

HETERODERA GLYCINES POPULATION DEVELOPMENT ON SOYBEAN TREATED WITH GLYPHOSATE

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ABSTRACT

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Soybean cyst nematode (*Heterodera glycines*) is a major yield limiting pest in all major soybean producing countries. In the last decade genetically modified soybean tolerant to glyphosate has become widely planted and postemergence application of glyphosate has increased exponentially. Genetically modified crops may affect nontarget microorganisms, either directly or indirectly as in the case of glyphosate application to tolerant crops. Glyphosate translocates to soybean roots where it might affect metabolic sinks such as syncytia produced by *Heterodera glycines*. In 2002-2004 glyphosate tolerant soybean DSR 320 susceptible to *H. glycines* and DSR 327 resistant to *H. glycines* were grown in three different fields and either sprayed with glyphosate at the recommended rate and time of application or not sprayed. Nematode reproduction and soybean yield were determined. In all three years, Pf and Pf/Pi were significantly greater on DSR 320 when compared with DSR 327. In 2004, when Pi exceeded the damage threshold, there was a cultivar x glyphosate interaction for Pf on DSR 320 with greater numbers of eggs recovered from glyphosate treated DSR 320. In 2002 and 2003 Pi was below the damage threshold and no differences in yield occurred between the resistant and susceptible cultivars. However, in 2003 a significant cultivar x glyphosate interaction was observed for yield of DSR 320. In 2004 a significant difference in yield between DSR 320 and DSR 327 was observed. Glyphosate application affected Pf of *H. glycines* eggs in 2004, the only year Pi exceeded the damage threshold. However, the increase Pf did not translate into crop loss.

Key words: *Glycine max*, glyphosate, *Heterodera glycines*, soybean, soybean cyst nematode.

RESUMEN

Noel, G. R., and L. M. Wax. 2009. Desarrollo de poblaciones de *Heterodera glycines* en soya tratada con glifosato. *Nematropica* 39:247-253.

El nematodo quiste de la soya (*Heterodera glycines*) es un factor limitante en la producción de soya en todos los principales países productores. Durante los últimos años, se ha aumentado la siembra de soya genéticamente modificada con tolerancia al glifosato y la consecuente aplicación de glifosato ha incrementado exponencialmente. Los cultivos genéticamente modificados pueden tener efectos directos o indirectos sobre los microorganismos. Algunos de los efectos indirectos son los causados por la aplicación de glifosato en cultivos tolerantes. El glifosato se trasloca a las raíces de la soya, en donde puede afectar los eventos de gasto metabólico tales como los sincicios producidos por *Heterodera glycines*. En 2002-2004, se cultivaron dos cultivares de soya tolerantes a glifosato, DSR 320, que susceptible a *H. glycines*, y DSR 327, resistente a *H. glycines*, en tres campos diferentes y se evaluaron con y sin aplicaciones de glifosato de acuerdo con las dosis y tiempos recomendados. Se determinó la reproducción de los nematodos y la producción de soya. En cada uno de los tres años, la Pf y Pf/Pi fueron mayores con DSR 320 que con DSR 327. En 2004, cuando la Pi excedió el umbral de daño, se observó una interacción de cultivar x glifosato para la Pf en DSR 320, con mayor cantidad de huevos en el cultivar DSR 320 tratado con glifosato. En 2002 y 2003, la Pi se mantuvo por debajo del umbral de daño y no se observaron diferencias en la producción entre el cultivar resistente y el susceptible. Sin embargo, en 2003 se observó una interacción significativa para la producción del cultivar DSR 320. En

2004, se observó una diferencia significativa en la producción entre el cultivar DSR 320 y el cultivar DSR 327. La aplicación de glifosato afectó la Pf de *H. glycines* en 2004, el único año cuando el Pi estuvo por encima del umbral de daño. Sin embargo, este aumento en la Pf no se tradujo en pérdidas de rendimiento.

Palabras clave: *Glycine max*, glifosato, nematodo quiste de la soya, *Heterodera glycines*, soya.

INTRODUCTION

Heterodera glycines Ichinohe is distributed throughout the major soybean (*Glycine max* (L.) Merr.) production areas of Asia, North America, and South America and is a major yield-limiting factor of soybean grown in these areas (Riggs, 2004). In North and South America glyphosate is used widely to control weeds in glyphosate-tolerant soybean. In the U.S.A. in 2006, 89% of the soybean planted was glyphosate tolerant (Brookes and Barfoot, 2008). Approximately 63% of the crop in Canada was glyphosate tolerant. In South America glyphosate-tolerant plantings of soybean ranged from 55% in Brazil to 90% in Paraguay and nearly 100% in Argentina and Uruguay.

