SUPPRESSION OF *HETERODERA GLYCINES* IN A SOYBEAN FIELD ARTIFICIALLY INFESTED WITH *PASTEURIA NISHIZAWAE*

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ABSTRACT

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In 1999, microplot soil infested with *Pasteuria nishizawae* was added to an ongoing tillage and crop rotation study to determine the effectiveness of *P. nishizawae* in managing *Heterodera glycines*. Population dynamics of *H. glycines* was monitored each year through 2005 as numbers of eggs per 100 cm^3 soil at planting (Pi) and at harvest (Pf) of soybean cultivars either resistant or susceptible to *H. glycines*. A reproduction factor, Pf/Pi (Pi > 0), also was calculated and soybean yield was determined. Infestation of field soil with *P. nishizawae* had significant effects on population dynamics of *H. glycines*. Overall, Pi was lower in *P. nishizawae*-infested plots than on non-infested plots, with means of 320 ± 80 and 580 ± 80 eggs/100 cm^3, respectively. Plots infested with *P. nishizawae* had similar or lower Pf than the non-infested plots, and Pf/Pi was, on average, lower in *P. nishizawae*-infested plots than on non-infested plots, 8.0 ± 2.1 compared with 14.0 ± 1.9. With only one exception in 2000, yield of soybean grown in *P. nishizawae*-infested plots was similar or greater than that of non-infested plots. The resistance or susceptibility of the soybean cultivar to *H. glycines* also had significant effects on population densities, since Pi, Pf, and Pf/Pi were similar or lower on the resistant cultivar than on the susceptible cultivar, except in 2005 when Pf and Pf/Pi were lower on the susceptible cultivar. In five of seven years, yield of the resistant cultivar was greater than that of the susceptible cultivar except in 2002 when yields were similar and 2005 when yield of the susceptible cultivar was greater. Tillage method had no effect on population densities of *H. glycines*. However, yield was higher following conventional tillage than for no tillage, with means of 2740 ± 40 versus 2520 ± 40 kg/ha. In this study, *P. nishizawae* was successfully transferred to a soybean field, became suppressive to *H. glycines*, and was associated with an increase in soybean yield.

Key words: Biological control, conservation tillage, genetic resistance, *Heterodera glycines*, no-tillage, *Pasteuria nishizawae*, soybean cyst nematode, suppressive soil.

RESUMEN

Noel, G. R., N. Atibalentja, and S. J. Bauer. 2010. Supresión de *Heterodera glycines* en un cultivo de soya infestado artificialmente con *Pasteuria nishizawae*. Nematropica 40:41-52.

En 1999, se adicionó suelo infestado con *Pasteuria nishizawae* a un estudio de rotaciones y labranzas para determinar la efectividad de este microorganismo en el manejo de *Heterodera glycines*. Se observó la dinámica poblacional de *H. glycines* cada año hasta el 2005, registrándose el número de huevos por 100 cm^3 de suelo al momento de la siembra (Pi) y al momento de la cosecha (Pf), utilizando cultivares de soya resistentes y susceptibles a *H. glycines*. Se calculó el factor de reproducción, Pf/Pi (Pi > 0), y se midió la producción de soya. La infestación del suelo con *P. nishizawae* tuvo efectos significativos en la dinámica poblacional de *H. glycines*. En general, la Pi fue más baja en los lotes infestados con *P. nishizawae* que en los lotes no infestados, con promedios de 320 ± 80 y 580 ± 80 huevos/100 cm^3, respectivamente. Los lotes infestados con *P. nishizawae* tuvieron Pf similar o más baja que los lotes no infestados, y el Pf/Pi fue, en promedio, menor en lotes infestados que en los no infestados, 8.0 ± 2.1 comparado con 14.0 ± 1.9. Con excepción del año 2000, el rendimiento del cultivo fue igual o mayor

