Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean

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Jhala, A. J., Malik, M. S. and Willis, J. B. 2015. **Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean**. Can. J. Plant Sci. **95**: 973–981. Acetochlor, an acetamide herbicide, has been used for many years for weed control in several crops, including soybean. Micro-encapsulated acetochlor has been recently registered for preplant (PP), pre-emergence (PRE), and post-emergence (POST) application in soybean in the United States. Information is not available regarding the sequential application of acetochlor for weed control and soybean tolerance. The objectives of this research were to determine the effect of application timing of micro-encapsulated acetochlor applied in tank-mixture with glyphosate in single or sequential applications for weed control in glyphosate-resistant soybean, and to determine its impact on soybean injury and yields. Field experiments were conducted at Clay Center, Nebraska, in 2012 and 2013, and at Waverly, Nebraska, in 2013. Acetochlor tank-mixed with glyphosate applied alone PP, PRE, or tank-mixed with flumioxazin, fomesafen, or sulfentrazone plus chlorimuron provided 99% control of common waterhemp, green foxtail, and velvetleaf at 15 d after planting (DAP); however, control declined to $\leq$40% at 100 DAP. Acetochlor tank-mixed with glyphosate applied PRE followed by early POST (V2 to V3 stage of soybean) or late POST (V4 to V5 stage) resulted in $\geq$90% control of common waterhemp and green foxtail, reduced weed density to $\leq$2 plants m^{-2} and biomass to $\leq$12 g m^{-2}, and resulted in soybean yields >3775 kg ha^{-1}. The sequential applications of glyphosate plus acetochlor applied PP followed by early POST or late POST resulted in equivalent weed control to the best herbicide combinations included in this study and soybean yield equivalent to the weed free control. Injury to soybean was <10% in each of the treatments evaluated. Micro-encapsulated acetochlor can be a good option for soybean growers for controlling grasses and small-seeded broadleaf weeds if applied in a PRE followed by POST herbicide program in tank-mixture with herbicides of other modes of action.

Key words: Acetamide, crop tolerance, post-emergence herbicides, residual herbicides, resistance management, sequential applications, tank-mixture, weed biomass

Jhala, A. J., Malik, M. S. et Willis, J. B. 2015. **Désherbage par applications séquentielles de microcapsules d'acétochlor dans le soja résistant au glyphosate et tolérance de la culture au traitement**. Can. J. Plant Sci. **95**: 973–981. On utilise l'acétochlor depuis de nombreuses années pour lutter contre les mauvaises herbes dans diverses cultures, dont le soja. Les États-Unis ont homologué depuis peu les microcapsules de cet herbicide à base d'acétamide comme traitement avant les semis, avant la levée et après la levée pour le soja. Toutefois, on ne possède pas d'informations sur le désherbage par applications séquentielles d'acétochlor, ni sur la tolérance du soja à un tel traitement. La présente recherche devait établir les conséquences du moment d'application d'un mélange de microcapsules d'acétochlor et de glyphosate en un traitement unique ou en traitement séquentiel pour lutter contre les mauvaises herbes dans les cultures de soja résistant au glyphosate; elle devait aussi préciser l'impact du traitement sur les dommages causés au soja et sur le rendement de la culture. Les expériences ont été réalisées au champ, à Clay Center (Nebraska), en 2012 et 2013, ainsi qu'à Waverly (Nebraska), en 2013. L'application du mélange d'acétochlor et de glyphosate avant les semis ou la levée, seul ou en combinaison avec de la flumioxazine, du fomesafen ou du sulfentrazone plus du chlorimuron détruit 99 % de l'amaranthe rugueuse, de la sétaire verte et de l'abutilon quinze jours après les semis. Cependant, le désherbage diminue à 40 % et moins cent jours après les semis. Le mélange d'acétochlor et de glyphosate appliqué avant la levée, puis au début (stade V2 ou V3 du soja) ou à la fin de la levée (stade V4 ou V5) détruit au moins 90 % de l'amaranthe rugueuse et de la sétaire verte, ce qui réduit la densité des adventices à un maximum de 2 plants par m^2 et leur biomasse à un maximum de 12 g par m^2, avec pour résultat un rendement du soja supérieur à 3 775 kg par hectare. Les applications séquentielles de glyphosate et d'acétochlor avant les semis, puis au début ou à la fin de la levée assurent une lutte équivalente aux meilleures combinaisons d'herbicides examinées dans le cadre de l'étude et débouchent sur un rendement en soja équivalent à celui de la parcelle témoin désherbée. Les traitements évalués causent moins de dix pour cent de dommages au soja. Les microcapsules d'acétochlor

