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Impact of Herbicides on *Heterodera glycines* Susceptible and Resistant Soybean Cultivars[1]

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Abstract: Several abiotic and biotic stresses can affect soybean in a growing season. *Heterodera glycines,* soybean cyst nematode, reduces yield of soybean more than any other pathogen in the United States. Field and greenhouse studies were conducted to determine whether preemergence and postemergence herbicides modified the reproduction of *H. glycines,* and to determine the effects of possible interactive stresses caused by herbicides and *H. glycines* on soybean growth and yield. *Heterodera glycines* reproduction factor (Rf) generally was less on resistant than susceptible cultivars, resulting in a yield advantage for resistant cultivars. The yield advantage of resistant cultivars was due to more pods per plant on resistant than susceptible cultivars. Pendimethalin reduced *H. glycines* Rf on the susceptible cultivars in 1998 at Champaign, Illinois, and in greenhouse studies reduced dry root weight of *H. glycines*-resistant and susceptible cultivars, therefore reducing Rf on the susceptible cultivars. The interactive stresses from acifluorfen or imazethapyr and *H. glycines* reduced the dry shoot weight of the resistant cultivar Jack in a greenhouse study. Herbicides did not affect resistant cultivars' ability to suppress *H. glycines* Rf; therefore, growers planting resistant cultivars should make herbicide decisions based on weeds present and cultivar tolerance to the herbicide.

Key words: *Glycine max,* herbicide, *Heterodera glycines,* interaction, nematode, reproduction, SCN, soybean, soybean cyst nematode.

Heterodera glycines Ichinohe, soybean cyst nematode, causes greater yield reduction in soybean (*Glycine max* (L.) Merr.) than any other pathogen in the United States. In 1998, the estimated yield reduction was more than 8.9×10^6 metric tons for the top 10 soybean-producing countries, with an estimated yield reduction of more than 7.5×10^6 metric tons in the United States alone (Wrather et al., 2001a, 2001b). Management of *H. glycines* is accomplished primarily with the use of crop rotation and resistant cultivars (Riggs and Niblack, 1999). Yield benefits of resistant cultivars have been demonstrated throughout the United States (Niblack et al., 1992; Todd et al., 1995; Young, 1996).

Herbicides have been reported to affect *H. glycines* in different ways. Egg hatch, root penetration, maturation, and reproduction of *H. glycines* are affected by different herbicides (Bostian et al., 1984a,b; Browde et al., 1994c; Kraus et al., 1982; Levene et al., 1998a,b; Riggs and Oliver, 1982; Schmitt et al., 1983; Sipes and Schmitt, 1989; Wong et al., 1993). Herbicides also have been reported to interact with *H. glycines* to increase stress to soybean. Browde et al. (1994a, 1994b) reported that soybean growing in plots with a low initial population density of *H. glycines* (184 eggs/100 cm^3 soil) and treated with a mixture of acifluorfen and bentazon had decreased stomatal conductance, increased visible herbicide injury, and reduced leaf area, plant height, pod number, pod dry weight, and grain yield compared with soybean not treated with herbicides. Most research on herbicide and *H. glycines* interactions has not included resistant cultivars.

The objectives of this research were to: (*i*) determine the effect of both preemergence (PRE) and postemergence (POST) herbicides on *H. glycines* reproduction and (*ii*) determine the effects of combinations of herbicides and *H. glycines* on susceptible and resistant soybean cultivars under field and greenhouse conditions.

Materials and Methods

Field study 1: A field study was conducted in 1998 and 1999 in Champaign, Illinois. The site was planted with a *H. glycines*-susceptible soybean cultivar in 1996 and with both susceptible and resistant soybean cultivars in 1997. The susceptible cultivars Asgrow AG3704 and Savoy and resistant cultivars Asgrow AG3904, Jack, Pioneer 9362, and Pioneer 9363 were planted on 19 May 1998 and 20 May 1999. The cultivar Pioneer 9362 was replaced with Pioneer 93B65 (resistant) in 1999 because seed was unavailable. Plots were planted 8 rows wide on 0.76-m centers, 7.3 m long, and later trimmed to 6.1 m. The two middle rows were used for harvesting, and all other rows except outer rows, were used for growth and yield data collection. Soil types at the site were a Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquolls) and a Flanagan silt loam (fine, montmorillonitic, mesic Aquic Argiudolls). The soil had pH 7.1, 4.5% organic matter, and 107, 60, and 271 kg/ha of N, P, and K, respectively.

There were five herbicide treatments. They consisted of a no-herbicide hand-weeded control, PRE-applied pendimethalin (Prowl, BASF Corp., Research Triangle Park, NC) at 1.39 kg a.i./ha; PRE-applied tank-mixture

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of dimethenamid (Frontier, BASF Corp., Research Triangle Park, NC) and metribuzin (Sencor, Bayer Corp., Kansas City, MO) at 1.47 and 0.42 kg a.i./ha, respectively; POST-applied acifluorfen (Blazer, BASF Corp., Research Triangle Park, NC) at 0.42 kg a.i./ha + 1% (v/v) crop oil concentrate (COC); and POST-applied imazethapyr (Pursuit, BASF Corp., Research Triangle Park, NC) at 0.07 kg a.i./ha + 1% (v/v) COC. Preemergence herbicides were applied after planting but before soybean plants emerged, and POST herbicides were applied at approximately growth stage V4 (Fehr et al., 1971). All herbicides were applied with a CO_2-pressurized hand sprayer with 8003 flat-fan nozzles (Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 liters/ha at 207 kPa pressure. Weeds that emerged after all herbicides were sprayed were removed by hand in all plots.

One soil core, 2.5-cm diam. × 20 cm, was collected from each row per plot in a zig-zag pattern and bulked. The numbers of *H. glycines* eggs per 100 cm^3 soil at planting (Pi) and at harvest (Pf) were determined. Cysts were extracted from 100-cm^3 samples by gravity-sieving with a 710-µm-pore sieve (no. 25) nested over a 250-µm-pore sieve (no. 60) and counted. Eggs were extracted and counted by methods described in Niblack et al. (1993). *Heterodera glycines* reproduction factor (Rf) was calculated by (Pf + 1) / (Pi + 1). The mean number of eggs per cyst was calculated by (number of eggs/100 cm^3 soil)/(number of cysts/100 cm^3 soil).

