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Short communication

Impact of glyphosate-resistant volunteer corn (*Zea mays* L.) density, control timing, and late-season emergence on yield of glyphosate-resistant soybean (*Glycine max* L.)

Parminder S. Chahal, Amit J. Jhala^{*}

Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68583-0915, USA

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ABSTRACT

Glyphosate-resistant (GR) volunteer corn is a troublesome weed in soybean fields in a corn-soybean rotation as well as in corn fields in a continuous corn production system. The objectives of this study were to evaluate the impact of (1) different densities of GR volunteer corn on soybean yields, present as individual plants or clumps, controlled at fourth trifoliate (V4), sixth trifoliate (V6), or full flowering (R2) soybean growth stages, and (2) late-season volunteer corn emergence on soybean yields, after being controlled at different soybean growth stages. Field experiments were conducted in 2013 and 2014 under irrigated conditions in Clay County, Nebraska, and under rain-fed conditions in Lancaster County, Nebraska, USA. To maintain the desired number of isolated volunteer corn plants (1250, 2500, 5000, and 10,000 plants ha^{-1}) and clumps (63, 125, 250, and 500 clumps ha^{-1}), individual seeds and/or corn ears were hand-planted in each plot based on their respective target densities. Volunteer corn was controlled with applications of clethodim at V4, V6, or R2 soybean growth stages. Late-season volunteer corn emergence had no effect on soybean yield with volunteer corn densities and control timings at both locations in 2013 and 2014. During the first year of study at Clay County, volunteer corn densities and control timings had no effect on soybean yield. When volunteer corn was left uncontrolled or controlled at the R2 soybean growth stage, yield was the lowest at highest isolated volunteer corn plants (10,000 plants ha⁻¹) plus clump density (500 clumps ha⁻¹) during the second year of study in Clay County (\leq 5068 kg ha⁻¹) and during both years of study in Lancaster County (\leq 1968 kg ha⁻¹).

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1. Introduction

The development and commercial cultivation of glyphosateresistant (GR) crops has allowed growers to apply glyphosate, a non-selective and foliar active herbicide, as over-the-top application in GR crops for broad-spectrum weed control. In the United States, 93% of soybean (*Glycine max* L.) and 85% of corn (*Zea mays* L.) planted in 2013 were GR (Green, 2014). Despite many economic and agronomic advantages to growers, a continuous cultivation of GR corn and soybean in rotation and an almost exclusive reliance on glyphosate for weed control in the Midwestern United States has raised several concerns, including the evolution of GR weeds and the management of GR volunteer corn in GR corn and soybean (Davis et al., 2008; Marquardt et al., 2012).

* Corresponding author. E-mail address: Amit.Jhala@unl.edu (A.J. Jhala). No-till agricultural system has been gained popularity because growers can maintain a profitable crop production by reducing labor and fuel input while also restricting topsoil erosion in agricultural fields (Brown et al., 1989; Griffith et al., 1986; Hairston et al., 1984). However, weed control under this system is primarily dependent on the use of herbicides (Buhler, 1988; Coffman and Frank, 1991; Koskinen and McWhorter, 1986). Similarly, the adoption of conservation and no-tillage systems in corn-soybean cropping systems has favored the survival of volunteer corn, usually through leftover corn seeds/ears on the soil surface or at shallow soil depths, whereas seeds are usually buried deep in the soil in conventional tillage systems (Steckel et al., 2009). Volunteer corn has the ability to germinate and emerge from seeds present at the soil surface as well as from seeds buried up to 15 cm in the soil (Chahal, 2014).

Volunteer corn, depending on density, may reduce soybean yield if not controlled. In Minnesota, a uniform corn density of 0.4 plants m^{-1} of soybean row caused a 14–49% yield reduction







depending on the location and year (Andersen et al., 1982). Wilson et al. (2010) reported that a volunteer corn density of 8750 and 17,500 plants ha⁻¹ reduced soybean yields by 10 and 27%, respectively, in Nebraska. Clumps of volunteer corn plants cause more soybean yield losses compared with individual plant. Andersen et al. (1982) reported a 31–83% reduction in soybean yield from volunteer corn clump densities increasing from 1 to 4 clumps spaced between every 2.4 m of soybean row.

