NRC Research Press

ARTICLE

Biologically effective rates of a new premix (atrazine, bicyclopyrone, mesotrione, and S-metolachlor) for preemergence or postemergence control of common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis*] in corn

D. Sarangi and A.J. Jhala

Abstract: A premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor was recently approved for broad-spectrum weed control in corn in the United States. Greenhouse and field experiments were conducted in 2015 and 2016 to evaluate the response of common waterhemp to various rates of the premix applied before emergence (preemergence, PRE) or after emergence (postemergence, POST) in corn. In greenhouse dose-response bioassays, PRE application of the premix at 975 g a.i. ha^{-1} provided 90% control (visual estimates) of common waterhemp at 28 d after treatment (DAT). The POST doses to control 90% (ED_{90}) of common waterhemp at 21 DAT were estimated as 1157 and 1838 g a.i. ha^{-1} for the 8–10, and 15–18 cm tall common waterhemp, respectively. Under field conditions, the premix applied PRE at the labeled rate (2900 g a.i. ha^{-1}) provided 98% and 91% control of common waterhemp at 14 and 63 DAT, respectively. The ED_{90} values for the in-field POST dose-response bioassay were 680 and 2302 g a.i. ha^{-1} at 14 DAT for the 8–10 and 15–18 cm tall common waterhemp, respectively. The root mean square error and the model efficiency coefficient values indicated a good fit for the prediction models. Spearman's correlation coefficient (r_S) showed that corn yield was positively correlated ($r_S \geq 0.55$; $P < 0.001$) with common waterhemp biomass reduction. The premix applied PRE provided higher corn yields compared with the premix applied POST. The new premix will provide an additional herbicide option with multiple effective modes of action to control common waterhemp in corn.

Key words: crop injury, dose-response, goodness of fit, plant height, resistance management.

Résumé : Un prémélange d'atrazine, de bicyclopyrone, de mésotrione et de *S*-métolachlor a récemment été homologué pour combattre un large spectre d'adventices dans les champs de maïs, aux États-Unis. En 2015 et 2016, les auteurs ont procédé à des expériences en serre et sur le terrain pour déterminer la réaction de l'amarante rugueuse au prémélange, appliqué à divers taux avant et après la levée, dans les cultures de maïs. Lors des essais dose-réponse en serre, l'application de 975 g de matière active par hectare avant la levée a détruit 90 % (estimation visuelle) de l'amarante rugueuse, 28 jours après le traitement. Les valeurs DE_{90} des applications post-levée s'établissent respectivement à 1157 et à 1838 g de matière active par hectare, 21 jours après l'application sur les plants d'amarante rugueuse de 8 à 10 et de 15 à 18 cm de hauteur. Sur le terrain, l'application du prémélange avant la levée au taux recommandé (2900 g de matière active par hectare) détruit respectivement 98 % et 91 % de l'amarante rugueuse, 14 et 63 jours après le traitement. Les valeurs DE_{90} établies lors de l'essai dose-réponse post-levée sur le terrain s'élèvent respectivement à 680 et à 2302 g de matière active par hectare, 14 jours après le traitement, pour les plants d'amarante rugueuse de 8 à 10 et de 15 à 18 cm de hauteur. L'écart-type et le coefficient d'efficacité indiquent un bon ajustement avec les modèles prévisionnels. Le coefficient de corrélation de Spearman (r_S) révèle qu'il existe une corrélation positive ($r_S \geq 0,55$; $P < 0,001$) entre le rendement du maïs et la réduction de la biomasse de l'amarante rugueuse. L'application du prémélange avant la levée augmente le rendement du maïs, comparativement à son application après la levée. Le nouveau prémélange offre une solution

Received 27 January 2017. Accepted 24 April 2017.

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*A.J. Jhala currently serves as an Associate Editor: peer review and editorial decisions regarding this manuscript were handled by Scott White.

de plus pour lutter contre l'amarante rugueuse dans les champs de maïs au moyen d'herbicides à modes d'action multiples. [Traduit par la Rédaction]

Mots-clés : dommages aux cultures, dose réponse, qualité de l'ajustement, hauteur des plants, gestion de la résistance.

Introduction

Crop yield loss due to weed interference is one of the major threats to optimum crop production and global food security. The application of herbicides to control weeds is not a new concept in crop production systems; however, extensive use of herbicide(s) with a single mode of action (MOA) has led to the evolution of herbicide-resistant weeds (Powles and Yu 2010). Since the first report of synthetic auxin-resistant spreading dayflower (*Commelina diffusa* Burm. f.) and wild carrot (*Daucus carota* L.) in 1957 (Hilton 1957; Switzer 1957; Heap 2017*c*), the number of herbicide-resistant weeds has increased rapidly (Délye et al. 2013). Globally, 251 weed species have been confirmed to be resistant to 23 (of the 26) known MOAs and to a total of 162 herbicides (Heap 2017*a*). In Nebraska, eight weed species (grass and broadleaf) have been confirmed to be resistant to at least one herbicide MOA group (Jhala 2017). Additionally, multiple herbicide resistance has also been reported in several weed species, including common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis*] (Sarangi et al. 2015), kochia [*Kochia scoparia* (L.) Schrad.] (Rana and Jhala 2016), and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Jhala et al. 2014; Chahal et al. 2017).

Tank mixing herbicides with multiple effective MOAs has been proposed as a strategy for managing herbicide-resistant weeds (Gressel and Segel 1990; Norsworthy et al. 2012), assuming that the mutation conferring resistance to a tank mix partner does not increase the fitness with the presence of another active ingredient with a distinct MOA (Diggle et al. 2003; Lagator et al. 2013). Wrubel and Gressel (1994) have listed criteria for effective tank mix partners: (*i*) a mixture should control the same weed spectra, (*ii*) the component-active ingredients should have a different target site but the same persistence, and (*iii*) the mixture partners must be degraded in plants via different mechanisms and preferably will employ a negative cross-resistance to the active ingredient(s) belonging to a specific MOA.

Acuron® (Syngenta Crop Protection, LLC, Greensboro, NC) was recently commercialized in the United States for broad-spectrum weed control with preplant, preemergence (PRE), or early postemergence (POST) application in field corn (Anonymous 2016). It is also labeled for seed corn; however, it is labeled only for PRE application in sweet corn and yellow popcorn. It is a premixture (hereafter referred to as a "premix") of atrazine (10.9% of total volume), bicyclopyrone (0.7%), mesotrione (2.6%), and S-metolachlor (23.4%). These four active ingredients have three different MOAs: photosystem II inhibitor (atrazine), 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor (bicyclopyrone and mesotrione), and very long-chain fatty acid elongases inhibitor (S-metolachlor). Bicyclopyrone, a new active ingredient, can provide soil-applied residual or POST weed control, and Acuron® was the first bicyclopyrone-containing product commercialized recently (Jhala and Sarangi 2016). For the last three decades, no herbicide with a new MOA has been commercialized for use in corn and soybean, therefore, combining existing herbicides is becoming an important practice for managing herbicide-resistant weeds (Duke 2012; Owen et al. 2015). The synergistic effects of certain herbicide active ingredients can improve weed control efficacy (Bollman et al. 2006); for example, synergy between atrazine and HPPD inhibitors has been reported for effective control of *Amaranthus* species (Hugie et al. 2008; Woodyard et al. 2009; Jhala et al. 2014).

