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Glyphosate-Resistant Soybean Cultivar Response to Glyphosate

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ABSTRACT

Glyphosate (*N*-(phosphonomethyl) glycine)-resistant (GR) soybean [*Glycine max* (L.) Merr.] technology is gaining acceptance in U.S. cropping systems, yet potential yield suppression from either cultivar genetic differentials, the GR gene/gene insertion process, or glyphosate is a concern. Other work shows that the GR gene/gene insertion process may suppress soybean yield. No one has reported the effects of glyphosate on a diverse group of commercially available GR soybean cultivars. In this study we evaluated one of the potential sources of GR yield suppression—the effect of glyphosate on yield, growth, and development of GR cultivars. Field experiments were conducted at four Nebraska locations with12 GR cultivars in 1998 and 13 GR cultivars in 1999. Soybean response to glyphosate, ammonium sulfate (AMS), and water application at 21 and 42 d after soybean emergence was compared with control plots treated with AMS and water in 1998. An additional control, water alone, was added in 1999. Grain yield among cultivars differed as expected with a range of 3.44 to 3.96 Mg ha^{-1} in the 2-yr averages. Glyphosate did not affect the majority of the soybean growth and development characteristics measured. Grain yield of GR soybean was not affected by glyphosate at any location or when averaged over locations. Two-year average grain yield of cultivars treated with glyphosate, AMS, and water was 3.74 Mg ha^{-1}; this was not different from 3.79 Mg ha^{-1} with AMS and water treatment.

The development of herbicide-resistant crops represents new weed control technology. Examples include soybean, corn (*Zea mays* L.), and canola (*Brassica napus* L.) resistant to glyphosate, the active ingredient of Roundup, and glufosinate (2-amino-4-(hydroxymethylphosphinyl)butanoic acid) (Moll, 1997; Rasche and Gadsby, 1997). Glyphosate-resistant (GR) soybean was one of the first major applications of genetic engineering in field crops (Delannay et al., 1995; Padgette et al., 1995). Growers have readily integrated herbicide-resistant crops into their production practices. Herbicide-resistant soybean were grown on 7, 17, 44, and 57% of the U.S. soybean area from 1996 to 1999, respectively (USDA, 1999; National Agric. Statistics Service, 1999). Most herbicide-resistant soybean cultivars are GR.

Although GR technology is gaining acceptance in U.S. cropping systems, 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 yield suppression may exist (Nelson et al., 1997, 1998, 1999; Minor, 1998; Oplinger et al., 1998; Harry Minor, Univ. of Missouri, personal communication, 1999).

Yield suppression may result from either (i) cultivar genetic differentials, (ii) the GR gene/gene insertion process (GR effect), or (iii) glyphosate (herbicide effect), or a combination of the three. Thus, in the first situation yield of GR cultivars may be suppressed relative to that of other cultivars simply because the GR gene was inserted in low yielding or older cultivars. We consider yield suppression associated with the GR effect or herbicide effect a greater potential problem than cultivar genetic differentials since the latter can be overcome by inserting the GR gene in high yielding parent lines. The relative importance of yield suppression due to cultivar genetic differentials should thus diminish with time. The GR effect and herbicide effect, however, could potentially handicap yields regardless of the cultivars used. Elmore et al. (2001) found that GR lines yielded 5% less than their non-GR sister lines. This documented that at least part of the yield suppression associated with GR soybean is the GR gene or its insertion.

Research with the first GR line, 40-3-2 and its progeny indicated that GR agronomic characteristics and yield were not affected by glyphosate applications up to twice the labeled rate [1.68 kg a.e. (acid equivalent) ha^{-1}] (Delannay et al., 1995). Glyphosate application in both vegetative and reproductive stages of the crop did not adversely affect the crop and the GR gene was stable over successive generations. The GR gene from 40-3-2 remains as the source for tolerance in current GR soybean cultivars (X. Delannay, personal communication, Dec. 1999).

Glyphosate-resistant soybean treated with glyphosate have yielded the same or better than GR soybean treated with conventional pre- or postemergence herbicides (Bennett et al., 1998; Hofer et al., 1998; Nelson and Renner, 1999). Observations of side-by-side comparisons in 1997 at the University of Nebraska South Central Research and Extension Center (SCREC) indicated that glyphosate with AMS (ammonium sulfate) may have delayed soybean flowering (R.W. Elmore, unpublished data, 1997). Ammonium sulfate enhances glyphosate activity and weed control. Nelson and Renner (1999) found that glyphosate with and without AMS resulted in similar yields and weed control with a single cultivar. No one has reported the effects of glyphosate

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Abbreviations: a.e., acid equivalent; AMS, ammonium sulfate; DAE, days after emergence; Gly, glyphosate; GR, glyphosate resistant; NEREC-HAL, Northeast Research and Extension Center–Haskell Agric. Lab.; R1, flowering; R7, physiological maturity; R8, harvest maturity; SCREC, South Central Research and Extension Center; WCREC, West Central Research and Extension Center.

