EFFECTS OF TILLAGE AND CROP SEQUENCE ON PARASITISM OF *HETERODERA GLYCINES* JUVENILES BY *HIRSUTELLA* SPP. AND ON JUVENILE POPULATION DENSITY

S. Chen* and S. Liu

University of Minnesota, Southern Research and Outreach Center, 35838 120th Street, Waseca, MN 56093-4521. *Corresponding author: chenx099@umn.edu

ABSTRACT

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The effect of tillage on parasitism of *Heterodera glycines* second-stage juvenile (J2) by the nematophagous fungi *Hirsutella rhossiliensis* and/or *Hirsutella minnesotensis* and on J2 population density was investigated at four sites in soybean-corn rotation and one site in soybean monoculture in Minnesota. Soil samples were taken at planting, in midseason, and at harvest in 2002, at planting, 1 and 2 months after planting, and at harvest in 2003, and 2 weeks and 2 months after planting, and at harvest in 2004. *Heterodera glycines* J2 were extracted, and the number and percentage of J2 parasitized by *Hirsutella* were determined. No significant differences in the percentages of parasitized J2 were observed between conventional tillage (CT) and no-tillage (NT) at all sampling dates, except that the percentage of J2 parasitized in NT was significantly higher than in CT during the soybean-growing season in 2003 at one site in the soybean-corn annual rotation. The percentage of J2 parasitized by *Hirsutella* was higher in soybean fields than in corn fields, and the percentage of J2 parasitized in midseason was generally higher than that at planting and harvest. Growing a resistant soybean cultivar in the preceding year reduced the percentage of J2 parasitized by *Hirsutella* in the following season. Conventional tillage increased the mid-season J2 population density during 2002-2004 soybean-growing seasons as compared with NT at two of the five sites.

Key words: biological control, crop rotation, *Heterodera glycines*, *Hirsutella minnesotensis*, *Hirsutella rhossiliensis*, nematophagous fungi, soybean cyst nematode, tillage.

RESUMEN

Chen, S., y S. Liu. Efectos de la labranza y la secuencia de cultivos sobre el parasitismo de juveniles de *Heterodera glycines* por *Hirsutella* spp. y sobre la densidad de población de juveniles. 2007. Nematropica 37:93-106.

Se estudió el efecto de la labranza sobre el parasitismo de juveniles de segundo estadio (J2) de *Heterodera glycines* por los hongos nematófagos *Hirsutella rhossiliensis* y/o *Hirsutella minnesotensis* y sobre la densidad de población de J2 en cuatro sitios con rotaciones de soya y maíz y un sitio con monocultivo de soya, en Minnesota. Se tomaron muestras de suelo al momento de la siembra, en la mitad del ciclo del cutivo y al momento de cosecha en 2002, al momento de la siembra, 1 y 2 meses después de la siembra, y al momento de cosecha en 2003, y 2 semanas y 2 meses después de la siembra, y al momento de cosecha en 2004. Se extrajeron J2 de *Heterodera glycines*, y se determinó el número y porcentaje de J2 parasitados por *Hirsutella*. No se observaron diferencias significativas en los porcentajes de J2 parasitados entre labranza tradicional (CT = conventional tillage) y labranza cero (NT = no-tillage) en todas las fechas de muestreo, pero el porcentaje de J2 parasitados en NT fue significativamente más alto que en CT para soya en 2003 en un lugar con rotación anual de soya-maíz. El porcentaje de J2 parasitados por *Hirsutella* fue más alto en campos de soya que en campos de maíz, y el porcentaje de J2 parasitados en la mitad del ciclo del cultivo fue generalmente más alto que al momento de siembra o cosecha. El uso de soya resistente en el año anterior redujo el porcentaje de J2 parasitados por *Hirsutella* en el ciclo siguiente. La labranza tradicional aumentó la densidad de población de J2 en la mitad del ciclo del cultivo en soya en 2002-2004 comparado con NT en dos de los cinco sitios.

Palabras clave: control biológico, *Heterodera glycines*, *Hirsutella minnesotensis*, *Hirsutella rhossiliensis*, hongo nematófago, labranza, nematodo quiste de la soya, rotación de cultivos.

INTRODUCTION

The soybean cyst nematode, *Heterodera glycines* Ichinohe, accounts for almost half of soybean yield loss due to diseases in the United States (Monson and Schmitt, 2004; Wrather *et al.*, 2003). The main options for managing the nematode include growing resistant cultivars and crop rotation (Chen *et al.*, 2001a; MacGuidwin *et al.*, 1995; Niblack, 2005; Niblack and Chen, 2004; Young, 1998). An integrated approach, however, is needed for long-term effective management. Biological control and some cultural measures such as tillage and soil fertility management can play important roles in *Heterodera glycines* management (Barker and Koenning, 1998; McSorley and Porazinska, 2001; Niblack and Chen, 2004), but much more research is needed for their practical and effective use.