Common waterhemp is a summer annual broadleaf weed native to North America (Waselkov and Olsen 2014) and is a predominant weed species in agricultural fields in the midwestern United States (Prince et al. 2012). A recent survey by the Weed Science Society of America listed common waterhemp among the top five troublesome weeds in the United States (Van Wychen 2016). In a study conducted in Illinois, Steckel and Sprague (2004) reported that the season-long interference of common waterhemp caused 74% corn yield loss, where 270 common waterhemp plants m^{-2} were allowed to compete beyond the V10 growth stage of corn. Common waterhemp is a prolific seed producer, developing a more persistent seed bank than many other annual weed species. Hartzler et al. (2004) reported that a single female common waterhemp plant competing with soybean throughout the growing season can set 300 000 to 2.3 million seeds. In a study conducted in Iowa, Buhler and Hartzler (2001) recovered 12% of common waterhemp seeds after 4 yr of seed burial. Moreover, a long-term seed longevity study conducted in Nebraska showed that 1% to 3% of tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer var. *tuberculatus*] seeds remained viable up to 17 yr after burial at a 20 cm depth (Burnside et al. 1996). Thus, favorable biological characteristics of common waterhemp, along with its adaptation to a wide variety of climatic conditions, have favored its persistence in agronomic crop production fields in the midwestern United States (Nordby et al. 2007; Sarangi et al. 2016). Common waterhemp has an extended emergence period: from the third week of May through mid-August in midwestern conditions

(Hartzler et al. 1999). Werle et al. (2014) classified common waterhemp as a late-emerging species for its emergence sequence and duration. Additionally, common waterhemp biotypes resistant to acetolactate synthase (ALS) inhibitors (Horak and Peterson 1995), photosystem II inhibitors (Anderson et al. 1996), protoporphyrinogen oxidase (PPO) inhibitors (Shoup et al. 2003), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors (i.e., glyphosate) (Legleiter and Bradley 2008), HPPD inhibitors (Hausman et al. 2011), and synthetic auxins (Bernards et al. 2012) have been confirmed in the United States.

Common waterhemp is a dioecious species and pollen movement under field conditions promotes rapid dispersal of resistance traits among different populations (Sarangi et al. 2017*b*). Furthermore, glyphosate-resistant (GR) common waterhemp is widely distributed (in 18 states; Heap 2017*b*) in the midwestern and southern United States, requiring strategies for effective management of this problem weed. Due to the evolution of herbicide resistance and the extended emergence pattern of common waterhemp, several studies have stated that residual herbicides with multiple MOAs (applied PRE) are the cornerstone for effective control (Legleiter and Bradley 2009; Tranel et al. 2011; Jhala et al. 2017; Sarangi et al. 2017*a*). Studies have shown that herbicide efficacy is dependent on application timing and weed growth stages (Falk et al. 2006; Chahal et al. 2015; Ganie et al. 2015; Soltani et al. 2016; Sarangi and Jhala 2017). Devlin et al. (1991) stated that registrants register high rates of herbicides to ensure acceptable weed control across a broad range of weed species, weed growth stages, and environmental conditions. Therefore, it is important to determine the biologically effective doses and the effect of application timing of a new herbicide for a specific weed species. Additionally, it is important to evaluate the selectivity of the crop in response to a range of herbicide doses applied PRE and (or) POST. The objectives of this study were to evaluate (*i*) the response of common waterhemp to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied PRE or POST at two growth stages (8–10 or 15–18 cm plant height) in greenhouse and field studies and (*ii*) the response of corn in terms of injury and yield as affected by application timing (PRE or POST) and premix dose.

Materials and Methods

Greenhouse dose-response studies

Plant materials

Glyphosate-resistant common waterhemp seeds were collected in 2012 from a soybean field in Lancaster County, NE, and their sensitivity to glyphosate and other herbicides was evaluated. A whole-plant dose-response bioassay conducted at the University of Nebraska-Lincoln showed that the GR common waterhemp biotype was 22- to 32-fold resistant to glyphosate compared with known glyphosate-susceptible (GS) common waterhemp biotypes (Sarangi et al. 2015). The same study also revealed that the GR biotype had a reduced sensitivity to POST applied ALS inhibitors. Seeds were stored in airtight polythene bags at 4 °C until experiments commenced.

Whole-plant dose-response bioassay

In 2015, two separate whole-plant dose-response bioassays were conducted under greenhouse conditions at the University of Nebraska-Lincoln to evaluate the response of GR common waterhemp to a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied PRE or POST. Each experiment was repeated in time under similar greenhouse conditions.

The soil used in the PRE dose-response study was collected from a field near Lincoln, NE, with no history of residual herbicide use. The soil texture was silt-loam with 6.1 pH, 22% sand, 54% silt, 24% clay, and 3% organic matter. Square plastic pots (10 cm × 10 cm × 12 cm) were filled with finely ground soil and 400 seeds (seed count was performed by taking the weight of samples of 200 seeds, as described by Sarangi et al. 2016) of GR common waterhemp were sprinkled on the soil surface. A thin layer (1 cm) of ground soil was spread on top of the seeds and the premix was applied on the day following common waterhemp planting. Water was sprinkled when necessary on the soil surface to ensure the germination of common waterhemp and to dissolve the premix in soil water, so that the herbicide could be taken up by the developing weed seedlings. The greenhouse was maintained at a 28/24 °C day/night temperature with a 16 h photoperiod using artificial halide lamps (600 μmol photon m^{-2} s^{-1}).

In a separate dose-response study, efficacy of the premix applied POST to the GR common waterhemp was evaluated. Common waterhemp seedlings were grown in 72-cell germination trays and transplanted at the first-true leaf stage into square plastic pots (mentioned above) containing a 1:3 mixture of soil to potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, QC). A single common waterhemp plant was allowed to grow in each pot and sufficient water and nutrients (24–8–16, Miracle-Gro Water Soluble All Purpose Plant Food, Scotts Miracle-Gro Products Inc., Marysville, OH) were supplied as needed. Greenhouse conditions were similar to those mentioned above in the PRE dose-response study. The premix was applied when GR common waterhemp plants were 8–10 or 15–18 cm tall.

The phytotoxicity of the premix on the GR corn hybrid (NK N69Q-3000 GT) was evaluated in the greenhouse dose-response study. Two seeds of the GR corn hybrid were planted in each plastic pot (described above) and kept in the same greenhouse mentioned above. Preemergence application of the premix was made on the day following corn planting, whereas POST applications were made when the corn was 18 or 30 cm in height. The studies were repeated in time using the same procedure.

