NRC Research Press

ARTICLE

Weed control and crop safety in sulfonylurea/glyphosate-resistant soybean

Zahoor A. Ganie and Amit J. Jhala

Abstract: A soybean trait resistant to sulfonylurea herbicides along with glyphosate (Bolt™ soybean) has been developed. Information is needed to determine herbicide programs for weed control and crop safety in this new multiple herbicide–resistant soybean trait. The objectives of this study were to evaluate weed control and crop safety in sulfonylurea/glyphosate-resistant soybean with herbicide programs, including but not limited to acetolactate synthase (ALS) inhibitors. Field experiments were conducted near Clay Center, NE, USA, in 2016 and 2017. Herbicide programs with multiple sites-of-action including rimsulfuron/thifensulfuron in mixture with flumioxazin, flumioxazin/chlorimuron, pyroxasulfone, chlorimuron/metribuzin, or saflufenacil/imazethapyr plus dimethenamid-P provided 91%–97% control of common waterhemp, velvetleaf, and common lambsquarters. Rimsulfuron and (or) thifensulfuron resulted in 92%–97% control of velvetleaf and common lambsquarters and 81%–87% common waterhemp control at 21 d after pre-emergence (PRE) (DAPRE) herbicide application. Soybean injury was transient and varied from 3% to 11% at 21 DAPRE and 14 d after post-emergence (POST) (DAPOST) herbicide application without causing yield loss. At 30 and 60 DAPOST, 87%–97% velvetleaf control and 92%–98% common lambsquarters control was achieved with herbicide programs tested (PRE, POST, or PRE followed by POST). Common waterhemp control at 30 and 60 DAPOST was not consistent between years. Weed density and biomass reduction were mostly similar to weed control achieved. Untreated control resulted in the lowest soybean yield (1811 kg ha^{-1}) in 2016 compared with 3406–4611 kg ha^{-1} in herbicide programs.

Key words: crop safety, herbicide-resistant, sulfonylurea herbicides, weed control.

Résumé : Il existe une nouvelle variété de soja (Bolt^{MC}) résistante aux herbicides à base de sulfonylurée et de glyphosate. Toutefois, on manque d'informations pour établir les programmes de désherbage chimique qui combattront les adventices sans nuire à sa culture. Les auteurs ont évalué la destruction des mauvaises herbes et la protection de la culture dans le cadre de programmes de désherbage chimique appliqués au soja résistant au sulfonylurée/glyphosate, y compris les herbicides à base d'inhibiteurs de l'acétolactate synthase (ALS), sans toutefois s'y restreindre. À cette fin, ils ont effectué des expériences sur le terrain près de Clay Center, au Nebraska, USA, en 2016 et 2017. Les programmes de désherbage portant sur de nombreux sites d'action à partir de rimsulfuron/thifensulfuron mélangé à du flumioxazine, de flumioxazine/chlorimuron, de pyroxasulfone, de chlorimuron/métribuzine ou de saflufénacil/imazéthapyr plus du diméthénamide-P ont détruit 91 à 97 % de l'amarante tuberculée, de l'abutilon et du chénopode blanc. Le rimsulfuron ou le thifensulfuron ont supprimé 92 à 97 % de l'abutilon et du chénopode blanc, ainsi que 81 à 87% de l'amarante tuberculée dans les parcelles témoin, 21 j après application de l'herbicide de prélevée (PRE — « pre-emergence »; DAPRE — « days after pre-emergence »). Les dommages causés au soja étaient passagers et ont varié de 3 à 11 %, 21 DAPRE et 14 j après celle des herbicides de post-levée (POST — « post-emergence »; DAPOST — « days after post-emergence »), sans perte de rendement. À 30 et 60 DAPOST, respectivement, les herbicides testés (PRE, POST, PRE puis POST) avaient détruit 87 à 97 % de l'abutilon dans la parcelle témoin et 92 à 98 % du chénopode blanc. La proportion d'amarante tuberculée détruite 30 et 60 DAPOST manquait d'uniformité d'une année à l'autre. La densité des adventices et la réduction de la biomasse sont dans une large mesure analogues au degré de désherbage obtenu. En 2016, ce sont les parcelles témoin non traitées qui ont produit le moins de soja (1811 kg ha^{-1}, contre de 3406 à 4611 kg ha^{-1} avec l'usage d'herbicides). [Traduit par la Rédaction]

Received 25 December 2019. Accepted 5 May 2020.

Z.A. Ganie and A.J. Jhala.* Department of Agronomy and Horticulture, University of Nebraska–Lincoln, 279 Plant Science Hall, P.O. Box 830915, Lincoln, NE 68583-0915, USA.

Corresponding author: Amit J. Jhala (email: Amit.Jhala@unl.edu).

*A.J. Jhala currently serves as a Technical Editor; peer review and editorial decisions regarding this manuscript were handled by Robert Gulden.

Mots-clés : protection des cultures, résistance aux herbicides, herbicides à base de sulfonylurée, lutte contre les mauvaises herbes.

Introduction

Soybean is an important crop grown on 120 million ha throughout the world with a production of 351 million metric tonnes (USDA-FAS 2018). The United States is the largest producer of soybean with the production of 80 million metric tonnes harvested from 31 million ha, amounting to a farm gate value of $15 billion in 2018 (USDA-NASS 2018). Weed interference is the major biotic restraint to soybean production, causing an average yield loss of 37% (Oerke 2006). Soltani et al. (2017) reported that, on average, a 52.1% yield loss is possible in soybean due to weed interference in the absence of weed management in the United States and Canada.