The active ingredient of glyphosate, N-(phosphonomethyl) glycine, inhibits synthesis of aromatic amino acids (eg. phenylalanine, tyrosine, and tryptophan) by translocating into metabolic sinks such as roots and possibly nematode feeding sites comprised of giant cells or syncytia (Duke, 1988). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphatase synthase, resulting in loss of energy in accumulation of 3-deoxy-D-arabino-heptulose-7-phosphate, hydrobenzoic acids and shikimate, and toxic accumulation of intermediates in the shikimic acid pathway (Fisher *et al.*, 1986; Jaworski, 1972; Zablutowicz and Reddy, 2004). Research on the nontarget effects of glyphosate on various microorganisms has not led to consistent results. *Meloidogyne javanica* (Treub) Chitwood was not

affected by application of glyphosate to stems of *Brassica napus* L. (McLeod *et al.*, 2002). In a field study, numbers of nodules induced by *Bradyrhizobium japonicum* (Kirchner) Jordan were not affected, but the nodule mass was reduced by 21-28% (Reddy and Zablutowicz, 2003). In addition leghemoglobin content was reduced by 8-10%. Under controlled conditions, nitrogen fixation was reduced by application of glyphosate, but field studies were inconclusive (Zablutowicz and Reddy, 2004). In a 2-year field study, treatment of soybean with glyphosate did not result in increased root colonization or disease severity caused by sudden death syndrome of soybean (SDS; causal organism *Fusarium virguliforme* Aoki, O'Donnell, Homma & Lattanzi (Njiti, *et al.*, 2003). In another field experiment, treatment of soybean with glyphosate resulted in increased foliar disease severity of SDS in 2 of 3 years, but there were no differences in root infection or yield due to glyphosate treatment (Santiago *et al.*, 2001).

Few studies have evaluated the effects of glyphosate application on nematode population dynamics and/or crop yield. In a greenhouse study no interrelationship of glyphosate application and *H. glycines* infection was found for soybean growth (Yang *et al.*, 2002). In that study population dynamics of *H. glycines* were not determined. In another greenhouse study, numbers of *H. glycines* 65 days after inoculation were not affected by application of glyphosate to soybean (Leon *et al.*, 2005). Nematode numbers and trophic groups did not

differ in response to glyphosate application in a corn/soybean production system (Liphadzi *et al.*, 2005). *Heterodera glycines* was not detected in that study. The purpose of the study reported herein was to determine whether glyphosate application affects numbers of *H. glycines* in naturally infested soybean fields and to determine response in yield resulting from application of glyphosate to soybean resistant or susceptible to *H. glycines*.

MATERIALS AND METHODS

Experiments were conducted in 2002 - 2004 in fields with a 20-year history of infestation with *H. glycines*. Populations in all three fields were classified as Hg Type 0. Soils in 2002 and 2003 were a Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls) with pH 6.0 and 3.9% OM in 2002 and pH 6.2 and 5.2% OM in 2003, and in 2004 the soil was a Dana silt loam (Fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls), with pH 6.1 and 4.2% OM. Two glyphosate resistant cultivars, DSR 320 susceptible to *H. glycines* and DSR 327 resistant to *H. glycines* (PI88788 source of resistance), were planted. The experimental design consisted of 12 replications arranged in a split-plot design with cultivars as main plots and glyphosate application as subplots. Experimental units were four 6-m long 76-cm wide rows.

Preplant applications of metolachlor and a premix of chlorimuron plus sulfentrazone ethyl were made at recommended rates using commercial equipment. A CO₂ pressurized spray rig mounted on a customized all terrain vehicle was used to apply glyphosate to each plot individually at recommended rates 6 weeks after planting (V3 to V4 growth stage (Pedersen, 2007)). No post emergence herbicide applications were made to control plots.