Field dose-response studies

Field dose-response bioassays were conducted in 2015 and 2016 at the South Central Agricultural Laboratory (40.57°N, 98.14°W, near Clay Center, NE) at the University of Nebraska-Lincoln. The soil texture at the experimental site was Crete silt loam (montmorillonitic, mesic, Pachic Argiustolls) with a pH of 6.5%, 17% sand, 58% silt, 25% clay, and 3% organic matter. The experimental site was primarily infested with common waterhemp. A GR corn hybrid (NK N69Q-3000 GT) was planted at 78 300 seeds ha^{-1} at 76 cm row spacing on 13 May 2015 and 24 May 2016. The experimental site was under a center-pivot irrigation system and the plots were irrigated when needed. The site was fertilized with 11–52–0 fertilizer at 112 kg ha^{-1} with an additional 202 kg ha^{-1} of N in the form of anhydrous ammonia applied in the spring. Preemergence and POST experiments were conducted separately to evaluate the response of common waterhemp and corn to the applications of the premix under field conditions.

Premix treatments

Greenhouse experiments were laid out in a randomized complete block design with a factorial arrangement of 10 herbicide treatments and two growth stages (for the POST dose-response bioassay). Each greenhouse bioassay had five replications and a single plastic pot was considered as an experimental unit. The premix rates were 0×, 0.031×, 0.062×, 0.125×, 0.25×, 0.5×, 1×, 1.5×, 2×, and 2.5× the labeled rate, which was 2900 g a.i. ha^{-1} (for 3% organic matter). The herbicide treatments were similar for the PRE and POST dose-response bioassays conducted in the greenhouse as well as in the field. A non-ionic surfactant (NIS, 0.25% *v*/*v*, Induce®, Helena® Chemical Company, Collierville, TN) was included in the POST herbicide treatments. In the greenhouse, the premix was applied using a single-tip spray chamber (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}.

The field experiments were laid out in a randomized complete block design with four replications and a plot size of 9 m × 3 m. The premix was applied 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}. Preemergence application of the premix was made on the day following corn planting. The air temperature during PRE application was 15 °C and 22 °C in 2015 and 2016, respectively. The POST application of the premix was made at two growth stages of common waterhemp (8–10 or 15–18 cm). The premix was applied to 8–10 cm tall common waterhemp plants on 5 June 2015 and 10 June 2016, when the corn was at the V3 growth stage (18 cm tall). Postemergence application of the premix to taller (15–18 cm) common waterhemp plants were made on 17 June 2015 and 20 June 2016, when corn was at the V5 growth stage (30 cm tall).

Data collection

In the greenhouse, GR common waterhemp control and emergence affected by PRE applications of the premix was assessed at 14, 21, and 28 d after treatment (DAT). Control data was estimated based on the severity of the injury symptoms (bleaching of leaf, chlorosis and necrosis, and plant death) compared with the nontreated control (i.e., 0× rate of the premix), using a scale ranging from 0% to 100%, where 0% means no control or injury and 100% means complete death of the plant. Percent reduction in weed density was determined using eq. 1. In the POST dose-response bioassay, GR common waterhemp control was estimated at 7, 14, and 21 DAT in the greenhouse using a 0% to 100% scale as described previously. Corn injury was determined at 14, 21, and 28 DAT by estimating the injury using a 0% to 100% scale as described previously. The surviving common waterhemp plants were cut at the base at 21 DAT and oven-dried at 65 °C until they reached a constant weight. The biomass data were converted into percent biomass reduction compared with the nontreated control (Sarangi et al. 2017*a*) using the equation

$$\text{aboveground biomass (or weed density) reduction (\%)} = [(C - B)/C] \times 100 \quad (1)$$

where C is the biomass (or weed density) of the nontreated control plot and B is the biomass (or density) of an individual treated plot.

In the field dose-response bioassay, control estimates and density of common waterhemp were estimated at 14, 21, 35, and 63 DAT and at corn harvest on a scale of 0% to 100% as described previously. Common waterhemp densities were recorded by counting the weed plants in two 0.25 m^2 quadrats placed randomly between two center rows of corn in each plot and presented as percent density reduction using eq. 1. Common waterhemp plants surviving the premix treatment were severed at the base at 63 DAT by placing two randomly selected quadrats (0.25 m^2) per plot from the two center corn rows. The biomass samples were oven-dried at 65 °C until they reached a constant weight and the percent reduction of aboveground biomass was calculated using eq. 1.

Phytotoxicity of the premix on GR corn was evaluated in the field dose-response bioassays (PRE and POST). At 14 and 21 DAT, corn injury was evaluated visually on a 0% to 100% scale based on leaf tissue bleaching, chlorosis and necrosis, malformation of leaves (unfurling), and plant stunting. Corn height was measured by averaging the height (from the base to the topmost visible leaf collar) of five randomly selected corn plants in each plot. Corn was harvested from the center two rows in each plot using a plot combine, and the grain yield was adjusted to 15.5% moisture content.

Statistical analysis

Data were subjected to analysis of variance using PROC GLIMMIX in SAS version 9.3 (SAS Institute Inc., Cary, NC) to perform the significance test. Premix treatments were considered fixed effects and 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 model (eq. 2) was used to determine the effective premix doses required to control common waterhemp (or reduce aboveground biomass and (or) density) by 50% and 90% (ED_{50} and ED_{90}) using the *drc* package in R (R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al. 2007):

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

where Y is the response variable (percent control or percent reduction in the aboveground biomass and (or) density); x is the herbicide dose; c and d are the lower limit (which was set to zero) and the estimated maximum value of Y, respectively; and e represents the herbicide doses resulting in 50% of Y (i.e., ED_{50}). The parameter b is the relative slope around the parameter e. Corn yield data was also regressed against herbicide doses using eq. 2.

Model goodness of fit

Goodness of fit parameters [root mean square error (RMSE) and model efficiency coefficient (EF)] were calculated to evaluate the model fit using eqs. 3 and 4:

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

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

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. Prediction of R^2 is an inadequate goodness measure for eq. 2, a nonlinear model (Spiess and Neumeyer 2010); therefore, Sarangi et al. (2016) suggested that reporting RMSE and EF would be better suited for a nonlinear function. A smaller RMSE value means better fit and an EF value closer to 1.00 means more accurate predictions.

Results and Discussion

Treatment × year (or treatment × experimental run for the greenhouse studies) interactions were nonsignificant ($P > 0.05$); therefore, data from both years and (or) experimental runs were combined.

Greenhouse dose-response studies

Preemergence dose-response study

The premix applied PRE at ≥2900 g a.i. ha^{-1} (i.e., 1× rate) provided ≥95% control of GR common waterhemp. The premix doses required for 50% and 90% control (ED_{50} and ED_{90}) of GR common waterhemp at 14 DAT were 181 and 1236 g a.i. ha^{-1}, respectively (Table 1). The response of common waterhemp to the premix was similar at later observation dates (Fig. 1A); for example, at 28 DAT, the biologically effective doses (ED_{50} and ED_{90}) were 195 and 975 g a.i. ha^{-1}, respectively, which were comparable to the doses required at 14 DAT. GR common waterhemp density reduction data concurred with control estimates and 97% density reduction was observed at the 1× rate (Table 1). Doses required to reduce GR common waterhemp density by 90% (ED_{90}) ranged between 644 and 704 g a.i. ha^{-1}, although there was no difference in the ED_{90} values between 14 and 28 DAT (Table 1; Fig. 1B). In a field experiment conducted in Missouri, Legleiter and Bradley (2009) reported that the PRE application of atrazine + mesotrione + S-metolachlor (1460, 190, and 1460 g a.i. ha^{-1}) provided 98% control of GR common waterhemp up to 90 DAT. The RMSE values for GR common waterhemp control and density reduction were <9.0 and the EF values were close to 1.00 (≥0.93) (Table 1), indicating a good fit for the log–logistic model.

