J2 density was low. At midseason about 6% of J2 in the SC plots were parasitized by Hr, and the percentage was lower than that in all other soybean plots except the first-year soybean following

TABLE 1. Effect of cropping sequence on change in density of second-stage juveniles (J2) of *Heterodera glycines* during the growing season.

Crop sequence[b]	Pf/Pi[a]	
	1997	1998
S1	ND	120.20 a
S2	43.01 b	11.00 bc
S3	12.62 bc	17.49 cd
S4	6.61 cd	1.65 de
S5	13.67 bc	0.59 e
SC	216.00 a	56.14 b
SS	2.51 de	1.28 de
C1	0.43 ef	0.14 e
C2	0.25 f	0.52 e
CS	0.21 f	0.20 e

[a] Change in J2 density is expressed as Pf/Pi, where Pf is the density at the end of the growing season (final population density) and Pi is the density at planting (initial population density). Values were arcsine-transformed for ANOVA. Presented numbers are means of non-transformed data with four replicates. Means in a column followed by a common letter are not significantly different according to an LSD test ($P = 0.05$).

[b] S1, S2, S3, S4, and S5 = first-, second-, third-, fourth-, and fifth-year soybean, respectively, following 5 years of corn. SC = soybean in annual rotation with corn. SS = soybean in monoculture. C1 and C2 = first-, and second-year corn, respectively, following 5 years of soybean. CS = corn in annual rotation with soybean. ND = no data for plots with low or no J2.

5 years of corn (S1). In 1998, 1% of J2 in SC plots were parasitized at planting. Parasitism reached 23% at midseason and 17% at end of season. The percentage of J2 parasitized in the SC plots was generally similar to other soybean plots except the S1 plots. In corn plots following soybean (C1 and CS), fungal parasitism recorded in 1998 decreased by the end of the growing season. Fungal parasitism was also observed in 1998 at planting in plots of second- and third-year corn (C2 and C3) and at midseason in C2 plots. In other corn plots the fungal parasitism was not determined because the J2 density was low.

Multiple regression analysis indicated that interaction between season and J2 density on percentage of J2 parasitized by Hr was significant. With combined data in the 2 years by season from all plots in which parasitism was measured, the relationship between J2 density and J2 parasitized is described in Figure 3. Percentage of J2 parasitized by Hr was positively related ($P < 0.01$) to J2 density at midseason and at end of season, but not at planting.

DISCUSSION

This study demonstrated that crop sequence affected parasitism of *H. glycines* by Hr. This effect may be attributed to the density-dependent relationship between the fungus and its host. Previous studies have reported that the fungus behaves as an obligate parasite in natural soil (Jaffee, 1992). Our observation that parasitism of *H. glycines* by Hr was density-dependent agrees with previous reports on other nematodes (Jaffee, 1993; Jaffee et al., 1989). In soybean plots, J2 increased. Consequently, the percentage of J2 parasitized by the fungus increased. When corn was planted after soybean, J2 density decreased and, as a result, parasitism by Hr decreased. In plots of third- to fifth-year corn (C3 to C5) and in plots of corn monoculture (CC), J2 were at a low or undetectable level. Although parasitism of the nematode by Hr was not determined in these plots and in other plots with low J2 density, the fungal population density would be near zero.

During the growing seasons, J2 density and percentage of J2 parasitized by Hr increased in some soybean plots, especially in S1 plots. Percentage of J2 parasitized by the fungus, however, differed among the three sampling dates. Percentage of J2 parasitized by Hr was higher at midseason than at end of season in most soybean plots although J2 density at end of season was higher than or similar to J2 density in midseason. The effect of sampling dates on fungal parasitism was probably due to the difference in temperature at different sampling dates. Nematodes may be more active at midseason and therefore more likely to encounter Hr conidia.