The yield loss in soybean varies with weed species composition, density of weed infestation (Cordes and Bauman 1984; Henry and Bauman 1989; Crook and Renner 1990; Baysinger and Sims 1991; Oliver et al. 1991; Fellows and Roeth 1992; Vail and Oliver 1993; Barnes et al. 2018), time of relative emergence between the crop and weeds (Hock et al. 2006), and a myriad of other crop management practices and environmental conditions (Jackson et al. 1985; Krausz et al. 2001). Herbicides provide an effective and economical tool for weed control and profitable soybean production (Bruff and Shaw 1992; Lanie et al. 1994; Muyonga et al. 1996). For example, Sweat et al. (1998) reported ≥90% control of *Amaranthus* species including common waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), redroot pigweed (*Amaranthus retroflexus* L.), and tumble pigweed (*Amaranthus albus* L.) with pre-emergence (PRE) application of acetolactate synthase (ALS) (imazaquin and imazethapyr), long-chain fatty acid (acetochlor, alachlor, and metolachlor), microtubule (pendimethalin and trifluralin), photosystem II (metribuzin), and (or) protoporphyrinogen (PPO) inhibitors (sulfentrazone). Prior to widespread adoption of glyphosate-resistant soybean, commonly used POST herbicides in soybean included ALS inhibitors (chlorimuron-ethyl, cloransulam, and (or) imazaquin) and PPO inhibitors such as fomesafen or lactofen (Vidrine et al. 1993; Krausz et al. 1998; Shaw et al. 1999; Rousonelos et al. 2012). ALS inhibitors were one of the most widely used herbicides because of their efficacy at low use rate, broad spectrum of weed control, soil residual activity, and low mammalian toxicities (Tranel and Wright 2002; Green 2007). The evolution of resistance to ALS inhibitors in early 1990s, followed by the commercialization of glyphosate-resistant soybean in 1996, reduced the use of other herbicides (Young 2006). As a result, glyphosate became the primary herbicide for post-emergence (POST) weed control in soybean, reducing the utility of other herbicides and consequently enhancing the evolution of glyphosate-resistant weeds (Givens et al. 2009; Ganie et al. 2016).

Despite the unique features of chemical weed control including high efficacy, rapid results, easy application, and low cost, the success of herbicides has been challenged by the evolution of herbicide-resistant weeds (Peterson et al. 2018). More importantly, POST herbicide options in soybean have been reduced due to the evolution of weeds resistant to ALS inhibitors, glyphosate, and recently PPO inhibitors (Heap 2014; Sarangi et al. 2019). Historically, control of herbicide-resistant weeds has been relatively easy due to the discovery and commercialization of herbicides with novel sites-of-action at regular intervals (Heap 2014). In last three and a half decades, however, there has been no breakthrough in herbicide discovery; this has resulted in a focus on herbicide-resistant weed management through the use of herbicide rotations, mixtures, and to some extent diversifying approaches including crop rotation, tillage, row spacing, and other cultural practices (Norsworthy et al. 2012; Ganie et al. 2016, 2017).

Herbicides applied alone, in rotations or mixtures, should warrant crop safety to avoid crop injury or yield losses (Preston 2005). Genetic engineering has been extensively employed to introduce herbicide-resistant traits in several crops, including soybean, to allow use of POST nonselective herbicides such as glyphosate and glufosinate (Green 2007) and more recently, dicamba (Spaunhorst et al. 2014) and 2,4-D choline (Ruen et al. 2017). Additionally, seed mutagenesis and traditional breeding strategies have been used to increase the level of resistance of soybean to ALS inhibitors, particularly herbicides in the sulfonylurea family (Sebastian et al. 1989; Green 2007). Sulfonylurea-resistant soybean allows the safe use of sulfonylurea herbicides such as thifensulfuron and chlorimuron and reduces the risk of soybean injury due to physical drift and carryover (Anderson and Simmons 2004). Though a widespread occurrence of weed resistance to ALS-inhibiting herbicides has limited the use of ALS inhibitors, this unique chemistry is still effective on several weed species at exceptionally low rates and is often useful as a synergistic or additive partner in herbicide premixtures (Beckie and Reboud 2009; Green and Owen 2011; Westwood et al. 2018). In addition, ALS inhibitors continue to have a major role for weed control in rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) that often results in physical drift from rice fields to adjacent soybean fields and carryover injury in wheat–soybean rotation, particularly in the southern United States (Nandula et al. 2009; Rana et al. 2014).

Recently, a new soybean cultivar, known as Bolt™ soybean, has been developed through traditional breeding approaches to provide enhanced resistance to sulfonylurea herbicides. Bolt™ soybean has the synergistic combination of *Als1* and *Als2* genes, providing an improved tolerance to sulfonylurea herbicides compared with previously available sulfonylurea-tolerant soybean (Walter et al. 2014). The objectives of this study were to evaluate weed control and crop safety in soybean with Bolt™ technology by selecting herbicide programs based on multiple sites-of-action, including ALS inhibitors.

Materials and Methods

Field experiments were conducted at South Central Agricultural Laboratory, University of Nebraska–Lincoln, Clay Center (40.52°N, 98.05°W), NE, USA, in 2016 and 2017. The soil type at the experimental site was a fine, smectitic, mesic Vertic Argiaquolls (Butler series) with a silt loam texture, with 17% sand, 58% silt, 25% clay, and 2.5% organic matter and a pH of 6.5. The experimental location was infested with a natural stand of common waterhemp (*A. rudis*), velvetleaf (*Abutilon theophrasti* Medik.), and common lambsquarters (*Chenopodium album* L.). Glyphosate-resistant soybean variety P38T61BR (DuPont Pioneer®) with enhanced resistance to sulfonylurea herbicides (Bolt™ Technology) was seeded on 13 May 2016 and 24 Apr. 2017. The experiment was arranged in a randomized complete block design with four replications. Individual plots were 3 m wide and 9 m long with four soybean rows spaced 0.76 m apart. The experimental location was under a lateral irrigation system and was irrigated as needed. Treatments included PRE followed by (fb) POST herbicide mixtures alongside PRE or POST herbicides alone, and an untreated control for comparison (Table 1). Labels were followed to decide herbicide rates (Table 1) and appropriate crop and (or) weed growth stages. Herbicides were applied using a CO_2-pressurized backpack sprayer with a four-nozzle boom using AIXR 110015 flat-fan nozzles (TeeJet Spraying Systems Co., Wheaton, IL, USA) calibrated to deliver 140 L ha^{-1} at a pressure of 276 kPa. PRE herbicides were applied a day after soybean seeding, and POST herbicides were applied at 22 d after PRE (DAPRE) when the average weed height was around 8–12 cm.