en los lotes infestados que en los no infestados. La resistencia o susceptibilidad de los cultivares de soya a *H. glycines* también tuvo un efecto significativo en las densidades de población, con Pi, Pf, y Pf/Pi similares o menores en el cultivar resistente que en el susceptible, excepto en 2005 cuando la Pf y Pf/Pi fueron más bajos en el cultivar susceptible. En cinco de los siete años, el rendimiento del cultivar resistente fue mayor que el del susceptible. En el 2002 los rendimientos fueron similares y en el 2005 el cultivar susceptible tuvo mayor rendimiento. El método de labranza no tuvo efecto sobre la densidad de población de *H. glycines*. Sin embargo, el rendimiento fue mayor con labranza convencional que con labranza cero, con promedios de 2740 ± 40 versus 2520 ± 40 kg/ha. En este estudio, se transfirió exitosamente *P. nishizawae* a un campo de soya, convirtiéndole en supresivo a *H. glycines*, y se observó un aumento en el rendimiento del cultivo.

Palabras clave: Control biológico, *Heterodera glycines*, labranza cero, labranza mínima, nematodo quiste de la soya, *Pasteuria nishizawae*, resistencia genética, suelo supresivo.

INTRODUCTION

Soybean cyst nematode, *Heterodera glycines* Ichinohe, infests areas of soybean (*Glycine max* (L.) Merr.) production worldwide and is a major yield limiting pest in many countries (Tanha Maffi *et al.*, 1999; Riggs, 2004). In the United States, crop loss due to *H. glycines* in 2006 was estimated at 31% or 2.8 million MT (Wrather and Koenning, 2008). In the U.S., management of *H. glycines* relies primarily on a 2-yr rotation with a nonhost and planting of resistant cultivars of which approximately 1350 are available in the U.S. (Shier, 2008). This production system does not adequately manage *H. glycines* as evidenced by the continuing crop loss of approximately 30% per annum during the last several years (Wrather and Koenning, 2008). Additional management practices, including biological control, are needed to further reduce crop loss.

Many potential natural enemies of *H. glycines* are known (Morgan Jones *et al.*, 1981; Ownley Gintis *et al.*, 1983; Carris and Glawe, 1989; Stiles *et al.*, 1990, 1993; Chen *et al.*, 1996; Chen, 2004), but there are few reports of successful biological control of *H. glycines* (Nishizawa, 1987; Atibalentja *et al.*, 1998; Chen and Liu, 2005; Chen, 2007). *Pasteuria nishizawae* ((Sayre, Wergin, Schmidt & Starr) Noel, Atibalentja & Domier) is an endospore and mycelia-forming endoparasitic bacterium of *H. glycines* (Sayre and Starr, 1985: Sayre *et al.*, 1991a, 1991b; Atibalentja *et al.*, 2004; Noel *et al.*, 2005). In Asia, *P. nishizawae* has been reported in Japan, South Korea, and The People's Republic of China (Nishizawa, 1986, 1987; Lee *et al.*, 1998; Peng and Zhang, 1999; Ma *et al.*, 2005). The bacterium was first reported in North America when it was found in microplots used to raise *H. glycines* for use in a breeding program for nematode resistant soybean (Noel and Stanger, 1994). Only recently have additional discoveries of *P. nishizawae* been reported in North America (Donald and Hewlett, 2009).

In a microplot study, *P. nishizawae* and *H. glycines* were shown to follow the Lotka - Volterra predator-prey model, and numbers of *H. glycines* were reduced below the damage threshold (Atibalentja *et al.*, 1998). Based on that study, an experiment was designed to determine whether suppressive soil containing *P. nishizawae* could be transferred to a field experiment to manage *H. glycines* and to increase soybean yield.