Postemergence dose-response study

The POST dose-response curves indicate that control of GR common waterhemp was affected ($P < 0.001$) by plant height at the time of herbicide application (Fig. 2A). The dose required for 50% control (ED_{50}) of 8–10 cm tall GR common waterhemp was 337 g a.i. ha^{-1} at 21 DAT; however, a higher dose (415 g a.i. ha^{-1}) was needed to achieve the same level of control when the plants were 15–18 cm tall (Table 2). Similarly, doses of the premix required to provide 90% control (ED_{90}) of GR common waterhemp were 1157 and 1838 g a.i. ha^{-1} for the 8–10 and 15–18 cm tall plants, respectively. Several studies have also reported the growth stage dependent response of GR common waterhemp to different premix herbicides. In a POST dose-response study conducted in the greenhouse, Chahal et al. (2015) reported that 1179 and 2480 g a.e. ha^{-1} of 2,4-dichlorophenoxyacetic acid choline + glyphosate were needed to provide 90% control of 10 and 20 cm tall GR common waterhemp, respectively. Similarly, Ganie et al. (2015) observed that higher doses of a premix of fluthiacet-methyl and mesotrione were required to control 20 cm tall common waterhemp plants compared with the 10 cm tall plants, where ED_{90}

Table 1. Estimation of the regression parameter and model goodness of fit for a log–logistic function[a] fitted to glyphosate-resistant common waterhemp control and density reduction in response to the preemergence application of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor and the estimation of the effective doses needed to control or reduce weed density by 50% (ED_{50}) and 90% (ED_{90}) in a greenhouse dose-response study conducted at the University of Nebraska-Lincoln.

	Regression parameter	Model goodness of fit				
DAT	*b* (±SE)	RMSE	EF	ED_{50} (±SE) (g a.i. ha^{-1})	ED_{90} (±SE) (g a.i. ha^{-1})	Predicted value (%) at the 1× rate[b]
Control of common waterhemp						
14	−1.2 (±0.12)	8.6	0.93	181 (±12)	1236 (±259)	95
28	−1.6 (±0.13)	7.5	0.95	195 (±9)	975 (±139)	95
Density reduction of common waterhemp						
14	−1.6 (±0.15)	8.4	0.94	152 (±8)	704 (±110)	97
28	−1.8 (±0.17)	8.9	0.93	167 (±9)	644 (±94)	97

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

[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 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 dose causing 50% control or density reduction (i.e., ED_{50}) in glyphosate-resistant common waterhemp. The parameter *b* is the relative slope around the parameter *e*.

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

Fig. 1. Glyphosate-resistant common waterhemp (A) control and (B) density reduction at 14 and 28 d after treatments (DAT) in response to the preemergence application of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor in a greenhouse dose-response study conducted at the University of Nebraska-Lincoln.

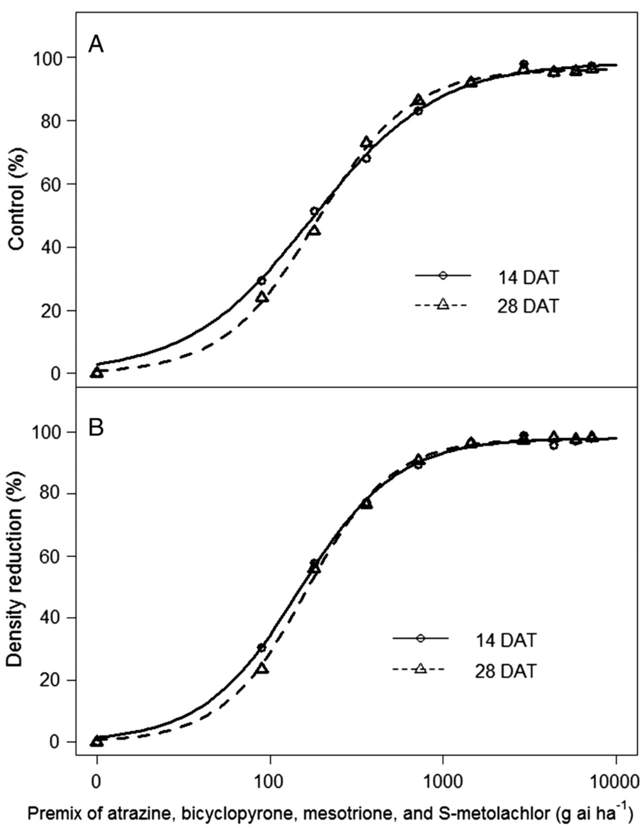


Fig. 2. Glyphosate-resistant common waterhemp (A) control and (B) aboveground biomass reduction at 21 d after treatment (DAT) in response to the postemergence application of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor on 8–10 or 15–18 cm tall plants in a greenhouse dose-response study conducted at the University of Nebraska-Lincoln.

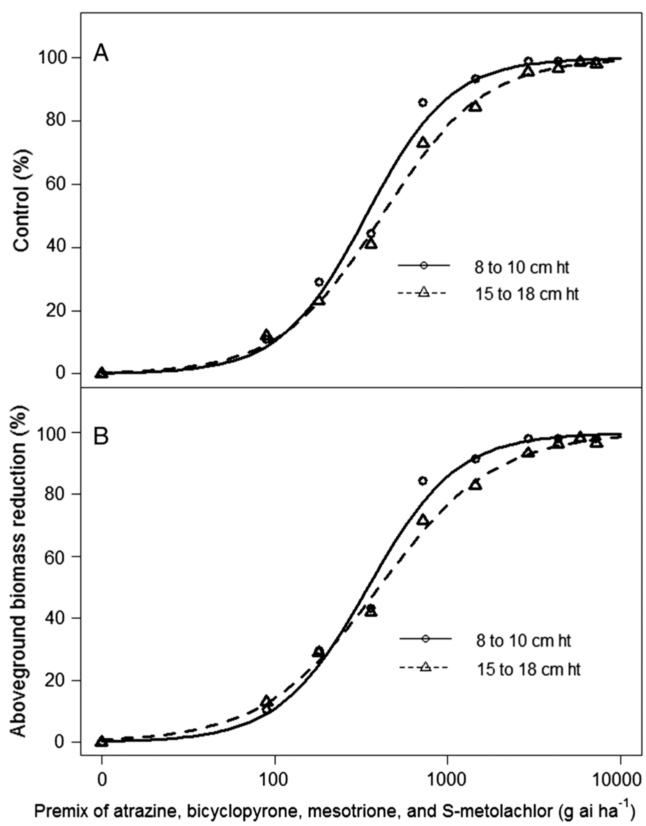


Table 2. Estimation (at 21 DAT) of the regression parameter and model goodness of fit for a log–logistic function[a] fitted to glyphosate-resistant common waterhemp control and aboveground biomass reduction in response to the postemergence applications of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor made at two different weed heights and the estimation of the effective doses needed to control or reduce aboveground biomass by 50% (ED_{50}) and 90% (ED_{90}) in a greenhouse dose-response study conducted at the University of Nebraska-Lincoln.