Abbreviations: **AMATA**, common waterhemp, *Amaranthus rudis* Sauer; **ABUTH**, green foxtail, *Setaria viridis* (L.) P. Beauv; **DAP**, days after planting; **DBP**, days before planting; **POST**, post-emergence application; **PP**, preplant application; **PRE**, pre-emergence application; **SETVI**, velvetleaf, *Abutilon theophrasti* Medik

pourraient constituer une bonne solution de rechange aux cultivateurs de soja pour lutter contre les graminées et les dicotylédones à petite graine quand l'application a lieu avant la levée et est complétée par un désherbage post-levée grâce à un mélange composé de microcapsules et d'herbicides à mode d'action différent.

Mots clés: Acétamide, tolérance des cultures, herbicides post-levée, herbicides résiduels, gestion de la résistance, applications séquentielles, mélange en citerne, biomasse des mauvaises herbes

After corn (*Zea mays* L.), soybean (*Glycine max* L. Merr.) is the most widely grown field crop in the United States (United States Department of Agriculture 2012). Weed management is an important component of soybean production to secure higher yields (Norsworthy 2003). Application of pre-emergence (PRE) and/or post-emergence (POST) herbicides is the primary method for weed control in soybean (Riar et al. 2013). The acetamides are widely used in corn, cotton (*Gossypium hirsutum* L.), sorghum [*Sorghum bicolor* (L.) Moench], and soybean for control of annual grasses and small-seeded broadleaf weeds. Acetochlor is listed as the fourth most commonly used herbicide in the United States by the United States Environmental Protection Agency (USEPA) (Grube et al. 2011). The solubility of acetochlor in water is 223 mg L^{-1} at 25°C and generally provides 3 to 5 wk of residual weed control, depending on application rate, soil type, weed pressure, and weather conditions (Parker et al. 2005). Acetochlor is primarily absorbed by emerging plant shoots and seedling roots, and is xylem-transported by acropetal movement (Boger et al. 2000). Susceptible grass weeds that do emerge appear twisted and malformed with leaves tightly rolled in the whorl that cannot unfurl properly, while broadleaf seedlings may have slightly cupped or crinkled leaves, especially under cold conditions (Weisshaar and Boger 1987).

Acetamide herbicides are usually applied PRE in corn and soybean; however, micro-encapsulated acetochlor has been recently registered for PP, PRE, and POST application in soybean (Anonymous 2011a). Acetochlor is sometimes applied 14 to 60 d before planting as an early preplant (EPP) application (Bunting et al. 2003), which may eliminate the need for an early spring burndown herbicide application (Stougaard et al. 1984). Several PRE-applied, residual herbicides, including acetochlor, have been registered in soybean that can be applied POST and have good crop safety (Nurse et al. 2007; Soltani et al. 2009). It is expected that microencapsulated acetochlor will improve crop safety and provide up to 30 to 40 d of residual weed control.

The application rate of micro-encapsulated acetochlor in soybean is 1.05 to 1.68 kg a.i. ha^{-1} in a single application depending on soil type and weed pressure (Anonymous 2011a). It can also be applied sequentially, with a maximum cumulative amount of 3.37 kg a.i. ha^{-1} per season. Weeds already emerged at the time of application are not controlled by acetochlor; therefore, it should be tank-mixed with a broad-spectrum herbicide such as glyphosate to control emerged weeds in glyphosate-resistant soybean. Weed control can be increased by delaying glyphosate applications to allow new weeds to emerge (Tharp and Kells 1999); however, delayed application can result in soybean yield reduction as a result of crop–weed competition during the initial growth stage. Therefore, tank-mixing glyphosate and acetochlor may provide better weed control compared with either herbicide applied alone. There is no information in the scientific literature on the effect of sequential applications of glyphosate plus acetochlor in respect to weed control and soybean tolerance.

Glyphosate has been used extensively for POST weed control in glyphosate-resistant crops for more than a decade; however, herbicide programs depending only on glyphosate resulted in the evolution of glyphosate-resistant weeds (Owen and Zelaya 2005). Therefore, soybean growers are tank-mixing herbicides with glyphosate to expand the utility of glyphosate-based herbicide programs (Young 2006). In addition, tank-mixing herbicides with different modes of action is one of the methods to control herbicide-resistant weeds (Beckie 2006; Norsworthy et al. 2012; Mellendorf et al. 2013). For example, acetochlor has been premixed with atrazine (Anonymous 2012a,b) as well as with clopyralid and flumetsulam for PRE weed control in corn (Anonymous 2011b).