MATERIALS AND METHODS

The experiment was established in 1999 in a tillage and crop rotation study ini-

tiated in 1994. Soil in the field was a Drummer silty clay loam (Typic Haplaquolls; fine silty, mixed mesic; 61.1% silt, 28.8% clay, and 10.1% sand), pH 6.6, and 6.0% OM. The population of *H. glycines* in the field was classified as Hg Type 0 (Niblack *et al.*, 2002). The average Pi for plots that received *P. nishizawae* infested soil and the controls were (means ± standard errors) 432 ± 132 and 520 ± 132/100 cm^3 soil, respectively. In 1999, rotation with maize was discontinued, and a monoculture of soybean was initiated. Plots planted previously with *H. glycines*-resistant cv. Linford (PI88.788 source) or with susceptible cv. Williams 82 continued to be planted with those cultivars (Bernard and Cremeens, 1988; Bernard and Noel, 1991). The experiment was conducted as a split-split plot in a randomized complete block design with three replications. Tillage method, either conventional or no tillage, was allocated to main plot, cultivar to subplot, and application of *P. nishizawae*-infested soil to sub-subplot (experimental unit), which was a four-row plot, 3-m wide × 12-m long. Each experimental unit was subdivided into two 3-m wide × 6-m long halves to increase the number of observations for yield and nematode data. Sub plots were separated by 12 m on both ends in the direction of tillage and planting, and sub-subplots were arranged so that runoff of rain water would flow from plots not infested with *P. nishizawae* to infested plots. The experiment was repeated over seven years (1999-2005) with no further randomization.

A no-till planter was pulled through the plot to mark each row of the experiment. Each 12-m-long row of the *P. nishizawae*-infested plots received 8 L of microplot soil naturally infested with *H. glycines* HG Type 0 and *P. nishizawae*. The equilibrium density of the microplot soil was 27 J2/100 cm^3 soil with 64% of J2 having at least one endospore adhering to the cuticle (Atibalentja *et al.*, 1998). Infested soil was incorporated with a strip-tillage implement in a 15- cm wide × 15-cm deep band. Controls did not receive strip tillage nor microplot soil. The experiment was planted each year with a no-till planter. In an attempt to delay contamination of control plots with *P. nishizawae*, tillage and planting operations and sampling of soil were completed in the control plots before moving to the infested plots. From 1999-2004, Linford and Williams 82 were planted. In 2005, the proprietary soybean cultivars K346RR SCN and Kruger K349RR, respectively, resistant (PI88.788 source) and susceptible to *H. glycines* were planted. Each year, immediately after planting and after harvest, 12 soil cores, each 2.5-cm-diameter × 15-cm-long, were taken in a zigzag pattern from the middle two rows of each half-experimental unit. Females and cysts of *H. glycines* were extracted from 100 cm^3 soil from each subsample, using the gravity sieving method (Cobb, 1918), to determine the number of eggs per 100 cm^3 soil at planting (Pi) and at harvest (Pf). A reproduction factor, Pf/Pi (Pi > 0), also was calculated for each subsample. Soybean yield (kg/ha) was determined by harvesting 5 m of the middle two rows of each half-experimental unit. Seed moisture was corrected to 13%. In 2005, soil samples were processed further, using centrifugal flotation (Jenkins, 1964) to extract J2 of *H. glycines* and verify the presence of *P. nishizawae*. The J2 were heat relaxed and preserved in 2% formalin before examination. All J2 extracted from each 100-cm^3 soil subsample were removed individually from a counting dish with the aid of a dissecting microscope, placed on temporary Cobb slides, and examined under an interference contrast microscope to determine attachment of *P. nishizawae* endospores onto the J2 cuticle.

The number of eggs and the reproduction factor were transformed to Log_{10} (X + 1) and data were averaged for each experimental unit prior to statistical analysis, using PROC MIXED (SAS Institute, Cary, NC). Since successive observations on the same experimental unit tend to be correlated, repeated measure analysis with autoregressive order one covariance structure was performed on the entire dataset from the 7-year-long experiment, based on the split-split plot model described above. In these analyses, replication, replication x tillage (main plot error), and replication x tillage x cultivar (subplot error) were treated as random effects, which in PROC MIXED are declared in the RANDOM rather than the MODEL statement. Unless specified otherwise, comparison of (least square) means were done at $P = 0.05$, using the default *t*-test. For comparison of years, contrast analysis was preferred to the examination of all possible pair-wise combinations. Thus, a contrast was made between year 2005, when proprietary soybean cultivars were used, and the previous years, when the older public cultivars were planted. A second contrast compared year 2000, when soybean suffered from a severe drought during pod development, and the other years in which Linford and Williams 82 were planted. Nematode data are shown in the original units instead of antilog of transformed data.

