

## Confirmation and Control of HPPD-Inhibiting Herbicide-Resistant Waterhemp (*Amaranthus tuberculatus*) in Nebraska

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Field and greenhouse experiments were conducted in Nebraska to (1) confirm the 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting resistant-waterhemp biotype (HPPD-RW) by quantifying the resistance levels in dose-response studies, and (2) to evaluate efficacy of PRE-only, POST-only, and PRE followed by POST herbicide programs for control of HPPD-RW in corn. Greenhouse dose-response studies confirmed that the suspected waterhemp biotype in Nebraska has evolved resistance to HPPD-inhibiting herbicides with a 2- to 18-fold resistance depending upon the type of HPPD-inhibiting herbicide being sprayed. Under field conditions, at 56 d after treatment,  $\geq 90\%$  control of the HPPD-RW was achieved with PRE-applied mesotrione/atrazine/S-metolachlor + acetochlor, pyroxasulfone (180 and 270 g ai ha<sup>-1</sup>), pyroxasulfone/fluthiacet-methyl/atrazine, and pyroxasulfone + saflufenacil + atrazine. Among POST-only herbicide programs, glyphosate, a premix of mesotrione/atrazine tank-mixed with diflufenzopyr/dicamba, or metribuzin, or glufosinate provided  $\geq 92\%$  HPPD-RW control. Herbicide combinations of different effective sites of action in mixtures provided  $\geq 86\%$  HPPD-RW control in PRE followed by POST herbicide programs. It is concluded that the suspected waterhemp biotype is resistant to HPPD-inhibiting herbicides and alternative herbicide programs are available for effective control in corn. The occurrence of HPPD-RW in Nebraska is significant because it limits the effectiveness of HPPD-inhibiting herbicides.

**Nomenclature:** Acetochlor, atrazine, glyphosate, clopyralid, dicamba, diflufenzopyr, dimethenamid-*P*, flumetsulam, fluthiacet-methyl, glufosinate, isoxaflutole, mesotrione, metribuzin, pyroxasulfone S-metolachlor, saflufenacil, rimsulfuron, tembotrione, thiencazone-methyl, topramezone, waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer, corn, *Zea mays* L.

**Key words:** 4-hydroxyphenylpyruvate dioxygenase, pigment inhibitors, PRE, POST, triketone, weed management, weed resistance.

Se realizaron experimentos de campo y de invernadero en Nebraska para (1) confirmar un biotipo de *Amaranthus tuberculatus* resistente a inhibidores de 4-hydroxyphenylpyruvate dioxygenase (HPPD) (HPPD-RW) cuantificando el nivel de resistencia con estudios de respuesta a dosis, y (2) evaluar la eficacia de programas de herbicidas para el control de HPPD-RW en maíz con sólo herbicidas PRE, sólo POST, y herbicidas PRE seguidos por POST. Los estudios de respuesta a dosis en invernadero confirmaron que el biotipo de *A. tuberculatus* en Nebraska ha evolucionado resistencia a herbicidas inhibidores de HPPD con 2 a 18 veces mayor resistencia dependiendo del tipo de herbicida inhibidor de HPPD que se aplicó. Bajo condiciones de campo, a 56 d después del tratamiento, se alcanzó  $\geq 90\%$  de control de HPPD RW con aplicaciones PRE de mesotrione/atrazine/S-metolachlor + acetochlor, pyroxasulfone (180 y 270 g ai ha<sup>-1</sup>), pyroxasulfone/fluthiacet-methyl/atrazine, y pyroxasulfone + saflufenacil + atrazine. Entre los programas de herbicidas con sólo POST, glyphosate, una premezcla de mesotrione/atrazine mezclados en tanque con diflufenzopyr/dicamba, o metribuzin, o glufosinate brindaron  $\geq 92\%$  control de HPPD-RW. Combinaciones de herbicidas efectivos con diferentes sitios de acción en mezclas brindaron  $\geq 86\%$  de control de HPPD-RW en programas de herbicidas PRE seguidos por POST. Se concluyó que el biotipo de *A. tuberculatus* es resistente a herbicidas inhibidores de HPPD y que hay programas de herbicidas alternativos disponibles para su control efectivo en maíz. La ocurrencia de HPPD-RW en Nebraska es significativa porque limita la efectividad de herbicidas inhibidores de HPPD.