Management of volunteer corn is challenging due to the ineffectiveness of pre-emergence, soil-applied herbicides registered in soybean (Beckett and Stoller, 1988), which provide only partial control (Chahal et al., 2014). Therefore, post-emergence application of acetyl-coenzyme A carboxylase (ACCase) inhibiting-herbicides is the only option for controlling GR volunteer corn in GR soybean (Beckett and Stoller, 1988; Beckett et al., 1992; Chahal et al., 2014; Deen et al., 2006; Marquardt and Johnson, 2013; Young and Hart, 1997). Indeed, the majority of growers control volunteer corn when it is visible above the soybean canopy, but this can result in early-season competition and may reduce yield depending on the density of the volunteer corn.

Soybean yield could be improved by identifying the critical period for controlling volunteer corn emerging early and late in the season. The critical period for weed control in soybean is longer under the no-till system starting from VC (unrolled unifoliate leaves) or V1 (1st trifoliate) to R1 or beginning flowering stage (Halford et al., 2001) compared with conventional tillage systems (VC to V4) at 2.5% yield loss (Van Acker et al., 1993). Density of a weed competing with crops throughout the season is an important factor in determining sovbean yield loss (Stoller et al., 1987): therefore, longer volunteer corn interference periods at higher densities might contribute to yield loss in soybean. Volunteer corn plants emerging late in the season could also provide competition in soybean and might result in yield loss. The effect of different volunteer corn densities on soybean yield has been studied and discussed in the literature (Andersen et al., 1982; Stoller et al., 1987; Wilson et al., 2010); however, scientific literature is not available about the integrated effect of volunteer corn densities, control timings, and late-season emergence on soybean yield. The objectives of this study were to determine the impact of (1) different densities of GR volunteer corn on soybean yields, present as individual plants or clumps, controlled at fourth trifoliate (V4), sixth trifoliate (V6), or full flowering (R2) soybean growth stages, and (2) late-season volunteer corn emergence on soybean yields after being controlled at different soybean growth stages.

2. Materials and methods

Field experiments were conducted at two locations in 2013 and 2014 at the South Central Agricultural Laboratory (SCAL) (40° 34' 12" N, 98° 7' 48" W), Clay Center, Clay County, Nebraska, and at Havelock Farm (40° 51' N, 96° 36' W), University of Nebraska-Lincoln, Lincoln, Lancaster County, Nebraska, USA. The soil texture at Clay County was silty clay loam with a pH of 6.5, 17% sand, 58% silt, 25% clay, and 2.5% organic matter, and the soil texture at Lancaster County was silty clay loam with a pH of 5.6, 19% sand, 54% silt, 27% clay, and 3% organic matter. The experimental site at Clay County was under irrigated conditions and at Lancaster County was under rain-fed/dryland conditions. Daily average temperature and daily total rainfall data for 2013 and 2014 growing season and the 30-year average (1983-2012) at both the experimental locations is provided in Figs. 1 and 2. Glyphosate-resistant soybean (Cv. 'Fontanelle 64R 20') was drilled in rows spaced 76 cm apart at a rate of 375,000 seeds ha^{-1} at Clay County (June 4, 2013 and May 19, 2014) and Lancaster County (June 17, 2013 and May 17, 2014). Whole or broken ear losses could occur up to 3–4% of the total crop

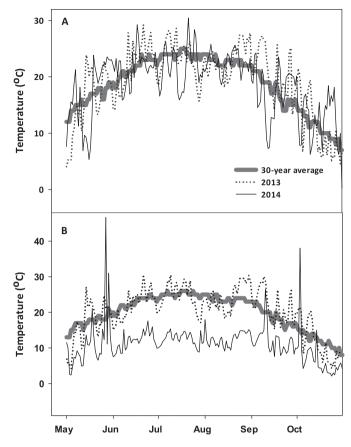


Fig. 1. Daily average air temperature (°C) in 2013 and 2014 growing season and the 30 year average (1983–2012) at (A) South Central Agricultural Laboratory (SCAL), Clay Center, Clay County and (B) Havelock Farm, University of Nebraska–Lincoln, Lincoln, Lancaster County, Nebraska, USA. Weather data were obtained from the High Plains Regional Climate Center (HPRCC; http://www.hprcc.unl.edu).