Weed control was estimated by visual observations of herbicide injury and growth suppression at 21 DAPRE and 30 and 60 d after POST (DAPOST) on a scale of 0%–100%, where 0% refers to no weed control and 100% refers to complete weed mortality in a plot. Similarly, soybean injury was recorded using a 0%–100% scale, where 0% referred to no soybean injury and 100% corresponded to complete soybean mortality. Density of observed weed species was recorded from three randomly selected 0.5 m^2 quadrats per plot from two middle soybean rows at 60 DAPOST. Similarly, biomass of weed species was assessed from three randomly selected 0.5 m^2 quadrats per plot at 60 DAPOST by harvesting the aboveground parts of weeds that survived herbicide treatments. Weeds harvested for biomass were placed in paper bags, dried in an oven for 72 h at 55 °C, and weighed (g). The weed biomass was converted into percent biomass reduction compared with untreated control as

$$(1)\qquad \text{Percent biomass reduction} = \left[\frac{(C-B)}{C}\right] \times 100$$

where C is the biomass of untreated control replicates and B is the biomass of an individual experimental plot. Soybean was harvested from the two central rows in each plot using a plot combine, and the yields were adjusted to 13% moisture content.

Statistical analysis

Data were subjected to an ANOVA using PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The treatments with zero response variables were not included in the analysis because it returns a non-estimation error. Before analysis, data were tested for normality of residuals using the PROC UNIVARIATE procedure in SAS. Year and treatment were considered as a fixed effect to determine if a year × treatment interaction existed or whether the data could be pooled between years. Percent weed control, crop injury, and biomass reduction data were processed into their decimal equivalents and analyzed using a linear predictor (eq. 2) and logit-link function (eq. 3) with beta distribution and Laplace method of approximation for maximum likelihood estimation. Soybean plant population and weed density data were analyzed following the same procedure but with negative binomial distribution, and yield data were analyzed using Gaussain distribution. Inverse link function was used to change data back from model scale to decimal equivalents; however, data are presented on original percent scale to facilitate correct biological interpretation of the results.

$$(2)\qquad \eta_{ij} = \log\left(\frac{\mu}{1-\mu}\right) = \mu + \tau_i + b_j$$

$$(3)\qquad \eta_{ij} = \text{logit}(\mu_{ij}) = \log[\mu_{ij}/(1-\mu_{ij})]$$

For the above equation, η_{ij} is the response or effect (percent weed control or biomass reduction, etc.) produced or caused by ith treatment (PRE and (or) POST herbicide program) in jth replication with $i = 1$–17 and $j = 1$–4; μ is the overall mean; τ_i is the effect of ith treatment; and b_j is effect of jth replication. The effect of the replications was considered as random effect with $b_j \sim \text{NI}(0, \sigma_b^2)$.

When the ANOVA indicated that treatment effects were significant, means were separated at $P \le 0.05$ using LSMEANS procedure with LINES command and Tukey–Kramer adjustment. Contrast analysis with a single degree of freedom were performed using LSESTIMATE command in SAS to compare ALS inhibitors vs. other

Table 1. Herbicides and application rates and timings for weed control and crop safety in sulfonylurea/glyphosate-resistant (Bolt™ soybean) soybean.

Herbicide program		Herbicide rate (g a.i. ha^{-1})		
PRE	POST	PRE	POST	Manufacturer
Rimsulfuron/thifensulfuron (Basis® Blend)	—	26	—	E.I. du Pont de Nemours and Company, Wilmington, DE, USA
	Chlorimuron/thifensulfuron (Synchrony® XP)	—	26	E.I. du Pont
	Glyphosate (Roundup PowerMax®)	—	1060	Monsanto Company, St. Louis, MO 63167, USA
Rimsulfuron/thifensulfuron	Glyphosate + chlorimuron/ thifensulfuron	26	1060 + 23	E.I. du Pont Monsanto Company E.I. du Pont
Rimsulfuron/thifensulfuron + flumioxazin (Valor® SX)	Glyphosate + chlorimuron/ thifensulfuron	26	1060 + 23	E.I. du Pont; FMC Corporation, Philadelphia, PA 19103, USA Monsanto Company
Rimsulfuron (Resolve® DF) + chlorimuron/ flumioxazin/thifensulfuron (Envive®)	Glyphosate + chlorimuron/ thifensulfuron	17 + 117	1060 + 23	E.I. du Pont Monsanto Company
Rimsulfuron/thifensulfuron + chlorimuron/ metribuzin (Canopy® Blend)	Glyphosate + chlorimuron/ thifensulfuron	26 + 306	1060 + 23	E.I. du Pont Monsanto Company
Flumioxazin + rimsulfuron + chlorimuron (Classic®)	Glyphosate + chlorimuron/ thifensulfuron	71 + 17 + 17	1060 + 23	FMC Corporation E.I. du Pont; Monsanto
Chlorimuron/flumioxazin/thifensulfuron	Glyphosate + chlorimuron/ thifensulfuron	117	1060 + 23	E.I. du Pont Monsanto Company; E.I. du Pont
Flumioxazin	Glyphosate + chlorimuron/ thifensulfuron	71	1060 + 23	FMC Corporation Monsanto Company; E.I. du Pont
Rimsulfuron	Chlorimuron/thifensulfuron	17	23	E.I. du Pont
Thifensulfuron (Harmony® SG)	Chlorimuron/thifensulfuron	7	23	E.I. du Pont
Rimsulfuron/thifensulfuron	Glyphosate	26	1060	E.I. du Pont Monsanto Company
Saflufenacil/imazethapyr (OpTill® PRO) + dimethenamid-P	Imazethapyr (Pursuit®)	95 + 525	70	BASF Corporation, Research Triangle Park, NC 27709, USA
Saflufenacil/imazethapyr + dimethenamid-P	Chlorimuron/thifensulfuron	95 + 525	23	BASF Corporation E.I. du Pont
Flumioxazin/pyroxasulfone (Fierce®)	Chlorimuron/thifensulfuron	200	23	BASF Corporation; E.I. du Pont