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Waterhemp is a summer, annual, broadleaf species native to the midwestern United States (Sauer 1967; Waselkov and Olsen 2014). Waterhemp has been identified as one of the most troublesome weeds over the past decade (Hager et al. 2002; Prince et al. 2012; Steckel and Sprague 2004). There are a variety of factors contributing to the rise of waterhemp as a problem weed, including adoption of no-tillage farming practices, extended germination period of waterhemp, decreased use of residual herbicides, and rapid spread of multiple herbicide resistance (Culpepper 2006; Felix and Owen 1999; Hartzler et al. 1999).

Waterhemp biotypes have evolved resistance to six herbicide site-of-action (SOA) groups, including acetolactate synthase inhibitors (Weed Science Society of America Site of Action [WSSA SAO] Group 2), synthetic auxins (WSSA SOA Group 4), triazines (WSSA SOA Group 5), 5-enolpyruvylshikimate-3-phosphate synthase inhibitors (WSSA SOA Group 9), and protoporphyrinogen oxidase inhibitors (WSSA SOA Group 14) (Bernards et al. 2012; Heap 2016a; Sarangi et al. 2015; Tranel et al. 2011). In addition, just in the last six years it was confirmed that waterhemp biotypes evolved resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (WSSA SOA Group 27) in Illinois (Hausman et al. 2011) and Iowa (McMullan and Green 2011). Therefore, new herbicide options to manage waterhemp are needed (Tranel et al. 2011). However, no new herbicide SOAs have been developed in recent years (Duke 2012).

The herbicides with the most recently developed SOA are the HPPD-inhibiting herbicides, introduced in the 1980s (Duke 2012; Mitchell et al. 2001). This herbicide group inhibits HPPD and causes bleaching of green tissues of susceptible plants (Grossmann and Ehrhardt 2007; Mitchell et al. 2001). Mesotrione, tembotrione, and topramezone are common POST-applied HPPD-inhibiting herbicides, primarily used in corn (Bollman et al. 2008; Gitsopoulos et al. 2010; Nurse et al. 2010; Sutton et al. 2002). The recent occurrence of a HPPD-inhibiting herbicide-resistant waterhemp biotype (HPPD-RW) has increased the complexity of waterhemp control in corn (McMullan and Green 2011).

Control of a waterhemp biotype in Illinois that is resistant to HPPD- and photosystem II-inhibiting herbicides was not achieved using a single active ingredient of foliar or soil-applied herbicide (Hausman et al. 2015; Hausman et al. 2013); therefore, there is a

need for including the use of mixtures in herbicide programs to control HPPD-RW. The benefits of using herbicide mixtures are well documented, including season-long weed control and a reduction in the risk of herbicide resistance (Beckie and Reboud 2009; Butts et al. 2016; Johnson et al. 2012; Kumar and Jha 2015; Loux et al. 2011).

Failure of a POST-applied HPPD-inhibiting herbicide to control waterhemp in a seed corn production operation was reported in eastern Nebraska in 2011. Therefore, we conducted a series of experiments to 1) confirm the presence of HPPD-RW and determine its level of resistance to POST-applied mesotrione, tembotrione, and topramezone in dose-response studies, and to 2) evaluate herbicide options for control of HPPD-RW based on PRE-only, POST-only, and PRE followed by (fb) POST herbicide programs. This information will be beneficial in the development of alternative herbicide programs for managing HPPD-RW in Nebraska.

## Materials and Methods

**Plant Materials.** In the fall of 2013, inflorescences of waterhemp plants that survived repeated mesotrione and tembotrione applications were collected from a field near Columbus, Platte County, NE, and used as the suspected HPPD-RW. Waterhemp inflorescences collected in the fall of 2014 from a field in Clay County, NE with a history of effective control using the recommended rate of HPPD-inhibiting herbicides were considered the HPPD-inhibiting herbicide-susceptible waterhemp biotype (HPPD-SW), and used in this study for a comparison. Inflorescences of waterhemp were dried for 2 wk at room temperature (25 C). The seeds were cleaned and stored at 5 C until used in the greenhouse study. Seeds were planted in 713-cm<sup>3</sup> plastic pots containing a commercial potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modest, Quebec, Canada). Emerged seedlings (1 cm) were transplanted into 164-cm<sup>3</sup> cone-tainers (Ray Leach "Cone-tainer" SC10®, Stuewe and Sons Inc, Tangent, OR 97389) containing identical commercial potting mix described above. Plants were supplied with adequate water and kept in greenhouse conditions at 28/22 C day/night temperature. Artificial lighting was provided using metal halide lamps (600 μmol photon m<sup>-2</sup> s<sup>-1</sup>) to ensure a 16-h photoperiod.

**Dose-Response Studies.** Greenhouse dose-response bioassays were conducted in 2015 at the University of Nebraska–Lincoln to determine the resistance levels of HPPD-RW and HPPD-SW sprayed with each of the three HPPD-inhibiting herbicides (mesotrione, tembotrione, and topramezone).