Fungal parasitism in this study was measured as the percentage of J2 with attached fungal conidia or filled with fungal mycelium. It was assumed that this measurement corresponded to observed mortality of J2 caused by fungal parasitism. Actual J2 mortality caused by the fungal parasitism may be different from the measurement if the duration of parasitized J2 in soil is different from that of unparasitized J2. If the J2 parasitized

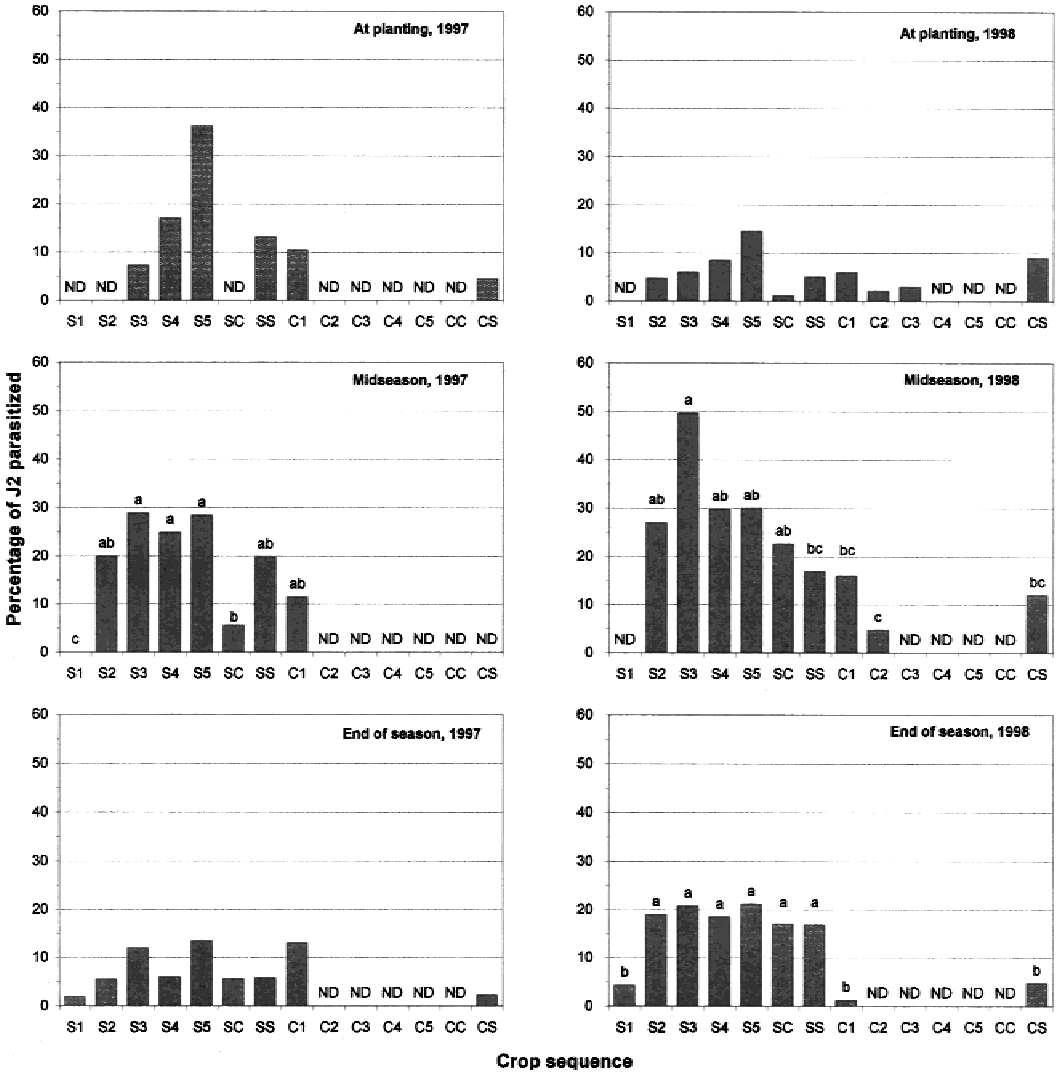

FIG. 2. Effect of various crop sequences on percentage of second-stage juveniles (J2) of *Heterodera glycines* parasitized by *Hirsutella rhossiliensis.* S1, S2, S3, S4, and S5 = first-, second-, third-, fourth-, and fifth-year soybean, respectively, following 5 years of corn. SC = soybean in annual rotation with corn. SS = soybean in monoculture. C1, C2, C3, C4, and C5 = first-, second-, third-, fourth-, and fifth-year corn, respectively, following 5 years of soybean. CS = corn in annual rotation with soybean. CC = corn in monoculture. ND = no data for plots with low or no J2. Values were arcsine-transformed for ANOVA. Presented numbers are non-transformed data with four replicates. Bars with a common letter are not significantly different ($P = 0.05$) according to an LSD test. No significant differences were observed at planting in 1997 and 1998 and at end of season in 1997.

by Hr degraded faster than unparasitized J2, actual mortality of J2 caused by the fungus should be higher than the observed percentage of J2 parasitized.