Note: Forward slash (/) and plus (+) are used to indicate an herbicide prepackaged mixture and tankmixture, respectively. Herbicide trade names are mentioned in the parenthesis at the first mention of herbicide active ingredients or their mixtures. PRE, pre-emergence; POST, post-emergence.

herbicide sites-of-action applied PRE, and another set of contrasts was run between ALS inhibitors applied PRE fb POST vs. the herbicide programs with multiple sites-of-action in PRE fb POST herbicide programs.

Results and Discussion

Year × treatment interactions were not significant for most variables including common waterhemp control at 21 DAPRE, control and density of velvetleaf and common lambsquarters, weed biomass reduction, soybean plant population, and soybean injury; therefore, data from both years were combined. Common waterhemp control at 30 and 60 DAPOST, common waterhemp density, and soybean yield were presented separately for both years because of significant differences between years.

Common waterhemp control

Saflufenacil/imazethapyr plus dimethenamid-P, flumioxazin applied PRE alone or in a mixture with rimsulfuron/thifensulfuron/chlorimuron, chlorimuron/metribuzin, or pyroxasulfone resulted in 94%–95% common waterhemp control 21 DAPRE (the forward slash "/" and "plus" are used to indicate an herbicide prepackaged mixture and a tankmixture, respectively). Jhala et al. (2017) reported 88% control of glyphosate-resistant common waterhemp following flumioxazin and 99% control with chlorimuron/thifensulfuron/flumioxazin or saflufenacil plus dimethenamid-P at 14 DAPRE. Similarly, rimsulfuron/thifensulfuron applied PRE provided 91%–93% control compared with 81% and 87% control with thifensulfuron and rimsulfuron applied alone, respectively (Table 2). Reduced efficacy with sulfonylurea herbicides was expected as Sarangi et al. (2015) previously reported that common waterhemp in eastern Nebraska has reduced sensitivity to ALS-inhibiting herbicides. However, in this study sulfonylurea herbicides applied PRE provided 81%–92% common waterhemp control, indicating some ALS inhibitors are still effective under specific field situations, especially applied PRE when weeds are emerging and are relatively more sensitive compared with established plants. In addition, ALS inhibitors are good tankmix partners for PPO-inhibiting herbicides as they reduce the selection pressure and delay the evolution of resistance.

Common waterhemp control with POST herbicides was inconsistent between years. For example, at 30 DAPOST 63%–94% common waterhemp control was achieved when flumioxazin-based mixtures were fb glyphosate plus chlorimuron/thifensulfuron applied POST in 2016 compared with 68%–88% control with a similar program in 2017. Herbicide programs with ALS inhibitors applied alone PRE or in a mixture with metribuzin fb glyphosate plus chlorimuron/thifensulfuron applied POST resulted in 86%–93% control in 2016 compared with an unacceptable control (60%–76%) in 2017 at 30 DAPOST (Table 2). On the contrary, Hutchinson (2007) reported ≥90% control of redroot pigweed (*A. retroflexus*), common lambsquarters (*C. album*), hairy nightshade (*Solanum physalifolium* Rusby), and green foxtail (*Setaria faberi* R.A.W. Herrm) in potato (*Solanum tuberosum* L.) throughout the season with flumioxazin plus metribuzin or rimsulfuron applied PRE. Similarly, saflufenacil/imazethapyr plus dimethenamid-P applied PRE fb imazethapyr or chlorimuron/thifensulfuron applied POST resulted in 71%–83% control in 2016 and 60%–82% control in 2017 at 30 DAPOST (Table 2). Moran et al. (2011) reported that 184–186 g a.i. ha^{-1} of saflufenacil plus dimethenamid-P applied alone or with glyphosate resulted in 95% control of common waterhemp. Sarangi et al. (2017) reported >90% control of glyphosate-resistant common waterhemp throughout the growing season in soybean with the use of saflufenacil/imazethapyr plus dimethenamid-P fb fomesafen plus glyphosate. Similarly, Chahal et al. (2018) reported 75%–86% control of Palmer amaranth, a closely related species of common waterhemp, with saflufenacil plus dimethenamid-P and with saflufenacil/pyroxasulfone applied PRE.

