

# PRODUCTION AGRICULTURE

## Glyphosate-Resistant Soybean Cultivar Yields Compared with Sister Lines

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### ABSTRACT

Herbicide-resistant crops like glyphosate resistant (GR) soybean [*Glycine max* (L.) Merr.] are gaining acceptance in U.S. cropping systems. Comparisons from cultivar performance trials suggest a yield suppression may exist with GR soybean. Yield suppressions may result from either cultivar genetic differentials, the GR gene/gene insertion process, or glyphosate. Grain yield of GR is probably not affected by glyphosate. Yield suppression due to the GR gene or its insertion process (GR effect) has not been reported. We conducted a field experiment at four Nebraska locations in 2 yr to evaluate the GR effect on soybean yield. Five backcross-derived pairs of GR and non-GR soybean sister lines were compared along with three high-yield, nonherbicide-resistant cultivars and five other herbicide-resistant cultivars. Glyphosate resistant sister lines yielded 5% (200 kg ha<sup>-1</sup>) less than the non-GR sisters (GR effect). Seed weight of the non-GR sisters was greater than that of the GR sisters (in 1999) and the non-GR sister lines were 20 mm shorter than the GR sisters. Other variables monitored were similar between the two cultivar groups. The high-yield, nonherbicide-resistant cultivars included for comparison yielded 5% more than the non-GR sisters and 10% more than the GR sisters.

SOYBEAN improvement through the incorporation of genetic resistance or tolerance is an accepted practice in soybean cultivar development for yield-limiting factors such as diseases (Athow, 1987) and nematodes (Riggs and Schmitt, 1987). A goal of plant breeders is to maintain the productivity of the parent line in the absence of the yield-limiting factor. Comparisons of near-isogenic lines with and without the tolerance or resistance genes are important to ascertain if grain yields are suppressed.

Phytophthora root rot (PRR, caused by *Phytophthora megasperma* f. sp. *glycinea* Kuan and Erwin) was one of the most destructive diseases of soybean (Athow, 1987). It provides a good case study for this discussion. In the early 1960s genetic resistance to PRR was incorporated into several cultivars through backcrossing programs resulting in near-isogenic lines (Athow, 1987). Several researchers using near-isogenic lines have reported that PRR-resistant lines perform the same as

PRR-susceptible lines in the absence of PRR (Caviness and Walters, 1971; Singh and Lambert, 1985; Wilcox and St. Martin, 1998). Singh and Lambert (1985) also reported no deleterious pleiotropic effects of the insertion of the gene for PRR resistance. Thus, no yield suppression was associated with the incorporation of the PRR genes into soybean cultivars.

Herbicide-resistant crops like glyphosate resistant (GR) soybean are gaining widespread acceptance in U.S. cropping systems. This technology has promise in areas where other herbicides cannot effectively control weeds. However, potential yield suppression associated with GR cultivars is a concern of producers and seed companies. Data from university soybean cultivar performance trials in several states suggest a yield suppression may exist with GR soybean (Minor, 1998; Nielsen, 2000; Nelson et al., 1997, 1998, 1999; Oplinger et al., 1998; H.C. Minor, Univ. of Missouri, personal communication, 1999). However, Delannay et al. (1995) stated that no yield suppression was associated with the GR gene. This statement was based on unpublished research where six pairs of isopopulations with and without the GR gene were compared (X. Delannay, personal communication, Dec. 1999). He concluded that GR cultivars should perform as well as conventional cultivars of equivalent maturities. The GR gene, CP4 EPSPS, from breeding line 40-3-2 tested in the Delannay et al. study remains as the source for resistance in current GR cultivars (X. Delannay, personal communication, Dec. 1999).