Leaf area index (LAI, leaf area per unit ground area) of each plot was measured nondestructively with an LAI-2000 plant canopy analyzer (LI-COR, Inc., Lincoln, NE) (Welles and Norman, 1991) at 2 weeks and 6 weeks after the POST herbicides were applied. At growth stages R3 and R8 (harvest maturity), 1 m of row from each plot was collected by cutting plant stems at the soil line (biomass sample). Biomass samples were dried under forced air at 43 °C for 7 days, weighed, and converted to grams per square meter. Sub-samples of three plants were separated into stems and pods, and the number of pods determined. Stem and pod samples were dried under forced air at 38 °C for 7 days and weighed. Pods were threshed mechanically and the seed was collected, weighed, and counted electronically. Yield components that included pods per plant, seed per pod, and weight per seed were calculated from these measurements. The entire two middle rows of each plot were harvested with a small plot combine. Harvested grain was weighed, and yield was adjusted to 13% moisture.

Plots were arranged in randomized complete blocks with four replications. Different randomizations were used each year. All *H. glycines* data were transformed to $\log_{10}(x + 1)$ values before statistical analysis. Years were analyzed separately because of the different cultivars used each year. Data were analyzed as a nested design where cultivars were nested with *H. glycines*-susceptible and resistant cultivar types. Analysis of variance (ANOVA) was calculated with the general linear models procedure (PROC GLM) of SAS (SAS Institute Inc., Cary, NC). Main effect means were compared by means of Fisher' least significant difference (LSD) at the 0.05 probability level. If there was a cultivar type x herbicide interaction, then least-square means of the interaction were compared using the PDIFF option in SAS at the 0.05 probability level.

Field study 2: A field study was conducted in 1999 and 2000 near Monmouth, Illinois. Different sites were used each year, and corn (*Zea mays* L.) was planted the year before experimentation. Soybean cultivars used in the study were *H. glycines*-susceptible Asgrow AG3002, Pioneer 93B01, and Siebens 2701, and *H. glycines*-resistant Pioneer 9363. All cultivars were glyphosate tolerant (Roundup-Ready) and were planted on 24 May 1999 and 15 May 2000. Plots were planted 4 rows wide on 0.76-m centers, 4.6 m long, and later trimmed to 3.4 m. The two middle rows were used for yield, and the outside rows were used for yield component data. The soil types were a Muscatine silt loam (fine-silty, mixed, mesic Aquic Hapludolls) and Sable silt loam (fine-silty, mixed, mesic Typic Hapludolls) in 1999 and 2000, respectively.

Glyphosate (Roundup Ultra, Monsanto Company, St. Louis, MO) was applied over the entire experiment for weed control at 1.12 kg a.i./ha prior to herbicide treatment. Herbicide treatments consisted of PRE-applied pendimethalin, POST-applied acifluorfen and imazethapyr, and a glyphosate-only control. Herbicide rates were the same as those described for the Champaign experiment. Herbicides were applied with a tractor-mounted sprayer with 8003 flat-fan nozzles calibrated to deliver 140 liters/ha. Procedures were conducted as described for field study 1 except that eggs per cyst were not calculated and biomass samples were not collected.

Plots were arranged in randomized complete blocks with four replications. *Heterodera glycines* data were transformed to $\log_{10}(x + 1)$ values and analyzed with PROC GLM. Years were analyzed separately due to year × treatment interactions and heterogeneous variances according to Levene' test for homogeneity. Data were analyzed as a nested design where cultivars were nested within *H. glycines*-susceptible and resistant cultivar types. Main effect means were compared by means of Fisher' LSD at the 0.05 probability level. If there was a cultivar type × herbicide interaction, then least-square means of the interaction were compared by means of the PDIFF option in SAS at the 0.05 probability level.

Field study 3: A field study was conducted at East Troy, Wisconsin, in 1997, 1998, and 1999. The soil type was a Matherton sandy loam (fine-loamy, mixed, mesic, Typic Endoaqualfs). The plots were at the same site in 1997 and 1999 but at an adjacent site in 1998. Plots were planted 20 May 1997, 20 May 1998, and 27 May 1999, and corn was the previously grown crop at the sites each year. Plots were planted 8 rows wide on 0.76-m centers, and 7.6 m long. Soil samples were collected, cysts were extracted, and eggs were enumerated as described for field study 1 except that cysts were extracted from soil with gravity-sieving on a 710-µm-pore sieve (no. 25) nested over a 180-µm-pore sieve (no. 80). Soybean cultivars susceptible to *H. glycines* used in this study were Asgrow AG2701, Novartis NK 24-92, and Dairyland DSR 250 STS in 1997; Asgrow AG2101, Novartis NK 24-92, and Dairyland DSR 220 STS in 1998; and Asgrow AG2101 and Asgrow AG2553 in 1999. Cultivars resistant to *H. glycines* were Asgrow AG2901, Pioneer 92B91, and Dairyland DSR 96319 in 1997; Asgrow AG2201, Pioneer 9234, and Dairyland DSR 246 STS in 1998; and Asgrow AG2201 and Pioneer 9234 in 1999.

Herbicide treatments consisted of a no-herbicide hand-weeded control, PRE-applied pendimethalin, POST-applied acifluorfen, POST-applied imazethapyr, and POST-applied glyphosate. Application rates of the herbicides were the same as in field study 1, except for glyphosate, which was applied at 1.12 kg a.i./ha. Glyphosate was applied to the glyphosate-tolerant (Roundup-Ready) cultivars only, which were Asgrow AG2701 and Asgrow AG2901 in 1997 and Asgrow AG2101 and Asgrow AG2201 in 1998 and 1999. Herbicides were applied with a tractor-mounted sprayer with 8003 flat-fan nozzles calibrated to deliver 187 liters/ha at 207 kPa pressure.