yield (Shay et al., 1993); therefore, it was assumed that the number of whole corn ears lost during the corn harvest usually comprises 5% of the individual corn seed density. To maintain the desired number of isolated volunteer corn plants (1250, 2500, 5000, and 10,000 plants ha^{-1}) and clump densities (63, 125, 250, and 500 ha^{-1}), individual corn seeds and whole ears were hand-planted in each plot based on their respective target densities at Clay County (June 13, 2013 and May 25, 2014) and Lancaster County (June 21, 2013 and May 23, 2014). A nontreated control with no volunteer corn seeds or ears planted was included for comparison.

The experiment was arranged in a split-split plot design with volunteer corn density treated as the main plot. The split-plot was volunteer corn control timings depending on soybean growth stages (V4, V6, or R2), and the split-split plot was late-season volunteer corn emergence. The split-split plot size at Clay and Lancaster County was 3×13 m and 3×15 m, respectively, and the treatments were replicated four times. In the split-plot, volunteer corn was allowed to compete with soybean until harvest or was controlled at the V4, V6, or R2 soybean growth stages by applications of clethodim (Select Max, Valent USA Corporation, Walnut Creek, CA 94596) at 76 g ai ha^{-1} at the V4 stage and 136 g ai ha^{-1} at the V6 and R2 soybean growth stages. Clethodim treatments were prepared in distilled water and mixed with nonionic surfactant (NIS, Induce, Helena Chemical Co., Collierville, TN) at 0.25% v/v. Prior to mixing clethodim, ammonium sulfate (AMS, DSM Chemicals North America Inc., Augusta, GA) was added to the distilled water at 2.5% wt/v. In the split-split plot, volunteer corn plants that

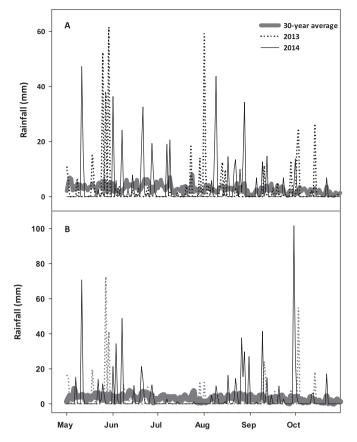


Fig. 2. Daily total rainfall (mm) in 2013 and 2014 growing season and the 30 year average (1983–2012) at (A) South Central Agricultural Laboratory (SCAL), Clay Center, Clay County and (B) Havelock Farm, University of Nebraska–Lincoln, Lincoln, Lancaster County, Nebraska, USA. Weather data were obtained from the High Plains Regional Climate Center (HPRCC; http://www.hprcc.unl.edu).

emerged after clethodim treatments were either allowed to grow until harvest or removed every fifteen days (in case of new emergence) until harvest using a hand hoe. Volunteer corn plants were 7–10 cm, 17–23 cm, and 45–60 cm tall at Clay County, and 5–8 cm, 14–17 cm, and 40–52 cm tall at Lancaster County when treated at the V4, V6, and R2 soybean growth stages, respectively, in 2013 and 2014.

To minimize competition from grass [giant foxtail (Setaria faberi Herrm.)] and broadleaf weeds [common waterhemp (Amaranthus rudis Sauer) and velvetleaf (Abutilon theophrati Medik.)], S-metolachlor (Dual-II Magnum, Syngenta Crop Protection, Greensboro, NC 27419) at 1.63 kg ai ha^{-1} and glyphosate (Touchdown, Syngenta Crop Protection) at 1.06 kg ae ha⁻¹ plus ammonium sulfate at 2.5% wt/v was applied pre-plant (2 days before soybean planting). Glyphosate was applied as a post-emergence application at Clay County (July 10, 2013 and June 20, 2014) and Lancaster County (July 7, 2013 and June 23, 2014) to avoid in-season competition with other grass and broadleaf weeds. The meaning of nontreated control plots in this project is without volunteer corn, but above mentioned herbicides were applied to keep them weed free as well as by manually removing weeds using a hand hoe. All herbicide applications were made by using a CO₂-pressurized backpack sprayer consisting of a four-nozzle boom fitted with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., P. O. Box 7900, Wheaton, IL 60189) calibrated to deliver 140 L ha⁻¹ at 276 kPa.