Yield suppression may result from either the GR gene/gene insertion, glyphosate (both individually or collectively are termed yield drag), or cultivar genetic differentiation (yield lag). Yield lag represents yield suppression due to the genetics of the cultivar or line in which the GR gene is inserted. Thus, yield of GR cultivars may lag behind that of other cultivars simply because the GR gene was inserted in lower yielding or older cultivars. Yield drag can result from either the GR gene or its insertion (GR effect) or the application of glyphosate (herbicide effect). We reported that glyphosate did not suppress grain yield of GR soybean cultivars and hence did not contribute to a yield drag (Elmore et al., 2001). Yield drag could also result from the GR

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**Abbreviations:** a.e., acid equivalent; GR, glyphosate resistant; LL, liberty link; NEREC-HAL, Northeast Research and Extension Center-Haskell Agric. Lab.; PRR, phytophthora root rot; R1, flowering; R7, physiological maturity; R8, harvest maturity; SCREC, South Central Research and Extension Center; STS, sulfonylurea-resistant soybean; WCREC, West Central Research and Extension Center.

**Table 1. Location, important activity dates, and water received. Nebraska, 1998–1999.**

Location	City	Year	Planting date	Emergence date	Irrigation applied	Rainfall†	Harvest date
Agronomy Farm	Lincoln	1998	25 May	1 June	none	299	20 Oct.
		1999	25 May	1 June	none	268	22 Oct.
NEREC-HAL‡	Concord	1998	27 May	3 June	40	421	23 Oct.
		1999	26 May	3 June	102	306	13 Oct.
SCREC§	Clay Center	1998	20 May	1 June	127	144	13 Oct.
		1999	26 May	5 June	233	341	16 and 22 Oct.
WCREC¶	North Platte	1998	26 May	1 June	3 applications per year††	375	13 Oct.
		1999	25 June	30 June		411	15 Oct.

† Growing season precipitation.

‡ Northeast Research and Extension Center–Haskell Ag Lab.

§ Univ. of Nebraska South Central Research and Extension Center.

¶ West Central Research and Extension Center.

†† Applications began on 20 July 1998 and 19 July 1999 and ended on 20 Aug. 1998 and 18 Aug. 1999. A gated-pipe, gravity irrigation system was used. Irrigation amounts are not available.

**Table 2. Soils at each location. Nebraska, 1998–1999 (see Table 1 for location abbreviations).**

Location	Years	Soil type	Soil classification
Agronomy Farm	1998 and 1999	Kennebec silt loam	Fine-silty, mixed, mesic, Cumulic Hapludolls
NEREC-HAL	1998 and 1999	Alcester silty clay loam	Fine-silty, mixed, mesic, Cumulic Haplustolls
SCREC	1998 and 1999	Hastings silt loam	Fine, montmorillonitic, mesic Udic Argiustoll
WCREC	1998 and 1999	Cozad and Hord silt loam	Coarse-silty, mixed, mesic Fluventic Haplustolls and Fine-silty, mixed, mesic Cumulic Haplustolls

gene or its insertion process (GR effect); evidence of this has not been reported.

We designed two experiments to test for yield drag: the effect of GR gene insertion on GR (reported in this paper) and the effect of glyphosate (Elmore et al., 2001). To evaluate the GR effect on yield and agronomic traits, field experiments were conducted at four Nebraska locations on five pairs of GR, non-GR sister lines in 1998, and on four pairs of GR, non-GR, sister lines in 1999. Eight other cultivars were included for comparison. We could not discern between yield drag associated with the GR gene itself or effects of its insertion in this study. Thus, reference to the GR effect could mean either or both of these possibilities.

## MATERIALS AND METHODS

Field experiments were planted at four Nebraska locations in 1998 and 1999 (Tables 1 and 2). Corn was grown before the experimental year in both years at all locations. Subplots were 4 to 0.76 m rows by 9.1 m in length. Seeding rate was 370 000 seed ha<sup>-1</sup>. Field preparation activities varied by location and year: Lincoln Agronomy farm 1998 and 1999—disk and field cultivate in spring; North East Research and Extension Center–Haskell Ag Lab 1998—disk and field cultivate in spring 1999—fall disk, spring disk and field cultivate; South Central Research and Extension Center 1998—two passes of mulch master (John Deere, Moline, IL) in spring; 1999—roto-tilled in spring; West Central Research and Extension Center: 1998 and 1999—ridge till. Plots were sprayed with the pre-emergence herbicide combination of metolachlor and metribuzin to help control weeds. Glyphosate was also applied at the West Central Research and Extension Center (WCREC) location to control emerged weeds (Table 3). The experiments were maintained weed-free by hand weeding. We used a randomized complete block experimental design with four replications at all locations except only three replicates were used at WCREC in 1999.