Weed control programs that include herbicides with multiple modes of action are needed due to the rapid increases in the number of herbicide-resistant weeds (Kaur et al. 2014). Soybean growers can benefit from micro-encapsulated acetochlor applied PRE or POST in tank-mix with other registered herbicides that provide multiple modes of action. A combination of foliar active and residual herbicides is particularly important for control of certain weed species such as common waterhemp that emerge throughout the growing season (Steckel et al. 2002). To develop weed management programs involving micro-encapsulated acetochlor in soybean, more information about the application timings of acetochlor at different growth stages of soybean is required. The objectives of this research were to determine weed control efficacy of micro-encapsulated acetochlor applied PP, PRE, and POST in tank-mixture with glyphosate, and to evaluate soybean tolerance and yield.

MATERIALS AND METHODS

Experimental Procedures

Field experiments were conducted at Clay Center, Nebraska, in 2012 and 2013 and at Waverly, Nebraska, in 2013. The soil at Clay Center was silty clay loam with a pH of 6.5, 17% sand, 58% silt, 25% clay, and 2.5% organic matter. The soil at Waverly was a silty clay loam and had a pH of 6.3, 19% sand, 56% silt, 25% clay, and 2.3% organic matter. The experiments were arranged in a randomized complete block design with four replications. The experiment was under no-till condition and corn was the previous crop at both sites.

Glyphosate-resistant soybean (cv. Pioneer 92Y70 at Clay Center and AG 2931 at Waverly) was seeded on May 07 and May 24 in 2012 and 2013, respectively, at Clay Center and on 2013 May 16 at Waverly. The seeds were seeded 3 cm deep in rows spaced 76 cm apart. The plot size was 3 × 9 m and comprised four soybean rows. Several treatments were compared for weed control and crop tolerance (Table 1). An untreated control and a weed-free control were included in each replicate for comparison. The application rates of herbicides other than acetochlor were selected based on the recommended labeled rates. Most of the acetochlor treatments were selected based on the recommended labeled rates; however, sequential application of acetochlor three times at 1.68 kg a.i. ha^{-1} (PP or PRE followed by early and late POST) were included for comparison and to determine crop safety.

Herbicides were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPa equipped with a four-nozzle boom fitted with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189). Herbicide treatments were applied PP (2012 Apr. 23 and 2013 May 09), PRE (2012 May 09 and 2013 May 25), early- POST (2012 Jun. 12 and 2013 Jun. 28), and late-POST (2012 Jul, 03 and 2013 Jul. 19) at Clay Center, Nebraska. Herbicide treatments were applied PP (2013 May 01), PRE (2013 May 17), early-POST (2013 Jun. 05), and late-POST (2013 Jun. 17) at Waverly, Nebraska. Herbicide treatments at each application timing were tank-mixed with glyphosate at 870 g a.e. ha^{-1} plus ammonium sulfate at 2% wt/vol. Weed-free control plots were maintained by application of glyphosate at 870 g a.e. ha^{-1} + ammonium sulfate 2% wt/vol when weeds were emerged in the plots throughout the growing season. The Clay Center site was under the central pivot irrigation system, so the crop was irrigated when required during the growing season, while the Waverly site received sufficient precipitation (>1.5 cm within 7 d of herbicide application at each application timing).

During both years, weed control was assessed visually on a scale of 0 to 100% (0% equals no control and 100% equals complete control) at 15, 30, 60, and 100 d after planting (DAP). Soybean population density was measured by counting the number of soybean seedlings emerged in a meter row length from two middle rows in each plot 10 to 12 d after soybean emergence. Herbicide injury symptoms on soybean, if any, were recorded based on a scale of 0 to 100% (0% equals no injury

Table 1. Herbicide treatments, application timing, and rates as well as products used in field experiments conducted in Nebraska, USA, in 2012 and 2013