RESULTS

Pasteuria nishizawae in infested microplot soil was successfully transferred to the field trial, as ascertained by microscopic observation of endospore-encumbered J2 from soil samples collected in 2005. In infested plots, the percentage of J2 with at least one *P. nishizawae* endospore attached to the cuticle ranged from 4% to 30%. Only one control plot was contaminated with *P. nishizawae* and the level of endospore attachment was 2% (one out of 61 J2 examined). Infestation of plots with *P. nishizawae* had significant effects ($P \leq 0.05$) on population densities of *H. glycines* (Table 1). Specifically, Pi of *H. glycines* was, on average, lower in *P. nishizawae*-infested plots than in non-infested plots, with means ± standard errors of 317 ± 83 and 557 ± 83 eggs/100 cm^3 soil, respectively. Infestation of field soil with *P. nishizawae* also had a significant effect on Pf of *H. glycines*, but this effect varied with year (Table 2). In 2000, 2002, and 2004, the Pf of *Pasteuria*-infested plots were lower than those of non-infested plots, compared with 1999, 2001, 2003, and 2005 when the differences were not significant, even though Pf of infested plots were numerically lower than those of the non-infested plots. With *P. nishizawae* infestation, Pf in 2005 was not different from the average Pf of 850 eggs/100 cm^3 soil in previous years (Table 2). Without infestation, Pf in 2005 was lower than the 1999-2004 average Pf of 2698 eggs/100 cm^3 soil. In 2000, Pf on *Pasteuria*-infested plots and non-infested plots were respectively, 392 and 2208 eggs/100 cm^3 soil, which did not differ from the 942 eggs/100 cm^3 soil and 2796 eggs/100 cm^3 soil for infested and non-infested plots during the 1999 and 2001 - 2004 period (Table 2). Overall, plots infested with *P. nishizawae* had lower Pf/Pi than the non-infested plots, with means ± standard errors of 8.0 ± 2.1 and 14.0 ± 1.9, respectively. A significant year × *P. nishizawae* interaction was observed for soybean yield, since in 1999 and 2001-2004 yields were similar between *P. nishizawae*-infested plots and non-infested plots, whereas in 2005 *P. nishizawae*-infested plots provided higher yield than the non-infested plots (Table 2). In 2000, however, yield of plots not infested with *P. nishizawae* was greater than that of infested plots. On both the *P. nish-*

Table 1. Summary analysis of variance for the effects of year, tillage, soybean cultivar, and *Pasteuria nishizawae* on numbers of *Heterodera glycines* eggs per 100 cm^3 soil at planting (Pi) and at harvest (Pf), the reproduction factor (Pf/Pi), and yield of soybean (kg/ha), 1999 - 2005.

		P > *F*			
Source of variation[z]	*df*	Pi	Pf	Pf/Pi	Yield
Year	6	<0.001	0.003	<0.001	<0.001
Tillage	1	ns	ns	ns	<0.001
Year × tillage	6	ns	ns	ns	ns
Cultivar	1	0.011	0.021	0.035	<0.001
Year × cultivar	6	0.022	<0.001	<0.001	<0.001
Tillage × cultivar	1	ns	ns	ns	ns
Year × tillage × cultivar	6	ns	ns	ns	ns
Pasteuria	1	0.010	<0.001	<0.001	ns
Year × *Pasteuria*	6	ns	0.039	0.053	<0.001
Tillage × *Pasteuria*	1	ns	ns	ns	ns
Year × tillage × *Pasteuria*	6	ns	ns	ns	ns
Cultivar × *Pasteuria*	1	ns	ns	ns	ns
Year × cultivar × *Pasteuria*	6	ns	ns	ns	ns
Tillage × cultivar × *Pasteuria*	1	ns	ns	ns	ns
Year × tillage × cultivar × *Pasteuria*	6	ns	ns	ns	ns
CV%		161	132	166	22

[z]Only fixed effects are shown, since random effects such as replication, replication × tillage (main plot error), replication × tillage × cultivar (subplot error), and the residual appear in the RANDOM rather than the MODEL statement in PROC MIXED (SAS Institute, Cary, NC).

izawae-infested plots and the non-infested plots, the 2005 yields were higher than the average yields of 2480 kg/ha on infested plots and 2466 kg/ha on non-infested plots in previous years. In contrast, the 2000 yields on *P. nishizawae*-infested plots and non-infested plots were lower than the average yields of 2633 kg/ha and 2551 kg/ha on these plots during 1999 and 2001-2004 (Table 2).