The extraction efficiency of the healthy and parasitized J2 was not determined. It is possible that J2 parasitized by Hr were more difficult to extract from soil because the mycelium developed from the nematode cadaver and adhesive materials on conidia could have made them difficult to separate from soil. *Heterodera schachtii* J2 infected with Hr rapidly became non-extractable (Jaffee et al., 1991). If this was also true in *H. glycines,* the reduced extraction efficiency would contribute to underestimation of the percentage of parasitized J2.

Soybean yield loss of either resistant or

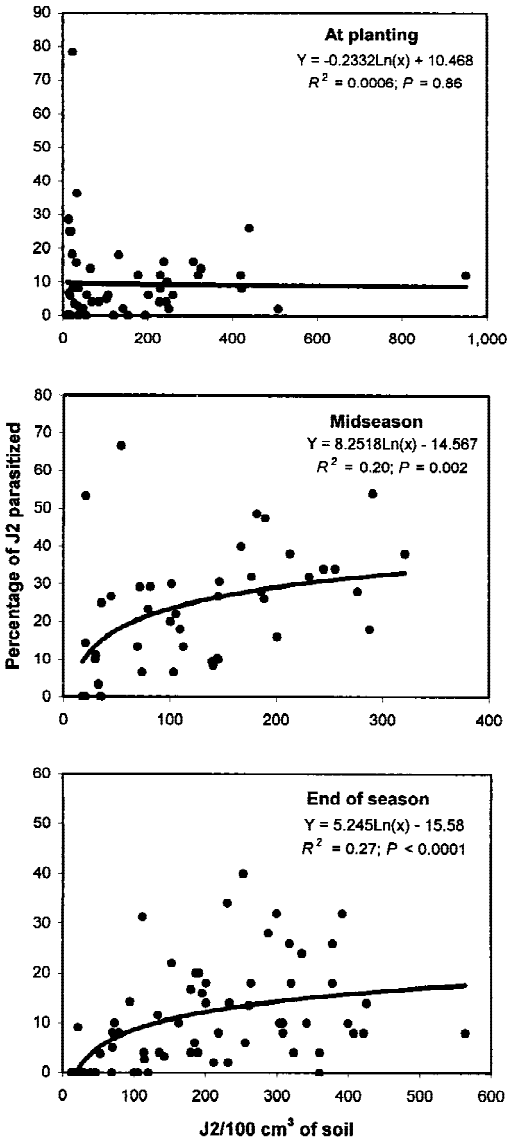

FIG. 3. Relationship between density of second-stage juveniles (J2) of *Heterodera glycines* and percentage of J2 parasitized by *Hirsutella rhossiliensis*.

susceptible cultivars is directly related to soybean cyst nematode density (MacGuidwin et al., 1995). Consequently, although Hr apparently did not reduce *H. glycines* densities to below a damaging level, suppression of the J2 density could reduce soybean yield loss caused by this nematode. Annual corn-soybean rotation is the most common cropping system in southern Minnesota. One year of corn reduced fungal initial inoculum for the next soybean season. The importance of Hr in soybean fields annually rotated with corn may be reduced in comparison with the fields with continuous soybean. Nevertheless, in plots of soybean annually rotated with corn, Hr increased in midseason and may have reduced J2 density and soybean yield loss caused by the nematode.

Variation of pathogenicity of Hr to nematodes exists among populations of the fungus within a nematode population and among nematode species (Tedford et al., 1994). *Hirsutella rhossiliensis* has a wide range of hosts (e.g., Sturhan and Schneider, 1980; Tedford et al., 1994). The fungus infected *Pratylenchus* spp. in vitro and in greenhouse tests (Timper and Brodie, 1994). *Pratylenchus* sp. and *Helicotylenchus* sp. were frequently encountered in corn plots, and they were also observed in soybean plots in the present study. Parasitism of these two nematodes by Hr was not observed. Further study, however, is needed to determine the host range of the Hr population in the present study.

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