Since the late 1980s, conservation tillage has been used increasingly in the United States to reduce soil erosion, preserve soil moisture during drought, improve water quality, increase organic matter, and reduce fuel costs (Fawcett and Towery, 2005; Noel and Wax, 2003). Tillage may affect nematode communities in soil, but no consistent benefit of using conservation tillage in managing plant-parasitic nematodes was observed in different studies (McSorley, 1998). The effects of tillage on *H. glycines* may depend on environmental factors in different geographical locations. In southern USA, a number of studies demonstrated that no-tillage practice reduced *H. glycines* population densities as compared with conventional or minimal tillage (Edwards *et al.*, 1988; Hershman and Bachi, 1995; Koenning *et al.*, 1995; Lawrence *et al.*, 1990; Tyler *et al.*, 1983, 1987). Workneh *et al.* (1999) reported that of all fields that were infested with *H. glycines*, the population densities were significantly lower in no-tillage fields than in fields that received some type of tillage in the North Central region. However, Niblack *et al.* (1999) reported no consistent effect of tillage on *H. glycines* in Missouri, and an increase in *H. glycines* reproduction in no-tillage as compared with conventional tillage has been reported in Illinois (Noel and Wax, 2003). Also, no effect of tillage on *H. glycines* egg population density was observed in a study conducted from 1993 to 1996 in Minnesota (Chen *et al.*, 2001b). A longer-term (5-10 years) effect of tillage on *H. glycines* in Minnesota was also minimal and inconsistent (Chen, 2007). The inconsistency of tillage effect on *H. glycines* was supported by an extensive study conducted from 1997 to 2000 in nine states in the North Central region of the USA and in Ontario, Canada (Atibalentja *et al.*, 2001).

The reasons for the variations in tillage effects on *H. glycines* have not been determined. It is possible that soil biotic and abiotic factors in different locations and fields accounted for the different responses of *H. glycines* populations to tillage treatments. Tyler *et al.* (1987) speculated that reduction of *H. glycines* in no-tillage could be partially attributed to the higher level of nematode parasites present in the long-term no-tillage plots. However, a subsequent study demonstrated that fungal parasitism of *H. glycines* eggs did not differ between no-tillage and conventional tillage (Bernard *et al.*, 1997).

The nematophagous fungi *Hirsutella rhossiliensis* Minter & Brady and *Hirsutella minnesotensis* Chen, Liu, & Chen are common parasites of the second-stage juveniles (J2) of *H. glycines* in Minnesota (Chen *et al.*, 2000; Liu and Chen, 2000). Parasitism of J2 by *H. rhossiliensis* and *H. minnesotensis* was detected in 43% and 14% of fields in the state, respectively. The fungi appeared to be highly pathogenic to *H. glycines* as they parasitized a high (up to 60%) percentage of J2 in some fields (Chen and Reese, 1999; Liu and Chen, 2000). In greenhouse tests, the two species effectively controlled *H. glycines* populations (Chen and Liu, 2005; Liu and Chen, 2005). Crop sequence affected the parasitism of J2 by *H. rhossiliensis* (Chen and Reese, 1999), but more studies are needed to determine effects of cultural practices on the fungal parasitism of the nematode. The objective of this study was to determine the effect of tillage and crop sequence on parasitism of *H. glycines* J2 by *Hirsutella* spp. and on J2 population density. Information on the effect of tillage on fungal parasitism of J2 may help understand the variability of the tillage impact on *H. glycines* populations.

MATERIALS AND METHODS

Field Plots

This study was conducted at five sites in three fields in Waseca County, MN. Sites N1 and N2 were established in a field in New Richland, initially used to study the effect of tillage and row spacing on *H. glycines* populations (Chen *et al.*, 2001b; Chen, 2007). The tillage treatment consisted of no-tillage (NT) and conventional tillage (CT) with moldboard plowing in the fall after harvesting corn, chisel plowing after harvesting soybean, and spring field cultivation prior to planting. The soil at the two sites was a Webster clay loam (fine-loamy, mixed, mesic, Typic Endoaquoll; 37.4% sand; 32.4% silt; 30.2% clay; 7.3% organic matter; pH 7.8 measured in 1998). Each experimental unit consisted of a plot 6 m long and 3 m wide with four rows of 76-cm row spacing.

Sites W1 and W2 were established in 1997 in a field near the city of Waseca that was used in another project for the study of tillage, crop sequences and row spacing effect on *H. glycines* (Donald *et al.*, 2000; Chen, 2007). The soil at the two sites was a Webster clay loam. The soil characteristics at W1 (39.4% sand; 30.6% silt; 30.1% clay; 6.0% organic matter; pH 7.1) and W2 (37.6% sand; 31.5% silt; 30.9% clay; 7.1% organic matter; pH 8.0) were analyzed in 2004. Each experimental unit consisted of a plot 9.1 m long and 4.6 m wide with six rows of 76-cm row spacing.