Soybean growth stages were carefully observed at regular intervals from the time of soybean emergence until the last application of clethodim at the R2 or full-flowering stage to control volunteer corn at desired soybean growth stages (V4, V6, or R2). On maturity, middle two rows of soybeans were harvested with a small-plot combine from 1.5×13 m and 1.5×15 m split—split plots at Clay County and Lancaster County, respectively, and yields were adjusted to 13% moisture content.

2.1. Statistical analyses

The PROC GLIMMIX procedure of SAS version 9.3 (SAS Institute Inc, Cary, NC) was used for data analysis. Data were analyzed separately for irrigated (Clay County) and rain-fed (Lancaster County) sites. For both the experimental sites, treatments (volunteer corn densities, control timing, and late-season emergence) and years were considered as fixed effects, while replications were considered as random effect. No significant year-by-treatment interaction for soybean yield was observed for the Lancaster County site (Table 1); therefore, soybean yield data were combined over years (Table 3). However, year-by-treatment interaction for soybean and presented separately (Table 2). Where the ANOVA indicated treatment effects were significant, means were separated at $P \le 0.05$ using Tukey–Kramer's pairwise comparison test.

3. Results and discussion

Late-season volunteer corn emergence had no effect on soybean yield at Clay County (P = 0.228) and Lancaster County research sites (P = 0.201) in 2013 and 2014 (Table 1); therefore, soybean yield data were combined across late-season volunteer corn emergence. Clethodim provided >90% control of volunteer corn plants and clumps when applied at the V4 or V6 soybean growth stages and >85% control at the R2 growth stage at 21 DAT (data not shown). Similarly, Marquardt and Johnson (2013) reported no difference in the control of different densities of volunteer corn with clethodim applied early (\leq 30 cm tall volunteer corn) or late (90 cm tall volunteer corn) in the season. In another study, 50–60 cm tall volunteer corn was controlled >90% with clethodim applied POST at a rate of 51 g ai ha⁻¹ (Alms et al., 2015). Additionally, Deen et al. (2006) reported that including surfactant improved the efficacy of clethodim for controlling GR volunteer corn.

Volunteer corn densities and control timings had no effect on soybean yield in Clay County in 2013 (Table 2). In 2014, soybean yield was not affected with volunteer corn density of \leq 5000 plants ha⁻¹ combined with \leq 250 clumps ha⁻¹ regardless of volunteer corn left uncontrolled or controlled at V4, V6, or R2 growth stage. At the highest density of volunteer corn plants (10,000 plants ha^{-1}) combined with 500 clumps ha^{-1} , soybean yield reduced to \leq 5265 kg ha⁻¹ when GR volunteer corn was left uncontrolled and controlled at V6 or R2 soybean stage, respectively, compared with volunteer corn at lower densities $(\leq 5000 \text{ plants ha}^{-1} \text{ combined with } \leq 250 \text{ clumps ha}^{-1})$, left uncontrolled, or controlled at V4, V6, or R2 soybean growth stages (5337 kg ha⁻¹) in 2014. The possible reason for the non-significant effect of volunteer corn densities and control timings on soybean yield at Clay County in 2013 could be the reduction of soybean yield almost to half for all the volunteer corn densities compared with 2014 due to hail and storm damage before the soybean harvest. Under dryland conditions at Lancaster County, soybean yield was reduced (<1880 kg ha⁻¹) at the highest volunteer corn (10,000 plants ha⁻¹) and clump densities (500 clumps ha⁻¹) left uncontrolled or controlled at R2 soybean growth stage compared with soybean yield at lower volunteer corn densities (\leq 5000 plants ha⁻¹ combined with \leq 250 clumps ha⁻¹), left

Table 1

P values from model analysis of the effect of glyphosate-resistant volunteer corn densities, control timings, and late-season emergence on glyphosate-resistant soybean yield in field experiments conducted at Clay County and Lancaster County, Nebraska, USA in 2013 and 2014.