Resistance or susceptibility of the cultivar to *H. glycines* also had significant effects on population densities of *H. glycines* and soybean yield, although the effects were confounded by interactions with year (Tables 1). In 1999, 2000, 2002, and 2004, Pi was lower in plots planted with the resistant Linford when compared with plots planted with the susceptible Williams 82 (Table 3). No significant differences in Pi were observed between these cultivars in 2001 and 2003, nor between the resistant K346RR SCN and the susceptible K349RR in 2005. The Pi on the latter two cultivars were higher than the average Pi of 119 eggs/100 cm^3 soil on Linford and 459 eggs/100 cm^3 soil on Williams 82, respectively (Table 3). The Pi in plots planted with Linford in 2000 was consistent with the average Pi of 118 eggs/100 cm^3 soil on this cultivar in other years, compared with Williams 82 on which Pi in 2000 was lower

Table 2. Effects of soil infestation with *Pasteuria nishizawae* (Pn +) versus no infestation (Pn -) on numbers of *Heterodera glycines* eggs per 100 cm^3 soil at harvest (Pf) and soybean yield over a seven-year period (1999 - 2005).

	Pf		Yield (kg/ha)	
Year	Pn +	Pn -	Pn +	Pn -
1999	1622 a[y]	2064 a	2733 a	2717 a
2000	392 b	2208 a	1717 b	2040 a
2001	911 a	3135 a	2576 a	2408 a
2002	353 b	1726 a	2638 a	2578 a
2003	886 a	3414 a	2602 a	2562 a
2004	938 b	3640 a	2616 a	2489 a
2005	825 a	1053 a	3747 a	3373 b
	Contrast estimates			
2005 vs 1999-2004	-25	-1645*[z]	1267**	907**
2000 vs 1999, 2001-2004	-550	-588	-916**	-511**

[y]In a given year, least square means of Pf and yield not followed by same letter are significantly different ($P \leq 0.05$).
[z]Figures followed by * and ** are significant at $0.01 < P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3. Effects of soybean cultivars, either resistant (Res) or susceptible (Sus) to *Heterodera glycines*, on numbers of eggs per 100 cm^3 soil at planting (Pi) and at harvest (Pf), the reproduction factor (Pf/Pi), and soybean yield over a seven-year period (1999 - 2005).

	Pi		Pf		Pf/Pi		Yield (kg/ha)	
Year	Res[x]	Sus	Res	Sus	Res	Sus	Res	Sus
1999	136 b[y]	817 a	975 a	2710 a	8.7 a	18.2 a	2957 a	2492 b
2000	125 b	309 a	767 a	1832 a	5.5 a	9.3 a	1988 a	1769 b
2001	157 a	175 a	579 b	3466 a	4.3 b	19.1 a	2677 a	2306 b
2002	38 b	229 a	450 b	1630 a	8.7 a	11.7 a	2686 a	2530 a
2003	53 a	49 a	928 b	3372 a	9.6 a	30.9 a	2862 a	2303 b
2004	205 b	1178 a	132 3b	3255 a	18.0 a	5.6 a	3019 a	2086 b
2005	1026 a	1620 a	1478 a	399 b	3.5 a	0.6 b	3435 b	3686 a
	Contrast estimates							
2005 vs. 1999-2004	907*[z]	1161**	641**	-2312**	-5.6	-15.2**	737**	1438**
2000 vs. 1999, 2001-2004	7	-180*	-84	-1055	-4.2	-7.8*	-852**	-574**