Table 1. Information pertinent to experiments conducted in Nebraska, 1998–1999.

Location	City	Year	Planting date	Emergence date	Irrigation applied	Rainfall	Harvest date
					mm	mm	
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	14 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 in both years; amounts not recorded	375	13 Oct.
		1999	25 May	2 June		411	15 Oct.

† Northeast Research and Extension Center–Haskell Ag Lab.
‡ Univ. of Nebraska South Central Research and Extension Center.
§ West Central Research and Extension Center.

relative to controls without glyphosate on a diverse group of commercially available GR cultivars.

We designed experiments to test for two possible sources of yield suppression: the effect of glyphosate herbicide application on GR soybean (herbicide effect, reported in this paper) and the effect of the GR gene insertion event (GR effect, see Elmore et al., 2001). Field experiments were conducted at four Nebraska locations with the intent to compare the effects of glyphosate with AMS and water to AMS with water on 12 cultivars in 1998. An additional cultivar and a control treatment of water only were added in 1999.

MATERIALS AND METHODS

Field experiments were conducted at four Nebraska locations in 1998 and 1999 (Table 1). Soils at the respective sites were: Agronomy Farm: Kennebec silt loam (fine-silty, mixed, mesic, Cumilic Hapludolls); Northeast Research and Extension Center-Haskell Ag Lab (NEREC-HAL): Alcester silty clay loam (fine-silty, mixed, mesic, Cumulic Haplustolls); South Central Research and Extension Center (SCREC): Hastings silt loam (fine, montmorillonitic, mesic Udic Argiustoll); and West Central Research and Extension Center (WCREC): Cozad and Hord silt loam (coarse-silty, mixed, mesic Fluventic Haplustolls and fine-silty, mixed, mesic Cumulic Haplustolls). Previous crop in both years at all locations was corn. Subplots consisted of four rows 0.76 m wide by 9.1 m in length. Seeding rate was 370 000 seed ha^{-1}. Field preparation activities are as follows: Agronomy farm 1998 and 1999—disk and field cultivate in spring; NEREC-HAL 1998—disk and field cultivate in spring 1999—fall disk, spring disk and field cultivate; SCREC 1998—two passes of Mulch Master (John Deere, Moline, IL) in spring 1999—rototilled in spring; WCREC: 1998 and 1999—ridge till.

Table 2. Herbicide application information by location and year. Nebraska, 1998–1999 (see Table 1 for location abbreviations).

Location	Year	Preemergence treatment†	1st glyphosate application‡	2nd glyphosate application‡
Agronomy Farm	1998	24 May	1 July	28 July
Agronomy Farm	1999	24 May	25 June	10 July
NEREC§	1998	27 May	1 July	16 July
NEREC§	1999	27 May	24 June	14 July
SCREC	1998	21 May	29 June	13 July
SCREC	1999	26 May	25 June	19 July
WCREC	1998	26 May¶	29 June	20 July
WCREC	1999	25 May‡‡	23 June	16 July

† Metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methyl ethyl)acetamide; 2.32 kg a.i. ha^{-1}).
‡ 0.84 kg a.e. ha^{-1}.
§ Clethodim, (*E*,*E*)-(±)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one, at 0.11 kg ha^{-1} and crop oil concentrate at 1.2 L ha^{-1} was applied on 25 June 1998 and 6 July 1999 for volunteer corn control.
¶ Metolachlor (1.8 kg a.i. ha^{-1}) and metribuzin (0.41 kg a.i. ha^{-1}).
‡‡ *s*-metolachlor, 1.4 kg a.i. ha^{-1}.

