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Response of glyphosate-resistant horseweed [*Conyza canadensis* (L.) Cronq.] to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor

D. Sarangi and A.J. Jhala

Abstract: A premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor has recently been commercialized for pre-emergence (PRE) and early post-emergence (POST) control of broadleaved and annual grass weeds in corn in the United States. Field and greenhouse dose-response bioassays were conducted in 2015 and 2016 to evaluate the response of glyphosate-resistant (GR) horseweed to this premix applied before or after emergence (PRE or POST). In a field PRE study, the 90% effective doses (ED_{90}) were 2613 and 2863 g a.i. ha^{-1} at 14 and 35 d after treatment (DAT), respectively, which were comparable to the labeled rate (2900 g a.i. ha^{-1}) of the premix. Under greenhouse conditions, POST applications made at the labeled rate to 8–10 or 15–18 cm diameter horseweed rosettes provided ≥97% control. The ED_{90} values for the in-field POST dose-response study were ≥3431 and ≥6717 g a.i. ha^{-1} for the 8–10 and 15–18 cm tall GR horseweed, respectively. At 14 DAT, the premix applied at the labeled rate provided 85% and 68% control of 8–10 and 15–18 cm tall GR horseweed, respectively. The root mean square error for the log-logistic model ranged from 4.2 to 9.2 and the model efficiency coefficient values were ≥0.94 (≈ 1.00), indicating a good fit for the prediction model. In conclusion, a new premix applied before emergence (PRE) will effectively control GR horseweed at the labeled rate compared with POST applications made to ≥8 cm tall plants.

Key words: growth stages, model goodness of fit, multiple modes of action, pre-emergence, resistance management.

Résumé : Un pré-mélange d'atrazine, de bicyclopyrone, de mesotrione et de S-métolachlore a récemment été introduit sur le marché américain pour lutter contre les dicotylédones et les herbacées annuelles dans les champs de maïs, avant et au début de la levée. En 2015 et 2016, les auteurs ont procédé à des analyses dose-réaction sur le terrain et en serre afin d'évaluer la réaction de la collinsie résistante au glyphosate (RG) à l'application du nouveau pré-mélange, avant ou après la levée. Pour obtenir la DE_{90} 14 et 35 jours après le traitement lors de l'étude sur le terrain, on a dû respectivement appliquer 2613 et 2863 g de matière active par hectare avant la levée, ce qui est comparable au taux recommandé sur l'étiquette du produit (2900 g de matière active par hectare). En serre, l'application du taux recommandé pour le traitement après la levée, quand le diamètre des rosettes de collinsie mesure 8–10 ou 15–18 cm de diamètre, détruit au moins 97 % des adventices. Pour obtenir la DE_{90} lors de l'étude dose-réaction du traitement post-levée sur le terrain, il a respectivement fallu appliquer ≥ 3431 g et ≥ 6717 g de matière active par hectare à la collensie RG de 8–10 et de 15–18 cm de hauteur. Quatorze jours après le traitement, l'application du pré-mélange au taux recommandé détruit respectivement 85 % et 68 % des collensies RG de 8–10 et de 15–18 cm de hauteur. L'écart-type du modèle logarithmique-logistique varie de 4,2 à 9,2 et son coefficient d'efficacité est d'au moins 0,94 (≈ 1,00), signe d'un bon ajustement du modèle prévisionnel. En conclusion, le nouveau pré-mélange autorise une lutte efficace contre la collensie RG quand le produit est appliqué avant la levée au taux recommandé sur l'étiquette, plutôt que quand on l'applique après la levée aux plants de 8 cm et plus. [Traduit par la Rédaction]

Mots-clés : stades de croissance, qualité d'ajustement du modèle, modes d'action multiples, prélevée, gestion de la résistance.

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Introduction

Glyphosate, a nonselective post-emergence (POST) herbicide, is the most commonly used herbicide globally. Commercialization of glyphosate-tolerant crops dramatically changed the pattern of glyphosate application in agriculture (Dill et al. 2008). Glyphosate-tolerant crop technology ensured a simplified and economical weed management program, where one or two POST application(s) of glyphosate provided broad-spectrum weed control, excellent crop safety, reduced herbicide carryover, and had no rotational restrictions for successive crops (Duke and Powles 2009; Green 2009). Since its first commercialization in 1974, more than 1.6 billion kg of glyphosate acid equivalent (a.e.) has been applied in the United States, two-thirds of which was applied in the last 10 yr (Benbrook 2016). In 2016, a majority of the corn and soybean acreage (89% and 94%, respectively) in the United States was planted with herbicide-tolerant varieties, primarily with glyphosate-tolerant technology [US Department of Agriculture-National Agricultural Statistics Service 2016].

Overreliance on glyphosate for weed control in glyphosate-tolerant crops has triggered the evolution of glyphosate-resistant (GR) weeds (Powles 2008). As of 2016, 35 weed species have been reported as GR from 27 countries, including 16 species from the United States (Heap 2016*b*). Horseweed [*Conyza canadensis* (L.) Cronq.] was the first GR weed species reported in the United States, as well as the first dicot species in the world reported resistant to glyphosate (VanGessel 2001). As of 2016, the presence of GR horseweed has been reported in 25 states in the United States with a geographic distribution ranging from Delaware to California (Heap 2016*a*). Occurrences of this problem weed have also been reported in 10 other countries, including Brazil, Canada, the People's Republic of China, and Spain (Cerdeira et al. 2011; Heap 2016*a*). In Nebraska, GR horseweed was first confirmed in 2006 (Knezevic 2007).

Horseweed is a winter or summer annual weed native to North America (Weaver 2001). It can be found in various environments including row-crop production fields, orchards, vineyards, field edges, and along roadsides and railway tracks (Davis et al. 2009; Hanson et al. 2009; Owen et al. 2009; Shrestha et al. 2010). It has a prolific seed-producing capability of 200 000 seeds plant^{-1} when grown at a density of 10 plants m^{-2}, with 80% of seeds able to germinate immediately after seed-shedding (Bhowmik and Bekech 1993; Loux et al. 2006). Because of its small seed size, conservation tillage practices including no-till practices, favor the germination and establishment of horseweed in agricultural fields (Brown and Whitwell 1988; Buhler 1992), leading to horseweed becoming one of the major weeds in glyphosate-tolerant crop production systems (Kruger et al. 2009). A study conducted in Minnesota and Iowa showed that the majority of horseweed emerged in the fall and survived the winter by forming rosettes, though significant spring and early summer emergence was also recorded in the same study (Buhler and Owen 1997). Similarly, Bhowmik and Bekech (1993) reported that horseweed seedling emergence was highest in late August to early September in Massachusetts, but emergence was also observed in spring and early summer. Previous studies indicated that early pre-plant (or burndown) and PRE applications of herbicide provided better control of horseweed compared with a sole reliance on POST herbicide programs (Wilson and Worsham 1988; Bruce and Kells 1990). Additionally, Owen et al. (2009) reported that tank-mixing burndown herbicides with one or more soil residual herbicides provided better control of GR horseweed in cotton (*Gossypium hirsutum* L.) compared with a program without residual herbicides. Therefore, it is important to control this problem weed in the fall or early spring to avoid potential competition with crops during summer, even though growers oftentimes do not plan their herbicide programs ahead of time and rely primarily on using POST herbicides when weeds are clearly visible.