[x]From 1999 to 2004, Linford was the resistant cultivar and Williams 82 was the susceptible cultivar, whereas in 2005 Kruger K346RR SCN was the resistant cultivar and Kruger K349RR was the susceptible cultivar.
[y]In a given year, least square means of Pi, Pf, Pf/Pi, and yield not followed by same letter are significantly different ($P \leq 0.05$).
[z]Figures followed by * and ** are significant at $0.01 < P \leq 0.05$ and $P \leq 0.01$, respectively.

than its average Pi of 489 eggs/100 cm^3 soil. Plots planted with the resistant Linford had a Pf similar to those planted with the susceptible Williams 82 in 1999 and 2000, compared with 2001-2004 when Pf was lower on Linford than on Williams 82 (Table 3). In 2005, Pf was lower on the susceptible K349RR than on the resistant K346RR SCN, which also had higher Pf than the average Pf of 837 eggs/100 cm^3 soil on Linford during the previous six years (Table 3). In contrast, Pf on K349RR was lower than the average of 2711 eggs/100 cm^3 soil on Williams 82. The Pf on the resistant Linford and the susceptible Williams 82 in 2000 were not different from the average Pf on these cultivars in other years, 851 eggs/100 cm^3 soil and 2887 eggs/100 cm^3 soil, respectively (Table 3). Reproduction of *H. glycines* on the resistant and susceptible cultivars was similar in most of the years, except in 2001 when Pf/Pi was lower on the resistant cultivar Linford than on the susceptible cultivar Williams 82, and in 2005 when Pf/Pi was lower on the susceptible cultivar K349RR than on the resistant cultivar K346RR SCN (Table 3). In addition, Pf/Pi of 3.5 on the resistant K346RR SCN did not differ significantly from the average Pf/Pi of 9.1 on Linford, but the Pf/Pi of 0.6 on the susceptible K349RR was significantly lower than the average Pf/Pi of 15.8 on Williams 82. Likewise, in 2000 Pf/Pi of 5.5 on Linford was not different from the average Pf/Pi of 9.7 on this cultivar in other years, as opposed to Williams 82 on which the Pf/Pi of 9.3 in 2000 was lower than its average Pf/Pi of 17.1 (Table 3). Soybean yield was higher for the resistant cultivar Linford than for the susceptible cultivar Williams 82, except in 2002 when no yield difference was observed (Table 3). In 2005, the resistant cultivar K346RR SCN provided lower yield than the susceptible cultivar K349RR, and the yield of these two proprietary cultivars were significantly higher than the average yield of the public cultivars used in previous years, 2698 kg/ha and 2248 kg/ha, respectively, for the resistant Linford and the susceptible Williams 82. The yields of both public cultivars in 2000 were significantly lower than their average in other years, 2840 kg/ha for Linford and 2343 kg/ha for Williams 82 (Table 3).

Tillage method had no significant effect on Pi, Pf, and Pf/Pi of *H. glycines* (Table 1). However, soybean yields were on average higher in conventionally tilled plots than in plots that were not tilled, with means ± standard errors of 2740 ± 44 and 2516 ± 44 kg/ha, respectively.

Repeated measure analysis showed that consecutive observations from the same experimental units were significantly correlated. The correlation coefficients were $\rho = 0.25$ ($P = 0.064$) for Pi, $\rho = -0.24$ ($P = 0.042$) for Pf/Pi, and $\rho = 0.44$ ($P < 0.001$) for soybean yield.

DISCUSSION

We have demonstrated that the suppressiveness of *P. nishizawae* to *H. glycines* is transferable, since applications of microplot soil infested with *P. nishizawae* to a non-suppressive field resulted in reductions in population densities of *H. glycines*. Infestation of field soil with *P. nishizawae* also provided similar or higher yield than non-infested soil, except in 2000 when, in spite of higher Pf, plots not infested with *P. nishizawae* had higher yield than infested plots. Soybean suffered from a severe drought during pod development in the summer of 2000, with only 37 mm of rainfall recorded during the 42-day period extending from 12 July to 22 August. This drought is believed to have stressed plants to such extent that the yield advantage of the non-infested plots was an artifact. In 2000, the yields of the resistant cultivar Lin-

ford and the susceptible cultivar Williams 82 were 30% and 25%, respectively, less than their average during 1999 and 2001-2004.