Spray treatments were applied topically to the soybean about 21 and 42 d after emergence (Table 2). Soybean growth stages for the first and second glyphosate applications were V2 to V5 and R1 to R3, respectively (Ritchie et al., 1996). Weather conditions were near normal both pre- and posttreatment at all locations. Treatments consisted of glyphosate at 0.84 kg a.e. ha^{-1} plus AMS at 2.7 kg a.i. ha^{-1} in water, and AMS at 2.7 kg ha^{-1} in water. Spray volumes of 187 L ha^{-1} were achieved with 8002 spray tips on tractor-mounted, compressed air sprayers. Plots other than those at WCREC were sprayed with the preemergence herbicide combination of metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; 2.32 kg a.i. ha^{-1}) and metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one; 0.51 kg a.i. ha^{-1}). At WCREC plots were sprayed with the preemergence herbicide combination of metolachlor (1.8 kg a.i. ha^{-1}), metribuzin (0.41 kg a.i. ha^{-1}), and glyphosate (0.84 kg a.e. ha^{-1}) in 1998. In 1999, s-metolachlor (1.4 kg a.i. ha^{-1}) was used preemergence at WCREC. The experiments were maintained weed-free by hand weeding.

A split-plot, randomized complete block experimental design was used with a factorial arrangement of treatments. Twelve GR soybean cultivars were included in 1998 and 13 GR cultivars were included in 1999 (Table 3). Cultivars with maturities adapted to all four locations were provided by some of the major seed companies in Nebraska. Each cultivar (main plot) was planted in two (1998) or three (1999) adjacent sub-

Table 3. Glyphosate-resistant soybean cultivars used in this study. Nebraska, 1998–1999.

Cultivar no.	Company	Glyphosate-resistant cultivars	Maturity group
1	Golden Harvest	H1280RR	2.9
2	Golden Harvest	H1357RR	3.5
3	Pioneer	92B05	2.0
4	Pioneer	92B51	2.5
5	Asgrow	AG2702	2.7
6	Asgrow	Ag3002	3.0
7	Northrup King	S28V8	2.8
8	Northrup King	S35F5	3.5
9	NU Pride-Excel	8355	3.5
10	Dyna Gro	187	2.5
11	Asgrow	A3601STS/RR	3.5
12	NC+	32RR	3.2
13 (1999 only)	Stine	3203-4	3.2

Table 4. Spray treatment effects on grain yield and other plant characteristics. Nebraska, 1998–1999.

	Grain yield		Flowering date		Physiological maturity		Mature plant height		Seed wt.
Spray treatment	1998–1999 8 Env.†	1999 4 Env.	1998–1999 6 Env.	1999 4 Env.	1998–1999 7 Env.	1999 4 Env.	1998–1999 8 Env.	1999 4 Env.	1999 2 Env.
	Mg ha^{-1}		d from 31 May		d from 31 May		mm		g/100
Gly‡	3.74*	3.65	57	54	112	112	963	986b	14.6a
AMS	3.79	3.73	57	54	112	112	969	994a	14.4b
Water	–	3.70	–	54	–	112	–	995a	14.6a
SE	0.16	0.03	16	0.1	2	0.2	3	3	0.1

* Means within the same column followed by the same letters or without letters are not different ($p \leq 0.05$).
† Env. = Total environments (locations × years) where data were collected.
‡ Spray treatments: Gly = glyphosate with ammonium sulfate (AMS) and water; AMS = AMS and water; Water = water alone.

plots (spray treatments) in four replicates. In 1998, one subplot was sprayed with glyphosate, AMS, and water, and the second subplot was sprayed with AMS and water. In 1999, a control treatment of water only was also included as a third subplot.

Crop development and plant height were monitored at 21, 42, and 56 days after emergence (DAE), and dates of flowering (R1), physiological maturity (R7), and harvest maturity (R8) were recorded (Ritchie et al., 1996). In addition, plant counts were taken during the vegetative stages and lodging scores were recorded at R8. The center two rows of each plot were harvested with a small plot harvester for yield and seed weight determination. Grain yield was adjusted to 130 g kg^{-1} moisture content.

Data were processed using SAS mixed models procedures (Littell et al., 1996). Cultivar and spray treatments were considered fixed effects. Locations, replicates, and their interactions with the fixed effects were considered random effects. Single degree-of-freedom comparisons were used to isolate the main effect of spray treatments in both years. Pair-wise comparisons of cultivar–spray treatment interactions were generated using the PDIFF option of the Least Squares Means statement of PROC Mixed in all analyses (Littell et al., 1996). Two sets of analyses were used for each variable because all cultivars and treatments were not included in both years. The first compared the first 12 cultivars and two spray treatments (glyphosate with AMS and water vs. AMS and water) over both years. The second analyses compared all 13 cultivars over all three spray treatments (glyphosate with AMS and water, AMS and water, and water alone) in 1999. All data presented are least squares adjusted means. Differences are significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Cultivar Effects

Many characteristics differed among cultivars when averaged over locations. These included plant height at 42 and 56 DAE, and growth stage at 56 DAE for 1999 (data not shown). These findings are common when cultivars from different seed companies and of different maturities are compared in experiments (Nelson et al., 1997, 1998, 1999).