Herbicide programs with multiple sites-of-action in mixtures at PRE fb POST provided 79%–97% control at 60 DAPOST in 2016, contrary to 50%–76% control in 2017 (Table 2). Inconsistency in common waterhemp control in 2017 compared with 2016, regardless of herbicide program, was due to higher precipitation resulting in relatively higher common waterhemp infestation at the experimental site in 2017. Partly, inconsistencies in common waterhemp control following glyphosate plus chlorimuron/thifensulfuron POST within a given year might be attributed to an occurrence of plants with segregating resistance to glyphosate and (or) ALS inhibitors that has been previously reported in the neighboring fields (Sarangi et al. 2015). Nevertheless, several studies have revealed that a mixture of soil residual herbicides along with the POST herbicide program with multiple effective sites-of-action is required for a season-long control of weed species such as common waterhemp, which typically has an extended window of emergence (Schultz et al. 2015; Jhala et al. 2017; Chahal et al. 2018).

Density of common waterhemp varied from 0 to 7 plants m^{-2} with flumioxazin plus rimsulfuron/thifensulfuron and (or) chlorimuron or pyroxasulfone fb glyphosate plus chlorimuron/thifensulfuron compared with 8 plants m^{-2} with ALS-inhibitors fb glyphosate in 2016 (Table 3). Common waterhemp density ranged from 8 to 12 plants m^{-2} with rimsulfuron or thifensulfuron applied PRE fb chlorimuron/thifensulfuron applied POST, and 6–7 plants m^{-2} with rimsulfuron/thifensulfuron applied PRE alone or chlorimuron/thifensulfuron applied POST alone (Table 3). In 2017, common waterhemp density varied from 17 to 22 plants m^{-2} with ALS-inhibiting herbicides applied alone PRE or POST or PRE fb POST compared with ≤10 plants m^{-2} with

Table 2. Common waterhemp control with sulfonylurea herbicides alone or in mixture with other site of action herbicides in sulfonylurea/glyphosate-resistant soybean in 2016 and 2017 in Nebraska.

Herbicide treatment	Application timing	Common waterhemp control (%)				
			2016		2017	
		21 DAPRE[a]	30 DAPOST	60 DAPOST	30 DAPOST	60 DAPOST
Untreated control	—	0	0	0	0	0
Rimsulfuron/thifensulfuron	PRE	92a	42de	76b	0	0
Chlorimuron/thifensulfuron	POST	0	66abcde	79b	23b	0
Glyphosate	POST	0	90ab	95a	47b	33de
Rimsulfuron/thifensulfuron fb glyphosate + chlorimuron/ thifensulfuron	PRE POST	91a	86abc	96a	60ab	40cd
Rimsulfuron/thifensulfuron + flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	95a	92ab	95a	88a	73ab
Rimsulfuron + chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	95a	63bcde	79b	83ab	59ab
Rimsulfuron/thifensulfuron + chlorimuron/metribuzin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	95a	93a	97a	76ab	60bc
Flumioxazin + rimsulfuron + chlorimuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	95a	94a	97a	85ab	76a
Chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	95a	91ab	96a	68ab	50abc
Flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	94a	92ab	95a	48b	50cde
Rimsulfuron fb chlorimuron/thifensulfuron	PRE POST	87a	47dce	69bc	0	0
Thifensulfuron fb chlorimuron/thifensulfuron	PRE POST	81a	27e	42c	0	0
Rimsulfuron/thifensulfuron fb glyphosate	PRE POST	93a	93a	97ab	44b	35cde
Saflufenacil/imazethapyr + dimethenamid-P fb imazethapyr	PRE POST	94a	83abc	93ab	60ab	40cd
Saflufenacil/imazethapyr + dimethenamid-P fb chlorimuron/thifensulfuron	PRE POST	95a	71abcd	90ab	82ab	74ab
Flumioxazin/pyroxasulfone fb chlorimuron/thifensulfuron	PRE POST	95a	89abc	94ab	89a	73ab
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0420

Note: Forward slash (/) and plus (+) are used to indicate an herbicide prepackaged mixture and tankmixture, respectively. Treatments with zero response variables for all replications were not included in statistical analysis. PRE, pre-emergence; POST, post-emergence; DAPRE, days after PRE; DAPOST, days after POST; fb, followed by. Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

[a]Year × treatment interactions were not significant for common waterhemp control at 21 DAPRE, therefore, data from both years were combined.

programs including multiple herbicide sites-of-action in PRE fb POST (Table 3). Rimsulfuron/thifensulfuron fb glyphosate or glyphosate only treatment had 14–15 plants m^{-2} at 60 DAPOST. Legleiter et al. (2009) reported reduction in common waterhemp density with flumioxazin or sulfentrazone applied PRE alone or fb lactofen, aciflourfen, and (or) glyphosate (≤5 plants m^{-2}) compared with glyphosate alone (38–70 plants m^{-2}). Schryver et al. (2017) reported that pyroxasulfone plus flumioxazin applied PRE reduced common waterhemp density by 96% compared with the untreated control, and a follow-up treatment with glufosinate resulted in a 100% reduction of common waterhemp biomass.