Acuron® (Syngenta Crop Protection, LLC, Greensboro, NC), a new broad-spectrum herbicide, is labeled for early pre-plant, PRE, and early POST applications in corn for controlling annual grasses and broadleaved weeds, including horseweed (Anonymous 2016). It is a mixture (hereafter referred to as "premix") of four active ingredients: atrazine (10.9% total premix volume), bicyclopyrone (0.7%), mesotrione (2.6%), and S-metolachlor (23.4%). Bicyclopyrone is a new active ingredient (a.i.) belonging to the 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor group and is expected to provide better control of grasses and hard-to-control broadleaved weeds such as giant ragweed (*Ambrosia trifida* L.) and common cocklebur (*Xanthium strumarium* L.). Use of herbicide premixes with multiple effective modes of action are encouraged to delay the evolution of herbicide-resistant weeds by reducing the selection pressure imposed on a weed population by a single herbicide mode of action (Wrubel and Gressel 1994; Diggle et al. 2003; Norsworthy et al. 2012). Sometimes, herbicide mixtures show improved efficacy for weed control due to their enhanced activity; for example, the synergistic effect of mesotrione and atrazine for controlling several weed species, including GR horseweed, has been documented (Armel et al. 2009; Walsh et al. 2012; Jhala et al. 2014). Because no herbicide active ingredient belonging to a new mode of action has come to the market in the last three decades (Duke 2012), tank mixtures and herbicide premixes with effective modes of action are recommended for the control of GR weeds (Beckie 2006; Sarangi et al. 2017).

Herbicide efficacy is highly dependent on the growth stages of a weed species and the timing of herbicide applications (Owen et al. 2009; Chahal et al. 2015; Ganie et al. 2015); therefore, it is important to conduct a dose-response bioassay to study the efficacy of a new premix applied at different growth stages of GR horseweed. The objectives of this study were to evaluate the response of GR horseweed to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied before or after emergence (PRE or POST) at two growth stages (8–10 or 15–18 cm rosette diameter or plant height) in greenhouse and field study.

Materials and Methods

Plant materials

In 2014, seeds of a known GR horseweed population were collected from the Lincoln Agronomy Farm (40.85°N, 96.62°W) at the University of Nebraska-Lincoln, Lincoln, NE. Seeds were stored in airtight polyethylene bags at 4 °C for 4 mo until the experiment commenced. The level of glyphosate resistance in this population ranged from 3 to 6 times that of glyphosate-susceptible (GS) horseweed population (Knezevic 2007).

Greenhouse dose-response studies

Greenhouse dose-response bioassay was conducted in 2015 to determine the response of GR horseweed to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST). Experiment was repeated in time, beginning 14 d after the first run. GR horseweed seedlings were grown in 72-celled germination trays containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, QC). The seedlings were then transferred at the 2–4 leaf stage to square plastic pots (10 cm × 10 cm × 12 cm) containing potting mix, with a single plant in each pot. The plants were supplied with adequate water and plant nutrients (24–8–16, Miracle-Gro Water Soluble All Purpose Plant Food, Scotts Miracle-Gro Products Inc., Marysville, OH) until the experiment commenced. A premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor was applied after emergence (POST) at two growth stages (8–10 or 15–18 cm rosette diameter) of GR horseweed. The greenhouse was maintained at a 20 °C/12 °C day/night temperature and artificial lights (600 μmol photon m^{-2} s^{-1}) were provided using metal halide lamps to ensure a 14-h photoperiod.

Greenhouse studies were laid out in a randomized complete block design with a factorial arrangement of ten herbicide rates and two growth stages. A single pot was considered as an experimental unit and five replications were maintained. The premix was applied at 10 rates (0, 0.031*x*, 0.062*x*, 0.125*x*, 0.25*x*, 0.5*x*, 1*x*, 1.5*x*, 2*x*, and 2.5*x*, where 1*x* is the labeled rate, 2900 g a.i. ha^{-1} for 3% soil organic matter) using a single-tip chamber sprayer (DeVries Manufacturing Corp., Hollandale, MN) fitted with an 8001E nozzle (TeeJet® Technologies, Spraying Systems Co., Wheaton, IL) calibrated to deliver 140 L ha^{-1} spray volume at 207 kPa pressure at a speed of 4 km h^{-1}. Post-emergence herbicide treatments included a non-ionic surfactant (NIS; Induce®, Helena® Chemical Company, Collierville, TN) at a rate of 0.25% *v*/*v*.

GR horseweed control was estimated visually at 7, 14, and 21 d after treatments (DAT) using a scale ranging from 0% to 100%, with 0% meaning no control or plant injury and 100% meaning complete plant death. Control was assessed visually based on the severity of the injury symptoms: chlorosis and necrosis, bleaching of the leaf tissues, stunting, and plant death; compared with the untreated control (i.e., 0*x* rate of premix). At 21 DAT, the surviving plants were severed at the base and oven-dried at 65 °C until they reached a constant weight. The aboveground biomass data was then converted into percent biomass reduction using eq. 1:

$$\text{Aboveground biomass reduction (\%)} = [(C - B)/C] \times 100 \tag{1}$$

where C is the biomass in the untreated control unit and B is the biomass in an individual treated experimental unit.