Because some herbicides could be applied only to certain cultivars, each herbicide-cultivar combination was considered a treatment. Therefore, there were 26 treatments in 1997 and 1998, and 18 treatments in 1999. Plots were arranged in randomized complete blocks with four replications. *Heterodera glycines* data were transformed to $\log_{10}(x + 1)$ values and analyzed with PROC GLM. Years were analyzed separately due to different cultivars being used each year and fewer treatments in 1999. Single-degree-of-freedom contrast statements were written to compare susceptible vs. resistant cultivars within and across all herbicide treatments for yield and Rf.

Greenhouse preemergence herbicide study: The study included three soybean cultivars, three PRE herbicide treatments, and *H. glycines* artificially infested and uninfested soil. Three seeds of soybean cultivar Jack (PI 88.788 source of resistance), Lee 74 (susceptible), or Savoy (susceptible) were planted into 1,000-cm^3 polypropylene pots containing a 2:1 sand:soil mixture. Pots were placed on a greenhouse bench and grown under a 16-hour photoperiod. The photosynthetically active radiation (PAR) was measured to be 434 µE/(m^2 sec) (LI-170 Quantum/Radiometer/Photometer, Lambda Instrument Corp., Lincoln, NE), and the temperature was 26 ± 3 °C. Pots were watered to saturation after planting, and twice daily thereafter. Prior to planting, seeds were treated with 2.7 ml/kg seed of Cell-Tech 2000 (Liphatech, Inc., Milwaukee, WI), which contains 2×10^9 viable cells of *Bradyrhizobium japonicum* per gram. After emergence, plants were thinned to one plant per pot.

Herbicide treatments in the PRE applied herbicide study were a no-herbicide control, pendimethalin at 1.39 kg a.i./ha, and a mixture of dimethenamid and metribuzin at 0.74 and 0.21 kg a.i./ha, respectively. Herbicides were applied directly to the soil immediately after planting in an automated spray chamber with an 80015EVS flat-fan nozzle (Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 liters/ha.

A population of *H. glycines* was collected from a field in Champaign, Illinois, and maintained on soybean cultivar Lee 74 in the greenhouse. When soybean plants were at growth stage V1, two holes approximately 0.5 cm diam. and 2 cm deep were made in the soil around the hypocotyls in each pot and were filled with a water-and-egg suspension containing approximately 1×10^4 eggs.

Plants and soil were removed from pots 30 days after soil was infested with *H. glycines*. The number of eggs was determined according to methods described for the field studies. Roots and shoots were separated, dried for 7 days under forced air at 38 °C, and weighed. The experiment was a $2 \times 3 \times 3$ factorial where the factors were *H. glycines* infestation, cultivars, and, herbicides, respectively. The experiment was a completely randomized design with three replications. The experiment was repeated once.

Data from the two trials were pooled. *Heterodera glycines* data were transformed to $\log_{10}(x + 1)$ values before statistical analysis. The ANOVA was determined with PROC GLM, and main effect means were compared by means of Fisher' LSD ($\alpha = 0.05$); however, if there was a significant ($P \leq 0.05$) interaction among factors, then least-square means were compared by means of the PDIFF option in SAS and were considered different when $P \leq 0.05$.

Greenhouse postemergence herbicide study: The soybean cultivars Jack (resistant) and Savoy (susceptible) were used in the postemergence herbicide study with the methods described above.

There were five herbicide treatments: a no-herbicide control, acifluorfen at a 1x and 2x rate (x = 0.42 kg a.i./ha), and imazethapyr at a 1x and 2x rate (x = 0.07 kg a.i./ha). Postemergence herbicides were applied with 1% (v/v) COC to soybean plants at the V2 growth stage with the automated spray chamber described above. Plants and soil were removed from pots either 30 or 60 days after soil was infested with *H. glycines* eggs. The number of eggs and number of shoot and root weights were determined with the methods described above. The study was a $2 \times 2 \times 2 \times 5$ factorial where the factors were *H. glycines* infestation, cultivars, time of plant removal, and herbicides, respectively. The experimental design was a completely randomized design with three replications. The experiment was repeated once. Data analysis was conducted as described for the preemergence herbicide study above.

Results

Field study 1: Differences ($P \leq 0.05$) were detected between cultivar types for the second LAI measurement, the second biomass measurement, pods per plant, seed weight, yield, Rf, and eggs per cyst in 1998 (Table 1). No differences among herbicide treatments were detected for any of the measured parameters in 1998. A cultivar type × herbicide interaction was detected for Rf in 1998. Differences were detected between cultivar types for the first LAI measurement, both biomass measurements, yield, Rf, and eggs per cyst in 1999. There were differences detected among herbicides for both biomass measurements and seed weight in 1999. A cultivar type × herbicide interaction was detected for the first LAI measurement in 1999. The mean Pi was 1,071 and 587 eggs/100 cm^3 soil and ranged from 0 to 6,350 and 0 to 4,000 eggs/100 cm^3 soil in 1998 and 1999, respectively.

In 1998, resistant cultivars had a greater LAI for the second measurement, biomass at the second measurement, pods per plant, and yield than susceptible cultivars but had a lower seed weight and fewer eggs per cyst (Table 2). Susceptible cultivars had a greater Rf than resistant cultivars within each herbicide treatment except pendimethalin. Within the pendimethalin treatment, the susceptible and resistant cultivars did not differ for Rf as each had a value of 1.0.

In 1999, resistant cultivars had a greater biomass for both measurements, pods per plant, and yield than susceptible cultivars but had a lower Rf value and fewer eggs per cyst (Table 3). The first biomass measurement revealed that plants growing in plots treated with dimethenamid + metribuzin or pendimethalin had a greater biomass than plants growing in the other plots. The second biomass measurement revealed that dimethenamid + metribuzin and pendimethalin-treated plots had greater biomasses than all other treatments except imazethapyr. Seed weight was greatest in plants treated with acifluorfen but did not differ from plants treated with imazethapyr. Susceptible cultivars had a greater LAI at the first measurement than resistant cultivars within each herbicide treatment except pendimethalin and acifluorfen, in which they did not differ.