Some cultivar characteristics were also affected by the location at which the cultivar was grown. These included seed yield, plant height at 21 DAE, flowering date, plant height at flowering, date of physiological maturity, date of harvest maturity, and lodging in 1999, and growth stage at 42 DAE and plant height at 56 DAE in the 2-yr data (data not shown). Again, differences among cultivars and locations are common when diverse cultivars are compared.

Spray Treatment Effects

Neither glyphosate nor AMS affected grain yield or the majority of the soybean growth and development characteristics measured (Table 4). We saw no significant visual injury. This confirms the grain yield data of Nelson and Renner (1999) with a single cultivar, but contradicts earlier observations of side-by-side compari-

Table 5. Spray treatment effects on glyphosate-resistant soybean cultivars. Averages of four locations. Nebraska, 1999.

	1999 data					
	Yield			Physiological maturity		
Cultivar†	Gly‡	AMS	Water	Gly	AMS	Water
	Mg ha^{-1}			d from 31 May		
1	3.76*	3.92	3.69	115	115	115
2	3.76	3.78	3.79	110	110	110
3	3.33	3.42	3.44	118	118	118
4	3.63	3.58	3.46	102	102	102
5	3.67	3.91	3.85	106	106	106
6	3.75	3.77	3.76	111	111	111
7	3.98	3.93	4.00	114	114	114
8	3.21	3.14	3.30	117	117	117
9	4.04	4.04	3.96	111	111	111
10	3.28	3.45	3.46	116	115	115
11	3.33	3.54	3.47	115a	115a	112b
12	3.95	4.04	3.94	110	110	110
13	3.82	4.01	4.02	113	113	113
Spray avg.	3.65	3.73	3.70	112	112	112
SE within cultivar among spray	0.088			0.41		
SE among spray avg.	0.032			0.23		

* Means within cultivar for each variable followed by the same letter or without letters are not different ($p \leq 0.05$).
† See Table 4 for complete cultivar descriptions.
‡ Spray treatments: Gly = glyphosate with ammonium sulfate (AMS) and water; AMS = AMS and water; Water = water alone.

Table 6. Spray treatment effects on glyphosate-resistant soybean cultivars. Averages of four locations. Nebraska, 1998 and 1999.

	2-yr data	
	Yield	
Cultivar†	Gly‡	AMS
	Mg ha^{-1}	
1	3.75*	3.85
2	3.71	3.73
3	3.52	3.57
4	3.66	3.67
5	3.81	3.94
6	3.96	3.98
7	3.98	3.99
8	3.54	3.53
9	3.95	3.91
10	3.44	3.58
11	3.56	3.62
12	3.85	3.88
Spray avg.	3.74	3.79
SE within cultivar between spray	0.064	
SE between spray avg.	0.163	

* Means within cultivar between the two treatments are not different ($p \leq 0.05$) for any of the cultivars.
† See Table 4 for complete cultivar descriptions.
‡ Gly = glyphosate with ammonium sulfate (AMS) and water; AMS = AMS and water.

sons at SCREC where flowering was delayed by glyphosate with AMS and water relative to conventional herbicides (R.W. Elmore, unpublished data, 1997). Flowering was neither affected by the glyphosate nor AMS in the present study (Table 4). However, plant height at physiological maturity in 1999 was reduced by 8 to 9 mm with glyphosate (Table 4). This finding was consistent across all locations but was not significant in the 2-yr analysis.

Cultivar as Affected by Spray Treatments

Grain yield of GR cultivars was neither affected by glyphosate at any location (data not shown) nor affected when averaged across locations (Tables 5 and 6). This was true in both the 1- and 2-yr analyses. Likewise, cultivar yield responses were consistent across the spray treatments. Physiological maturity of most of the cultivars was likewise not affected by the spray treatments; however, there were a few differences among some cultivars (Table 5 and 6). For example, physiological maturity of cultivar 11 was delayed with both glyphosate and AMS relative to the water alone treatment (Table 5). No other cultivar was affected in this way. Grain yield was not affected.

CONCLUSIONS

Although grain yield and plant characteristics varied among cultivars, in most cases they were not affected by glyphosate. Glyphosate did not affect yield of cultivars evaluated and thus did not contribute to yield suppression.

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