Velvetleaf control

Rimsulfuron applied alone or with thifensulfuron resulted in 95%–96% control of velvetleaf compared with 89% control with thifensulfuron alone at 21 DAPRE. Barnes and Oliver (2004) reported 80%–96% velvetleaf control with chloransulam at 18 g ha^{-1}. The PRE herbicide programs with multiple sites-of-action including rimsulfuron/thifensulfuron plus flumioxazin with or without chlorimuron and (or) metribuzin, and saflufenacil/imazethapyr plus dimethenamid-P, provided 96%–97% control of velvetleaf at 21 DAPRE (Table 3). Although flumioxazin, rimsulfuron, or thifensulfuron applied alone resulted in 89%–95% velvetleaf control, herbicide mixtures should be used to reduce the selection pressure of weed species due to single herbicide site-of-action (Beckie and Reboud 2009). A recent example to signify the consequences of same selection pressure over time is the confirmation of PPO-inhibitor-resistant common waterhemp in Nebraska (Sarangi et al. 2019). Aulakh and Jhala (2015) reported ≥92% velvetleaf control at 15 DAPRE following flumioxazin alone or with chlorimuron, sulfentrazone plus chloransulam, imazethapyr or metribuzin, and saflufenacil plus dimethenamid-P. At 30 and 60 DAPOST, rimsulfuron/thifensulfuron applied PRE, chlorimuron/thifensulfuron or glyphosate applied POST, and PRE fb POST herbicide programs provided 91%–97% velvetleaf control (Table 4). Though PRE- or POST-only herbicide programs provided effective velvetleaf control, PRE fb POST herbicides programs including multiple herbicide sites-of-action should be preferred to extend the viability of herbicide options. Aulakh and Jhala (2015) reported 91%–98% velvetleaf control at harvest with sulfentrazone plus chloransulam or metribuzin and flumioxazin plus chlorimuron fb glufosinate alone or with pyroxasulfone or *S*-metolachlor or acetochlor. Similarly, Hartzler and Battles (2001) reported 90% reduction in velvetleaf biomass and seed production with glyphosate at 840 g a.e. ha^{-1}. Velvetleaf density in the untreated control was 7 plants m^{-2} and varied from 0 to 3 plants m^{-2} in several similar herbicide programs.

Common lambsquarters control

PRE herbicides including rimsulfuron, thifensulfuron, and rimsulfuron/thifensulfuron applied alone or in a mixture with flumioxazin, flumioxazin plus chlorimuron or chlorimuron/metribuzin, flumioxazin alone, and saflufenacil/imazethapyr plus dimethenamid-P provided 93%–97% control of common lambsquarters at 21 DAPRE (Table 4). Irrespective of PRE herbicide program, glyphosate plus chlorimuron/thifensulfuron applied POST resulted in 94%–96% control of common lambsquarters at 30 and 60 DAPOST. Similarly, a comparable control was achieved with imazethapyr or chlorimuron/thifensulfuron and chlorimuron/thifensulfuron applied POST following saflufenacil/imazethapyr plus dimethenamid-P applied PRE (Table 4). Likewise, Isaacs et al. (2002) reported 95% control of common lambsquarters at 26 d after treatment with rimsulfuron/thifensulfuron applied POST at 18 g ha^{-1}. Density of common lambsquarters in untreated control (10 plants m^{-2}) was greater compared with all herbicide programs (1–4 plants m^{-2}) (Table 3).

Weed biomass reduction

Year × treatment interactions were significant; therefore, biomass data were presented separately for each year. In 2016, most of the PRE fb POST herbicide programs with multiple sites-of-action resulted in 60%–91% reduction in weed biomass compared with the untreated control (Table 3). Glyphosate resulted in 89% biomass reduction compared with 53% reduction with chlorimuron/thifensulfuron applied POST and 25% reduction with rimsulfuron applied PRE fb chlorimuron/thifensulfuron applied POST. Similarly, in 2017, PRE fb POST herbicide programs with multiple sites-of-action resulted in greater weed biomass reduction varying from 44% to 74% compared with <27% reduction with ALS inhibitors applied alone PRE, POST, or PRE fb POST (Table 3). Similarly, Jhala et al. (2017) reported more variability in common waterhemp biomass with 63%–97% biomass reduction with PRE fb POST herbicide programs compared with ≤65% reduction with a POST herbicide program in soybean. Contrast analysis indicated that weed control programs with only sulfonylurea herbicides resulted in 22%–49% weed biomass reduction compared with 66%–89% reduction with programs based on multiple herbicide sites-of-action in PRE and in POST herbicide programs (Table 5).

Soybean stand and injury

Soybean stands varied from 15 to 17 plants m^{-1} row length without a significant difference between treatments, indicating no effect of PRE herbicides on soybean emergence (Table 6). Soybean injury ranged from 4% to 11% at 21 DAPRE with few differences between treatments. At 21 DAPRE, soybean injury with rimsulfuron/thifensulfuron plus flumioxazin was

Table 3. Aboveground biomass reduction, and density of common waterhemp, velvetleaf and common lambsquarters with sulfonylureas alone or in mixture with other site-of-action herbicides in sulfonylurea/glyphosate-resistant soybean in 2016 and 2017 in Nebraska.

		Weed biomass reduction (%)		Weed density (no. of plants m^{-2})			
				Common waterhemp		Velvetleaf[a]	Common lambsquarters[a]
		60 DAPOST		60 DAPOST			
Herbicide treatment	Application timing	2016	2017	2016	2017	60 DAPOST	
Untreated control	—	0	0	18a	26a	7a	10a
Rimsulfuron/thifensulfuron	PRE	65bcdef	0	7bcd	21ab	3b	4b
Chlorimuron/thifensulfuron	POST	53cd	23bcbc	6bcd	22a	2b	3b
Glyphosate	POST	89ab	44abc	1de	14cd	1b	2b
Rimsulfuron/thifensulfuron fb glyphosate + chlorimuron/ thifensulfuron	PRE POST	89ab	44abc	3cde	11cde	1.5b	0
Rimsulfuron/thifensulfuron + flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	91a	70ab	0	4fg	1b	0
Rimsulfuron + chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	87abc	74a	7bcd	9defg	2b	0
Rimsulfuron/thifensulfuron + chlorimuron/metribuzin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	91a	51abcd	2cde	10def	1b	0
Chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	91a	70ab	0	3g	0	0
Flumioxazin/chlorimuron/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	84abcd	64abcd	4cde	9defg	1b	1b
Flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	88ab	51abc	2cde	13cd	1b	2b
Rimsulfuron fb chlorimuron/thifensulfuron	PRE POST	25d	18c	8bc	21ab	3b	3b
Thifensulfuron fb chlorimuron/thifensulfuron	PRE POST	74abcdef	27bc	12ab	17abc	3b	2b
Rimsulfuron/thifensulfuron fb glyphosate	PRE POST	91a	44abc	8bc	15bcd	2b	0
Saflufenacil/imazethapyr + dimethenamid-P fb imazethapyr	PRE POST	80abc	46abc	2cde	14cd	2b	0
Saflufenacil/imazethapyr + dimethenamid-P fb chlorimuron/thifensulfuron	PRE POST	60bcd	59abc	7bcd	5efg	3b	0
Flumioxazin + pyroxasulfone fb chlorimuron + thifensulfuron	PRE POST	87abc	54abc	2cde	6efg	2b	2b
P value		<0.0465	<0.2499	<0.0001	<0.0001	0.035	<0.0001