Site W3 was located at the University of Minnesota Southern Research and Outreach Center research farm in Waseca and has been in no-tillage and monoculture of soybean for a number of years prior to this study. The soil at W3 was Nicollet clay loam (fine loamy, mixed, mesic, Aquic Hapludoll; 29.9% sand; 37.9% silt; 32.2% clay; pH 7.6). In 2003, plots were established for NT and CT. The CT was fall chisel plowing after harvest and spring cultivation prior to planting. Each experimental unit consisted of a plot 7.3 m long and 3.1 m wide with four rows of 76-cm row spacing.

A number of herbicides were used for preemergence and post-emergence weed control according to weed species and pressure in different years, and the same herbicides were used across all treatments within a site in a year.

The nematophagous fungi *H. rhossiliensis* was present at all sites, and *H. minnesotensis* was present at N1, N2, W1, and W2.

Experimental Design and Data Collection

This study was carried out during 2002-2004 by collecting data from the selected plots in the existing experimental plots originally used for other research projects (N1, N2, W1, and W2) and from the additional W3 site. In 2002, the data were collected from N1, N2, W1, and W2 sites. The experiment was a split-plot design with two tillage treatments (NT and CT) as main plots and three soil sampling dates as subplots. The two sites in the same field (N1 and N2 in New Richland, and W1 and W2 in Waseca) were alternately grown to soybean and corn every year, and the plots that were in corn/susceptible soybean annual rotations were selected for this study. In 2002, corn was planted on 30 May at N1 site and on 18 May at W1 site, and soybean 'Sturdy' was planted on 30 May at N2 site and on 15 may at W2 sites. A sample composed of 20 cores of soil was taken from the root zone in the two central rows of each plot with a 2.5-cm-diameter soil probe to a 20 cm depth at planting, 2 months after planting, and at harvest. Four replicates were included for N1 and N2 sites and six replicates were used at W1 and W2 sites.

In 2003, data were collected from the W1, W2, and W3 sites, and the experimental design was the same as in 2002 except for sampling dates. At W1 and W2 sites, the same plots that were in corn/susceptible soybean annual rotations were sampled. Soybean 'Sturdy' was planted on 15 May at the W1 site and corn was planted on 1 May at W2 site. At W3 site, the susceptible soybean 'Pioneer brand 92B13' was planted on 29 May. Soil samples were taken at planting, 1 month and 2 months after planting, and at harvest. Six replicates were included at all sites.

In 2004, data were collected from the W1 and W2 sites, and the experiment was a split-split-plot design with two tillage treatments (NT and CT) as main plots, two crop sequence treatments as sub-plots, and three sampling dates as sub-subplots. The crop sequences included in this study were CSS and CRS at W1 site and SCS and RCS at W2 site. The letters C, S, R in the crop sequences represent corn, susceptible soybean 'Sturdy' and resistant soybean 'Freeborn', respectively; the first, second, and third letters represent crops in 2002, 2003, and 2004, respectively. Soybean was planted on 7 May at both sites. Soil samples were taken at 2 weeks, 2 months after planting, and at harvest. Six replicates were included.

Each soil sample was thoroughly mixed and passed through 5-mm-aperture sieve. A subsample of 100 cm^3 soil was used to extract *H. glycines* J2 with a sucrose-flotation and centrifugation technique (Jenkins, 1964). The number of J2 was determined. All J2 parasitized by *Hirsutella* were counted in 2002 and 2003, and the parasitized J2 were counted from the first 50 J2 examined in 2004. Any J2 with one or more attached *Hirsutella* conidia or J2 colonized with fungal mycelium were considered as being parasitized by the fungi.

Data Analysis

The percentage of J2 parasitized by fungi were degree-arcsine ($x^{0.5}$)-transformed and J2 densities were $\ln(x + 1)$-transformed to normalize or improve the homogeneity of variance for statistical analysis. Because the sampling dates differed among years, the data were analyzed separately by year. To determine any similarity of the results among sites, the data of all combinations of sites within a year were initially analyzed with split-plot analysis of variance (ANOVA) with blocks (replicates) within site using SAS general linear model (GLM), although crop sequences were the same only between N1 and W1 and between N2 and W2 in 2002. Except for the percentages of nematodes parasitized by fungi in N2 and W2 sites, interactions of the site with treatments, either tillage, crop sequence (2004 only), and/or sampling dates, were significant for every combination of sites. The data were further analyzed by site and year and the ANOVA data are presented. When interactions between factors were significant within a site, further ANOVA at individual treatment levels of factors were performed. Means were compared with the Fisher's least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS

Fungal Parasitism

In most cases, tillage did not affect parasitism of J2 by *Hirsutella* spp. (Tables 1-3). In 2003, however, percentage of J2 parasitized by *Hirsutella* was slightly higher in NT (16%) than CT (11%) in the soybean plots at W1.