Field dose-response studies

The in-field PRE and POST dose-response experiments were conducted at the Lincoln Agronomy Farm at the University of Nebraska-Lincoln in 2015 and 2016. The field had been under glyphosate-tolerant corn and soybean production with reliance on glyphosate for weed control for the last several years. GR horseweed was the predominant weed species at the experimental site along with sparsely distributed summer annuals such as common lambsquarters (*Chenopodium album* L.) and common sunflower (*Helianthus annuus* L.). The texture of the soil was silt-loam with a pH of 5.6, 19% sand, 54% silt, 27% clay, and 3% organic matter. Herbicide was applied at the same rates as described in the greenhouse experiment, using a handheld CO_2-pressurized backpack sprayer equipped with AIXR 110015 flat fan nozzles (TeeJet® Technologies) calibrated to deliver 140 L ha^{-1} at 276 kPa at a constant speed of 4.8 km h^{-1}. The plots were 3 m wide × 9 m long and the experiments were laid out in a randomized complete block design with four replications. Pre-emergence treatments were applied in the third week of April to simulate the usual PRE application timing in corn in Nebraska. The PRE application of this herbicide also worked as a burndown treatment for the horseweed seedlings that emerged in the fall and early spring, which were at the rosette stage and just beginning to bolt. The number of horseweed seedlings present at the time of PRE application ranged from 18 to 35 plants m^{-2}. In a factorial experiment, the POST application of the premix was made at two growth stages (8–10 or 15–18 cm tall) of the GR horseweed. As the plants were varying in their growth stages under field conditions, plant height (from the soil surface) was measured by averaging the height of 100 randomly selected horseweed plants across the field. The experiments were conducted under no-crop (bare ground) and rain-fed (dryland) conditions without any supplemental irrigation.

Control of GR horseweed was estimated visually at 14, 21, 35, 49, and 63 DAT on a scale of 0%–100% as described previously. In the PRE dose-response bioassay, horseweed densities were recorded by counting the number of living horseweed plants in two 0.25 m^2 quadrats placed randomly in each plot and percent density reduction compared with the untreated control was estimated. GR horseweed plants surviving the herbicide application were cut at the soil surface at 63 DAT from two randomly selected 0.25 m^2 quadrats and placed in an oven at 65 °C. Percent reduction in aboveground biomass was then estimated using eq. 1.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC) to perform a test of significance ($P < 0.05$) for years or experimental runs, treatments, and their interactions. Treatments were considered fixed effects, whereas years (experimental runs) and blocks (nested within year) were considered random effects in the model. The contribution of the random effect (along with the interactions between random and fixed effects) was quantified to check its significance. A four-parameter log-logistic function (eq. 2) was used to determine the biologically effective doses of the premix required to control the GR horseweed by 50% and 90% (ED_{50} and ED_{90}) using the *drc* package in R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al. 2007)

(2) $$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$$

where Y is the response variable (percent control/injury, or percent reduction in the aboveground biomass/density), x is the herbicide dose, c and d are the lower limit (which is 0) and the estimated maximum value of Y, respectively, and e represents the herbicide doses causing 50% control or aboveground biomass (or density) reduction (i.e., ED_{50}) in GR horseweed. The parameter b is the relative slope around the parameter e.

Model goodness of fit

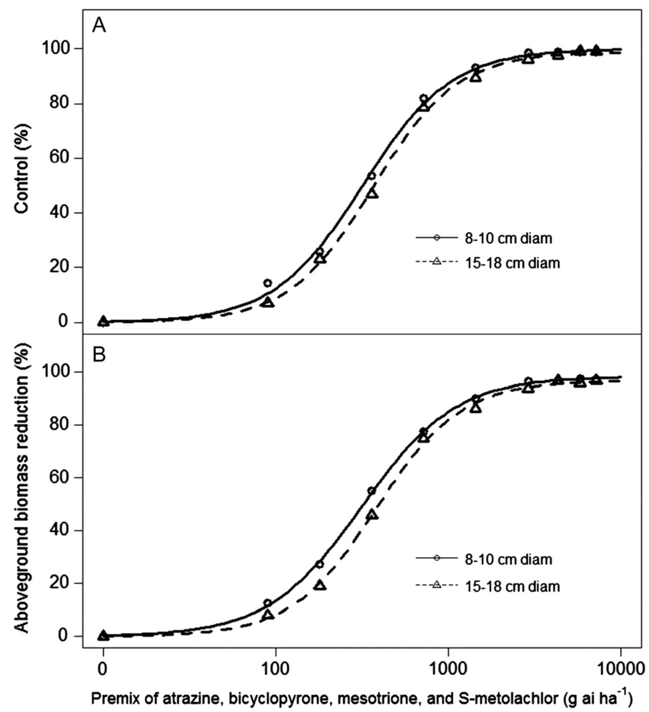
Lack-of-fit test was performed in the *drc* package in the R software (described previously) to check the fit for the model. Additionally, root mean square error (RMSE) and modelling efficiency coefficient (EF) were calculated by using eqs. 3 and 4:

(3) $$\text{RMSE} = \left[\frac{1}{n}\sum_{i=1}^{n}(P_i - O_i)^2\right]^{1/2}$$

(4) $$\text{EF} = 1 - \left[\sum_{i=1}^{n}(O_i - P_i)^2 / \sum_{i=1}^{n}(O_i - \bar{O}_i)^2\right]$$

where P_i is the predicted value, O_i is the observed value, $\bar{O}_i$ is the mean observed value, and n is the total number of observations. Sarangi et al. (2016) and Spiess and Neumeyer (2010) noted that the evaluation of R^2 is an inadequate fitness measure for a nonlinear model (e.g., eq. 2); therefore, reporting RMSE and EF are essential. A smaller RMSE value means better fit, and an EF value closer to 1.00 means more accurate predictions.

Fig. 1. Glyphosate-resistant horseweed (A) control and (B) aboveground biomass reduction at 21 d after treatment (DAT) in a greenhouse dose-response study conducted at the University of Nebraska-Lincoln with a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) at two growth stages (8–10 or 15–18 cm diameter).



Results and Discussion

Treatment × year (or treatment × experimental run for the greenhouse studies) interactions were not significant ($P > 0.05$); therefore, data from both years (or from both experimental runs) were combined.

Greenhouse dose-response studies

The responses of GR horseweed to the POST application of the premix differed slightly for two growth stages: 8–10 and 15–18 cm rosette diameter (Fig. 1A). The dose required for 90% (ED_{90}) control of GR horseweed at 8–10 cm rosette diameter was 1165 g a.i. ha^{-1}; however, the ED_{90} value was 1330 g a.i. ha^{-1} for the 15–18 cm rosette diameter (Table 1). GR horseweed control was ≥97% at 21 DAT when the premix was applied after emergence (POST) at a 1*x* rate (i.e., the labeled rate, 2900 g a.i. ha^{-1}) regardless the growth stages. The comparison of the biologically effective doses for horseweed control showed that the ED_{50} values differed slightly ($P < 0.05$) for the two growth stages, but that the ED_{90} values were similar ($P = 0.25$), meaning that GR horseweed control was independent of growth stage at the higher doses of the premix. Similarly, the results from

Table 1. Regression parameter estimates and goodness of fit (RMSE and EF) for a four-parameter log-logistic function[a] fitted to glyphosate-resistant (GR) horseweed control and aboveground biomass reduction (at 21 DAT) with post-emergence applications of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor; and estimation of the biologically effective doses required to control (or, to reduce the aboveground biomass of) GR horseweed by 50% (ED_{50}) and 90% (ED_{90}).