Each study had a completely randomized design with four replications and was repeated twice. Separate experiments were conducted for the HPPD-RW and the HPPD-SW. The treatments were arranged in a factorial treatment design with 3 herbicides and 6 rates. The herbicide rates for the HPPD-RW were 0, 0.5×, 1×, 2×, 4×, and 8×, and for the HPPD-SW were 0, 0.25×, 0.5×, 0.75×, 1×, and 2×, where 1× represents either 105 g ai ha<sup>-1</sup> mesotrione (Syngenta Crop Protection, Research Triangle Park, NC 27709) plus 1% v/v of crop oil concentrate (Agri-Dex®, Helena Chemical Co., Collierville, TN 38017) and 20.5 g L<sup>-1</sup> of ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA 30901); 92 g ai ha<sup>-1</sup> tembotrione (Bayer Crop Science, Research Triangle Park, NC 27709) plus 1% v/v methylated seed oil (Noble®, Winfield Solutions, Shoreview, MN 55126) and 20.5 g L<sup>-1</sup> ammonium sulfate; or 24.5 g ai ha<sup>-1</sup> topramezone (AMVAC, Los Angeles, CA 90023) plus 1% v/v methylated seed oil and 20.5 g L<sup>-1</sup> ammonium sulfate.

Herbicide treatments were applied with a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN 56045) fitted with an 8001 E nozzle (Spraying Systems Co., North Avenue, Wheaton, IL 60139), calibrated to deliver 140 L ha<sup>-1</sup> spray volume at 210 kPa at a speed of 3.7 km h<sup>-1</sup>. Waterhemp control was assessed visually 21 d after treatment (DAT) using a scale of 0% to 100% (where 0 indicates no injury and 100 indicates plant death). Control ratings were based on symptoms such as bleaching, necrosis, and stunting of plants compared to non-treated plants. Aboveground biomass was harvested at 21 DAT from each experimental unit and oven-dried at 65 C until reaching constant weight before weight of biomass was recorded. The biomass (g) data were converted into biomass reduction (%) compared with the non-treated experimental unit as:

$$\% \text{ HPPD-RW biomass reduction} = [(\bar{E} - B) / \bar{E}] \times 100, \quad [1]$$

where  $\bar{E}$  represents the mean biomass (g) of the non-treated experimental unit replicates, and

$B$  represents the biomass (g) of an individual treated experimental unit.

The effective dose needed to suppress the population by 50% (ED<sub>50</sub>) and 90% (ED<sub>90</sub>) for HPPD-RW and HPPD-SW was determined using the three-parameter log-logistic curve of the drc package of the R statistical environment (Knezevic et al. 2007):

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

In this model,  $Y$  is the control (%) or biomass reduction (%),  $d$  is the upper limit, and  $e$  represents the ED<sub>50</sub> value. The parameter  $b$  is the relative slope around the parameter  $e$ , and  $x$  is the herbicide dose in g ai ha<sup>-1</sup>.

The resistance level was calculated by dividing the effective dose (ED<sub>50</sub>) of the HPPD-RW by the effective dose of the HPPD-SW. The resistance level indices for the respective effective dose between the HPPD-RW and the HPPD-SW were compared using the EDcomp (or SI) function of package drc in R software (Ritz and Streibig 2005). The EDcomp function compares the ratio of effective doses using  $t$ -statistics, where  $P$ -value < 0.05 indicates that herbicide ED<sub>50</sub> values are different between the HPPD-RW and the HPPD-SW. The Fligner-Killeen test of homogeneity was used to test the assumption of constant error variance among data sets. This is a non-parametric test, which can detect departures from normality in data (Conover et al. 1981).

**Efficacy of Herbicide Programs on HPPD-RW.** Field experiments were conducted in 2013 and 2014 at a Platte County field location near Columbus, NE (41.64°N, 97.58°W) where the HPPD-RW was reported. The soil type at the study location was a silty clay loam (12% sand, 60% silt, 28% clay) with 3.3% organic matter and a pH of 6.8. Glyphosate- and glufosinate-tolerant hybrid corn ‘Golden Harvest H-9138’ was seeded at 79,280 seeds ha<sup>-1</sup> in rows spaced 76 cm apart on May 16, 2013 and May 22, 2014. Monthly mean air temperature and total precipitation data during the study periods are provided (Table 1). Experiments were conducted in a randomized complete block design with three replications and 10, 6, and 16 treatments for PRE-only, POST-only, and PRE fb POST herbicide programs, respectively (Tables 2, 3, and 4). A 3 by 7.6 m plot was considered an experimental unit.