	Regression parameter	Model goodness of fit				
GR waterhemp height (cm)	*b* (±SE)	RMSE	EF	ED_{50} (±SE) (g a.i. ha^{-1})	ED_{90} (±SE) (g a.i. ha^{-1})	Predicted value (%) at the 1× rate[b]
Control of common waterhemp						
8–10	−1.8 (±0.08)	5.8	0.98	337 (±10)	1157 (±69)	98
15–18	−1.5 (±0.06)	5.9	0.97	415 (±13)	1838 (±128)	95
Aboveground biomass reduction of common waterhemp						
8–10	−1.7 (±0.09)	5.9	0.98	346 (±12)	1273 (±107)	97
15–18	−1.3 (±0.07)	8.1	0.95	401 (±19)	2178 (±217)	93

Note: GR, glyphosate-resistant; SE, standard error of the mean; RMSE, root mean square error; EF, modelling efficiency coefficient.

[a]$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where *Y* is the response variable (percent control or percent reduction in 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 biomass reduction (i.e., ED_{50}) in glyphosate-resistant common waterhemp. The parameter *b* is the relative slope around the parameter *e*.

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

Table 3. Monthly mean air temperature and total precipitation during the 2015 and 2016 growing seasons and the 30-yr average at Clay Center, NE.[a]

	Mean temperature (°C)			Total precipitation (mm)		
Month	2015	2016	30-yr average	2015	2016	30-yr average
May	15.6	15.9	16.1	98.8	137.4	134.1
June	22.6	24.8	21.8	216.2	10.9	101.3
July	24.4	24.6	24.2	150.4	66.3	104.1
August	22.5	23.4	23.1	47.5	55.4	94.2
September	22.1	19.8	18.4	39.9	90.2	58.9
October	13.9	14.3	11.5	29.2	46.5	55.9
Annual	11.8	12.2	10.3	795.5	623.8	730.3

[a]Air temperature and precipitation data were obtained from High Plains Regional Climate Center (2017).

values were 78.3 and 144.0 g a.i. ha^{-1} for the 10 and 20 cm tall plants, respectively.

The dose-response curves for the aboveground biomass reduction showed a similar trend to the GR common waterhemp control. Biomass reductions with the 1× rate of the premix were estimated as 97% and 93% for the 8–10 and 15–18 cm tall common waterhemp plants, respectively (Fig. 2B). The biologically effective doses (ED_{50} and ED_{90}) needed for aboveground biomass reduction of the 8–10 cm tall common waterhemp plants were 346 and 1273 g a.i. ha^{-1}, respectively, whereas 401 and 2178 g a.i. ha^{-1} were needed for the 15–18 cm tall plants (Table 2). The RMSE values for the POST dose-response studies conducted under greenhouse conditions ranged between 5.8 and 8.1 and the EF values were ≥0.95. Similarly, in a POST dose-response bioassay (in the greenhouse), evaluating the response of GR horseweed [*Conyza Canadensis* (L.) Cronq.] to the same premix, Sarangi and Jhala (2017) reported RMSE values of ≤5.6 and EF values of ≥0.98.

Field dose-response studies

Preemergence dose-response study

Mean temperature and total precipitation data at the research site showed that there was adequate moisture and favorable temperatures in both years necessary for the premix activity (Table 3). Preemergence application of the premix was highly effective and its application at the labeled rate (2900 g a.i. ha^{-1}) resulted in 98% and 91% control of common waterhemp at 14 and 63 DAT, respectively (Table 4). Similarly, in a field experiment conducted in Nebraska, Sarangi and Jhala (2017) reported that the PRE application of the premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor provided 91% control of GR horseweed at 14 DAT. Under field conditions, the dose required to control common waterhemp

Table 4. Estimation of the regression parameter and model goodness of fit for a log–logistic function[a] fitted to common waterhemp control, density, and aboveground biomass reduction in response to the preemergence application of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor and the estimation of the effective doses needed to control or reduce weed density by 50% (ED_{50}) and 90% (ED_{90}) in a field dose-response study conducted in 2015 and 2016 at Clay Center, NE.

	Regression parameter	Model goodness of fit				
DAT	b (±SE)	RMSE	EF	ED_{50} (±SE) (g a.i. ha^{-1})	ED_{90} (±SE) (g a.i. ha^{-1})	Predicted value (%) at the 1× rate[b]
Control of common waterhemp						
14	−1.3 (±0.12)	5.2	0.97	94 (±5)	586 (±93)	98
35	−1.4 (±0.20)	9.1	0.92	149 (±11)	1173 (±369)	93
63	−1.3 (±0.19)	11.0	0.89	251 (±27)	2796 (±1152)	91
Density reduction of common waterhemp						
14	−1.3 (±0.17)	7.3	0.95	102 (±7)	555 (±115)	98
63	−1.2 (±0.19)	12.2	0.88	274 (±35)	2824 (±1255)	90
Aboveground biomass reduction of common waterhemp						
63	−1.1 (±0.20)	13.4	0.85	229 (±33)	2389 (±1178)	91

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

[a]$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where Y is the response variable (control or reduction in the density or 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 density reduction or aboveground biomass reduction (i.e., ED_{50}) of common waterhemp. The parameter b is the relative slope around the parameter e.