Parasitism of J2 by *Hirsutella* changed over a growing season. In general, fungal parasitism of J2 declined over the corn-growing season (Figs. 1A, 2A). In 2003 corn season at W2, however, fungal parasitism of J2 2 months after planting was higher than that 1 month after planting and at harvest (Fig. 2D). When soybean was grown following corn, parasitism of J2 by *Hirsutella* generally increased in midseason. Parasitism at the end of season was lower than (Fig. 2B) or similar to (Figs. 1B, 2C, 2F) that in midseason. In 2004, in plots with soybean following soybean at W1, parasitism of J2 by *Hirsutella* 2 weeks after planting and 2 months after planting was higher than at harvest for the sequence with *H. glycines*-susceptible cultivar in 2003, but there was no difference for the sequence with resistant cultivar in 2003 (Fig. 2E). Fungal parasitism of J2 2 weeks after planting in 2004 was higher following susceptible soybean than resistant soybean (Fig. 2E). In monocultures of soybean at W3 in 2003, parasitism of J2 by the fungus was higher in early and midseason than late season (Fig. 3).

J2 Population

Tillage affected J2 population density only in soybean-growing seasons at W1 and W2, and the effects differed between years and soil sampling dates (Tables 1-3, Fig. 4).

Fig. 1. Parasitism of *Heterodera glycines* second-stage juveniles (J2) by *Hirsutella* spp. in corn-soybean annual rotation at N1 and N2 in New Richland in 2002. Bars are means of two tillage treatments with four replicates, and the lines above the bars represent standard errors. Values followed by the same letter within the same graph are not different at $P \geq 0.05$ according to LSD test.