Parameters	Clay county (irrigated)	Lancaster county (rain-fed)
	P-value	
Volunteer corn density	0.004	0.034
Control timings	0.039	0.010
Volunteer corn density*Control timings	0.001	<0.0001
Late-season emergence	0.228	0.201
Volunteer corn density*Late-season emergence	0.643	0.943
Control timings*Late-season emergence	0.691	0.762
Volunteer corn density*Control timings*Late-season emergence	0.848	0.440
Year	<0.0001	0.483
Volunteer corn density*Year	0.026	0.383
Control timings*Year	0.007	0.071
Volunteer corn density*Control timings*Year	0.034	0.606
Late-season emergence*Year	0.620	0.204
Volunteer corn density*Late-season emergence*Year	0.641	0.866
Control timings*Late-season emergence*Year	0.569	0.420
Volunteer corn density*Control timings*Late-season emergence*Year	0.982	0.792

Table 2

Effect of glyphosate-resistant volunteer corn densities, control timings, and lateseason emergence on glyphosate-resistant soybean yield in field experiments conducted in Clay County, Nebraska, USA under irrigated conditions in 2013 and 2014.^a

Volunteer corn density ^b		Control timing ^c	Soybean y	Soybean yield ^d	
			2013	2014	
plants ha ⁻¹	clumps ha^{-1}		kg ha ⁻¹		
0	0	_	2936a	5683a	
1250	63	No	3067a	5621a	
1250	63	V4	2691a	5453a	
1250	63	V6	2960a	5474a	
1250	63	R2	2827a	5617a	
2500	125	No	2789a	5511a	
2500	125	V4	2929a	5337a	
2500	125	V6	2815a	5459a	
2500	125	R2	2936a	5420a	
5000	250	No	2697a	5564a	
5000	250	V4	2956a	5528a	
5000	250	V6	2860a	5485a	
5000	250	R2	3046a	5448a	
10,000	500	No	2901a	4994b	
10,000	500	V4	2765a	5417a	
10,000	500	V6	2785a	5265ab	
10,000	500	R2	2812a	5068b	
P-value	_	-	0.5044	0.0086	

^a Year-by-treatment interaction was significant; therefore, soybean yield data are presented separately for both years.

^b Desired individual volunteer corn plant densities were maintained in the soybean field by planting individual volunteer corn seeds/kernels. Whole corn ears were planted at 5% of individual kernel density to maintain clumps of volunteer corn in soybean field.

^c Abbreviations: No, no control of volunteer corn plants; V4, V6, R2, volunteer corn plants were controlled at fourth trifoliate, sixth trifoliate, or full-flowering soybean growth stages, respectively.

^d Means within columns with common letter(s) are not significantly different according to Tukey–Kramer's pair-wise comparison test at P \leq 0.05. In 2013, soybean yield was reduced almost to half for all the volunteer corn densities compared with 2014 due to hail and storm damage before the soybean harvest and that could have resulted for the non-significant effect of volunteer corn densities and control timings on soybean yield in 2013.

uncontrolled, or controlled at V4, V6, or R2 soybean growth stages (\geq 2322 kg ha⁻¹) (Table 3). In contrast, Marquardt and Johnson (2013) reported no difference in soybean yield at different densities of volunteer corn (500–160,000 plants ha⁻¹) controlled early or later in the season. Similar to irrigated conditions at Clay County, soybean yield was not affected at Lancaster County when volunteer corn density was \leq 5000 plants ha⁻¹ combined with \leq 250 clumps ha⁻¹ regardless of volunteer corn left uncontrolled or

Table 3

Effect of glyphosate-resistant volunteer corn densities, control timings, and lateseason emergence on glyphosate-resistant soybean yield in field experiments conducted in Lancaster County, Nebraska, USA under rain-fed (dryland) conditions in 2013 and 2014.^a