Cultivars grown are shown in Table 4. Entries 1 to 3 were included based on their tolerance to glufosinate (Liberty Link,

LL) and chlorimuron/thifensulfuron (sulfonylurea-resistant soybean, STS). Entries 4 to 6 were included because of their high yield in the Univ. of Nebraska's 1997 cultivar tests (Nelson et al., 1997). Entries 7 to 8 were included since they were also in the companion study (Elmore et al., 2001); these cultivars were provided by two of the major seed companies in Nebraska based on their maturities and yield. Entries 1 to 8 were included as checks. Backcross-derived BC3 and BC4 sister line pairs were provided by two seed companies. The lines were chosen based on appropriate maturities for our locations. Unfortunately, isogenic lines of non-GR and GR cultivars or lines were not available.

Flowering date (R1), physiological maturity (R7), and harvest maturity (R8) (Ritchie et al., 1996) were recorded at several of the locations. In addition, stand counts were taken during the vegetative stages, plant height at R7, and lodging scores were recorded at R8. Seed weights were recorded at three locations in 1999. The center two rows of each plot

**Table 3. Preplant and preemergence herbicide application information by location and year. Nebraska, 1998–1999 (see Table 1 for location abbreviations).**

Location	Year	Herbicide	Application date	Rate
Agronomy Farm	1998	metolachlor	24 May	kg ha <sup>-1</sup>
		+		2.3
Agronomy Farm	1999	metribuzin	24 May	+
				0.51 a.i.
NEREC†	1998		27 May	
			27 May	
SCREC	1998		21 May	
			26 May	
WCREC	1998	metolachlor	26 May	1.8
		+		+
WCREC	1999	metribuzin	25 May	0.41 a.i.
		+		+
WCREC	1999	glyphosate	25 May	0.84 a.e.
		s-metolachlor		1.4 a.i.
WCREC	1999	+		+
		glyphosate		0.84 a.e.

† Clethodim and crop oil concentrate were applied at 0.11 kg ha<sup>-1</sup> + 1.10 l ha<sup>-1</sup> on 25 June 1998 and 6 July 1999 for volunteer corn control.

**Table 4. Cultivars used in this study, Nebraska, 1998–1999.**

No.	Company	Cultivar or line	Notes	Grain yield†
				Mg ha <sup>-1</sup>
1	Asgrow	2704-LL	Liberty‡/STS§ resistant	3.48
2	Pioneer	9323-ST5	STS resistant	3.51
3	Golden Harvest	H1359-ST5	STS resistant	3.09
4	Hoegemeyer	232	Normal- high yield	3.91
5	Desoy	2343	Normal- high yield	3.82
6	M/W Genetics	2711	Normal- high yield	3.88
7	Pioneer	92B51	GR¶	3.47
8	Asgrow	AG3002	GR	3.81
9	NC+	2.4N	Non-GR Sister of #10	3.77a*
10	NC+	2.5RR	GR	3.45b
11	NC+	3.2N	Non-GR Sister of #12	3.64a
12	NC+	3.2RR	GR	3.48a
13	Stine (1998 only)	EX25N	Non-GR Sister of #14	4.14a
14	Stine (1998 only)	EX25RR	GR	3.78b
15	Stine	2170	Non-GR Sister of #16	3.69a
16	Stine	2174	GR	3.44a
17	Stine	2250	Non-GR Sister of #18	3.61a
18	Stine	2254	GR	3.55a

\* Sister pairs followed by the same letter are not different ( $P \leq 0.05$ ) based on single-degree-of-freedom comparisons.

† Two-year, four-location means except for 1 yr means for entries 13 and 14.

‡ Resistant to Liberty, glufosinate.

§ STS, soybean resistant to chlorimuron/thifensulfuron.

¶ Glyphosate resistant soybean. Entries 7 and 8 were grown in the experiments discussed in Elmore et al., 2001.