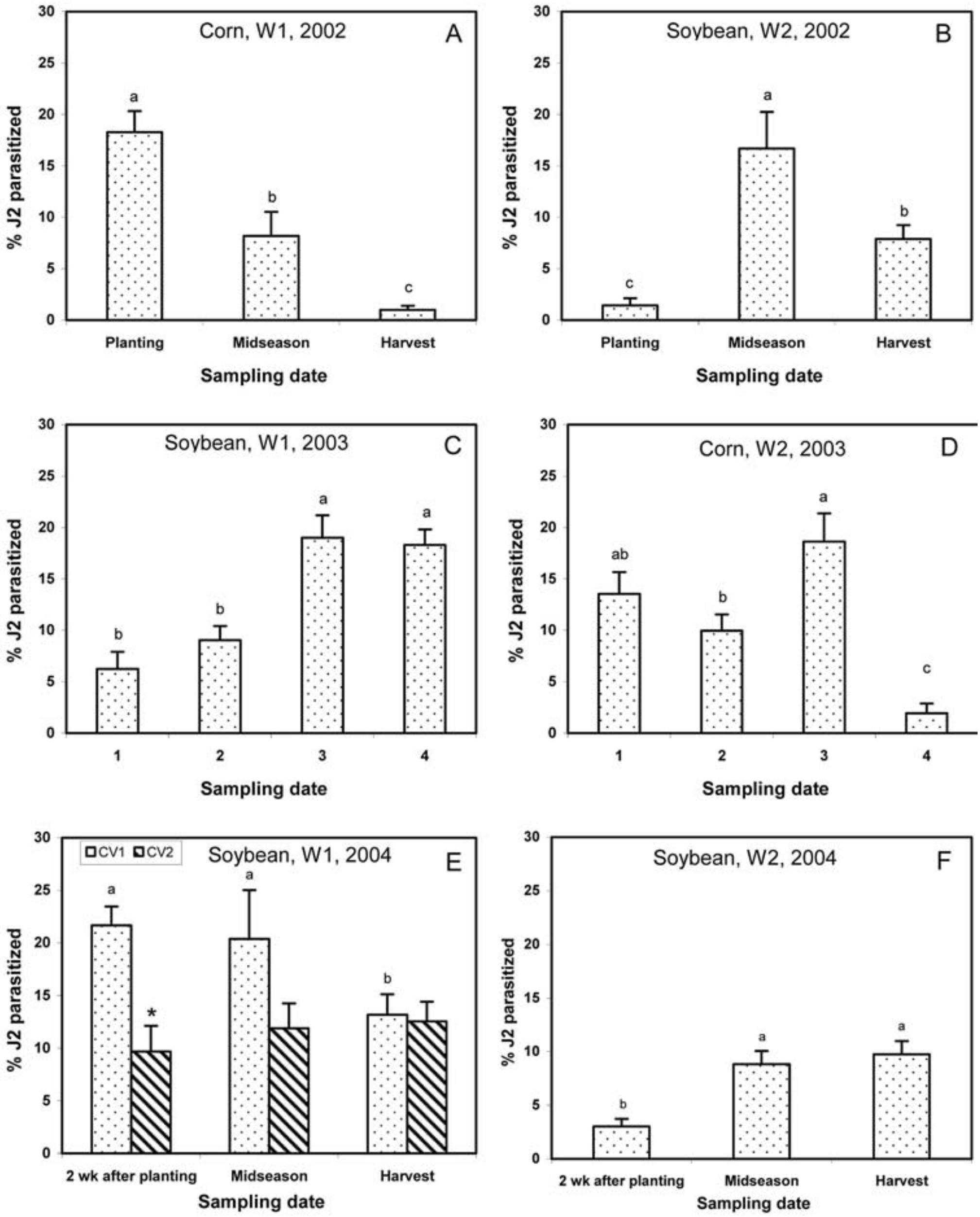

Fig. 2. Parasitism of *Heterodera glycines* second-stage juveniles (J2) by *Hirsutella* spp. at W1 and W2 in Waseca during 2002-2004. Sampling dates 1, 2, 3, and 4 represent at planting, 1 month after planting, 2 months after planting, and at harvest, respectively. CV1 and CV2 represent resistant and susceptible soybean cultivars, respectively, in the previous year (2003).The bars in A-E are means of two tillage treatments with six replicates, and the bars in F are means of two tillage treatments and two crop sequences with six replicates. The lines above the bars are standard errors. Values followed by the same letters or without letter among the different sampling dates within the same graph and same cultivar (E) are not different at $P \geq 0.05$ according to LSD test. * in graph E indicates difference at $P < 0.05$ between CV1 and CV2 with the same sampling date.

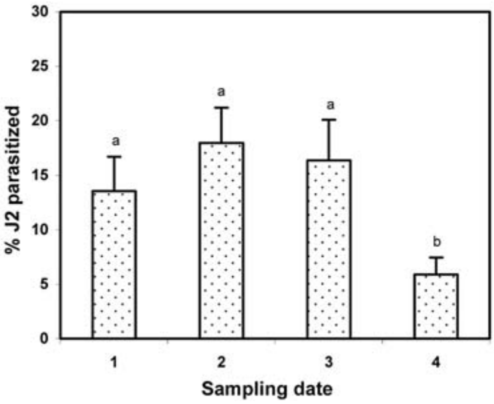

Fig. 3. Parasitism of *Heterodera glycines* second-stage juveniles (J2) by *Hirsutella* spp. in soybean monoculture at W3 in Waseca in 2003. Bars are means of two tillage treatments with six replicates, and the lines above the bars represent standard errors. Values followed by the same letter are not different at $P \geq 0.05$ according to LSD test.

Conventional tillage increased J2 population density midseason (2 months after planting) in all soybean years compared with NT at W1 and W2 (Fig. 4C, F, G, and H), except 2002 at W2 (Fig. 4B, $P = 0.07$). However, higher J2 population densities in NT than CT were observed 1 month after planting soybean in 2003 at W1 (Fig. 4C). There was no difference in J2 population density at planting and harvest between the two tillage treatments.

The population density of J2 differed between sampling dates. In 2002, J2 densities at corn planting were higher than at midseason and end of season (Figs. 4A, 5A). In the 2003 corn-growing season at W2, J2 density was highest at planting, intermediate at 2 months after planting, and lowest 1 month after planting and at harvest (Fig. 4D). In the growing seasons of susceptible soybean following corn, J2 density increased in midseason as compared with the initial population density in both tillage treatments at N2 in 2002 (Fig. 5B) and at W1 in 2003 (Fig. 4C), and in CT at W2 in 2002 (Fig. 4B) and 2004 (Fig. 4G). The end-season J2 densities were similar to (Figs. 4G, 5B) or lower than (Fig. 4B, C) midseason population densities. In 2004 at W1, no difference was seen in J2 density among the three sampling dates in plots where susceptible soybean was grown in 2003, but the density was higher at the end-season than midseason and at planting in plots where resistant soybean was grown in 2003 (Fig. 4E). In the plots where resistant soybean was grown in 2002 (RCS) at W2, the J2 population density in 2004 increased throughout the growing season in both NT and CT treatments (Fig. 4H). In contrast, J2 population density in monoculture of susceptible soybean increased 1 month after planting as compared with 2 weeks after planting, and declined in midseason and at the end of season (Fig. 6).

The J2 population density 2 weeks after planting in 2004 at W2 was higher in plots grown in 2002 with susceptible soybean (Fig. 4G) than with resistant soybean (Fig. 4H) (statistical comparison not shown). The difference became insignificant in the midseason and the end of season. Similarly, at W1, susceptible soybean in 2003 resulted in higher J2 population density at planting and midseason in 2004 as compared with resistant soybean in 2003, but the difference was not significant at the end of season (Fig. 4E).

DISCUSSION

This study suggests that the activities of *Hirsutella* spp. are similar in the no-tillage and conventional tillage. In a previous study, it was demonstrated that tillage had limited effect on fungal parasites of *H. glycines* eggs (Bernard *et al.*, 1997). Unlike *Hirsutella* spp., which are apparently obligate parasites of nematodes in soil (Jaffee and Zehr, 1985), most fungi associated with nematode eggs are facultative para-

Table 1. Analysis of variance of percentage of *Heterodera glycines* second-stage juveniles (J2) parasitized by *Hirsutella* spp. and J2 population density in response to tillage during corn and soybean-growing seasons in 2002 in southern Minnesota.