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

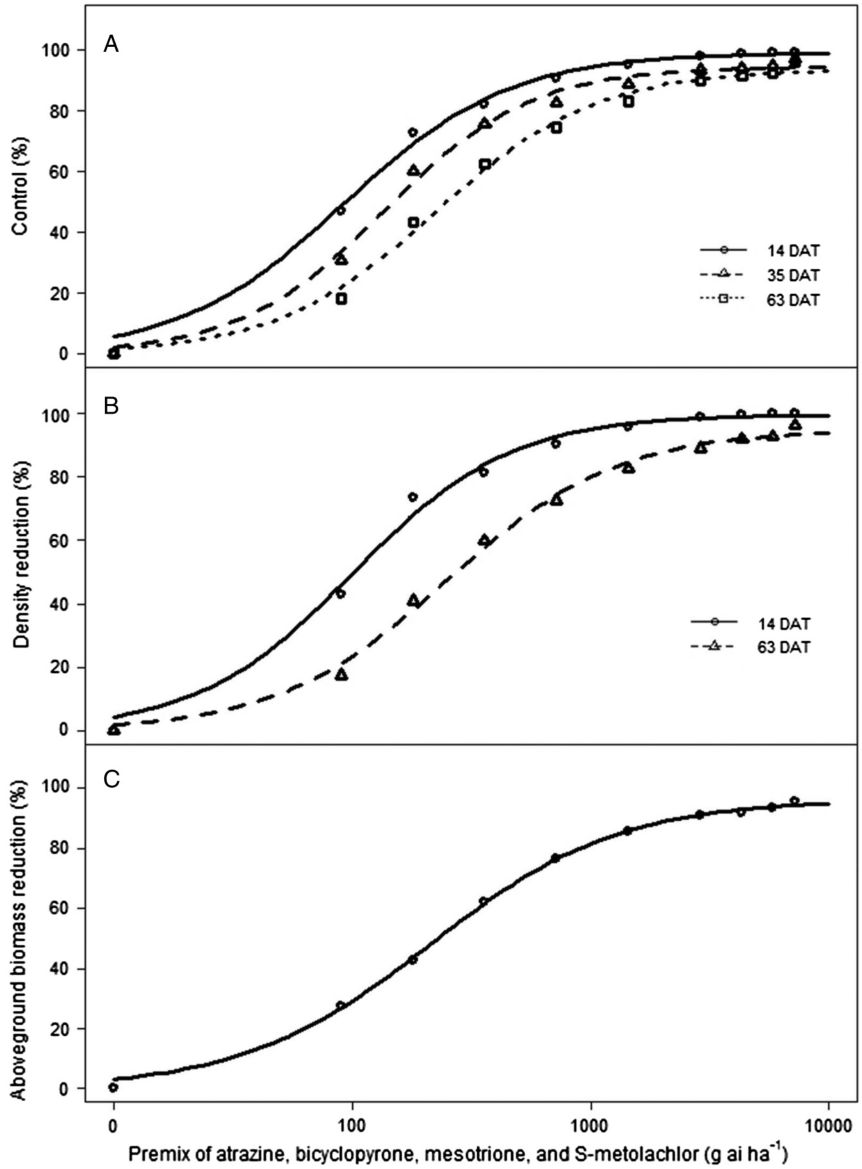
by 90% was increased as the time from premix application to estimation of control increased. The ED_{90} values were 586 and 1173 g a.i. ha^{-1} at 14 and 35 DAT, respectively; however, the dose increased to 2796 g a.i. ha^{-1} at 63 DAT (Table 4). In a field experiment conducted in Ontario, Soltani et al. (2009) reported that PRE application of atrazine + mesotrione + S-metolachlor at the labeled rate provided 92% control of common waterhemp at 28 DAT; however, control decreased to 88% at 70 DAT. In a dose-response study in Nebraska, Knezevic et al. (2009) reported that PRE application of pyroxasulfone at 152 g a.i. ha^{-1} provided 90% control of common waterhemp at 28 DAT; however, higher doses (≥198 g a.i. ha^{-1}) were required to achieve similar levels of control beyond 45 DAT. Similarly, Adams et al. (2014) reported that the premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied PRE at the labeled rate provided ≥97% control of common waterhemp throughout the season. In a field experiment conducted in Kansas, Shoup and Al-Khatib (2004) reported that PRE application of S-metolachlor + mesotrione at 707 and 210 g a.i. ha^{-1} provided ≥93% control of common waterhemp throughout the season. In Ontario, PRE application of S-metolachlor and (or) atrazine + mesotrione at the labeled rate resulted in ≥99% control of common waterhemp at 70 DAT and reduced weed biomass by 100% under field conditions (Vyn et al. 2006).

Common waterhemp density and aboveground biomass reduction data showed a similar trend as the control estimates (Figs. 3B and 3C). The ED_{90} values for the density reduction were 555 and 2824 g a.i. ha^{-1} at 14 and 63 DAT, respectively (Table 4). Preemergence application of the premix at 1× (2900 g a.i. ha^{-1}) rate resulted in a 91% reduction in biomass at 63 DAT under field conditions. The doses of the premix required to reduce the aboveground biomass by 50% and 90% (ED_{50} and ED_{90}) were 229 and 2389 g a.i. ha^{-1}, respectively (Table 4). The goodness of fit parameters (RMSE and EF) showed a good fit for the PRE dose-response curves in this study. The RMSE values ranged between 5.2 and 13.4 for common waterhemp control, density, and aboveground biomass reduction. The model efficiency coefficient (EF) values were estimated as ≥0.85 (Table 4).

Postemergence dose-response study

Dose-response curves indicated that POST application of the premix provided better control of the 8–10 cm tall common waterhemp plants compared with the 15–18 cm tall plants (Figs. 4A, 4B, and 4C). The premix doses required to achieve 90% (ED_{90}) control of the 8–10 cm tall common waterhemp plants were 680, 1308, and 1830 g a.i. ha^{-1} at 14, 35, and 63 DAT, respectively (Table 5). Similarly, there was an increasing trend with time progression in the ED_{50} values for the 8–10 cm tall common waterhemp plants. The premix applied POST at the labeled rate to plants at smaller heights (8–10 cm tall) resulted in ≥95% common waterhemp control (up to 63 DAT) under field conditions. In a field study conducted in Iowa, Owen et al. (2012) reported that early POST application of atrazine + glyphosate + mesotrione + S-metolachlor provided 99% control of common waterhemp 5 wk after treatment.

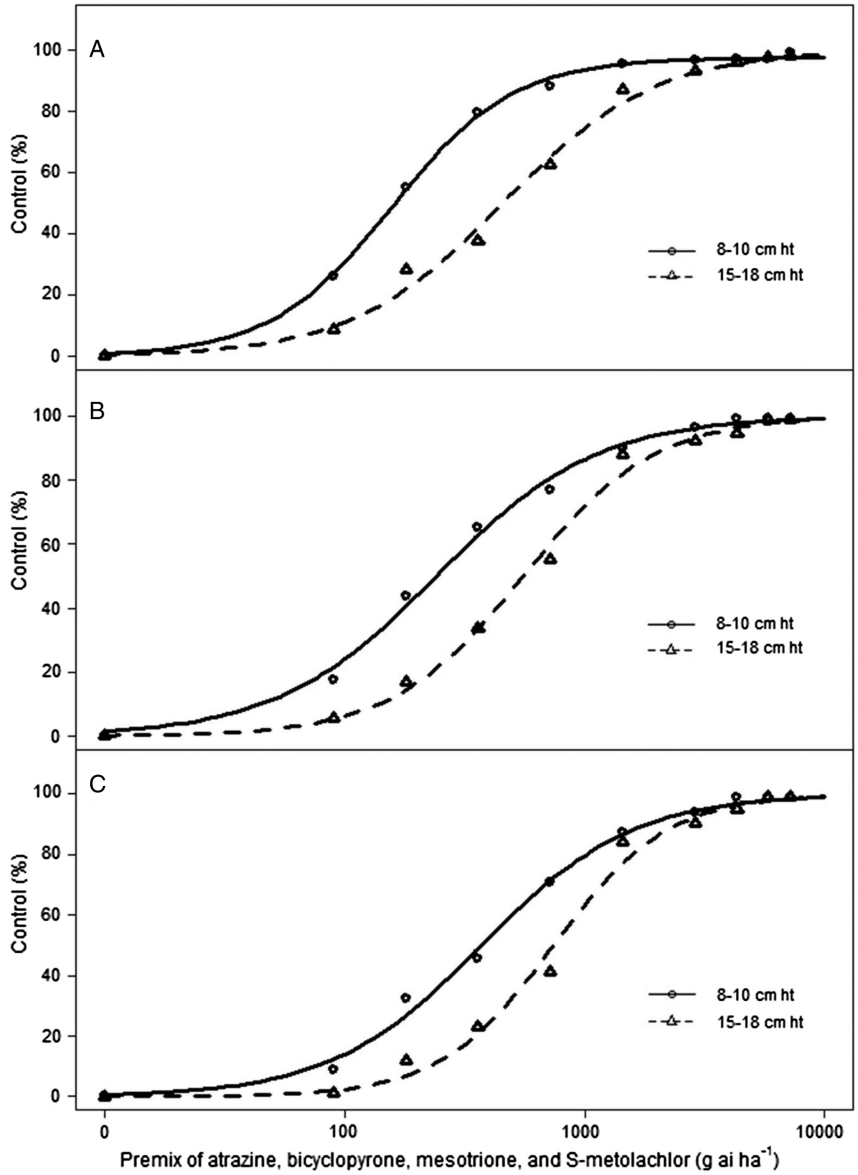
Fig. 3. Common waterhemp (A) control, (B) density reduction, and (C) aboveground biomass reduction at 14, 35, and 63 d after treatments (DAT) in response to the preemergence application of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor in a field dose-response study conducted in 2015 and 2016 in Clay Center, NE.