Note: Forward slash (/) and plus (+) are used to indicate an herbicide prepackaged mixture and tankmixture, respectively. Treatments with zero response variables for all replications were not included in statistical analysis. PRE, pre-emergence; POST, post-emergence; DAPRE, days after PRE; DAPOST, days after POST; fb, followed by. Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

[a]Year-by-treatment interactions were not significant for velvetleaf and common lambsquarters density, therefore, data from both years were combined.

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Table 4. Velvetleaf and common lambsquarters control with sulfonylureas alone or in mixture with other site of action herbicides in sulfonylurea/glyphosate-resistant soybean in 2016 and 2017 in Nebraska.

Herbicide treatment	Application timing	Velvetleaf control (%)			Common lambsquarters control (%)		
		21 DAPRE	30 DAPOST	60 DAPOST	21 DAPRE	30 DAPOST	60 DAPOST
Untreated control	—	0	0	0	0	0	0
Rimsulfuron/thifensulfuron	PRE	92a	91a	90b	93a	98a	94a
Chlorimuron/thifensulfuron	POST	0	94a	91b	0	92a	95a
Glyphosate	POST	0	94a	94ab	0	95a	95a
Rimsulfuron/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	96a	95a	94a	95a	94a	95a
Rimsulfuron/thifensulfuron + flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	97a	97a	95a	97a	98a	96a
Rimsulfuron + chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	96a	97a	94a	97a	98a	95a
Rimsulfuron/thifensulfuron + chlorimuron/metribuzin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	97a	97a	94a	96a	98a	96a
Flumioxazin + rimsulfuron + chlorimuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	97a	96a	96	97a	97a	96a
Chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	97a	96a	95a	97a	96a	95a
Flumioxazin fb glyphosate + chlorimuron/ thifensulfuron	PRE POST	95a	96a	93a	95a	97a	93a
Rimsulfuron fb chlorimuron/thifensulfuron	PRE POST	95a	96a	87ab	96a	93a	93a
Thifensulfuron fb chlorimuron/thifensulfuron	PRE POST	89a	97a	95a	95a	98a	96a
Rimsulfuron/thifensulfuron fb glyphosate	PRE POST	95a	92a	94a	95a	93a	95a
Saflufenacil/imazethapyr + dimethenamid-P fb imazethapyr	PRE POST	97a	96a	96a	96a	96a	97a
Saflufenacil/imazethapyr + dimethenamid-P fb chlorimuron/thifensulfuron	PRE POST	97a	97a	96a	97a	97a	95a
Flumioxazin/pyroxasulfone fb chlorimuron/ thifensulfuron	PRE POST	96a	96a	95a	97a	97a	97a
P value		0.0002	0.2513	0.2090	0.8621	0.062	0.8927

Note: Forward slash (/) and plus (+) are used to indicate an herbicide prepackaged mixture and tankmixture, respectively. Treatments with zero response variables for all replications were not included in statistical analysis. PRE, pre-emergence; POST, post-emergence; DAPRE, days after PRE; DAPOST, days after POST; fb, followed by. Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

Table 5. Contrast analysis to compare weed control, biomass reduction, and soybean yield with sulfonylureas alone vs. other site of-action herbicides in sulfonylurea/glyphosate-resistant soybean in Nebraska.

Treatment	Common waterhemp		Velvetleaf		Common lambsquarters		% Biomass reduction		Yield	
	21 DAPRE	30 DAPOST	21 DAPRE	30 DAPOST	21 DAPRE	30 DAPOST	2016	2017	2016	2017
SUs vs. OSOA herbicides	89 vs. 94 NS	—	93 vs. 95 NS	—	95 vs. 96 NS	—	—	—	—	—
SUs PRE/POST vs. MSOA PRE/POST	—	56 vs. 87* (2016) 0 vs. 80** (2017)	—	96 vs. 97	—	95 vs. 97**	49 vs. 89*	22 vs. 66**	3107 vs. 4036*	392 vs. 1900*

Note: PRE, pre-emergence; POST, post-emergence; DAPRE, days after PRE; DAPOST, days after POST; SUs, sulfonylurea herbicides; OSOA, other site-of-action herbicides; MSOA, xxxxx; NS, not significant. * and ** represent significant differences based on P value ($P < 0.05$) and ($P < 0.01$), respectively, determined using a single degree of freedom contrast analysis in SAS.

11% compared with 4% injury with sulfonylurea herbicides (rimsulfuron/thifensulfuron plus chlorimuron/metribuzin) (Table 6). Similarly, crop injury at 14 DAPOST varied from 1% to 10%; however, soybean injuries were transient and alleviated by 60 DAPOST and were not reflected in soybean yield (Table 6).