**Table 5. Non-glyphosate-resistant sister lines (non-GR sisters) yielded more than their GR sisters averaged over all locations and 2 yr. Growth and development of these two variety groups differed.**

Variety group (entry numbers in each group)	Yield	Flowering days fr 31 May	1999 Seed wt.	Lodging at R7†	Plant height at Mat. (R7)	Maturity (R7) days fr 31 May	Maturity (R8) days fr 31 May	Plant density × 1000	Grain moisture
		Mg ha <sup>-1</sup>	g/100		mm			plants ha <sup>-1</sup>	%
Non-GR Sisters (9,11,15,17)	3.68a	43.6a	14.7a	1.6a	860b	111.9a	120.4a	266a	10.0a
GR Sisters (10,12,16,18)	3.48*	43.7a	14.1b	1.4a	880a	112.7a	121.7a	267a	10.0a
SE	0.08	0.6	0.2	0.1	14	0.5	0.9	11	0.4
No. of locations reporting data: 1998/1999	4/4	2/4	0/3	4/4	4/4	3/4	3/1	4/4	4/4

\* Means followed by the same letter within a column are similar ( $P \leq 0.05$ ). Means were separated using single-degree-of-freedom comparisons.

† 1 to 5 scale with 1 = erect and 5 = prostrate.

were harvested with a small plot harvester for yield and seed weight determination.

Data were processed with SAS mixed models procedures (Littell et al., 1996). Cultivar was considered a fixed effect. Locations and replicates and their interactions with the fixed effect were treated as random effects. Single-degree-of-freedom comparisons were used to isolate cultivar grouping differences: LL/STS vs. STS; STS vs. High yield; LL/STS vs. High yield; LL/STS vs. all GR; STS vs. all GR; High yield vs. non-

GR sisters; non-GR sisters vs. GR sisters; GR cultivars (7 and 8) vs. GR sisters; High yield vs. all GR; High yield vs. GR (7 and 8); 9 vs. 10; 11 vs. 12; 13 vs. 14; 15 vs. 16; and 17 vs. 18. We also used correlation to compare grain yields of GR and non-GR sister lines.

Three sets of analyses were used for each variable. The first compared all entries except 13 and 14 over both years. The second and third analyses included all entries in 1998 and 1999, respectively, since entries 13 and 14 were not available

**Table 6. Herbicide-resistant varieties yielded less than the nonherbicide-resistant check varieties averaged over locations and 2 yr.**

Cultivar group (entry numbers in each group)	Yield	Flowering date fr 31 May	1999 Seed wt.	Lodging at R7†	Plant height at Mat. (R7)	Maturity (R7) fr 31 May	Maturity (R8) fr 31 May	Plant density × 1000	Grain moisture
		Mg/ha	g/100		mm			plants/ha	%
Liberty Link(LL)/STS (1)	3.48bc*	43.0b	13.5c	1.4b	860b	113.9b	124.0ab	275a	10.1b
STS (2,3)	3.30c	46.2a	14.0bc	1.7a	1010a	116.6a	126.6a	271a	11.7a
All glyphosate resistant cultivars (7,8,10,12,16,18)	3.53b	43.8b	14.2b	1.4b	890b	112.7b	121.6b	263a	10.0b
Nonherbicide-resistant controls (4,5,6)	3.87a	43.1b	15.0a	2.0a	870b	109.9c	119.3c	277a	9.9b
SE‡	0.13	1.0	0.3	0.2	24	0.8	1.6	19	0.7
No. of locations reporting data: 1998/1999	4/4	2/4	0/3	4/4	4/4	3/4	3/1	4/4	4/4

\* Means followed by the same letter within a column are similar ( $P \leq 0.05$ ). Means were separated using single-degree-of-freedom comparisons.

† 1 to 5 scale with 1 = erect and 5 = prostrate.

‡ Standard errors of the mean (SE) are the greatest encountered for the individual single-degree-of-freedom comparisons among means in each column. In all cases this was the SE associated with the LL/STS vs. STS single-degree-of-freedom comparison.