Field study 2: Differences ($P \leq 0.05$) were detected between cultivar types for both LAI measurements, pods per plant, and Rf in 1999 (Table 4). Differences were detected among herbicide treatments for both LAI measurements and pods per plant in 1999. A cultivar type × herbicide interaction was detected for pods per plant in 1999. There were differences detected between cultivar types for pods per plant, seeds per pod, seed weight, and Rf in 2000. No differences among herbicides or interactions were detected in 2000. The mean Pi was 424 and 1,166 eggs/100 cm^3 soil and ranged from 0 to 2,100 and 0 to 6,200 eggs/100 cm^3 soil in 1999 and 2000, respectively.

In 1999, resistant cultivars had a lower LAI at the first measurement and a lower Rf value than susceptible cultivars but a greater LAI at the second measurement (Table 5). Plants treated with only glyphosate had the greatest LAI at the first measurement but did not differ from those treated with imazethapyr. Plants treated with glyphosate or acifluorfen had the greatest LAI at the second measurement but did not differ from those treated with imazethapyr. Susceptible and resistant cultivars did not differ within herbicide treatments except when only glyphosate was applied. When only glyphosate was applied, resistant cultivars had 70 pods per plant compared with susceptible cultivars, which had 44 pods per plant.

In 2000, resistant cultivars had more pods per plant than susceptible cultivars but had fewer seeds per pod, lower seed weight, and a lower Rf value (Table 6). There were no differences among herbicides or any cultivar type × herbicide interactions.

Field study 3: From the results of the ANOVA, treatments differed ($P \leq 0.05$) for both yield and Rf in 1997, for yield in 1998, and for Rf in 1999.

TABLE 1. Probability values associated with *F*-tests from an analysis of variance of data collected from Champaign, Illinois experiments (field study 1).

Year	Source of variation	LAI 1[a]	LAI 2[b]	Biomass 1[c]	Biomass 2[d]	Pods per plant	Seeds per pod	Seed wt.	Yield	Rf	Eggs per cyst
1998	Type[e]	0.2050	0.0021	0.3959	0.0001	0.0001	0.9352	0.0360	0.0001	0.0001	0.0001
	Herb[f]	0.3974	0.5869	0.8547	0.4184	0.2623	0.3507	0.8374	0.9699	0.2817	0.6707
	Type × Herb	0.6538	0.8955	0.8077	0.3091	0.5368	0.9886	0.1333	0.8950	0.0384	0.1996
1999	Type	0.0002	0.2288	0.0002	0.0001	0.0047	0.3036	0.9186	0.0001	0.0001	0.0001
	Herb	0.2334	0.1486	0.0001	0.0109	0.0655	0.1291	0.0087	0.3726	0.9715	0.3654
	Type × Herb	0.0148	0.3389	0.3131	0.4857	0.6419	0.5437	0.6317	0.0650	0.8330	0.3263

[a] Leaf area index (LAI) recorded 2 weeks after POST herbicides were applied.
[b] LAI recorded 6 weeks after POST herbicides were applied.
[c] Biomass (dry weight of all above-ground soybean plant parts) measured at R3 growth stage.
[d] Measured at R8 growth stage.
[e] Cultivar type based on susceptibility to *Heterodera glycines.*
[f] Herbicide treatments.

TABLE 2. Effect of cultivar type and herbicide on leaf area index, biomass, pods per plant, seeds per pod, seed weight, yield, Rf, and eggs per cyst at Champaign, Illinois, in 1998 (field study 1).

Cultivar type[a]	Herbicide[b]	LAI 1[c]	LAI 2[d]	Biomass 1 (g/m^2)[e]	Biomass 2 (g/m^2)[f]	Pods per plant	Seeds per pod	Seed wt. (mg)	Yield (kg/ha)	Rf	Eggs per cyst[g]
Susc.	—	1.9 a	4.2 b	437 a	579 b	44 b	1.9 a	141 a	2,416 b	—	109 a
Res.	—	1.8 a	4.5 a	448 a	700 a	61 a	1.9 a	137 b	2,892 a	—	51 b
—	Handweed	1.9 a	4.4 a	440 a	676 a	55 a	1.8 a	138 a	2,655 a	—	76 a
—	Dim + Met	1.9 a	4.5 a	450 a	660 a	59 a	1.9 a	138 a	2,727 a	—	66 a
—	Pendimeth	1.9 a	4.3 a	460 a	640 a	59 a	1.9 a	138 a	2,752 a	—	54 a
—	Acifluorf	1.9 a	4.5 a	434 a	670 a	55 a	2.0 a	139 a	2,762 a	—	53 a
—	Imazethap	1.7 a	4.3 a	437 a	661 a	48 a	1.9 a	140 a	2,789 a	—	99 a
Susc.	Handweed	—	—	—	—	—	—	—	—	6.3 a	—
Res.	Handweed	—	—	—	—	—	—	—	—	1.0 b	—
Susc.	Dim + Met	—	—	—	—	—	—	—	—	4.0 a	—
Res.	Dim + Met	—	—	—	—	—	—	—	—	0.5 b	—
Susc.	Pendimeth	—	—	—	—	—	—	—	—	1.0 b	—
Res.	Pendimeth	—	—	—	—	—	—	—	—	1.0 b	—
Susc.	Acifluorf	—	—	—	—	—	—	—	—	4.5 a	—
Res.	Acifluorf	—	—	—	—	—	—	—	—	0.2 b	—
Susc.	Imazethap	—	—	—	—	—	—	—	—	3.4 a	—
Res.	Imazethap	—	—	—	—	—	—	—	—	0.9 b	—

Data are means of four replicates. Means within a column followed by a common letter are not different ($P \leq 0.05$). Nematode data were transformed to $\log_{10}(x + 1)$ values before statistical analysis, but untransformed means are reported in the table.

[a] Cultivar type was based on resistance to *Heterodera glycines*. Susceptible cultivars were Asgrow AG3704 and Savoy; resistant cultivars were Asgrow AG3904, Jack, Pioneer 9362, and Pioneer 9363.

[b] Herbicide treatments consisted of a handweeded control, a preemergence (PRE) applied tank-mixture of dimethenamid and metribuzin, PRE applied pendimethalin, postemergence (POST) applied acifluorfen, and POST applied imazethapyr.