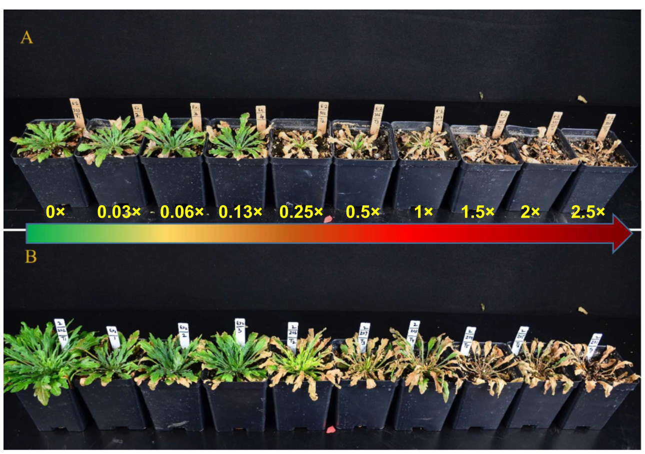
GR horseweed growth stages	Regression parameter	Model goodness of fit		ED_{50} (± SE) (g a.i. ha^{-1})	ED_{90} (± SE) (g a.i. ha^{-1})	Predicted value (%) at 1*x* rate[b]
	b (± SE)	RMSE	EF			
Control of GR horseweed						
8–10 cm rosette diameter	−1.7 (± 0.07)	5.3	0.98	318 (± 9)	1165 (± 68)	98
15–18 cm rosette diameter	−1.8 (± 0.07)	4.3	0.99	370 (± 9)	1330 (± 85)	97
Aboveground biomass reduction of GR horseweed						
8–10 cm rosette diameter	−1.6 (± 0.08)	5.3	0.98	322 (± 11)	1385 (± 126)	96
15–18 cm rosette diameter	−1.8 (± 0.10)	5.6	0.98	395 (± 14)	1615 (± 153)	94

Note: DAT, days after treatment; EF, modelling efficiency coefficient; RMSE, root mean square error; SE, standard error of mean.

[a]$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where *Y* is the response variable (percent control or percent reduction in the aboveground biomass), *x* is the herbicide dose, *c* and *d* are the lower limit (which is 0) and the estimated maximum value of *Y*, respectively, and *e* represents the herbicide dose causing 50% control or aboveground biomass reduction (i.e., ED_{50}) in GR horseweed. The parameter *b* is the relative slope around the parameter *e*.

[b]Premix labeled rate (1*x*) = 2900 g a.i. ha^{-1}.

Fig. 2. Dose-response of glyphosate-resistant horseweed at 21 d after treatment (DAT) to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) to (A) 8–10 cm, and (B) 15–18 cm rosette diameter. [Colour online.]



a glyphosate dose-response study conducted in Mississippi showed that the rosette size of horseweed had little effect on the ED_{50} values for the GR and glyphosate-susceptible biotypes (Koger et al. 2004). Kruger et al. (2008) also reported that the control of horseweed with an application of 2,4-D was not affected by rosette size, which ranged between 0.5 and 10 cm diameter. Figure 2 illustrates a visual demonstration of the dose-response of GR horseweed to the premix, when applied to the 8–10 cm rosette diameter (Fig. 2A), and to the 15–18 cm diameter (Fig. 2B).

Aboveground biomass reduction data showed that the maximum biomass reduction was ≥97% (compared with the untreated control) for both growth stages of GR horseweed (Fig. 1B). The premix doses required for 90% reduction (ED_{90}) in the aboveground biomass at 21 DAT were 1385 and 1615 g a.i. ha^{-1}, for 8–10 and 15–18 cm rosette diameter, respectively (Table 1). Aboveground biomass reduction at the 1*x* rate of the premix was predicted as 96% and 94% for the 8–10 and 15–18 cm rosette diameter, respectively. The RMSE values for control estimates and biomass reduction ranged from 4.3 to 5.6 and the EF values were ≥0.98 (≈ 1.00), indicating a good fit of the model (Table 1). Similarly, Sarangi et al. (2016) reported a RMSE value of 5.4–11.6 and an EF value 0.83–0.97 during validation of a log-logistic model for common waterhemp (*Amaranthus rudis* Sauer) plant height in response to water stress.

Field dose-response studies

Pre-emergence dose-response study

In the field studies, precipitation was adequate in both years to activate the premix applied to the soil (Table 2). The dose-response curves showed that at 14 DAT, 90% control of the GR horseweed was obtained with 2613 g a.i. ha^{-1} of the premix applied before emergence (PRE); however, higher doses were required to achieve the same level of control at 35 DAT (2863 g a.i. ha^{-1}) and 63 DAT (3187 g a.i. ha^{-1}) (Fig. 3A; Table 3). Reduction in residual activity later in the season required higher doses of premix applied before emergence (PRE) to control GR horseweed under field conditions. Pre-emergence application of the premix at the 1*x* rate resulted in ≥90% control of GR horseweed initially, and the control ratings were reduced later in the season. However, 88% control was observed even after 2 mo of PRE application at the labeled rate (Table 3). Figure 4 also shows that the PRE application of the premix was highly effective and

Table 2. Monthly mean air temperature and total precipitation during the 2015 and 2016 and 30-yr average at the Lincoln Agronomy Farm at the University of Nebraska-Lincoln.[a]

	Mean temperature (°C)			Total precipitation (mm)		
Month	2015	2016	30-yr average	2015	2016	30-yr average
March	6.3	8.6	4.9	19.6	23.6	41.1
April	12.2	12.8	11.0	50.5	111.0	70.4
May	16.4	17.1	17.1	276.9	137.7	116.3
June	23.0	26.0	22.8	194.6	14.7	104.6
July	25.1	25.4	25.3	60.7	118.6	79.2
Annual	11.8	12.8	11.0	1045.5	734.1	726.7

[a]Mean air temperature and total precipitation data were obtained from the National Oceanic and Atmospheric Administration (2015).