As a result of a significant year × *P. nishizawae* interaction, Pf was lower on *P. nishizawae* infested plots than on non-infested plots in some years but not in others. This finding is consistent with the cyclic nature of the *P. nishizawae H. glycines* interrelationship described by the Lotka-Volterra predator-prey model of population dynamics (Atibalentja *et al.*, 1998). An obligate parasite such as *P. nishizawae* would not eliminate its host at the risk of its own annihilation (Ciancio, 1995). Thus, *P. nishizawae* and *H. glycines* maintain a dynamic equilibrium with populations of both organisms increasing and decreasing asynchronously with each other over time.

An argument could be made that a biocontrol agent other than *P. nishizawae* was responsible for the suppressiveness of the soil transferred from microplots to the field plot. It is possible to isolate a myriad of fungi from cysts, but isolation does not necessarily mean the fungi are pathogenic to *H. glycines* (Morgan-Jones *et al.*, 1981; Ownley Gintis *et al.*, 1983; Carris and Glawe, 1989; Carris *et al.*, 1989). The cyst-colonizing fungi *Diheterospora chlamydosporia* (Goddard) Barron & Onions (Synonym: *Verticillium chamydosporium* Goddard), *Paraphoma radicina* (McAlp) Morgan-Jones & White, *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson, *Stagonospora heteroderae* Carris, Glawe & Morgan-Jones, *Fusarium virguliforme* (Akoi, O'Donnell, Homma & Lattanzi) and *Fusarium oxysporum* Schlect. were isolated from *H. glycines* in Illinois soybean fields suppressive to the nematode (Carris *et al.*, 1989). These fungi were used in a greenhouse study in nonsterile field soil to determine their potential as pathogens of *H. glycines* (Stiles *et al.*, 1993). None was pathogenic. The soil used in that study was the same soil type as the one in which *P. nishizawae* was first found, but was from an area of close proximity not infested with *H. glycines* and presumably also not infested with *P. nishizawae*. Detection of *P. nishizawae* in only one control plot was surprising. We expected transfer of endospores or infected nematode life stages by dispersal of soil on farm equipment, wind, or rain. However, designing the experiment to minimize runoff of rainfall and conducting farming practices on non-infested plots first was effective in delaying contamination of control plots. Earlier reports have discussed the difficulty of maintaining *Pasteuria*-free control plots adjacent to artificially infested plots in field research (Giblin-Davis, 2000; Kariuki and Dickson, 2007).

Ideally, a suspension of known concentration of *P. nishizawae* endospores would have been used to infested the field soil at the beginning of this study, and the *P. nishizawae* quantified each year in terms of numbers of endospores per gram of soil. At the time of initiation of this experiment, attempts to devise suitable techniques of mass-production of *Pasteuria* spp. had not been successful (Bishop and Ellar, 1991; Williams *et al.*, 1989; Hewlett *et al.*, 2002). For limited amounts of inoculum, cysts of *H. glycines* filled with *P. nishizawae* endospores are often crushed in a small volume of water to produce crude endospore suspensions. However, cysts developing from diseased females of *H. glycines* are fragile and rupture easily in soil before they can be collected. Therefore, *P. nishizawae* could not be obtained in sufficient numbers to infest field plots as can be done with *P. penetrans* associated with *Meloidogyne* spp. inside roots of host plants. At present, there is no practical and reliable method to quantify numbers of endospores of *Pasteuria* spp. in soil. An early attempt used differential centrifuga-

tion, but the method was too laborious to use routinely in a large field study (Davies *et al.*, 1990). In addition, determination of *H. glycines* and *P. nishizawae* population dynamics as done for the microplot study reported previously was prohibitively labor intensive. Only recently were the preliminary results of a real-time PCR assay for the detection of *P. nishizawae* in soil reported (Atibalentja *et al.*, 2008). Therefore, we decided to prove transfer of *P. nishizawae* only upon determination of crop loss.