Immediately after planting and after harvest, 12 2.5-cm-diameter × 15-cm-long soil cores were taken in a zig-zag pattern from the middle two rows of each experimental unit. Soil from each experimental unit was bulked and 250 cm³ shaken overnight in water on a rotary shaker. Cysts were extracted from the suspension by gravity sieving using nested 850 and 250 μm-pore sieves (Cobb, 1918). Cysts were removed individually from the washings, crushed, and eggs counted with the aid of a dissecting microscope. The number of eggs/250 cm³ soil was determined at planting (Pi) and at harvest (Pf) and Pf/Pi was calculated. Yield (kg/ha) was determined by harvesting 5 m of the middle two rows. Seed moisture was corrected to 13%. The number of eggs was transformed (Log₁₀), and nematode and yield data were analyzed using PROC MIXED (SAS Institute, Cary, NC). Plots below the level of detection at planting were not assigned a value and not included in the analysis.

RESULTS

In 2002, Pi ranged from 0 to 1,110 (\bar{x} = 160) eggs/250 cm³ with 27 of the 48 plots being below the detection level. The maximum number of cysts recovered was three. In samples taken at harvest 9 of 48 experimental units remained with no detectable cysts. Of these, only one was planted to *H. glycines*-susceptible DSR 320. In 2003, 31 of the 48 plots had no detectable cysts at planting. Pi ranged from 0 to 930 (\bar{x} = 70) eggs/250 cm³, and the maximum number of cysts recovered was four. At harvest 15 of the plots (all planted to *H. glycines*-resistant DSR 327) continued to have no detectable cysts. In 2004, Pi ranged from 0 to 2,820 (\bar{x} = 360) eggs/250 cm³ with seven plots being below the detection level. At harvest six plots (all planted to resistant DSR 327) were below the detection level. There were

no significant differences in Pi among treatments in any of the three years.

In all three years, Pf was greater ($P = 0.001$) on susceptible DSR 320 when compared with resistant DSR 327 (Tables 1 and 2). Similarly, Pf/Pi was greater on DSR 320 when compared with DSR 327 in 2002-2004 ($P = 0.03$ in 2002, and $P = 0.006$ in 2003 and 2004). In none of the 3 years was there a main effect of glyphosate on Pf/Pi, nor were there any cultivar \times glyphosate interactions. There was no main effect of glyphosate on Pf in any of the 3 years, but there was a cultivar \times glyphosate interaction in 2004 ($P = 0.05$). The comparison of Pf between DSR 320 with no glyphosate application and DSR 320 to which glyphosate was applied differed at $P = 0.05$. When application of glyphosate was compared within *H. glycines*-resistant DSR 327 there was no affect on Pf ($P = 0.35$; Table 2).

Yield was affected by *H. glycines* in 2004 when *H. glycines*-resistant DSR 327 had greater yield than *H. glycines*-susceptible DSR 320 ($P = 0.001$; Tables 1 and 2). There were no differences in yield between the two cultivars in 2002 and 2003. In none of the 3 years was there a main effect of glyphosate application on yield. However in 2003, there was a cultivar \times glyphosate interaction ($P = 0.05$), and the probability level for the comparison of glyphosate application within DSR 320 was $P = 0.06$. The comparison of glyphosate application within DSR 327 was not significant ($P = 0.36$; Table 2).

DISCUSSION

In the first 2 years of the 3-year experiment, numbers of *H. glycines* were below the damage threshold established for Illi-

Table 1. Effects of glyphosate treatment on numbers of *Heterodera glycines* and yield of DSR 320 susceptible and DSR 327 resistant to *H. glycines*, 2002-2004.

Cultivar	Glyphosate	Pf	Pf/Pi	Yield (kg/ha)
2002				
DSR 320	-	1,840	13	3,190
DSR 320	+	1,160	40	3,030
DSR 327	-	200	5	3,220
DSR 327	+	290	20	3,220
2003				
DSR 320	-	1,030	12	3,390
DSR 320	+	960	14	3,290
DSR 327	-	180	1	3,340
DSR 327	+	80	1	3,390
2004				
DSR 320	-	910	14	3,320
DSR 320	+	1,600	6	3,400
DSR 327	-	280	2	3,720
DSR 327	+	160	1	3,690

Table 2. Summary analysis of variance table and interactions for the effects of soybean cultivars DSR 320 susceptible and DSR 327 resistant to *Heterodera glycines* and glyphosate treatment on Pf and Pf/Pi of *H. glycines*, and yield 2002–2004.