		New Richland sites				Waseca sites				
		N1 (corn)		N2 (soybean)			W1 (corn)		W2 (soybean)	
Source	df	MS	*F*-value	MS	*F*-value	df	MS	*F*-value	MS	*F*-value
					Fungal parasitism					
Replicate (R)	3	115		47		5	94		41	
Tillage (T)	1	130	1.4	31	1.6	1	18	0.4	0.1	0
R × T	3	91		20		5	51		124	
Sample date (D)	2	1393	35.9***	358	8.2**	2	1365	41.4***	1084	26.7***
T × D	2	72	1.9	2	0.1	2	59	1.8	2	0
R × T × D	12	39		44		20	33		41	
					J2 population density					
Replicate (R)	3	0.8		1.8		5	0.3		0.9	
Tillage (T)	1	0.5	2 .4	0.8	4.4	1	0.1	0.2	1.3	4.1
R × T	3	0.2		0.2		5	0.6		0.3	
Sample date (D)	2	13.1	15.6***	1.9	9.6**	2	5.3	17.6***	1.1	2.0
T × D	2	1.1	1.3	0.2	0.8	2	0.3	1.0	2.1	3.8*
R × T × D	12	0.8		0.2		20	0.3		0.6	

The data of percentage of fungi parasitized were transformed with degree-arcsine ($x^{0.5}$) and J2 population densities (J2/100 cm^3 soil) were transformed with ln (x +1) before being subject to split-plot analysis of variance. df stands for degrees of freedom. MS stands for mean square. *, **, and *** represent significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

sites (Chen and Dickson, 2004). Based on the previous study and our study, tillage may have limited effect on fungal parasitism of *H. glycines,* thus the tillage effect on the nematode population densities of *H. glycines* may not be due to the effect on fungal parasitism of the nematode. However, fungal parasitism may be affected by many other soil factors, such as soil pH, moisture, texture, organic matter, and temperature (Jaffee *et al.*, 1994; Jaffee and Zasoski, 2001; Tedford *et al.*, 1995a). For example, we may speculate that conventional tillage may increase J2 movement due to increased soil temperature and oxygen concentration, and increase chance of infection by *Hirsutella* (Tedford *et al.*, 1995b). In the present study, a slightly higher percentage of J2 parasitized by *Hirsutella* in NT than CT was observed in the soybean plots only at one site and in one year. The reasons for the different results in the different years and sites were unclear. Further studies are needed to determine the interactive effects of tillage and soil factors on the nematodes.

This study demonstrated that seasonal change in the percentage of J2 parasitized by *Hirsutella* spp. was dependent on the type of crops planted. The percentage of J2 parasitized generally declined over corn-growing seasons and increased dur-

Table 2. Analysis of variance of percentage of *Heterodera glycines* second-stage juveniles (J2) parasitized by *Hirsutella* spp. and J2 population density in response to tillage during corn and soybean-growing seasons in 2003 in Waseca, southern Minnesota.

		Sites					
		W1 (soybean)		W2 (corn)		W3 (soybean)	
Source	df	MS	*F*-value	MS	*F*-value	MS	*F*-value
			Fungal parasitism				
Replicate (R)	5	73		20		192	
Tilliage (T)	1	246	11.0*	36	1.4	35	0.1
R × T	5	22		26		270	
Sample date (D)	3	495	16.1***	926	17.6***	313	4.4*
T × D	3	31	1.0	109	2.1	42	0.6
R × T × D	30	31		53		70	
			J2 population density				
Replicate (R)	5	1.0		0.4		1.2	
Tilliage (T)	1	0.1	0.6	1.5	6.2	0.2	1.0
R × T	5	0.1		0.2		0.2	
Sample date (D)	3	7.6	77.5***	11.0	55.1***	5.6	14.9***
T × D	3	2.7	27.4***	0.4	1.8	0.6	1.6
R × T × D	30	0.1		0.2		0.4	

The data of percentage of fungi parasitized were transformed with degree-arcsine ($x^{0.5}$) and J2 population densities (J2/100 cm^3 soil) were transformed with ln (x +1) before being subject to split-plot analysis of variance. df stands for degrees of freedom. MS stands for mean square. *, and *** represent significance at $P < 0.05$, and $P < 0.001$, respectively.

ing soybean-growing seasons. Overall, the percentages of J2 parasitized by the fungi were higher in the soybean-growing season than in corn-growing season. These results agree with the previous study (Chen and Reese, 1999), and may be attributed to the density-dependent relationship between the fungi and the nematodes (Jaffee *et al.*, 1989). Soil temperature can be one of the major factors for the difference in fungal parasitism at different sampling times. At planting and harvest, the soil temperatures were generally low, which may have inhibited fungal and J2 activities. Therefore, the percentage of J2 parasitized by the fungi would be generally higher in mid-season than at planting and harvest. The effects of cultivars (*H. glycines*-resistant and *H. glycines*-susceptible) in the preceding year on the fungal parasitism of J2 may also be attributed to the density-dependent parasitism, because J2 densities were higher in plots following the susceptible soybean than the resistant soybean.