Tillage method *per se*, or in interaction with cultivar and/or *P. nishizawae*, had no effect on population densities of *H. glycines*. Thus, numbers of *P. nishizawae* endospores or attachment to J2, if affected at all by tillage method, were sufficient enough to reduce nematode population densities. In a microplot study artificially infested with *P. penetrans* endospores and *M. incognita* J2, higher percentages of J2 were encumbered with endospores under no-tillage than under tillage (20-cm deep rotary tillage) and tillage plus irrigation at planting (Talavera *et al.*, 2002). Numbers of J2 at planting and at harvest did not differ, but after fallow they were higher under no-tillage compared with tillage and tillage plus irrigation.

The proprietary cultivars planted in 2005 had a higher yield potential than the older public cultivars planted the first six years of the experiment. The lower Pf of *H. glycines* associated with Kruger 349RR was unexpected. When purchased, Kruger 349RR was marketed as susceptible to *H. glycines*. A common practice in the Midwest is to label some if not all "susceptible" cultivars as being susceptible when in fact they may have moderate to low levels of resistance *to H. glycines*. Subsequent to termination of the experiment reported herein, we learned Kruger 349RR was considered as "moderately susceptible". In addition, Kruger 346RR SCN does not provide the level of resistance to *H. glycines* found in Linford (Noel, unpubl.; VIPS, 2005). It is apparent the population of *H. glycines* in plots planted to the resistant cultivar had partially defeated the resistance in Kruger 346RR SCN. These plots had been planted to Linford in 1992, 1994, 1996, and 1998, followed by a monoculture of PI88.788 resistance in 1999-2005 when Linford was planted in 1999-2004 followed by Kruger 346RR SCN in 2005.

The absence of a cultivar × *P. nishizawae* interaction in this study suggests that the effects of *P. nishizawae* on populations of *H. glycines* and soybean yield were independent of type of cultivar. This result is significant for implementing an IPM strategy utilizing biological control with *P. nishizawae* as one component to manage *H. glycines*. *Pasteuria nishizawae* would curb populations of *H. glycines* not only in years when the susceptible cultivar is planted, but also complement resistant cultivars when introduced into the rotation. Reducing numbers of *H. glycines* on the resistant cultivar would increase the durability of resistance. Presence of *P. nishizawae* coupled with moderately susceptible or moderately resistant cultivars may provide a yield advantage when compared with highly resistant cultivars that express a yield penalty associated with their resistance. Crop rotation with a non-host of *H. glycines* was not investigated. The efficiency of maize in reducing numbers of *H. glycines* in one year of rotation, as practiced in the Midwestern U.S., is highly variable (Atibalentja *et al.*, 2001; Porter *et al.*, 2001; Noel and Wax, 2003) and would have unknown effects on the population dynamics of *P. nishizawae*.

The experiment reported herein provided proof of concept that *P. nishizawae* can reduce populations of *H. glycines* sufficiently under field conditions to provide an increase in soybean yield. Our results expand those of previous studies demon-

strating the transferability of *Pasteuria* spp.-mediated soil suppressiveness to other plant-parasitic nematodes (Stirling, 1984; Brown *et al.*, 1985; Chen *et al.*, 1996; Giblin-Davis, 2000; Kariuki and Dickson, 2007). In the future, successful *in vitro* culture of *P. nishizawae* may allow production and application of large numbers of endospores that will reduce populations of *H. glycines* in the same manner as an efficacious nematicide (Hewlett *et al.*, 2002). This study also demonstrated *P. nishizawae* can affect numbers of *H. glycines* associated with a resistant cultivar. How populations of *H. glycines* are affected by *P. nishizawae* during crop rotation will be an interesting subject to investigate in future studies. Of particular interest will be determining the effects on *P. nishizawae* and *H. glycines* when planting an *H. glycines* - susceptible cultivar every fourth year of the rotation sequence. A molecular assay for detection and quantification of *P. nishizawae* in soil is being developed in order to more accurately determine concentrations of endospores in soil in future field experiments and to conduct surveys (Atibalentja *et al.*, 2008).

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