Treatment[z,y]	Application timing[z]	Rate	Product name	Manufacturer
		(kg a.e. or a.i. ha^{-1})		
Weed-free control (glyphosate)[x]	PRE *fb* EPOST *fb* LPOST	0.87	Roundup PowerMax	Monsanto Company
Glyphosate *fb* glyphosate	EPOST *fb* LPOST	0.87 *fb* 0.87	Roundup PowerMax	Monsanto Company
Acetochlor	14 DBP	1.68	Warrant	Monsanto Company
Acetochlor	PRE	1.68	Warrant	Monsanto Company
Acetochlor	PRE	3.37	Warrant	Monsanto Company
Acetochlor + fomesafen	PRE	1.68 + 0.21	Warrant + Reflex	Monsanto Company + Syngenta Crop Protection
Acetochlor + flumioxazin	PRE	1.68 + 0.11	Warrant + Valor SX	Monsanto Company + Valent Corporation
Acetochlor + sulfentrazone + chlorimuron-ethyl	PRE	1.68 + 0.29	Warrant + Authority XL	Monsanto Company + FMC Corporations
Flumioxazin + chlorimuron *fb* acetochlor	PRE fb EPOST	0.126 *fb* 1.68	Valor XLT *fb* Warrant	Valent Corporation *fb* Monsanto Company
Acetochlor *fb* acetochlor	PRE *fb* EPOST	1.68 *fb* 1.68	Warrant *fb* Warrant	Monsanto Company
Acetochlor *fb* acetochlor	PRE *fb* LPOST	1.68 *fb* 1.68	Warrant *fb* Warrant	Monsanto Company
Acetochlor *fb* acetochlor *fb* acetochlor	PRE *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	Warrant *fb* Warrant *fb* Warrant	Monsanto Company
Acetochlor *fb* acetochlor *fb* acetochlor	14 DBP *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	Warrant *fb* Warrant *fb* Warrant	Monsanto Company

[z]Abbreviations: DBP, days before planting; *fb*, followed by; EPOST, early post-emergence; LPOST, late post-emergence.
[y]All herbicide treatments at each application timings were tank-mixed with glyphosate at 0.87 kg a.e. ha^{-1} + ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA) at 2% wt/vol.
[x]Weed-free plots were maintained by application of glyphosate at 0.87 kg a.e. ha^{-1} + ammonium sulfate 2% wt/vol when weeds were emerged in the plots throughout the growing season.

and 100% equals complete death of soybean plant) at 7 and 14 d after herbicide treatment. Weed density was determined from two randomly selected 0.25-m^2 quadrats per plot at 15, 30, 60, and 100 DAP. The weeds that survived herbicide treatments were cut at the stem base close to the soil surface from two randomly selected 0.25-m^2 quadrats per plot, placed in paper bags, dried in an oven for 72 h at 65°C, and the weight was recorded. Soybean was harvested using a plot combine and yields were adjusted to 13% moisture content.

Statistical Analyses

Data were subjected to ANOVA using the PROC MIXED procedure in SAS software version 9.3 (SAS Institute Inc, Cary, NC). Data were tested for normality with the use of PROC UNIVARIATE. Weed control, density, and biomass data were arcsine square-root transformed before analysis; however, back-transformed data are presented with the mean separation based on transformed data. Year, experimental site, and herbicide treatments were considered fixed effects, while replication was considered a random effect in the model. If the year-by-treatment, and site-by-treatment interaction was non-significant, data from the 2 yr and sites were combined. However, if the year-by-treatment or site-by-treatment interaction was significant, data were analyzed separately by year and site. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ using Tukey–Kramer's pairwise comparison test.

RESULTS AND DISCUSSION

Year-by-treatment and site-by-treatment interactions for weed control, weed density, and biomass were not significant; therefore, data were combined and presented. The primary weed species present, which were common to all sites, were common waterhemp, green foxtail, and velvetleaf. Soybean population density was not affected by acetochlor applied alone 14 d before planting (DBP), PRE, or in tank-mix with fomesafen, flumioxazin, or sulfentrazone plus chlorimuron-ethyl (Table 2). All herbicide treatments resulted in 99% control of common waterhemp, green foxtail, and velvetleaf at 15 DAP, except when glyphosate was applied alone. However, weed control varied between treatments at 60 DAP. For example, acetochlor applied 14 DBP resulted in 70, 68, and 72% control of common waterhemp, green foxtail, and velvetleaf, respectively. Acetochlor applied alone at 1.68 or 3.37 kg a.i. ha^{-1} or in tank-mix with flumioxazin, fomesafen, or sulfentrazone plus chlorimuron resulted in $\leq$82% control of common waterhemp and green foxtail, with no difference among them compared with acetochlor applied sequentially. Acetochlor applied PRE followed by early-POST (V2 to V3 stage of soybean) or late-POST (V4 to V5 stage of soybean) resulted in $\geq$95% control of common waterhemp, green foxtail, and velvetleaf, and was comparable with weed-free treatment and acetochlor applied PP or PRE followed by early and late POST (Table 2).