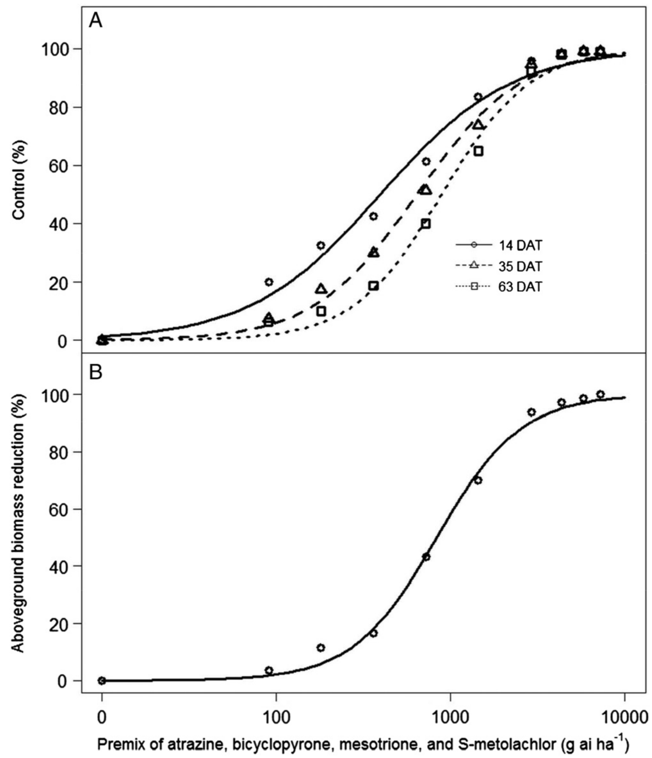
premix applied at the labeled rate or greater (Figs. 4C, 4D) resulted in more than 90% control of GR horseweed at 21 DAT. In a field study conducted in Wisconsin, Buhler (1991) reported that residual control of giant foxtail (*Setaria faberi* Herrm.) and velvetleaf (*Abutilon theophrasti* Medik.) with atrazine + S-metolachlor (at 2200 + 2800 g a.i. ha^{-1}) reduced significantly beyond 21 DAT. Similarly, Stephenson et al. (2004) reported that morning-glory (*Ipomoea* L. sp.) control reduced beyond 4 wk after PRE applications of atrazine + S-metolachlor or mesotrione alone.

Plant density data followed a trend similar to that of the control estimates (data not shown). The aboveground biomass reduction curve at 63 DAT showed that 90% biomass reduction in GR horseweed was achieved with an application of premix at the labeled rate (1*x*) (Fig. 3B). The herbicide dose required to reduce aboveground biomass by 90% was 2826 g a.i. ha^{-1}, which concurred with the biologically effective doses required for control of GR horseweed (Table 3). The RMSE values ranged from 7.3 to 9.1 and the EF values were ≥ 0.94 (Table 3), showing a good fit of the four-parameter log-logistic model.

Post-emergence dose-response study

Field application of the premix was made when horseweed was 8–10 and 15–18 cm tall, simulating the early and mid-POST herbicide applications in corn. The premix doses required for 90% control of 8–10 cm tall GR horseweed were 4309, 3469, and 3431 g a.i. ha^{-1} at 14, 35, and 63 DAT, respectively (Table 4). Post-emergence application of the premix to ≥8 cm tall plants caused relatively slow herbicide activity inside the plants and ≥90% control of GR horseweed was obtained only with the higher doses (>2900 g a.i. ha^{-1} or >1*x* rate) (Fig. 5), resulting in higher ED_{90} values at 14 DAT compared with 35 DAT. In contrast, premix applied at a reduced rate (<1*x*) caused immediate burning to the shoot tips of the

Fig. 3. Glyphosate-resistant horseweed (A) control at 14, 35, and 63 d after treatment (DAT), and (B) aboveground biomass reduction in a field dose-response study with a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied before emergence (PRE) in 2015 and 2016.



8–10 cm tall GR horseweed, which stimulated additional branching (lateral) from the stem after 21 DAT and hardened off the plants, lowering the control ratings and increasing the ED_{50} values. Mellendorf et al. (2013) reported that lower doses of saflufenacil applied after emergence (POST) to ≥5 cm tall GR horseweed plants resulted in shoot regrowth at 28 DAT, lowering the control ratings at later stages.

Figures 6 and 7 illustrate the visual demonstration of GR horseweed control at 35 DAT when the premix was applied after emergence (POST) to two growth stages (8–10 or 15–18 cm height). Post-emergence-control of GR horseweed was dependent on the growth stage ($P < 0.001$); therefore, a higher degree of control was observed in the 8–10 cm tall horseweed plants compared with the 15–18 cm tall plants (Fig. 5). Several studies have previously reported growth stage-dependent responses of GR weed species to certain POST herbicides: for example, Chahal et al. (2015) reported that the growth stage of GR common waterhemp, giant ragweed (*A. trifida*), and kochia [*Kochia scoparia* (L.) Schrad.] significantly affected control and biomass reduction in response to a premix of 2,4-D choline and glyphosate. Ganie et al. (2015) also showed that higher doses of a premix of fluthiacet-methyl and mesotrione were required for control of

Table 3. Regression parameter estimates and goodness of fit (RMSE and EF) for a four-parameter log-logistic function[a] fitted to glyphosate-resistant (GR) horseweed control, and aboveground biomass reduction with pre-emergence applications of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor; and estimation of the biologically effective doses required to control (or reduce the aboveground biomass of) GR horseweed by 50% (ED_{50}) and 90% (ED_{90}).

		Model goodness of fit				
DAT	Regression parameter [*b* (± SE)]	RMSE	EF	ED_{50} (± SE) (g a.i. ha^{-1})	ED_{90} (± SE) (g a.i. ha^{-1})	Predicted value (%) at 1*x* rate[b]
Control of GR horseweed						
14	–1.2 (± 0.09)	7.7	0.95	394 (± 31)	2613 (± 386)	91
35	–1.5 (± 0.11)	7.3	0.96	639 (± 41)	2863 (± 347)	90
63	–1.7 (± 0.14)	7.4	0.97	887 (± 53)	3187 (± 355)	88
Aboveground biomass reduction of GR horseweed						
63	–1.8 (± 0.19)	8.9	0.95	833 (± 58)	2826 (± 382)	90

Note: DAT, days after treatment; EF, modelling efficiency coefficient; RMSE, root mean square error; SE, standard error of mean.

[a]$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where *Y* is the response variable (percent control or percent reduction in the aboveground biomass), *x* is the herbicide dose, *c* and *d* are the lower limit (which is 0) and the estimated maximum value of *Y*, respectively, and *e* represents the herbicide doses causing 50% control or aboveground biomass reduction (i.e., ED_{50}) in GR horseweed. The parameter *b* is the relative slope around the parameter *e*.