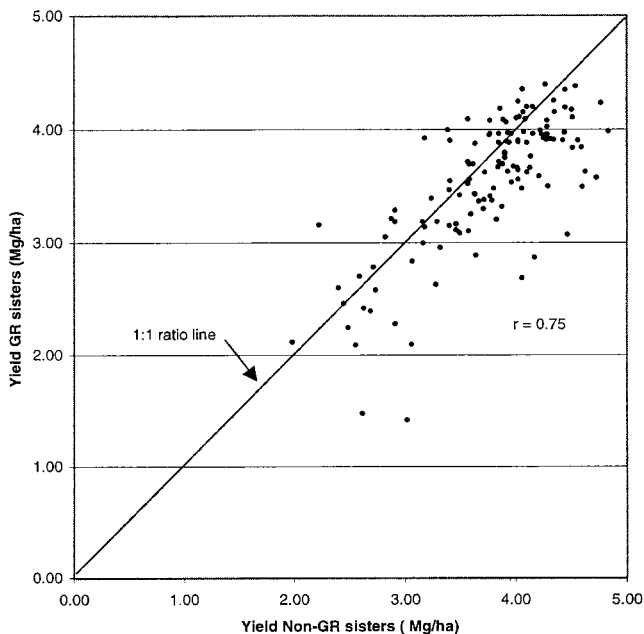


Fig. 1. Yield of non-GR (glyphosate resistant) sisters compared with their respective GR sisters at four locations in 2 yr. Each marker represents yield data of sister line pairs from the same replicate, location, and year. University of Nebraska, 1998 and 1999.

in 1999. Data presented are least squares adjusted means. Differences mentioned are significant at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

On average, non-GR sister lines yielded 5% ( $200 \text{ kg ha}^{-1}$ ) more than the GR sisters when averaged over all locations and both years (Table 5). Non-GR sister grain yields were greater than those of their associated GR sisters in two of the five pairs (Table 4). Results were similar in the single-year analyses (data not shown). Grain yields of sister-line pairs are shown in Fig. 1. The greater number of data points to the right of the 1:1 ratio line indicates that the non-GR sisters yielded more on the average than their GR sister counterparts. This reinforces the previous statement on the average soybean yields of the non-GR sisters relative to the GR sisters in the individual years and in the 2-yr analysis (Table 5).

Seed weight of the non-GR sisters was greater than that of the GR sisters (in 1999) and the non-GR sister lines were 20 mm shorter than the GR sisters (Table 5). Other variables monitored were similar between the two cultivar groups. The GR sisters yielded the same as the average of entries 7 and 8, two GR cultivars included in the study for comparison (data not shown). Entries 7 and 8 yielded the same as other GR cultivars in another study with other GR cultivars (Elmore et al., 2001). Yield of the GR check cultivar entry 8 was similar to those of the high-yield, nonherbicide-resistant cultivars (entries 4, 5, and 6). In addition, although no statistical comparisons were possible, yields of the highest yielding GR cultivars in the other study (Elmore et al., 2001) were similar to those of the high-yield, nonherbicide-resistant cultivars in this study (data not shown). The high-yield, nonherbicide-resistant cultivars (entries

4, 5, and 6) yielded 5% more than the non-GR sisters (Tables 5 and 6). This 5% difference is a yield lag. The GR gene in the GR sisters therefore reduced soybean yield 5% compared to the non-GR sisters and 10% when compared to high-yield, nonherbicide-resistant cultivars.

The high-yield, nonherbicide-resistant checks in the study (entries 4, 5, and 6) also yielded the same or more than the other herbicide-resistant cultivars included in the experiment (Tables 4 and 6). The average yield of all seven GR cultivars was similar to that of the LL/STS cultivar (entry 1), and greater than that of the average of the two STS cultivars (entries 2 and 3). A comparison of the means of the STS cultivars shows that entry 3 yielded less than the other STS cultivar, entry 2, as well as the other herbicide-resistant cultivars (Table 4). Herbicide-resistant cultivars yielded from the same to 15% less than the nonherbicide-resistant cultivars included in these studies (Table 4 and 6).