Postemergence application of the premix to 15–18 cm tall common waterhemp plants resulted in higher values for the biologically effective doses (ED_{50} and ED_{90}) compared with application to the 8–10 cm tall plants. The comparison also showed that the ED values were dependent on plant height ($P < 0.001$) at 14 and 35 DAT; however, the ED_{90} values at 63 DAT were similar ($P = 0.06$) for the 8–10 and 15–18 cm tall common waterhemp plants. Several studies have reported the growth stage dependent response of different weed species to herbicides and that contact herbicides such as acifluorfen, fomesafen, and glufosinate are more effective on weeds at their early growth stages (Coetzer et al. 2002; Hager et al. 2003; Falk et al. 2006), although there are several reports available on the growth stage dependent efficacy of systemic herbicides such as 2,4-dichlorophenoxyacetic acid, glyphosate, and mesotrione (Krausz et al. 1996; Chahal et al. 2015; Soltani et al. 2016; Yu and McCullough 2016). The ED_{50} values for the taller plants (15–18 cm) were estimated as 458, 556, and 745 g a.i. ha^{-1} at 14, 35, and 63 DAT, respectively (Table 5). Doses of >2200 g a.i. ha^{-1} were required to achieve 90% control (ED_{90}) of the 15–18 cm tall common waterhemp plants under field conditions. Continuous emergence of common waterhemp throughout the growing season and regrowth from the stem at lower doses (≤0.5× rate) decreased control estimates after 21 DAT. The 1× rate of the premix provided 92% to 93% control of 15–18 cm tall common waterhemp up to 63 DAT.

The aboveground biomass reduction data were comparable with the control estimates at 63 DAT (Fig. 5).

Fig. 4. Common waterhemp control at (A) 14, (B) 35, and (C) 63 d after treatment (DAT) in response to the postemergence application of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor in a field dose-response study conducted in 2015 and 2016 in Clay Center, NE.



The biologically effective doses required for 50% control of the 8–10 and 15–18 cm tall common waterhemp were 353 and 684 g a.i. ha^{-1}, respectively, compared with 1583 and 2748 g a.i. ha^{-1} for 90% control of the 8–10 and 15–18 cm tall common waterhemp plants, respectively (Table 5). The RMSE values estimated for the POST dose-response models ranged from 9.1 to 14.1. The model efficiency coefficient (EF) values were ≥0.89, showing a good fit for the prediction model. Ganie and Jhala (2017), validating a four-parameter logistic function to evaluate the response of common ragweed population (*Ambrosia artemisiifolia* L.) to glyphosate application, reported RMSE values ranging from 7.3 to 19.1 and EF values ranging between 0.77 and 0.95.

Corn response and yield

Greenhouse dose-response study

No injury symptoms were observed in corn after PRE-application of the premix at the labeled rate (2900 g a.i. ha^{-1}), indicating excellent crop safety. Similarly, Jain et al. (2015) noted that the PRE application of the premix is safe on corn. Low level corn injury (5%) consisting of stunting and leaf bleaching was observed with PRE application at the highest dose (2.5× rate or 7200 g a.i. ha^{-1}); however, the symptoms were transitory and dissipated by 4 wk after treatment (data not shown). The premix applied POST to 15-cm-tall corn plants caused no injury at the labeled rate and <10% injury at the ≥2× rate (data not shown). Corn injury was 12% and

Table 5. Estimation of the regression parameter and model goodness of fit for a log–logistic function[a] fitted to common waterhemp control and aboveground biomass reduction in response to the postemergence applications of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor made at two different weed heights and the estimation of the effective doses needed to control or reduce aboveground biomass by 50% (ED_{50}) and 90% (ED_{90}) in a field dose-response study conducted in 2015 and 2016 at Clay Center, NE.

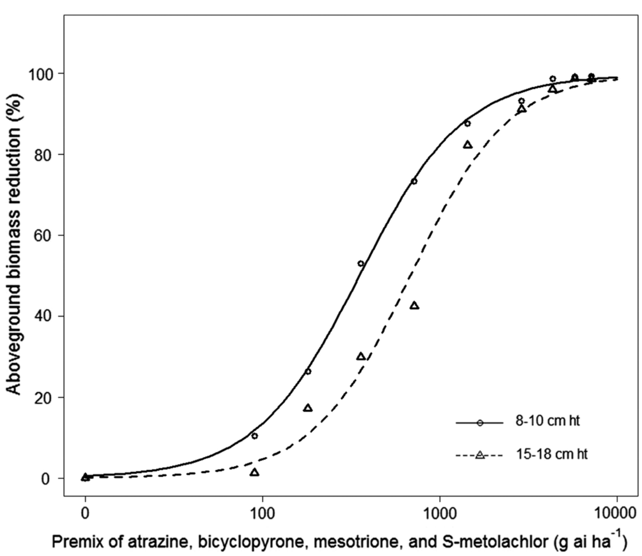
DAT	Plant height (cm)	Regression parameters *b* (±SE)	Model goodness of fit RMSE	 EF	ED_{50} (±SE) (g a.i. ha^{-1})	ED_{90} (±SE) (g a.i. ha^{-1})	Predicted value (%) at the 1× rate[b]
Control of common waterhemp							
14	8–10	−1.7 (±0.19)	9.1	0.93	162 (±10)	680 (±124)	97
	15–18	−1.4 (±0.09)	9.9	0.93	458 (±29)	2302 (±292)	93
35	8–10	−1.3 (±0.15)	9.9	0.92	242 (±21)	1308 (±314)	96
	15–18	−1.6 (±0.16)	12.7	0.90	556 (±41)	2230 (±338)	93
63	8–10	−1.4 (±0.10)	9.9	0.93	373 (±23)	1830 (±236)	95
	15–18	−1.8 (±0.19)	11.6	0.92	745 (±46)	2471 (±321)	92
Aboveground biomass reduction of common waterhemp							
63	8–10	−1.5 (±0.18)	11.2	0.92	353 (±31)	1583 (±367)	96
	15–18	−1.6 (±0.17)	14.1	0.89	684 (±57)	2748 (±438)	91

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

[a]$Y = c + \{d - c/1 + \exp[b(\log x - \log e)]\}$, where *Y* is the response variable (control or reduction in 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}) of common waterhemp. The parameter *b* is the relative slope around the parameter *e*.