Residual activity of acetochlor applied PP or PRE alone or in tank-mix with flumioxazin, fomesafen, or sulfentrazone plus chlorimuron declined and resulted in poor weed control ($\leq$60%) by 100 DAP. For example, one pass acetochlor treatments resulted in $\leq$35% control of common waterhemp and velvetleaf and $\leq$55% control of green foxtail compared with a two-pass acetochlor program that resulted in $\geq$92, 75, and 88% control of common waterhemp, velvetleaf, and green foxtail, respectively, at 100 DAP (Table 2). Persistence of acetochlor is affected by many factors, including soil organic matter, microbial degradation, and leaching (Beestman and Deming 1974; Gish et al. 1995). Several studies reported that one-pass weed control programs are insufficient to provide season-long weed control in soybean, and that PRE followed by POST herbicide programs are required, especially to control weeds that emerge later in the season, such as common waterhemp (Nurse et al. 2007; Stewart et al. 2011). Acetochlor applied PRE followed by early- or late-POST was comparable with weed-free control, acetochlor applied sequentially three times, as well as flumioxazin plus chlorimuron applied PRE followed by acetochlor plus glyphosate applied POST. Hausman et al. (2013) reported 93 and 88% control of common waterhemp 30 and 60 DAT, respectively, with acetochlor applied at 3.36 kg a.i. ha^{-1}. Acetochlor applied sequentially (three times) at 1.68 kg a.i. ha^{-1} in a tank-mix with glyphosate provided season-long weed control and had excellent crop safety; however, it cannot be recommended because the rate of acetochlor exceeds the maximum labeled rate of 3.37 kg a.i. ha^{-1} in a year (Anonymous 2011a). It is also highly unlikely that soybean growers will apply a residual herbicide three times in a season. However, soybean injury was <10% and transient even with three time applications of acetochlor in a season (data not shown).

Weed densities differed between herbicide treatments (Table 3). The untreated control had the highest common waterhemp, green foxtail, and velvetleaf density at 15 DAP and it was comparable with glyphosate-only treatment because glyphosate was not yet applied at this time. All acetochlor treatments resulted in 99% weed control; therefore, no weeds were present at 15 DAP. However, weeds emerged later in the season because residual activity of acetochlor cannot be usually expected >50 d (Anonymous 2011a). For example, acetochlor applied PP or PRE at any rate, or in tank-mixed with fomesafen, flumioxazin, or sulfentrazone plus chlorimuron resulted in 10 to 13, 5 to 19, and 5 to 7 plants m^{-2} of common waterhemp, velvetleaf, and green foxtail, respectively, compared with acetochlor applied sequentially 60 DAP ($\leq$1 plant m^{-2}) and it was comparable with flumioxazin plus chlorimuron applied PRE followed by micro-encapsulated acetochlor plus glyphosate applied POST and weed-free control (Table 3). A similar trend was observed at 100 DAP for weed

Table 2. Effect of herbicide treatments on soybean density and weed control at 15, 60, and 100 d after planting (DAP) in glyphosate-resistant soybean in field experiments conducted at Clay Center in 2012 and 2013 and Waverly, Nebraska, USA, in 2013. Year-by-treatment and site-by-treatment interaction for weed control was not significant; therefore, data were combined[z]

				15 DAP[zx]			60 DAP[zx]			100 DAP[zx]		
Treatment[y]	Application timing	Rate	Soybean density[x]	AMATA	ABUTH	SETVI	AMATA	ABUTH	SETVI	AMATA	ABUTH	SETVI
		(kg a.e. or a.i. ha^{-1})	(plants m^{-1})	(%)								
Unntreated control[w]	–	–	23*a*	0	0	0	0	0	0	0	0	0
Weed-free control[v]	–	–	24*a*	100*a*	100*a*	100*a*	100*c*	100*c*	100*c*	100*d*	100*e*	100*e*
Glyphosate *fb* glyphosate	EPOST *fb* LPOST	0.87 *fb* 0.87	25*a*	0*b*	0*b*	20*b*	99*c*	99*c*	99*c*	75*d*	78*c*	90*d*
Acetochlor	14 DBP	1.68	22*a*	99*a*	99*a*	99*a*	70*a*	68*a*	72*a*	0*a*	0*a*	0*a*
Acetochlor	PRE	1.68	24*a*	99*a*	99*a*	99*a*	80*b*	67*a*	82*b*	30*b*	0*a*	0*a*
Acetochlor	PRE	3.37	25*a*	99*a*	99*a*	99*a*	80*b*	80*b*	79*b*	35*b*	0*a*	60*c*
Acetochlor + fomesafen	PRE	1.68 + 0.21	23*a*	99*a*	99*a*	99*a*	81*b*	80*b*	80*b*	37*b*	30*b*	0*a*
Acetochlor + flumioxazin	PRE	1.68 + 0.11	24*a*	99*a*	99*a*	99*a*	82*b*	79*b*	81*b*	30*b*	35*b*	40*b*
Acetochlor + sulfentrazone + chlorimuron-ethyl	PRE	1.68 + 0.29	23*a*	99*a*	99*a*	99*a*	82*b*	78*b*	78*b*	48*c*	0*a*	55*c*
Flumioxazin + chlorimuron *fb* acetochlor	PRE fb EPOST	0.126 *fb* 1.68	25*a*	99*a*	99*a*	99*a*	99*c*	98*c*	98*c*	97*e*	90*d*	89*d*
Acetochlor *fb* acetochlor	PRE *fb* EPOST	1.68 *fb* 1.68	25*a*	99*a*	99*a*	99*a*	96*c*	97*c*	95*c*	92*e*	75*c*	89*d*
Acetochlor *fb* acetochlor	PRE *fb* LPOST	1.68 *fb* 1.68	23*a*	99*a*	99*a*	99*a*	96*c*	99*c*	97*c*	93*e*	78*c*	88*d*
Acetochlor *fb* acetochlor *fb* acetochlor	PRE *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	22*a*	99*a*	99*a*	99*a*	99*c*	98*c*	98*c*	95*e*	90*d*	90*d*
Acetochlor *fb* acetochlor *fb* acetochlor	14 DBP *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	23*a*	99*a*	99*a*	98*a*	99*c*	98*c*	97*c*	97 e	90*d*	89*d*