[b]Premix labeled rate (1*x*) = 2900 g a.i. ha^{-1}.

Fig. 4. Response of glyphosate-resistant horseweed at 21 d after treatment (DAT) to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied before emergence (PRE) at the rates of (A) 0*x*, (B) 0.5*x*, (C) 1*x*, and (D) 2*x*, where 1*x* = the labeled rate, 2900 g a.i. ha^{-1} for 3% soil-organic matter. [Colour online.]

20 cm tall GR weeds compared with 10 cm tall GR weeds. In this study, control of GR horseweed was ≥85% when the premix was applied after emergence (POST) at the labeled rate (1*x*) to the 8–10 cm tall plants; whereas control reduced to 68% for the 15–18 cm tall plants at 14 DAT and further declined to 54% at 63 DAT (Table 4). The ED_{90} values for the taller (15–18 cm) horseweed plants were 7655, 7350, and 6717 g a.i. ha^{-1} at 14, 35, and 63 DAT, respectively. Estimated effective doses greater than 7200 g a.i. ha^{-1} (the highest dose of the premix tested in this study) had limited biological meaning for GR horseweed control. Figure 7C shows that the lateral branching after application of the premix was observed in the 15–18 cm tall GR horseweed, even at the higher doses (≥1*x*). The aboveground biomass reduction for GR horseweed was comparable with control estimates at 63 DAT (Fig. 8). The biologically effective herbicide dose for 90% biomass reduction in the 8–10 cm tall GR horseweed was estimated as 4244 g a.i. ha^{-1}; however, the value was higher (ED_{90} = 10 337 g a.i. ha^{-1}) for the 15–18 cm tall horseweed plants (Table 4).

The RMSE and EF values for the POST dose-response study conducted under field conditions ranged from 4.2 to 9.2 and 0.95 to 0.99, respectively. Werle et al. (2014), validating a sigmoidal prediction function for emergence of summer annual weed species, reported an RMSE value ranging from 3.7 to 14.9 with an EF value from 0.82 to 0.99. Therefore, the goodness-of-fit

Table 4. Regression parameter estimates and goodness of fit (RMSE and EF) for a four-parameter log-logistic function[a] fitted to glyphosate-resistant (GR) horseweed control and aboveground biomass reduction with post-emergence applications of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor; and estimation of the biologically effective doses required to control (or, to reduce the aboveground biomass of) GR horseweed by 50% (ED_{50}) and 90% (ED_{90}).

DAT	GR horseweed growth stages	Regression parameters [b (± SE)]	Model goodness of fit RMSE	EF	ED_{50} (± SE) (g a.i. ha^{-1})	ED_{90} (± SE) (g a.i. ha^{-1})	Predicted value (%) at 1x rate[b]
Control of GR horseweed							
14	8–10 cm tall	−1.2 (± 0.09)	7.4	0.96	672 (± 48)	4309 (± 604)	85
	15–18 cm tall	−1.5 (± 0.10)	6.4	0.97	1716 (± 89)	7655 (± 756)	68
35	8–10 cm tall	−1.5 (± 0.11)	7.1	0.97	827 (± 50)	3469 (± 392)	87
	15–18 cm tall	−1.8 (± 0.11)	5.2	0.98	2235 (± 82)	7350 (± 506)	62
63	8–10 cm tall	−2.3 (± 0.27)	9.2	0.95	1307 (± 85)	3431 (± 406)	86
	15–18 cm tall	−2.5 (± 0.26)	4.2	0.99	2720 (± 140)	6717 (± 874)	54
Aboveground biomass reduction of GR horseweed							
63	8–10 cm tall	−2.1 (± 0.19)	7.3	0.97	1468 (± 78)	4244 (± 407)	80
	15–18 cm tall	−2.1 (± 0.28)	5.5	0.98	2508 (± 233)	10 337 (± 2794)	57

Note: DAT, days after treatment; EF, modelling efficiency coefficient; RMSE, root mean square error; SE, standard error of mean.

[a]$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where Y is the response variable (percent control or percent reduction in the aboveground biomass), x is the herbicide dose, c and d are the lower limit (which is 0) and the estimated maximum value of Y, respectively, and e represents the herbicide doses causing 50% control or aboveground biomass reduction (i.e., ED_{50}) in GR horseweed. The parameter b is the relative slope around the parameter e.

[b]Premix labeled rate (1x) = 2900 g a.i. ha^{-1}.

parameters estimated in this study indicate a good fit for the prediction model for the POST dose-response study.