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

Fig. 5. Aboveground biomass reduction of common waterhemp at 63 d after treatment (DAT) in response to postemergence applications of a premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor in a field dose-response study conducted in 2015 and 2016 in Clay Center, NE.

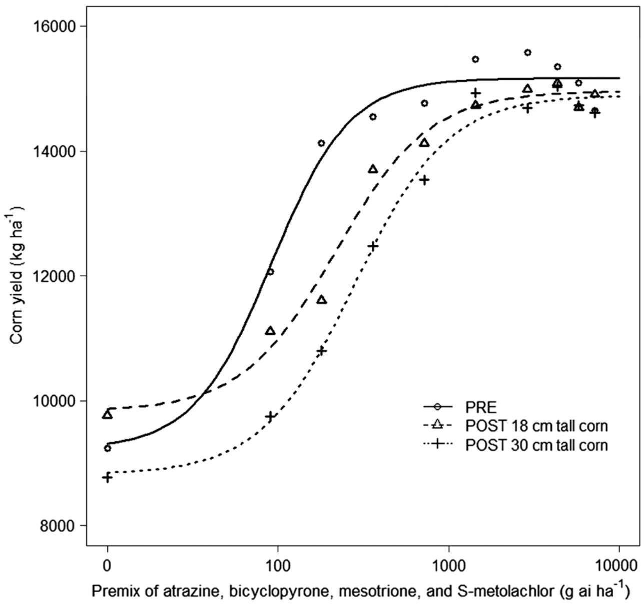


23% at 21 DAT with the POST applications made to 30-cm-tall corn at the 1.5× and 2.5× rates, respectively (data not shown).

Field dose-response study

Similarly to the greenhouse corn dose-response study, PRE application of the premix did not cause any crop injury at 14 DAT. Similarly, Armel et al. (2003) reported that PRE application of mesotrione + atrazine at 160 and 560 g a.i. ha^{-1} showed <13% corn injury at 21 DAT. Nurse et al. (2010) also reported that mesotrione injury to corn was <10% at 14 DAT of PRE application. Postemergence application of the premix at 7200 g a.i. ha^{-1} (2.5× rate) to 18-cm-tall corn plants caused 6% corn injury at 14 DAT; however, plants recovered from the injury later in the season. There was no corn injury when the premix was applied at the labeled rate (2900 g a.i. ha^{-1}) to the 18- or 30-cm-tall corn; however, increasing doses (>1×) resulted in 7%–16% corn injury at 14 DAT to 30-cm-tall corn (data not shown). Johnson et al. (2002) reported that early- and mid-POST applications of mesotrione + atrazine at 140 and 253 g a.i. ha^{-1} caused 5% to 12% corn injury at 7 DAT but corn plants were able to recover from the injury by 28 DAT. Foliar activities of mesotrione and atrazine are highly dependent on air temperature, relative humidity, and moisture and it is reported that wet foliage before and after atrazine application, along with cold weather, can severely injure corn plants (Thompson et al. 1970; Johnson and Young 2002). Therefore, the percent injury may vary for different locations and environments compared with the results obtained in this study.

Fig. 6. Corn yield response to preemergence (PRE) or postemergence (POST) applications of different doses of the premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor on 18- and 30-cm-tall corn in a field dose-response study conducted in 2015 and 2016 in Clay Center, NE.



Spearman's correlation coefficient (r_S) showed that corn yield was highly correlated ($r_S \geq 0.55$; $P < 0.001$) with common waterhemp biomass reduction in PRE and POST dose-response experiments (data not shown). Corn yield from both years were combined for each experiment and plotted against the premix doses (Fig. 6). Overall, PRE application of the premix resulted in higher yield compared with the POST applications. In the POST studies, no herbicides were applied until the V3/V5 stage of corn, which likely resulted in corn yield reduction due to early-season weed interference. Steckel and Sprague (2004) observed that common waterhemp emerging before the V6 corn stage can produce maximum weed biomass and cause significant reduction in corn yield. Preemergence application of the premix at the labeled rate (1× rate) resulted in maximum corn yield (15 580 kg ha^{-1}), which was comparable with all other treatments except doses ≤360 g a.i. ha^{-1}. Postemergence applications of the premix at 4330 g a.i. ha^{-1} (1.5×) resulted in the highest corn yield (15 094 kg ha^{-1} for 18-cm-tall corn and 15 031 kg ha^{-1} for 30-cm-tall corn); however, it was comparable to the yield obtained at the ≥1440 g a.i. ha^{-1} (≥0.5× rate) premix dose (data not shown).

Practical implications

This is the first report with detailed dose-response bioassays evaluating the response of common waterhemp to the premix of atrazine, bicyclopyrone, mesotrione, and S-metolachlor applied PRE or POST in corn. Results showed that the premix applied PRE or early POST at the labeled rate (2900 g a.i. ha^{-1}) provided >90% control of common waterhemp with no corn injury. Common waterhemp has a prolonged emergence period, therefore the inclusion of soil-residual herbicides (with multiple effective MOAs) in herbicide programs can effectively control this problem weed. Often, growers are unable to apply PRE herbicides for reasons such as wet soil or other unfavorable conditions. The efficacy of the premix tested in this study was excellent when applied early POST, providing an option for POST control of common waterhemp that reduces the weed biomass production per unit area. The label instructions should not be violated, especially the season-maximum application rates of the premix (2900 g a.i. ha^{-1} for ≥3% organic matter content) or for its active ingredients (Anonymous 2016). The premix also contains a crop safener, benoxacor, and application (PRE or POST) of the premix at the labeled rate showed excellent corn safety in this study. Therefore, it is expected that the new premix commercialized recently in the United States can be used for controlling several problem weed species (Montgomery et al. 2015; Jhala and Sarangi 2016; Sarangi and Jhala 2017), including common waterhemp in corn.

A common waterhemp biotype from Illinois has recently evolved resistance to ALS inhibitors, photosystem-II inhibitors, PPO inhibitors, HPPD inhibitors, and synthetic auxins, leaving no POST herbicide option (other than glyphosate) for controlling this problem weed in GR corn and soybean (Heap 2017*b*). Moreover, GR common waterhemp is widespread in the midwestern United States. Therefore, this premix should be used wisely to delay the evolution of resistance, especially for the new active ingredient, bicyclopyrone. It is known that most HPPD inhibitors tank-mixed with atrazine at a reduced rate provide a synergistic effect (Johnson et al. 2002; Hausman et al. 2011); however, the premix should never be applied at sublethal doses for a particular growth stage of any weed species because this will increase the chances of evolution of weed resistance due to stress-induced mutations (Gressel 2011). It is concluded that the premix applied PRE or early POST, even at relatively lower doses (compared with the labeled rate) in GR corn can effectively control common waterhemp; however, late POST application of this herbicide should be avoided to reduce the chances of early-season crop–weed competition and possible corn yield loss.

Acknowledgements

The authors would like to thank A.S. Franssen from Syngenta Crop Protection, LLC, for his support in this study. We also appreciate the help of I. Rogers and I. Schleufer in this project.

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