[c] Leaf area index (LAI) recorded 2 weeks after POST herbicides were applied.

[d] LAI recorded 6 weeks after POST herbicides were applied.

[e] Biomass (dry weight of all above-ground soybean plant parts) measured at R3 growth stage.

[f] Measured at R8 growth stage.

[g] Number of eggs per cyst was calculated by (number of eggs/100 cm^3 soil)/(number of cysts/100 cm^3 soil).

TABLE 3. Effect of cultivar type and herbicide on leaf area index, biomass, pods per plant, seeds per pod, seed weight, yield, Rf, and eggs per cyst at Champaign, Illinois, in 1999 (field study 1).

Cultivar type[a]	Herbicide[b]	LAI 1[c]	LAI 2[d]	Biomass 1 (g/m^2)[e]	Biomass 2 (g/m^2)[f]	Pods per plant	Seeds per pod	Seed wt. (mg)	Yield (kg/ha)	Rf	Eggs per cyst[g]
Susc.	—	—	3.9 a	533 b	630 b	47 b	1.7 a	152 a	3,050 b	6.1 a	94 a
Res.	—	—	4.1 a	604 a	706 a	54 a	1.7 a	152 a	3,406 a	0.7 b	33 b
—	Handweed	—	4.1 a	513 b	654 b	50 a	1.7 a	147 b	3,236 a	2.7 a	71 a
—	Dim + Met	—	4.2 a	648 a	714 a	53 a	1.7 a	148 b	3,392 a	2.5 a	54 a
—	Pendimeth	—	4.1 a	622 a	720 a	57 a	1.7 a	151 b	3,300 a	2.4 a	46 a
—	Acifluorf	—	3.7 a	559 b	638 b	47 a	1.6 a	161 a	3,265 a	2.7 a	38 a
—	Imazethap	—	4.0 a	561 b	675 ab	52 a	1.8 a	153 ab	3,243 a	2.2 a	59 a
Susc.	Handweed	2.7 a	—	—	—	—	—	—	—	—	—
Res.	Handweed	2.2 b	—	—	—	—	—	—	—	—	—
Susc.	Dim + Met	2.7 a	—	—	—	—	—	—	—	—	—
Res.	Dim + Met	2.2 b	—	—	—	—	—	—	—	—	—
Susc.	Pendimeth	2.2 b	—	—	—	—	—	—	—	—	—
Res.	Pendimeth	2.3 b	—	—	—	—	—	—	—	—	—
Susc.	Acifluorf	2.4 b	—	—	—	—	—	—	—	—	—
Res.	Acifluorf	2.2 b	—	—	—	—	—	—	—	—	—
Susc.	Imazethap	2.5 ab	—	—	—	—	—	—	—	—	—
Res.	Imazethap	1.9 c	—	—	—	—	—	—	—	—	—

Data are means of four replicates. Means within a column followed by a common letter are not different ($P \leq 0.05$). Nematode data were transformed to $\log_{10}(x + 1)$ values before statistical analysis, but untransformed means are reported in the table.

[a] Cultivar type was based on resistance to *Heterodera glycines*. Susceptible cultivars were Asgrow AG3704 and Savoy; resistant cultivars were Asgrow AG3904, Jack, Pioneer 9362, and Pioneer 9363.

[b] Herbicide treatments consisted of a handweeded control, a preemergence (PRE) applied tank-mixture of dimethenamid and metribuzin, PRE applied pendimethalin, postemergence (POST) applied acifluorfen, and POST applied imazethapyr.

[c] Leaf area index (LAI) recorded 2 weeks after POST herbicides were applied.

[d] LAI recorded 6 weeks after POST herbicides were applied.

[e] Biomass (dry weight of all above-ground soybean plant parts) measured at R3 growth stage.

[f] Measured at R8 growth stage.

[g] Number of eggs per cyst was calculated by (number of eggs/100 cm^3 soil)/(number of cysts/100 cm^3 soil).

TABLE 4. Probability values associated with *F*-tests from an analysis of variance of data collected from Monmouth, Illinois experiments (field study 2).

Year	Source of variation	LAI 1[a]	LAI 2[b]	Pods per plant	Seeds per pod	Seed wt.	Yield	Rf
1999	Type[c]	0.0023	0.0001	0.0001	0.8819	0.4058	0.1955	0.0001
	Herb[d]	0.0009	0.0016	0.0045	0.8083	0.7196	0.3630	0.7273
	Type × Herb	0.5925	0.1284	0.0132	0.8244	0.7609	0.5808	0.3626
2000	Type	0.0706	0.9691	0.0003	0.0383	0.0270	0.1355	0.0024
	Herb	0.0872	0.6335	0.2985	0.9153	0.8947	0.1341	0.9800
	Type × Herb	0.2982	0.8281	0.6865	0.9484	0.6165	0.8313	0.9102

[a] Leaf area index (LAI) recorded 2 weeks after POST herbicides were applied.
[b] LAI recorded 6 weeks after POST herbicides were applied.
[c] Cultivar type based on susceptibility to *Heterodera glycines*.
[d] Herbicide treatments.

Resistant cultivars generally had greater yields than susceptible cultivars within each herbicide treatment and across all herbicide treatments in 1997 (Table 7). In 1998 and 1999, resistant cultivars yielded better than the susceptible cultivars only when averaged across all herbicide treatments.

The mean Pi for each year was 490, 1,849, and 1,918 eggs/100 cm^3 soil for 1997, 1998, and 1999, respectively. Reproduction of *H. glycines* was greater on susceptible cultivars than on resistant cultivars within each herbicide treatment and across all herbicide treatments in 1997 (Table 7). In 1998 the Rf was greater on the susceptible cultivars than on the resistant cultivars only when averaged across all herbicide treatments. In 1999, the Rf was generally greater on susceptible cultivars compared to resistant cultivars within herbicide treatments and across all herbicide treatments.

Greenhouse preemergence herbicide study: There was a cultivar × herbicide interaction ($P \leq 0.05$) for *H. glycines* Rf, dry shoot weight, and dry root weight. Infestation by *H. glycines* did not affect either dry shoot or root weights in this study, and did not interact with cultivar or herbicide factors.