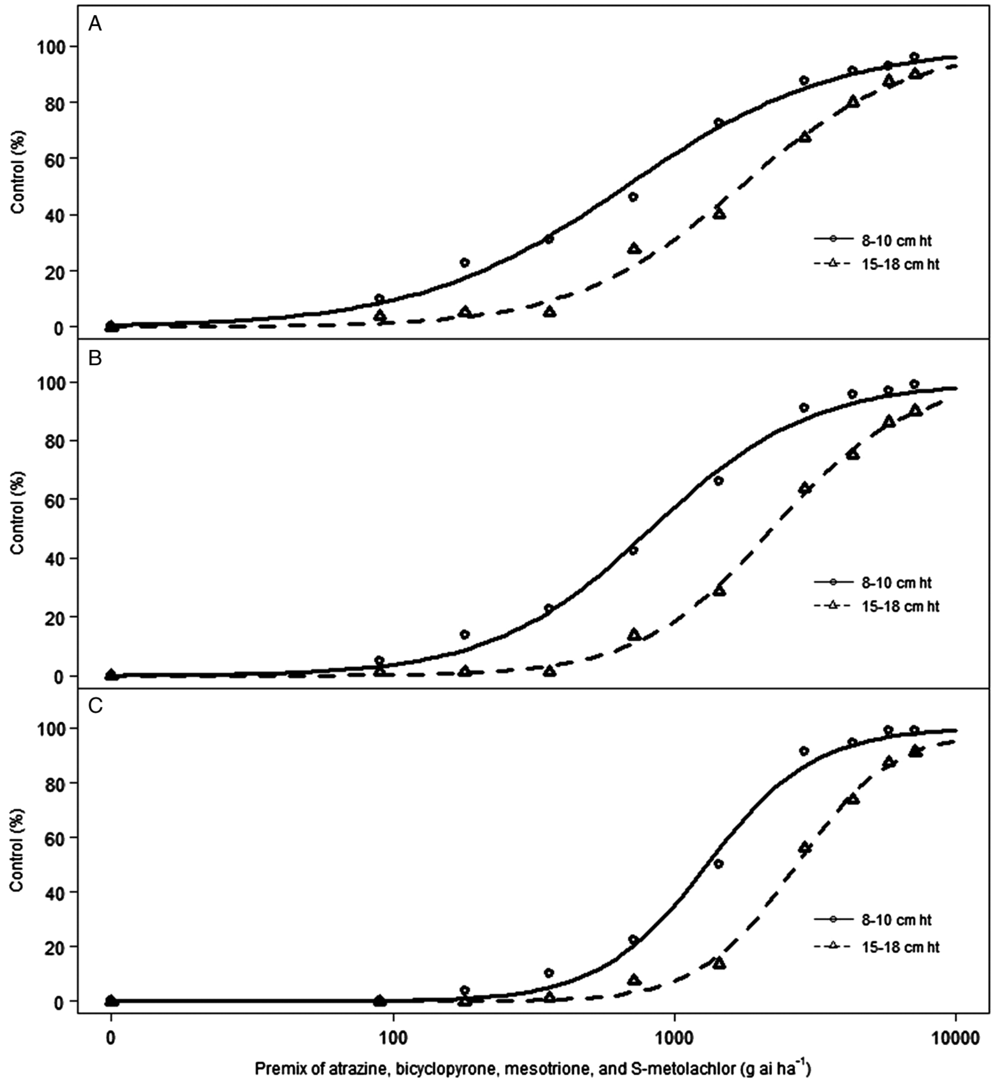
Results from this study showed that the premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) to GR horseweed plants at a rosette diameter of ≤18 cm using the labeled rate provided >90% control under greenhouse conditions. The field study showed that the premix applied before emergence (PRE) at the labeled rate (2900 g a.i. ha^{-1}) provided ≥90% control of GR horseweed up to 35 DAT; whereas the POST dose-response studies revealed that the biologically effective doses (ED_{90}) to control GR horseweed varied with the growth stages (or plant height), with control estimates never reaching 90% with premix application at the labeled rate for ≥8 cm tall plants. The premix contains 29% (by weight) atrazine in total a.i., with the herbicide label noting that cumulative atrazine use rates in a single growing season should not exceed 2800 g a.i. ha^{-1} (Anonymous 2016). These instructions should not be violated to reduce the risk of atrazine contamination in waterbodies and drinking water (Lakshminarayan et al. 1996) and to reduce the chances of atrazine carryover to sensitive crops in subsequent years (Brecke et al. 1981). The herbicide label also notes that the premix rate can vary depending on the soil texture and organic matter: for example, the maximum premix of 2400 g a.i. ha^{-1} is recommended for soil with <3% organic matter, however the season-maximum application rate should not exceed 2900 g a.i. ha^{-1} (Anonymous 2016).

Practical implications

The premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor has been recently commercialized and is expected to be used for controlling herbicide-resistant and problem weeds in corn. The use of herbicides with multiple effective modes of action is necessary to manage the increasing problem of GR weeds, including horseweed. Results of the field dose-response study suggested that the ED_{90} value(s) for the PRE application were recorded as 2800 g a.i. ha^{-1} at 35 DAT, while they were 3400 and 7350 for POST applications made to 8–10 and 15–18 cm tall horseweed plants, respectively, and the POST ED_{90} values were higher than the labeled rate (2900 g a.i. ha^{-1}) for the premix. The new premix tested in this study contains benoxacor, a crop safener believed to provide good crop safety as well (Bernards et al. 2006; Riechers et al. 2010).

Field dose-response studies were conducted under bare ground (no-crop) conditions; therefore, competition from crops for light, moisture, and nutrients was absent. Additionally, insufficient ground coverage may have increased the soil temperature, which is considered to enhance the emergence of horseweed under field conditions: for example, Nandula et al. (2006) showed that the increase in day temperature from 18 °C to 24 °C increased the germination of horseweed significantly under greenhouse conditions, meaning that the biologically effective doses of the premix may vary under crop conditions compared with the bare-ground studies due to variable weed density.

Fig. 5. Glyphosate-resistant horseweed control at (A) 14, (B) 35, and (C) 63 d after treatment (DAT) in a field dose-response study with a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) to 8–10 or 15–18 cm tall horseweed in 2015 and 2016.



Horseweed biotypes resistant to other herbicide modes of action [e.g., acetolactate synthase (ALS) inhibitors, photosystem I (PS I) inhibitors, and photosystem II (PS II) inhibitors] have been reported in North America, including several biotypes with multiple resistances (Smisek et al. 1998; Weaver et al. 2004; Trainer et al. 2005; VanGessel et al. 2006; Eubank et al. 2012). The majority of GR horseweed populations in eastern Nebraska are also resistant to ALS-inhibitors; thus, occurrences of weeds resistant to multiple herbicides significantly reduce the number of effective herbicide options (Busi et al. 2013; Sarangi et al. 2015; Jhala et al. 2017). Weed management programs relying on use of herbicide(s) with the same mode of action increase selection pressure, furthering the evolution of herbicide-resistant weeds (Norsworthy et al. 2012). Before the commercialization of glyphosate-tolerant crop technology, atrazine was the most widely used herbicide by corn growers in the United States; even by 2014, 55% of corn acres planted were treated with atrazine, while 38% were treated with glyphosate (US Department of Agriculture-National Agricultural Statistics Service 2015; Benbrook 2016). Moreover, atrazine-resistant horseweed biotypes were confirmed in several European countries and in an orchard in the United States (Heap 2016*a*). A Palmer amaranth (*Amaranthus palmeri* Watson) biotype from Nebraska was confirmed to be resistant to both atrazine and HPPD inhibitors in a continuous seed corn production field (Jhala et al. 2014). Similarly, a common waterhemp biotype from northeastern Nebraska was also confirmed for HPPD inhibitor resistance (Oliveira et al. 2017). Recently, Chahal et al. (2017) reported that a GR Palmer amaranth biotype from Thayer County, NE, showed a reduced sensitivity to atrazine and ALS

Fig. 6. Response of glyphosate-resistant horseweed at 35 d after treatment (DAT) to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) to the 8–10 cm tall plants at the rates of (A) 0*x*, (B) 1*x*, and (C) 2*x*, where 1*x* = the labeled rate, 2900 g a.i. ha^{-1}. [Colour online.]