Herbicide treatments were applied with a CO<sub>2</sub>-pressurized backpack sprayer calibrated to deliver 140 L ha<sup>-1</sup> aqueous solution at 172 kPa (PRE) and

Table 1. Mean monthly air temperature and total precipitation in field experiments conducted in 2013 and 2014 near Columbus, NE.

Month <sup>a</sup>	Temperature			Total precipitation		
	2013	2014	50-y avg.	2013	2014	50-y avg.
	C			mm		
May	15	16	17	83	96	112
June	21	21	22	120	200	118
July	23	22	25	29	108	81

<sup>a</sup>Abbreviations: Weather data were obtained from the High Plains Regional Climate Center (HPRCC; <http://www.hprcc.unl.edu>).

240 kPa (POST) with a 2 m spray boom through Turbo TeeJet<sup>®</sup> 11002 (PRE) and 110015 (POST) flat fan sprayer nozzles at a speed of 4.3 km h<sup>-1</sup>. The PRE herbicides were applied on May 17, 2013 and May 23, 2014, and the POST herbicides were applied when the HPPD-RW was 8 to 10 cm tall. The HPPD-RW control was visually assessed at 30, 41, and 56 DAT (PRE-only); 7, 14, and 21 DAT (POST-only); and 30 d after PRE (DAPRE), and 32 d after POST (DAPOST) (PRE fb POST) on a scale ranging from 0%, indicating no control, to 100%, indicating complete control. HPPD-RW density was determined at 56 DAT (PRE), 35 DAT (POST), and 32 DAPOST (PRE fb POST) by counting waterhemp within 0.25 m<sup>2</sup> quadrats arbitrarily

placed between the middle two corn rows in each experimental unit. The HPPD-RW densities in the non-treated experimental unit averaged of 196 and 344 plants m<sup>-2</sup> in 2013 and 2014, respectively. The HPPD-RW density (plants m<sup>-2</sup>) data were expressed as HPPD-RW density reduction (%) and compared with the non-treated experimental unit as follows:

$$\% \text{ HPPD-RW density reduction} = [(\hat{C} - D) / \hat{C}] \times 100 \quad [3]$$

Where  $\hat{C}$  is the mean HPPD-RW density (plants m<sup>-2</sup>) of the non-treated experimental unit replicates, and  $D$  is the HPPD-RW density (plants m<sup>-2</sup>) of an individual treated experimental unit.

ANOVA was performed using PROC GLIMMIX in SAS version 9.3 (SAS Institute Inc., Cary, NC 27513). Fligner-Killeen tests of homogeneity of variances between years and treatment-by-year interactions were conducted. HPPD-RW control (%) and density reduction (%) were analyzed with beta distribution with ilink function to meet assumptions of residual variance analysis. If ANOVA indicated significant treatment effects, means were separated at  $P \leq 0.05$  with Fisher's protected LSD test.

Table 2. List of PRE-only herbicides used for control of HPPD-inhibiting herbicide-resistant waterhemp in field experiments conducted in 2013 and 2014 near Columbus, NE.

Treatment	Herbicide <sup>a</sup>	Trade name	Rate	Manufacturer
1	Acetochlor/flumetsulam/clopyralid	SureStart <sup>®</sup>	g ai ha <sup>-1</sup> 1,490	Dow Agrosciences, Indianapolis, IN 46268
2	Mesotrione/atrazine/ <i>S</i> -metolachlor + acetochlor	Lumaz EZ <sup>®</sup> + Harness <sup>®</sup>	2,780 + 1,470	Syngenta Crop Protection, Research Triangle Park, NC 27709 + Monsanto Company, St. Louis, MO 63167
3	Pyroxasulfone	Zidua <sup>®</sup>	90	BASF Corporation, Research Triangle Park, NC 27709
4	Pyroxasulfone	Zidua <sup>®</sup>	180	BASF Corporation
5	Pyroxasulfone	Zidua <sup>®</sup>	270	BASF Corporation
6	Pyroxasulfone/fluthiacet-methyl/ atrazine	Anthem ATZ <sup>®</sup>	1,260	FMC Corporation, Philadelphia, PA 19104.
7	Pyroxasulfone + saflufenacil + atrazine	Zidua <sup>®</sup> + Sharpen <sup>®</sup> + AAtrex <sup>®</sup>	149 + 75 + 560	BASF Corporation + BASF Corporation + Syngenta Crop Protection
8	Saflufenacil/dimethenamid- <i>P</i> + dimethenamid- <i>P</i>	Verdict <sup>®</sup> + Outlook <sup>®</sup>	730 + 263	BASF Corporation + BASF Corporation
9	Thiencarbazone-methyl/isoxaflutole + atrazine	Corvus <sup>®</sup> + AAtrex <sup>®</sup>	129 + 1,800	Bayer CropScience, Research Triangle Park, NC 27709 + Syngenta Crop Protection

<sup>a</sup>Abbreviations: Herbicide premix (/); herbicide tankmix (+).