The Rf on the susceptible cultivars Lee 74 and Savoy growing in soil treated with pendimethalin was reduced compared with the no-herbicide controls, and was not different than the Rf on the resistant cultivar Jack (Table 8). Reproduction of *H. glycines* was greater on the susceptible cultivars compared with the resistant cultivar when there was no herbicide applied and when dimethenamid + metribuzin was applied to soil in which Lee 74 was growing. Dimethenamid + metribuzin did not affect Rf on Lee 74 compared to the no-herbicide control; however, seedlings of Savoy growing in soil treated with dimethenamid + metribuzin in both *H. glycines*-infested and uninfested soil died. This occurred because Savoy is sensitive to the herbicide metribuzin (Nickell, pers. comm.).

TABLE 5. Effect of cultivar type and herbicide on leaf area index, pods per plant, seeds per pod, seed weight, yield, and Rf at Monmouth, Illinois, in 1999 (field study 2).

Cultivar type[a]	Herbicide[b]	LAI 1[c]	LAI 2[d]	Pods per plant	Seeds per pod	Seed wt. (mg)	Yield (kg/ha)	Rf
Susc.	—	2.0 a	3.4 b	—	1.5 a	139 a	2,684 a	13.6 a
Res.	—	1.8 b	4.2 a	—	1.5 a	142 a	2,559 a	4.0 b
—	Glyphosate	2.2 a	3.9 a	—	1.5 a	137 a	2,743 a	14.4 a
—	Pendimeth	1.8 c	3.3 b	—	1.4 a	142 a	2,564 a	7.5 a
—	Acifluorf	1.9 bc	3.7 a	—	1.4 a	140 a	2,642 a	9.9 a
—	Imazethap	2.1 ab	3.6 ab	—	1.5 a	140 a	2,662 a	12.6 a
Susc.	Glyphosate	—	—	44 cde	—	—	—	—
Res.	Glyphosate	—	—	70 a	—	—	—	—
Susc.	Pendimeth	—	—	54 b	—	—	—	—
Res.	Pendimeth	—	—	55 b	—	—	—	—
Susc.	Acifluorf	—	—	40 e	—	—	—	—
Res.	Acifluorf	—	—	50 bcde	—	—	—	—
Susc.	Imazethap	—	—	53 de	—	—	—	—
Res.	Imazethap	—	—	53 bcd	—	—	—	-

Data are means of four replicates. Means within a column followed by a common letter are not different ($P \leq 0.05$). Nematode data were transformed to $\log_{10}(x + 1)$ values before statistical analysis, but untransformed means are reported in the table.
[a] Cultivar type was based on resistance to *Heterodera glycines*. Susceptible cultivars were Asgrow AG3002, Pioneer 93B01, and Siebens 2701; the resistant cultivar was Pioneer 9363.
[b] Glyphosate was applied over the entire experimental area for weed control before postemergence (POST) herbicide treatments were applied. Herbicide treatments consisted of a glyphosate-only control, preemergence applied pendimethalin, POST applied acifluorfen, and POST applied imazethapyr.
[c] Leaf area index (LAI) recorded 2 weeks after POST herbicides were applied.
[d] LAI recorded 6 weeks after POST herbicides were applied.

TABLE 6. Effect of cultivar type and herbicide on leaf area index, pods per plant, seeds per pod, seed weight, yield, and Rf at Monmouth, Illinois, in 2000 (field study 2).

Cultivar type[a]	Herbicide[b]	LAI 1[c]	LAI 2[d]	Pods per plant	Seeds per pod	Seed wt. (mg)	Yield (kg/ha)	Rf
Susc.	—	4.1 a	5.4 a	42 b	2.0 a	125 a	3,362 a	17.7 a
Res.	—	3.8 a	5.4 a	53 a	1.8 b	120 b	3,262 a	3.7 b
—	Glyphosate	4.4 a	5.4 a	42 a	2.0 a	122 a	3,451 a	15.0 a
—	Pendimeth	3.8 a	5.2 a	45 a	1.9 a	124 a	3,260 a	16.3 a
—	Acifluorf	3.9 a	5.4 a	48 a	1.9 a	123 a	3,329 a	16.7 a
—	Imazethap	4.1 a	5.4 a	45 a	1.9 a	126 a	3,308 a	9.2 a

Data are means of four replicates. Means within a column followed by a common letter are not different ($P \leq 0.05$). Nematode data were transformed to $\log_{10}(x + 1)$ values before statistical analysis, but untransformed means are reported in the table.

[a] Cultivar type was based on resistance to *Heterodera glycines*. Susceptible cultivars were Asgrow AG3002, Pioneer 93B01, and Siebens 2701; the resistant cultivar was Pioneer 9363.

[b] Glyphosate was applied over the entire experimental area for weed control before postemergence (POST) herbicide treatments were applied. Herbicide treatments consisted of a glyphosate-only control, preemergence applied pendimethalin, POST applied acifluorfen, and POST applied imazethapyr.

[c] Leaf area index (LAI) recorded 2 weeks after POST herbicides were applied.

[d] LAI recorded 6 weeks after POST herbicides were applied.

Seedlings of Lee 74 growing in dimethenamid + metribuzin-treated and pendimethalin-treated soil had a lower dry shoot weight than seedlings growing in soil not treated with an herbicide (Table 8). Seedlings of Savoy and Jack growing in soil treated with pendimethalin also had lower dry shoot weights than seedlings growing in soil not treated with an herbicide. Seedlings of Lee 74, Savoy, and Jack had lower dry root weights than seedlings growing in soil not treated with a herbicide.

Greenhouse postemergence herbicide study: Reproduction of *H. glycines* was not affected ($P \leq 0.05$) by the POST herbicides; however, cultivar did affect Rf. The Rf on Savoy were 27.7 and 90.9, 30 and 60 days after soil was infested with *H. glycines,* respectively; the Rf on Jack were 1.2 and 3.9, 30 and 60 days after soil was infested, respectively.

There was a three-way interaction among cultivar, herbicide, and *H. glycines* infestation for dry shoot weight at 30 days after *H. glycines* infestation. At 60 days

TABLE 7. Effect of herbicide treatment on yield and Rf on susceptible vs. resistant soybean cultivars at East Troy, Wisconsin, in 1997, 1998, and 1999 (field study 3).