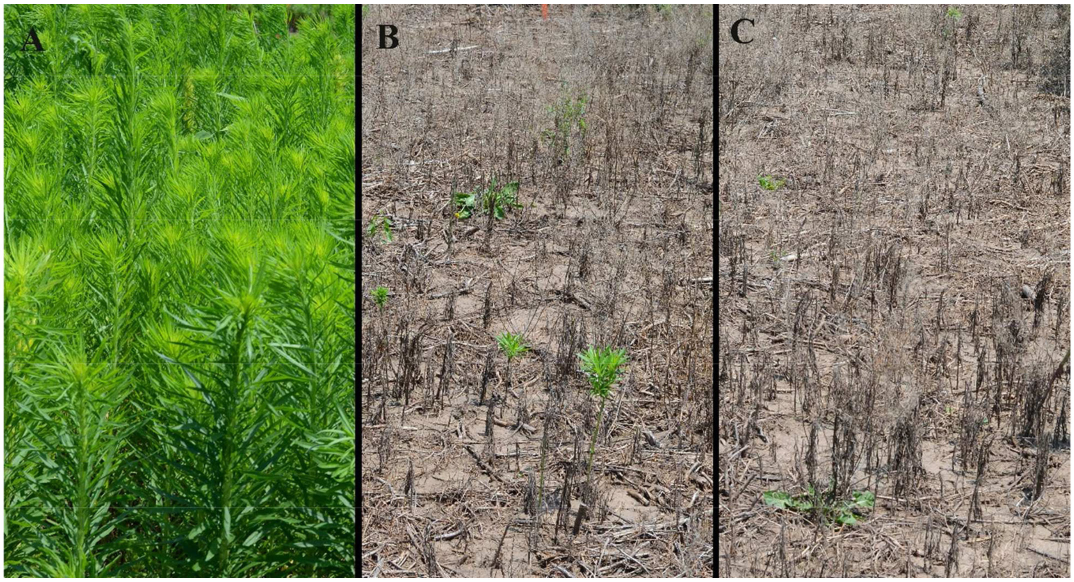
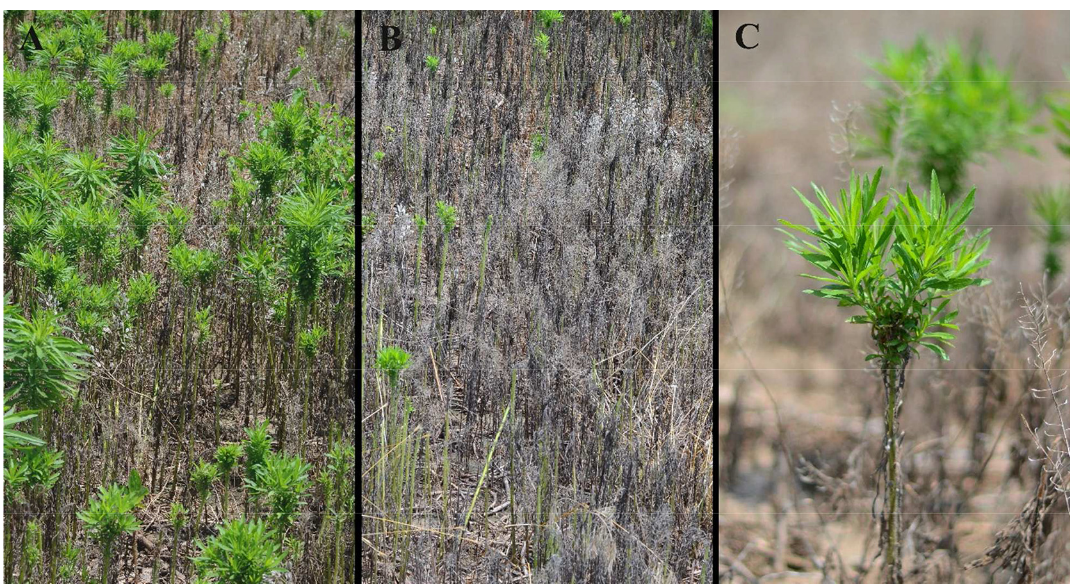


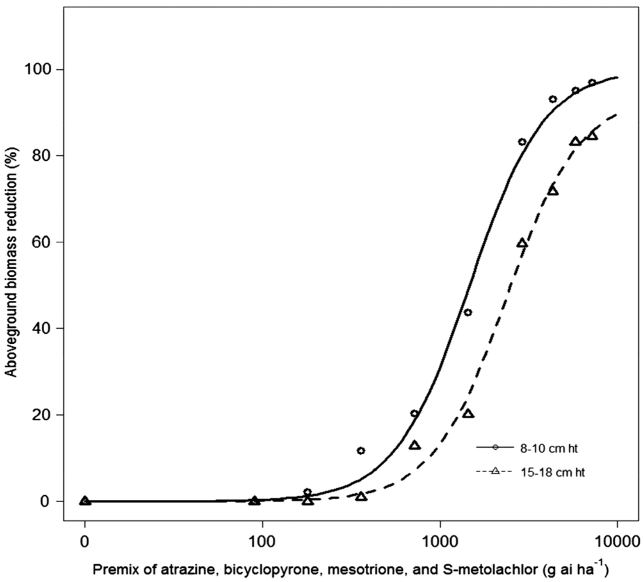
Fig. 7. Response of glyphosate-resistant horseweed at 35 d after treatment (DAT) to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) to the 15–18 cm tall plants at the rates of (A) 1*x*, and (B) 2*x*, where 1*x* = the labeled rate, 2900 g a.i. ha^{-1}. (C) Injury on the shoot tip of horseweed at the 1*x* rate of the premix caused additional branching from the stem. [Colour online.]



inhibitors. Therefore, the premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor should be used in rotation with other herbicide modes of action to prevent the evolution of resistance against this herbicide, including the new a.i., bicyclopyrone.

Gressel (2011) mentioned that the application of herbicide at sublethal doses may accelerate the evolution of resistance due to stress-induced mutations. Therefore, the premix should not be used at sublethal rates for a particular growth stage of any weed species. Results suggested that the application of premix at the labeled rate as a pre-plant burndown in spring (at the rosette stage) or before emergence (PRE) can effectively control GR horseweed populations. Similarly, Davis et al. (2009)

Fig. 8. Aboveground biomass reduction of glyphosate-resistant horseweed at 63 d after treatment (DAT) in a field dose-response study with a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied after emergence (POST) to 8–10 or 15–18 cm tall horseweed in 2015 and 2016.



reported that spring-applied pre-plant residual herbicides were most effective for season-long control of GR horseweed. Previous research conducted under no-till conditions in Indiana suggested that the timing of horseweed emergence could be variable (Davis and Johnson 2008); therefore, selection of herbicide application timing could be an important factor to achieve optimum control of GR horseweed. Shrestha et al. (2007) and VanGessel et al. (2009) also noted that herbicide efficacy is dependent on the horseweed growth stage. It can be concluded that the premix tested in this study will be a good fit for GR horseweed management programs in corn.

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