Soybean yield

Soybean yield in 2016 was comparable among herbicide programs varying from 3364 to 4667 kg ha^{-1}, with the exception of 2809 kg ha^{-1} with thifensulfuron applied PRE fb chlorimuron/thifensulfuron applied POST (Table 6). Soybean yield in 2017 was relatively lower compared with 2016, most likely due to high common waterhemp infestation; however, effects of the herbicide program were evident (Table 6). For example, PRE fb POST programs with multiple herbicide sites-of-action resulted in a soybean yield of 895–2078 kg ha^{-1} compared with ≤546 kg ha^{-1} with ALS inhibitors. Similarly, Sarangi and Jhala (2019) reported lower yields in 2017 compared with 2016 in a conventional soybean study conducted at the same research location. One of the reasons for a higher infestation of the *Amaranthus* spp. later in the season at this location might be due to higher precipitation in 2017 growing season compared with 2016. Similarly, contrast analysis indicated greater soybean yield with PRE fb POST herbicide programs based on multiple effective herbicide sites-of-action compared with ALS inhibitors alone, and the results were in consensus with the estimates of common waterhemp control and weed biomass reduction (Table 5).

Conclusion

Results of this study showed that sulfonylureas such as rimsulfuron/thifensulfuron in mixture with flumioxazin, saflufenacil plus dimethenamid-P, or metribuzin provided effective early season weed control and crop safety in soybean with Bolt™ technology. In addition, the residual activity of sulfonylurea herbicides provides an option to include an additional site-of-action for other residual herbicides such as flumioxazin, pyroxasulfone, saflufenacil plus dimethenamid-P, or metribuzin to reduce selection pressure. Although weed species resistant to ALS inhibitors are widespread, there are several weed species sensitive to various herbicides in this group, making them an effective partner in herbicide mixtures as they result in synergistic or additive interactions and expand the weed control spectrum.

Bolt™ technology permits zero-day planting of soybean following the preplant application of sulfonyurea herbicides as an additional tool to control glyphosate-resistant weeds in zero or no-till cropping systems (Leadoff® 2017). Additionally, soybean with enhanced sulfonylurea resistance offers greater crop safety in areas with an increased risk of drift from ALS-inhibiting herbicides or in double cropping systems, including soybean after wheat (Bond et al. 2015).

Table 6. Soybean stand count and yield following sulfonylureas alone or in mixture with other site of action herbicides in sulfonylurea/glyphosate-resistant soybean in 2016 and 2017 in Nebraska.

Treatment	Application timing	Soybean Plant population (no. m^{-1})	Injury (%) 21 DAPRE	Injury (%) 14 DAPOST	Yield (kg ha^{-1}) 2016	Yield (kg ha^{-1}) 2017
Untreated control	—	16a	0	0	1811c	286c
Rimsulfuron/thifensulfuron	PRE	17a	5abc	5ab	3686ab	546bc
Chlorimuron/thifensulfuron	POST	15a	5abc	5ab	3566ab	295bc
Glyphosate	POST	16a	6abc	4ab	4297a	1508ab
Rimsulfuron/thifensulfuron fb glyphosate + chlorimuron/ thifensulfuron	PRE POST	16a	4bc	3ab	4611a	895bc
Rimsulfuron/thifensulfuron + flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	15a	11a	10a	4175ab	2363a
Rimsulfuron + chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	15a	8abc	6ab	3364ab	1761ab
Rimsulfuron/thifensulfuron + chlorimuron/metribuzin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	16a	4bc	1b	4372a	1242abc
Flumioxazin + rimsulfuron + chlorimuron fb glyphosate + chlorimuron/thifensulfuron	PRE POST	16a	7abc	4ab	4088ab	2515a
Chlorimuron/flumioxazin/thifensulfuron fb glyphosate + chlorimuron/ thifensulfuron	PRE POST	15a	7abc	5ab	4182ab	1622ab
Flumioxazin fb glyphosate + chlorimuron/thifensulfuron	PRE POST	15a	8abc	6ab	4667a	2078ab
Rimsulfuron fb chlorimuron/thifensulfuron	PRE POST	16a	4bc	4ab	3406ab	534bc
Thifensulfuron fb chlorimuron/thifensulfuron	PRE POST	16a	6abc	6ab	2809b	250c
Rimsulfuron/thifensulfuron fb glyphosate	PRE POST	16a	6abc	5ab	3815ab	749bc
Saflufenacil/imazethapyr + dimethenamid-P fb imazethapyr	PRE POST	15a	8abc	6ab	3721ab	1407abc
Saflufenacil/imazethapyr + dimethenamid-P fb chlorimuron/ thifensulfuron	PRE POST	16a	7abc	7ab	4095ab	1799ab
Flumioxazin/pyroxasulfone fb chlorimuron/thifensulfuron	PRE POST	15a	10ab	7ab	4411a	1573ab
P value		>0.05	0.0003	0.0668	0.0005	0.0113

Note: Forward slash (/) and plus (+) are used to indicate an herbicide prepackaged mixture and tankmixture, respectively. Treatments with zero response variables for all replications were not included in statistical analysis. PRE, pre-emergence; POST, post-emergence; DAPRE, days after PRE; DAPOST, days after POST; fb, followed by. Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

Currently, Bolt™ technology is available with glyphosate-resistant soybean and is expected to be available with glyphosate plus dicamba-resistant soybean, which will further expand the scope of this technology. It is important to reiterate and emphasize that integrated weed management approaches based on diversity and a process of decision support are indispensable for long-term weed control sustainability (Swanton and Weise 1991; Harker and O'Donovan 2013; Ganie et al. 2016, 2017).

Acknowledgements

We appreciate help of Irvin Schleufer, Ian Rogers, Parminder Chahal, Debalin Sarangi, and Ethann Barnes in this project. We thank Corteva Agriscience for providing seed of Bolt™ soybean for this project. This work was partially supported by the USDA National Institute of Food and Agriculture, Hatch Project No. NEB-22-396, and USDA NIFA funded Nebraska Extension Implementation Program. No conflicts of interest have been declared.

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