Conventional tillage consistently increased J2 number in midseason of soybean crop in one field (W1 and W2) as compared with NT, but there was no difference between CT and NT in the other two fields. The reason for the difference among fields is unclear. At W1 and W2, there was no difference in initial and final egg population

Table 3. Analysis of variance of percentage of *Heterodera glycines* second-stage juveniles (J2) parasitized by *Hirsutella* spp. and J2 population density during soybean-growing season in 2004 in response to tillage and preceding soybean cultivars in southern Minnesota.

		Sites			
		W1		W2	
Source	df	MS	*F*-value	MS	*F*-value
		Fungal parasitism			
Replicate (R)	5	69	1.4	64	1.3
Tillage (T)	1	0	0.0	28	1.2
R × T	5	116		25	
Crop sequence (C)	1	830	5.2*	220	4.1
T × C	1	148	0.9	64	1.2
R × T × C	10	160		54	
Sample date (D)	2	15	0.3	791	16.3***
T × D	2	43	0.9	114	2.4
C × D	2	193	4.0*	49	1.0
T × C × D	2	74	1.5	122	2.5
R × T × C × D	40	48		48	
		J2 population density			
Replicate (R)	5	2.2	5.3	0.9	2.8
Tillage (T)	1	8.1	6.7*	3.5	25.1**
R × T	5	1.2		0.1	
Crop sequence (C)	1	23.9	20.6**	4.8	8.7*
T × C	1	0.1	0.1	1.8	3.2
R × T × C	10	1.2		0.6	
Sample date (D)	2	4.4	10.2***	24.7	76.1***
T × D	2	0.5	1.1	2.8	8.5***
C × D	2	7.3	17.2***	4.4	13.5***
T × C × D	2	0.1	0.1	0.0	0.1
R × T × C × D	40	0.4		0.3	

The data of percentage of fungi parasitized were transformed with degree-arcsine ($x^{0.5}$) and J2 population densities (J2/100 cm^3 soil) were transformed with ln (x +1) before being subject to split-plot analysis of variance. df stands for degrees of freedom. MS stands for mean square. *, **, and *** represent significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

densities (Chen, 2007). Although the midseason egg population density was not determined, it is unlikely that there was a difference in egg population density in the soybean-growing season between the two tillage treatments at these sites. Therefore, the increase in J2 number in CT compared with NT at midseason was probably not due to different egg population densities. It is possible that disturbance of soil in CT