Source of variation or interaction	2002			2003			2004		
	Pf	Pf/Pi	Kg/ha	Pf	Pf/Pi	Kg/ha	Pf	Pf/Pi	Kg/ha
Cultivar	0.001	0.03	ns	0.001	0.006	ns	0.001	0.006	0.001
Glyphosate	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cultivar × glyphosate	ns	ns	ns	ns	ns	0.05	0.05	ns	ns
DSR 320 - × DSR 320 +	ns	ns	ns	ns	ns	0.06	0.05	ns	ns
DSR 327 - × DSR 327 +	ns	ns	ns	ns	ns	ns	ns	ns	ns

nois (Noel, 2008). Low numbers of nematodes in 2002 and 2003 were unexpected. Soil in the fields may have become suppressive since these fields were known to have suffered crop loss previously and had been sampled. In 2002 and 2003, respectively, 18% and 64% of plots remained below the detection level at harvest. However, the large reproductive capacity of *H. glycines* was illustrated with increases in Pf/Pi on susceptible DSR 320 ranging from 12 to 40 in 2002 and 2003. These values would be much higher if a numerical value of 1 had been assigned to plots from which no eggs were recovered at planting rather than not use those plots for nematode analysis. Even with minimal observations the first two years, differences in Pf and Pf/Pi between the two cultivars were significant all 3 years. Only in 2004 was there an indication of glyphosate having an effect on numbers of *H. glycines* when greater numbers of eggs were associated with DSR 320 treated with glyphosate when compared with the untreated DSR 320. Unfortunately, 2004 was the only year in which sufficient numbers of eggs were recovered at planting in almost all plots to have a valid test of *H. glycines* population development. With sufficient numbers of nematodes in only 1 of 3 years, it is not possible to determine if the differences observed in 2004 are valid or an

artifact. An increase in numbers of eggs due to glyphosate would not be surprising. Glyphosate accumulates in metabolic sinks (Duke, 1988), and presumably syncytia produced by *H. glycines*. Interruption of the shikimate pathway by glyphosate might lead to increased establishment of syncytia upon which *H. glycines* feeds by interfering with production of compounds related to the natural defense system of the soybean plant (Lambert *et al.*, 2005; Pline-Srnic, 2005). Such interruption in normal function of the shikimate pathway leading to establishment of *H. glycines* feeding sites could lead to increases in numbers of cysts and eggs. However, in a greenhouse study, glyphosate treatment of cv. AG 2401 (susceptible to *H. glycines* and resistant to glyphosate) inoculated with *H. glycines* did not affect numbers of *H. glycines* 65 days after inoculation (Leon *et al.*, 2005). In that paper the authors based their study in part on field observations by farmers indicating a relationship between postemergence application of herbicides and *H. glycines* in reducing soybean growth and yield. We did not observe any symptoms in 2003 or 2004 that indicated an interaction between glyphosate and *H. glycines*. Since glyphosate is applied worldwide on fields infested with *H. glycines*, additional experimentation is required to determine whether or not an

interaction occurs between the two and what environmental and edaphic factors if any might contribute.

2004 was the only year in which there was sufficient numbers of *H. glycines* to cause yield reduction in the susceptible DSR 320. Reports indicate incorporation of glyphosate tolerance into soybean may have resulted in reduced yield potential (Oplinger *et al.*, 1998; Elmore *et al.*, 2001b). There also is evidence indicating of loss in yield potential of 200 kg/ha in soybean cvs. carrying both glyphosate tolerance and *H. glycines* resistance (Geisler *et al.*, 2002). Among *H. glycines* susceptible cultivars yield was 400 kg/ha higher for conventional cultivars when compared with glyphosate tolerant cultivars. In that study no nematode numbers were reported. In 2 of 3 years of the experiment reported herein, yield was greater than the county average determined by the National Agricultural Statistics Service (USDA, 2009). If a yield penalty resulting from incorporation of glyphosate tolerance into the elite cultivars used in these experiments occurred, it was not obvious. In addition, there was no yield penalty associated with the dual resistance to *H. glycines* and tolerance to glyphosate in DRS 327. Overall yield in our three experiments was quite similar, and the cultivar \times glyphosate interaction observed in 2003 was unexpected in view of evidence indicating glyphosate application does not affect yield (Delannay *et al.*, 1995; Elmore *et al.*, 2001a). The interaction observed was as a result of glyphosate treated DSR 320 decreasing in yield by 100 kg/ha and treated DSR 327 increasing by 50 kg/ha. In the absence of confirming data the interaction cannot be supported.

During the 3 years of experimentation we found indications that glyphosate application may increase numbers of *H. glycines* on susceptible cultivars, but this increase did not result in crop loss. However, addi-

tional research is required to confirm these observations. Until additional research is done, soybean producers should continue to use cropping systems and herbicide options that are the most economically beneficial to their overall farming operations.

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