Volunteer corn density ^b		Control timing ^c	Soybean yield ^d	
plant ha ⁻¹	clumps ha^{-1}		kg ha ⁻¹	
0	0	_	2416a	
1250	63	No	2453a	
1250	63	V4	2435a	
1250	63	V6	2338a	
1250	63	R2	2402a	
2500	125	No	2370a	
2500	125	V4	2548a	
2500	125	V6	2352a	
2500	125	R2	2459a	
5000	250	No	2356a	
5000	250	V4	2322a	
5000	250	V6	2402a	
5000	250	R2	2558a	
10,000	500	No	1876b	
10,000	500	V4	2392a	
10,000	500	V6	2431a	
10,000	500	R2	1968b	
P-value	_	-	0.0165	

^a Year-by-treatment interaction was not significant; therefore, data were combined over years.

^b Desired individual volunteer corn plant densities were maintained in the soybean field by planting individual volunteer corn seeds/kernels. Whole corn ears were planted at 5% of individual kernel density to maintain clumps of volunteer corn in soybean.

^c Abbreviations: No, no control of volunteer corn plants; V4, V6, R2, volunteer corn controlled at fourth trifoliate, sixth trifoliate, or full-flowering soybean growth stages, respectively.

 d Means within columns with common letter(s) are not significantly different according to Tukey–Kramer's pair-wise comparison test at P \leq 0.05.

controlled at V4, V6, or R2 growth stage.

Volunteer corn in soybean fields are usually composed of isolated as well as clumps of several corn plants, but clumps are often more competitive than individual plants at a particular density (Andersen et al., 1982). Beckett and Stoller (1988) reported soybean yield losses of 21 and 51% at volunteer corn clump densities of 5380 and 10,760 clumps ha⁻¹, respectively. In this study, volunteer corn clump densities of \leq 500 clumps ha⁻¹ were maintained; therefore, clumps along with individual plants did not play an important role in causing soybean yield reduction, except at the highest isolated volunteer corn plant density (10,000 plants ha⁻¹) combined with the highest number of clumps (500 ha^{-1}).

Most of the late-emerging volunteer corn, after being controlled at different control timings (V4, V6, or R2), were emerged from clumps rather than from individual plants. Thus, relatively lower clump densities (\leq 500 clumps ha⁻¹) have accounted for a lower soybean response to late-emerged volunteer corn in terms of yield, as more competition could have resulted at higher volunteer corn clump densities (>500 clumps ha⁻¹). Similarly, no effect of volunteer corn competition at lower densities (\leq 5000 plants ha⁻¹) was observed on soybean yield when controlled at different timings. A more significant soybean yield loss might have occurred with higher volunteer corn plants and clump densities as reported by Beckett and Stoller (1988) and Alms et al. (2015).

Results of this study indicated that volunteer corn control timings did not impact soybean yield at lower volunteer corn densities (\leq 5000 isolated plants and <500 clumps ha⁻¹); however, an early application of clethodim is advisable from an insect-resistance management viewpoint if volunteer corn plants also express the Bacillus thuringiensis (Bt) trait. Volunteer corn plants expressing the Bt gene provide extra selection pressure to the targeted insect pests against the Bt toxin (Krupke et al., 2009). Additionally, volunteer corn encourages the survival and dispersal of corn rootworm by acting as a host plant and providing feeding options for rootworm larvae in soybean field (Shaw et al., 1978), thus limiting the benefits of corn-soybean rotation (Krupke et al., 2009; Marguardt et al., 2012). To reduce the risk of corn rootworms, the interference of volunteer corn during harvesting, and the contamination of harvested sovbeans from volunteer corn seeds, volunteer corn plants should be controlled even if they do not reduce sovbean vields (Deen et al., 2006). The ACCase-inhibitors should be tank-mixed with herbicides belonging to different modes of action, such as glufosinate in glufosinate-resistant soybean (Chahal and Ihala, 2015). In summary, an integrated volunteer corn management program could be adopted that may include herbicides, crop rotation, and improved cultural agronomic practices to maximize control and reduce the potential for the evolution of herbicideresistant weeds (Chahal et al., 2015).

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