Herbicide[a]	Cultivar type[b]	Yield (kg/ha) 1997	Yield (kg/ha) 1998	Yield (kg/ha) 1999	Rf[c] 1997	Rf[c] 1998	Rf[c] 1999
Handweeded	Susceptible	2,625	4,388	2,374	10.5	3.8	159.4
	Resistant	3,303	4,605	2,897	1.2	1.3	5.4
	$P > F$	0.001	0.133	0.092	0.001	0.074	0.001
Pendimethalin	Susceptible	2,698	4,173	2,401	20.0	3.0	71.5
	Resistant	3,337	4,257	2,952	1.6	2.3	10.7
	$P > F$	0.001	0.561	0.053	0.001	0.149	0.079
Acifluorfen	Susceptible	2,769	4,296	2,545	11.9	3.3	38.3
	Resistant	3,352	4,573	3,018	2.2	1.5	5.3
	$P > F$	0.001	0.056	0.095	0.001	0.108	0.026
Imazethapyr	Susceptible	2,594	4,140	2,751	9.1	5.4	74.2
	Resistant	3,315	4,324	2,943	1.1	3.7	10.9
	$P > F$	0.001	0.199	0.091	0.001	0.258	0.001
Glyphosate[d]	Susceptible	2,776	4,215	2,679	12.3	3.3	61.8
	Resistant	3,263	4,443	3,077	2.3	1.5	13.8
	$P > F$	0.056	0.359	0.317	0.005	0.207	0.043
Across all herbicide treatments	Susceptible	2,680	4,247	2,536	13.0	3.8	83.2
	Resistant	3,322	4,440	2,956	1.6	2.2	8.7
	$P > F$	0.001	0.006	0.001	0.001	0.002	0.001

Data are means of four replicates. Values of $P > F$ were obtained from single-degree-of-freedom contrast statements.

[a] Herbicide treatments consisted of a handweeded control, preemergence (PRE) applied pendimethalin, postemergence (POST) applied acifluorfen, POST applied imazethapyr, and POST applied glyphosate.

[b] Cultivar type was based on resistance to *Heterodera glycines*. Susceptible cultivars were Asgrow AG2701, Novartis NK 24–92, and Dairyland DSR 250 STS in 1997; Asgrow AG2101, Novartis NK 24–92, and Dairyland DSR 220 STS in 1998; and Asgrow AG2101 and Asgrow AG2553 in 1999. Resistant cultivars were Asgrow AG2901, Pioneer 92B91, and Dairyland DSR 96319 in 1997; Asgrow AG2201, Pioneer 9234, and Dairyland DSR 246 STS in 1998; and Asgrow AG2201 and Pioneer 9234 in 1999.

[c] Rf, reproduction factor data of *Heterodera glycines* were transformed to $\log_{10}(x + 1)$ values before statistical analysis, but untransformed means are reported in the table.

[d] Glyphosate was applied only to the glyphosate-tolerant (Roundup-Ready) cultivars, which were Asgrow AG2701 and Asgrow AG2901 in 1997 and Asgrow AG2101 and Asgrow AG2201 in 1998 and 1999.

TABLE 8. The effect of different preemergence herbicide treatments on *Heterodera glycines* reproduction on three soybean cultivars growing in infested soil in a greenhouse, and the effect of herbicides on dry shoot and root weights of three cultivars averaged over *H. glycines*-infested and uninfested soil in the greenhouse 30 days after infestation.

Cultivar	Herbicide treatment[a]	Rf[b]	Dry shoot weight (g)	Dry root weight (g)
Susceptible				
Lee 74	None	6.1 bc	3.8 a	1.8 a
	Dim + Met	4.8 b	2.7 cd	1.6 a
	Pendimethalin	0.3 a	2.3 d	0.7 b
Savoy	None	7.1 c	3.5 ab	2.1 a
	Dim + Met	NA[c]	NA	NA
	Pendimethalin	0.7 a	1.0 e	0.3 b
Resistant				
Jack	None	0.2 a	3.1 abc	2.1 a
	Dim + Met	0.3 a	3.0 bcd	1.8 a
	Pendimethalin	0.0 a	1.3 e	0.8 b

Data are means of two trials combined (six total replicates). Pots (1,000-cm^3 volume) were infested with 1×10^4 eggs of *Heterodera glycines*. Means within a column followed by a common letter are not different according to the PDIFF option in SAS used to compare least-square ($P \leq 0.05$).

[a] Herbicide treatments, applied directly to the soil immediately after planting, consisted of a no-herbicide control; a mixture of dimethenamid and metribuzin at 0.74 and 0.21 kg a.i./ha, respectively; and pendimethalin at 1.39 kg a.i./ha.

[b] Rf, reproduction factor of *Heterodera glycines,* were transformed to $\log_{10}(x + 1)$ values before statistical analysis, but untransformed means are presented in the table.

[c] Not applicable because all seedlings of cv. Savoy in dimethenamid + metribuzin-treated soil died due to sensitivity to metribuzin.

after soil was infested with *H. glycines,* dry root weight was affected by cultivar and an interaction between cultivar and *H. glycines* infestation occurred. The dry shoot weight at 60 days after *H. glycines* infestation was affected by cultivar and by *H. glycines* infestation, with Jack and Savoy having dry shoot weights of 7.9 and 6.3 g, respectively, and plants with *H. glycines* having a dry shoot weight of 5.8 g compared with plants without *H. glycines* having a dry shoot weight of 8.5 g.

The cultivar Jack did not incur a reduction in dry shoot weight from *H. glycines* in the pots not treated with herbicides (Table 9). Conversely, dry shoot weight of Jack was reduced in the *H. glycines*-infested pots compared with the uninfested pots when acifluorfen at the 1x rate or imazethapyr at the 2x rate was applied. For Savoy, dry shoot weight was reduced by the presence of *H. glycines* in the no-herbicide control, acifluorfen 1x rate treatment, and acifluorfen 2x rate treatment.