[z]Abbreviations: AMATA, common waterhemp, ABUTH, velvetleaf; DAP, days after planting; DBP, days before planting; *fb*, followed by; EPOST, early POST; LPOST, late POST; SETVI, green foxtail.
[y]Acetochlor treatments at each application timings were tank-mixed with glyphosate at 870 g a.e. ha^{-1} + ammonium sulfate 2% wt/vol.
[x]Data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.
[w]Control (0%) data from untreated control plots were not included in analysis.
[v]Weed-free control plots were maintained by application of glyphosate at 870 g a.e. ha^{-1} + ammonium sulfate 2% wt/vol when weeds were emerged in the plots throughout the growing season.
a–e Means within columns with no common letter(s) are significantly different according to Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

Table 3. Effect of herbicide treatments on weed density at 15, 60, and 100 d after planting (DAP) and weed biomass in glyphosate-resistant soybean in field experiments conducted at Clay Center in 2012 and 2013 and Waverly, Nebraska, USA, in 2013. Year-by-treatment and site-by-treatment interaction for weed density was not significant; therefore, data were combined[z]

			Density at 15 DAP[yx]			Density at 60 DAP[yx]			Density at 100 DAP[yx]			
Treatment[z]	Application timing	Rate	AMATA	ABUTH	SETVI	AMATA	ABUTH	SETVI	AMATA	ABUTH	SETVI	Weed biomass[w]
		(kg a.e. or a.i. ha^{-1})	(no. m^{-2})									(g m^{-2})
Untreated control	–	–	15*a*	10*a*	9*a*	21*a*	20*a*	12*a*	30*a*	25*a*	14*a*	450*a*
Weed-free control[w]	–	–	0*b*	0*b*	0*b*	0*c*	0*d*	0*c*	0*d*	0*c*	0*c*	0*e*
Glyphosate *fb* glyphosate	EPOST *fb* LPOST	0.87 *fb* 0.87	12*a*	9*a*	10*a*	0*c*	0*c*	0*c*	8*c*	2*c*	0*c*	90*d*
Acetochlor	14 DBP	1.68	0*b*	0*b*	0*b*	10*b*	18*a*	6*b*	23*a*b	24*a*	13*a*	445*a*
Acetochlor	PRE	1.68	0*b*	0*b*	0*b*	11*b*	9*b*	7*b*	19*b*	26*a*	12*a*	280*b*
Acetochlor	PRE	3.37	0*b*	0*b*	0*b*	10*b*	5*c*	6*b*	15*bc*	16*b*	12*a*	280*b*
Acetochlor + fomesafen	PRE	1.68 + 0.21	0*b*	0*b*	0*b*	13*b*	19*a*	5*b*	12*bc*	15*b*	7*b*	190*c*
Acetochlor + flumioxazin	PRE	1.68 + 0.11	0*b*	0*b*	0*b*	10*b*	11*b*	6*b*	9*c*	14*b*	8*b*	180*c*
Acetochlor + sulfentrazone + chlorimuron-ethyl	PRE	1.68 + 0.29	0*b*	0*b*	0*b*	11*b*	10*b*	5*b*	20*b*	24*a*	8*b*	175*c*
Flumioxazin + chlorimuron *fb* acetochlor	PRE *fb* EPOST	0.126 *fb* 1.68	0*b*	0*b*	0*b*	0*c*	0*d*	0*c*	1*d*	0*c*	0*c*	7*e*
Acetochlor *fb* acetochlor	PRE *fb* EPOST	1.68 *fb* 1.68	0*b*	0*b*	0*b*	0*c*	1*d*	1*c*	2*d*	2*c*	1*c*	10*e*
Acetochlor *fb* acetochlor	PRE *fb* LPOST	1.68 *fb* 1.68	0*b*	0*b*	0*b*	0*c*	1*d*	0*c*	1*d*	1*c*	0*c*	12*e*
Acetochlor *fb* acetochlor *fb* acetochlor	PRE *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	0*b*	0*b*	0*b*	0*c*	0*d*	0*c*	1*d*	0*c*	1*c*	8*e*
Acetochlor *fb* acetochlor *fb* acetochlor	14 DBP *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	0*b*	0*b*	0*b*	0*c*	0*d*	0*c*	1*d*	1*c*	0*c*	5*e*