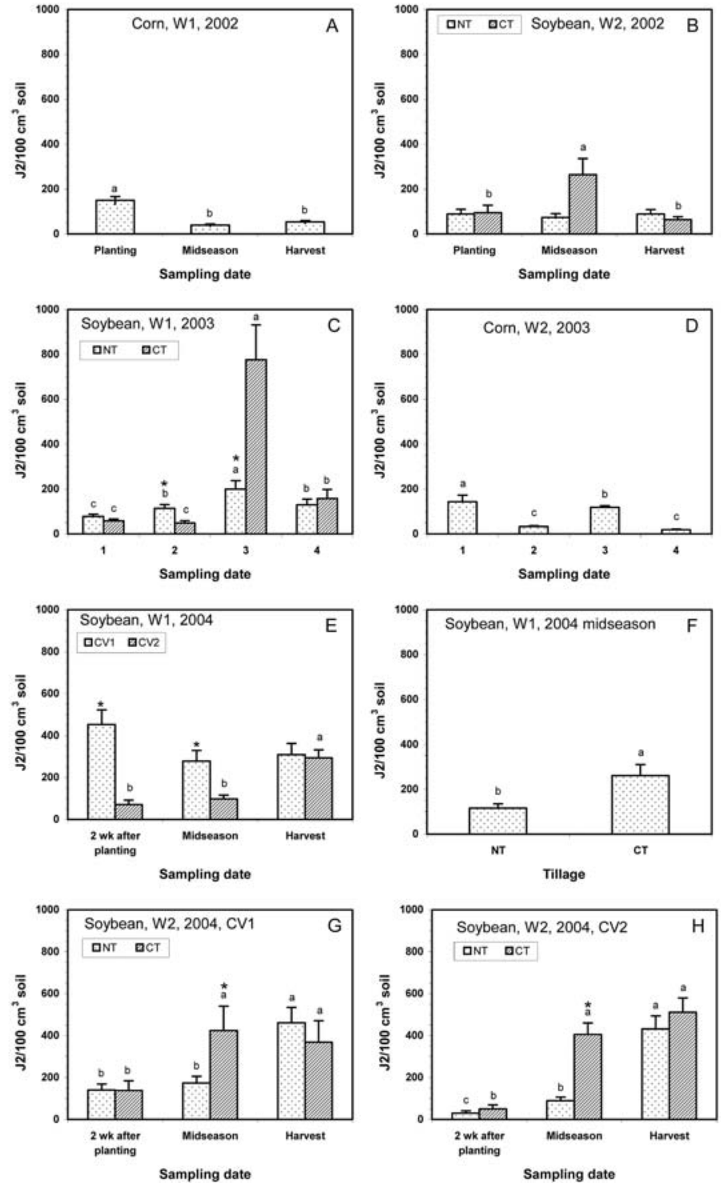

Fig. 4. Population density of *Heterodera glycines* second-stage juveniles (J2) at W1 and W2 in Waseca during 2002-2004. Sampling dates 1, 2, 3, and 4 represent at planting, 1 month, and 2 months after planting, and at harvest, respectively. NT and CT represent no-tillage and conventional tillage, respectively. CV1 and CV2 represent resistant and susceptible soybean cultivars, respectively, in the previous soybean year (2003 W1 site, E; or 2002 W2 site, G and H). The bars in A, D, and E are means of two tillage treatments with six replicates. The bars in B, C, G., and H are means of six replicates, and the bars in F are means of two treatments of preceding soybean cultivars with six replicates. The lines above the bars are standard errors. Values followed by the same letters or without letter among the different sampling dates within the same graph (A, D, and F) or within the same tillage in the same graph (B, C, G, and H), or with the same cultivar (E) are not significantly different at $P \geq 0.05$ according to LSD test. * indicates difference at $P < 0.05$ between NT and CT (C, G and H) or between CV1 and CV2 (E) with the same sampling date.

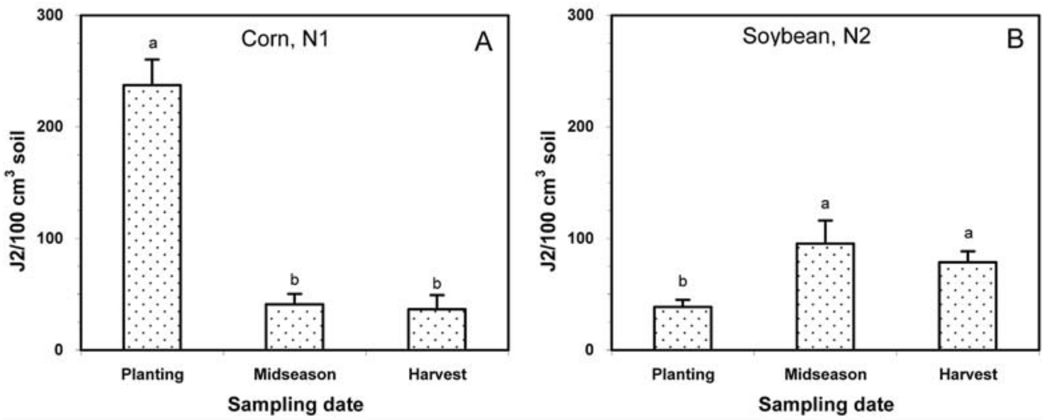

Fig. 5. Population density of *Heterodera glycines* second-stage juveniles (J2) in corn-soybean annual rotation at N1 and N2 in New Richland in 2002. Bars are means of two tillage treatments with four replicates, and the lines above bars represent standard errors. Values followed by the same letters within the same graph are not different at $P \geq 0.05$ according to LSD test.

enhanced *H. glycines* hatch under the soil conditions in W1 and W2. Further studies are needed to confirm this speculation and soil factors affecting *H. glycines* hatch.

In conclusion, tillage had little and no effect on the percentage of J2 parasitized by *Hirsutella* spp. Percentage of J2 parasitized by fungi was higher in soybean-growing season than in corn-growing season, and the highest level of parasitizism was generally in the midseason of the soybean growing year. Growing resistant soybean resulted in lower fungal parasitism in the following season. Conventional tillage increased J2 population density in the midseason at two sites, but the effect was not observed in the other three sites. Further studies are needed to determine *H. glycines* J2 and egg population dynamics during the soybean-growing season in the CT and NT cropping systems.

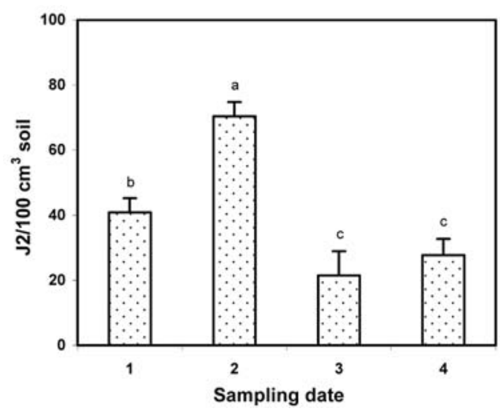

Fig. 6. Population density of *Heterodera glycines* second-stage juveniles (J2) in soybean monoculture at W3 in Waseca in 2003. Bars are means of two tillage treatments with six replicates, and the lines above the bars represent standard errors. Values followed by the same letter are not different at $P \geq 0.05$ according to LSD test.

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