DISCUSSION

The number of pods per plant on the resistant cultivars was generally greater than on the susceptible cultivars. Browde et al. (1994b) measured pods per plant on a susceptible cultivar in plots with different levels of *H. glycines* Pi and compared the different levels for number of pods per plant, but found no differences ($P \leq 0.05$); however, when a mixture of acifluorfen and bentazon was applied to the soybean plants, the herbicides and level of Pi interacted ($P \leq 0.10$) to decrease the number of pods per plant. In the studies presented herein, the numbers of pods per plant were generally greater in the resistant cultivars; however, at Monmouth, Illinois (field study 2), in 1999, the resistant cultivars had a greater number of pods only in the glyphosate-only treated plots. Application of herbicides other than glyphosate may have provided enough additional stress to the resistant cultivars to lower the pod numbers. Other reports have shown that pod numbers decreased in susceptible cultivars compared with resistant cultivars in *H. glycines*-infested soil (Heatherly and Young, 1993; Long and Todd, 2001; Todd et al., 1995). Yield compensation due to the fewer number of pods on the susceptible cultivars by the other yield component parameters (seeds per pod and weight per seed) occurred infrequently. This is important information about the effects of *H. glycines* on soybean, and breeders can use to select cultivars that not only limit *H. glycines* reproduction but also set greater pod numbers in the presence of *H. glycines.*

In the greenhouse, the interactive stresses of *H. glycines* and the POST herbicides decreased the dry shoot weight of both susceptible and resistant cultivars. This agrees with results reported by Browde et al. (1994a;

TABLE 9. Effect of postemergence herbicide and *Heterodera glycines* stress on the dry shoot weight of a susceptible (Savoy) and a resistant (Jack) cultivar 30 days after infesting soil with *H. glycines* in the greenhouse.

Cultivar	Herbicide[a]	*H. glycines* infested[b]	Dry shoot weight (g)
Susceptible			
Savoy	None	No	3.3 ab
		Yes	2.4 defg
	Acifluorfen 1x rate	No	3.5 a
		Yes	2.7 bcde
	Acifluorfen 2x rate	No	3.1 abc
		Yes	1.9 fg
	Imazethapyr 1x rate	No	3.3 ab
		Yes	2.7 bcde
	Imazethapyr 2x rate	No	2.7 bcde
		Yes	2.6 cde
Resistant			
Jack	None	No	3.0 abcd
		Yes	3.1 abc
	Acifluorfen 1x rate	No	3.5 a
		Yes	2.3 efg
	Acifluorfen 2x rate	No	2.5 def
		Yes	2.5 def
	Imazethapyr 1x rate	No	3.0 abcd
		Yes	2.6 cde
	Imazethapyr 2x rate	No	2.9 abcde
		Yes	1.8 g

Data are means of two trials combined (six total replicates). Means within a column followed by a common letter are not different according to the PDIFF option in SAS used to compare least-square means ($P \leq 0.05$).

[a] Herbicides were applied at the V2 growth stage and consisted of a no-herbicide control, acifluorfen at a 1x and a 2x rate (x = 0.42 kg a.i./ha), and imazethapyr at a 1x and a 2x rate (x = 0.07). Crop oil concentrate at 1% v/v was applied with the herbicide treatments as an adjuvant.

[b] Pots (1,000-cm^3 volume) were infested with 1×10^4 eggs of *Heterodera glycines.*

1994b), in which the interactive stresses of *H. glycines* and an herbicidal mixture of acifluorfen and bentazon decreased soybean growth and yield. It is important for growers and researchers to realize that these stresses can interact, and that managing these problems requires an integrated approach.

Pendimethalin reduced *H. glycines* Rf on the susceptible cultivars in field study 1 at Champaign, Illinois, in 1998. A reduction in Rf on susceptible cultivars also occurred in the greenhouse when pendimethalin was applied. Pendimethalin also was found to reduce the dry shoot weights and the dry root weights of all cultivars in the greenhouse. It is likely that the stress from pendimethalin reduced root growth, thus modifying host-fitness of the susceptible cultivars due to reduction in numbers of successful nematode infection sites. Browde et al. (1994c) reported a reduction in *H. glycines* population with the application of an herbicidal mixture of acifluorfen and bentazon, and attributed it to herbicide injury limiting root growth. Other stresses on soybean have been shown to reduce the *H. glycines* population. Stresses from some nematicides have been shown to stunt soybean roots initially, thus providing fewer infection sites for *H. glycines* (Schmitt et al, 1983). Alston et al. (1993) reported decreased *H. glycines* population densities due to weed stress on soybean. Russin et al. (1989) reported decreased *H. glycines* population densities due to stress on soybean caused by infection of *Diaporthe phaseolorum* var *caulivora,* the stem canker fungus. Other factors other than stress on the host may be involved in herbicides affecting *H. glycines* populations. Wong et al. (1993) reported that the herbicide acifluorfen directly suppressed *H. glycines* egg hatching in vitro. Levene et al. (1998b) reported that eggs from soybean treated with acifluorfen or bentazon were inhibited from hatching and attributed it to increased glyceollin production in the soybean plants (Levene et al., 1998a). Further research is needed to evaluate the effect of pendimethalin on egg hatching and glyceollin production.

Herbicides did not increase the *H. glycines* Rf of the resistant cultivars in any of the studies reported in this article. Sipes and Schmitt (1989) reported that the cultivar Centennial in plots treated with alachlor had more mature females than Centennial in plots not treated with alachlor. Griffin and Anderson (1979) also reported the same phenomenon with a *Meloidogyne hapla*-resistant alfalfa (*Medicago sativa*) cultivar, which had increased galling caused by *M. hapla* in plots treated with the herbicide ethyl dipropylthiocarbamate (EPTC). Neither alachlor nor EPTC was evaluated in our studies for their effects on soybean growth or *H. glycines*. From our research, it is recommended that the highest-priority decision for growers to make when managing *H. glycines* in a field is the choice to plant a resistant cultivar. Herbicides did not affect resistant cultivars' ability to suppress *H. glycines* Rf. The decision of what herbicide to use should be a secondary-priority decision based on the type of weeds present and on the cultivar's tolerance to the herbicide.

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