## CONCLUSIONS AND IMPLICATIONS

Yields were suppressed with GR soybean cultivars. Our other work showed that there was no effect of glyphosate on GR cultivars (Elmore et al., 2001). The work reported here demonstrates that a 5% yield suppression was related to the gene or its insertion process and another 5% suppression was due to cultivar genetic differential. Producers should consider the potential for 5 to 10% yield differentials between GR and non-GR cultivars as they evaluate the overall profitability of producing soybean. Cultivar choices are best based on (i) previous weed pressure and success of control measures in specific fields, (ii) the availability and cost of herbicides, (iii) availability and cost of herbicide-resistant cultivars, and (iv) yield, and not solely on whether cultivars are herbicide resistant. Based on our results from this study and those of Elmore et al., 2001, the yield suppression appears associated with the GR gene or its insertion process rather than glyphosate itself.

## ACKNOWLEDGMENTS

We thank the Nebraska Soybean Board, who partially supported the research. Technical support personnel at the various locations were: Clay Center—Sharon Hachtel, George Hoffmeister, Jr., Ralph Klein, Perry Ridgeway, Irv Schleufer, and Sandy Sterkel; Lincoln—Greg Dorn and John Eis; North Platte—Jeff Goulis; Concord—Lisa Lunz and Ray Brentlinger. We appreciate their efforts!

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## Downsizing an Integrated Crop Management Field Study Affects Economic and Biological Results

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### ABSTRACT

In recent years, there has been increased interest in long-term, field-scale cropping systems research to improve pest management, to protect air and soil quality, and to increase or maintain growers' profits. However, these studies require large tracts of land, sizeable labor forces, and substantial inventories of equipment, which make them very expensive to conduct. Because of recent concerns about reducing field research costs, this study compares economic and biological results from an original complete 6-yr integrated cropping management (ICM) systems field study to results from several downsized experiments, which were components of the complete study. Compared with the original ICM study, the downsized experiments reduced the number of treatment replications from four to three, reduced the number of crop rotation cycles from two to one (from 6 to 3 yr), or only grew one crop per rotation each year. The effect of downsizing on the profitability analysis and the statistical (biological) analysis were similar. Reducing replications altered both profitability and biological conclusions less than reducing the number of rotation cycles. Reducing crop rotation cycles markedly altered treatment profitability rankings compared with the complete study. Growing only one crop in a rotation per year was the most detrimental to biological results and entirely precluded computing mean annual cropping system profitability. This empirical study supports the importance of replicating treatments fully over time, over space, and over crop rotational positions.

LONG-TERM, LARGE-SCALE FIELD STUDIES that address integrated pest management (IPM) and/or integrated crop management (ICM) agro-ecosystems are important for several reasons. When properly designed

and correctly conducted, long-term field studies identify profitable cropping systems; assess conservation tillage practices; obtain information on direct, residual, and cumulative treatment effects; identify strategies for erosion control and pest management; and develop regulations for reduced pesticide use (Cady, 1991; Young et al., 1994a, 1994c; Alldredge and Young, 1995). These studies generally involve several agencies and must be interdisciplinary or multidisciplinary to identify important interacting agronomic, economic, and biological relationships (Martin et al., 1991; Schweizer et al., 1988; Young et al., 1994c). Integrated crop management studies are rarely attempted because they are expensive to conduct; require large areas of land; and utilize considerable labor to plant, harvest, and maintain plots, and to collect data (Cady, 1991; Young et al., 1994b). Reducing the size of long-term field studies is desirable if downsizing does not sacrifice biological and economic information. There are numerous ways in which researchers can downsize long-term, large-scale ICM studies. These include reducing treatments, data collected, plot size, disciplines involved, as well as decreasing replications, duration of the study, and land area required based on number of crops grown each year within a rotation. For example, the original proposal for the Pacific Northwest (PNW) ICM study called for 432 subplots rather than the final 144. The proposal was reduced by eliminating a tillage regime and fertility rates (Young et al., 1994b).

Statistical theory guides researchers in determining sample sizes for testing hypotheses or estimating parameters (Neter et al., 1996). Most sample size information is available for simple experimental designs, but may

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**Abbreviations:** WML, weed management level; ww, winter wheat; sw, spring wheat; sb, spring barley; sp, spring pea; MP, main plot; rep., replication; PNW, Pacific Northwest; EMS, error mean square; LSD, least significant difference; ICM, integrated crop management; IPM, integrated pest management.