[z]All herbicide treatments at each application timing were tank-mixed with glyphosate at 870 g a.e. ha^{-1} + ammonium sulfate 2% wt/vol.
[y]Abbreviations: AMATA, common waterhemp, ABUTH, velvetleaf; DBP, days before planting; *fb*, followed by; EPOST, early post-emergence; LPOST, late post-emergence; SETVI, green foxtail.
[x]Data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.
[w]Weed-free control plots were maintained by application of glyphosate at 870 g a.e. ha^{-1} + ammonium sulfate 2% wt/vol when weeds wereemerged in the plots throughout the growing season.
a–e Means within columns with no common letter(s) are significantly different according to Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

Table 4. Effect of herbicide treatments on soybean yield in field experiments conducted at Clay Center in 2012 and 2013 and Waverly, Nebraska, USA, in 2013[z]

Treatment[yx]	Application timing[yx]	Rate	Soybean yield	
			Clay Center	Waverly
		(kg a.e. or a.i. ha^{-1})	-------------(kg ha^{-1})------------	
Untreated control	–	–	2100*a*	1402*a*
Weed-free control[w]	–	–	4630*d*	3805*d*
Glyphosate *fb* glyphosate	EPOST *fb* LPOST	0.87 *fb* 0.87	3700*c*	2750*b*
Acetochlor	14 DBP	1.68	2588*b*	2820*b*
Acetochlor	PRE	1.68	3263*c*	2800*b*
Acetochlor	PRE	3.37	3665*c*	3250*c*
Acetochlor + fomesafen	PRE	1.68 + 0.21	3547*c*	2775*b*
Acetochlor + flumioxazin	PRE	1.68 + 0.11	3719*c*	2,740*b*
Acetochlor + sulfentrazone + chlorimuron-ethyl	PRE	1.68 + 0.29	3704*c*	2780*b*
Flumioxazin + chlorimuron *fb* acetochlor	PRE fb EPOST	0.126 *fb* 1.68	4440*d*	3810*d*
Acetochlor *fb* acetochlor	PRE *fb* EPOST	1.68 *fb* 1.68	4473*d*	3800*d*
Acetochlor *fb* acetochlor	PRE *fb* LPOST	1.68 *fb* 1.68	4321*d*	3775*d*
Acetochlor *fb* acetochlor *fb* acetochlor	PRE *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	4225*d*	3820*d*
Acetochlor *fb* acetochlor *fb* acetochlor	14 DBP *fb* EPOST *fb* LPOST	1.68 *fb* 1.68 *fb* 1.68	4536*d*	3733*d*

[z]Year-by-treatment interaction was not significant for soybean yield; therefore, data were combined; however, site-by-treatment interaction was significant; therefore yield data for Clay Center and Waverly are presented separately.
[y]Abbreviations: DBP, days before planting; *fb*, followed by; EPOST, early post-emergence; LPOST, late post-emergence.
[x]Acetochlor treatments at each application timing were tank-mixed with glyphosate at 870 g a.e. ha^{-1} + ammonium sulfate 2% wt/vol.
[w]Weed-free control plots were maintained by application of glyphosate at 0.87 kg ae ha^{-1} + ammonium sulfate 2% wt/v when weeds were emerged in the plots throughout the growing season.
a–d Means within columns with no common letter(s) are significantly different according to Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

density. In a 2-yr study, Hausman et al. (2013) reported fewer than five common waterhemp plants per square meter with application of acetochlor at 60 DAT. Soybean injury was < 10% in any acetochlor treatment that indicated excellent crop tolerance with micro-encapsulated acetochlor applied once or even in sequential applications (data not shown). Results of weed control and weed density were reflected in weed biomass, where untreated control had the highest biomass (450 g m^{-2}). Acetochlor applied sequentially resulted in weed biomass ≤ 12 g m^{-2} and was comparable with weed-free control and flumioxazin plus chlorimuron applied PRE followed by micro-encapsulated acetochlor plus glyphosate applied POST. No difference was observed between acetochlor applied early-POST (V2 to V3 stage) or late-POST (V4 to V5 stage) for weed control, density, biomass, and soybean injury.

A significant site-by-treatment interaction for soybean yield occurred; hence, soybean yield results are presented separately for Clay Center and Waverly (Table 4). Soybean yield at Clay Center was higher than Waverly because irrigation provided more consistent moisture, which resulted in higher soybean yield. All herbicide treatments resulted in higher yields compared with the untreated control. Acetochlor applied alone PP or PRE at recommended (1.68 kg a.i. ha^{-1}) or higher rate (3.37 kg a.i. ha^{-1}), or in tank-mixed with flumioxazin, fomesafen, or sulfentrazone plus chlorimuron resulted in lower yields (2588 to 3719 kg ha^{-1}) compared with acetochlor applied sequentially (3775 to 4473 kg ha^{-1}). No yield difference was observed between acetochlor applied PRE followed by early-POST (V2 to V3 stage) or late-POST (V4 to V5 stage) tank-mixed with glyphosate. In fact, yield was comparable with flumioxazin plus chlorimuron applied PRE followed by micro-encapsulated acetochlor plus glyphosate applied POST and the weed free control. Therefore, POST application timing of acetochlor with glyphosate should be decided based on presence of weeds at the time of application, weed species density and composition based on historical field records.

Micro-encapsulated acetochlor is now labeled for PP, PRE, and POST application (up to the R2 growth stage) in soybean. Results of this study conclude that micro-encapsulated acetochlor applied PRE followed by early or late POST in tank-mixture with glyphosate provided season-long weed control, reduced weed density and biomass, and secured greater soybean yields. Similar results were observed when flumioxazin plus chlorimuron applied PRE followed by micro-encapsulated acetochlor plus glyphosate applied POST. This herbicide program includes herbicides with four modes of action, a highly effective herbicide program for resistance management. The new, expanded label of acetochlor may now provide soybean growers the flexibility to manage grasses and small-seeded broadleaf weeds in a two-pass weed control program. In addition, results from this study conclude that a one-pass weed control program of glyphosate plus acetochlor applied PRE will not provide more than 4 to 6 wk residual weed control. Therefore, a sequential program is required to control late-emerging weeds such as common waterhemp that has an extended emergence pattern. The time of weed emergence is one of the most important factors in determining the magnitude of crop yield losses (Myers et al. 2004); therefore, herbicide application timing should be planned based on weed emergence and weed size to provide season-long weed control that results in maximum soybean yields (Hilgenfeld et al. 2004). Several studies reported that the addition of a residual herbicide with POST herbicide later in the season improves weed control, and reduces weed seed production, as well as providing an additional mode of action that reduces selection pressure for weed resistance (Gonzini et al. 1999; Tharp and Kells 2002; Koger et al. 2007). Thus, micro-encapsulated acetochlor will be a good additional tool to include in PRE or POST herbicide program in soybean.

This is the first report of micro-encapsulated acetochlor tank-mixed with glyphosate applied at different timings for weed control in glyphosate-resistant soybean. Results from this study conclude that the sequential application of glyphosate plus acetochlor provides excellent full-season control of common waterhemp, velvetleaf, and green foxtail. Despite widespread use of acetochlor for the past several years, only one weed species, Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*), has evolved resistance to acetochlor in the United States (Heap 2014). Therefore, micro-encapsulated acetochlor can be a component of a diversified weed management program in soybean.

For example, Hausman et al. (2013) reported >90% control of a 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitors-resistant common waterhemp with acetochlor. In this study, flumioxazin plus chlorimuron applied PRE followed by micro-encapsulated acetochlor plus glyphosate applied POST resulted in season-long (100 DAP) weed control and higher soybean yield. Herbicide programs are needed that include herbicides with different modes of action because repeated use of any herbicide leads to the evolution of herbicide-resistant weeds (Owen and Zelaya 2005; Powles and Yu 2010). Indeed, growers should adopt an integrated weed management approach that includes crop rotation, use of herbicides with different modes of action, and improved agronomic practices (Norsworthy et al. 2012). More research is required to evaluate the compatibility and efficacy of micro-encapsulated acetochlor applied in tank-mixture with PRE and POST soybean herbicides commonly used by growers.

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