Table 3. List of POST-only herbicides used for control of HPPD-inhibiting herbicide-resistant waterhemp in field experiments conducted in 2013 and 2014 near Columbus, NE.

Treatment	Herbicide <sup>a</sup>	Trade name	Rate	Manufacturer	Adjuvant <sup>b</sup>
			g ai(ae) ha <sup>-1</sup>		
1	Glyphosate	Touchdown Total®	1,320	Syngenta Crop Protection	AMS
2	Mesotrione/atrazine + diflufenzopyr/dicamba	Callisto Xtra® + Status®	650 + 196	Syngenta Crop Protection + BASF Corporation	COC + AMS
3	Mesotrione/atrazine + glufosinate	Callisto Xtra® + Liberty 280®	650 + 595	Syngenta Crop Protection + Bayer Crop Science	AMS
4	Mesotrione/atrazine + metribuzin	Callisto Xtra® + TriCor DF®	650 + 210	Syngenta Crop Protection + United Phosphorus, King of Prussia, PA 19406	COC + AMS
5	Mesotrione + fluthiacet-methyl	Callisto® + Cadet®	105 + 7	Syngenta Crop Protection + FMC Corporation	COC + AMS

<sup>a</sup>Abbreviations: Herbicide premix (/); herbicide tankmix (+).

<sup>b</sup>AMS, ammonium sulfate (20.5 g L<sup>-1</sup>; DSM Chemicals North America Inc., Augusta, GA 30901); COC, crop oil concentrate (1% v/v; Agridex, Helena Chemical Co., Collierville, TN 38017).

## Results and Discussion

**Dose-Response Studies.** Error variance among data sets was constant. Treatment-by-experiment interaction was not significant; therefore data were combined.

Dose-response studies confirmed that the waterhemp biotype was resistant to POST-applied HPPD-inhibiting herbicides (mesotrione, tembotrione, and topramezone). The labeled rate of mesotrione (105 g ha<sup>-1</sup>) provided less than 40% control of the HPPD-RW (Figure 1A). In addition, a mesotrione rate of 342 g ha<sup>-1</sup> was needed to achieve 50% (ED<sub>50</sub>) control of the HPPD-RW (Table 5), which is thirteen times the rate required to control HPPD-SW. The ED<sub>90</sub> was not calculated because 90% control was not achieved even with the maximum rate (840 g ha<sup>-1</sup>) of mesotrione tested in this study. A similar trend was evident with tembotrione (Figure 2A) and topramezone (Figure 3A), with the resistance level, based on ED<sub>50</sub> values, estimated as 6- and 3-fold higher for HPPD-RW than for HPPD-SW, respectively. In contrast, the HPPD-SW demonstrated sensitivity to all three HPPD-inhibiting herbicides applied POST: 90% control (ED<sub>90</sub>) was achieved with the labeled rates (Table 5).

Dose-response curves based on biomass reduction (%) suggest the same resistance level as do the data based on control (%) estimates (Figures 1B, 2B, and 3B; Table 6). Based on biomass reduction (%), the HPPD-SW was most resistant to mesotrione (18-fold), followed by tembotrione (5-fold), and topramezone (2-fold). Higher resistance levels to

mesotrione are likely due to longer use history of mesotrione-based products for weed control at the research site.

McMullan and Green (2011) reported waterhemp resistant to mesotrione in Iowa, but at a lower level of resistance (8-fold). Differences in fold-level resistance between reported biotypes may partly be due to variation in the sensitivity of the susceptible population used in the study, and may also be due to the fact that the waterhemp biotype from Iowa was also acetolactate synthase- and triazine-resistant. In addition, HPPD herbicide resistance level may be influenced by plant height at the time of application. The Iowa waterhemp biotype height at the time of application was 3 to 5 cm, compared to 8 to 10 cm in this study. Furthermore, multiple resistant populations of waterhemp in Illinois also exhibited various resistance levels to mesotrione, ranging 10- to 35-fold, depending upon the susceptible population used for comparison (Hausman et al. 2011). Palmer amaranth resistant to HPPD-inhibiting herbicides has also been confirmed in Nebraska (Jhala et al. 2014) and Kansas (Thompson et al. 2012). In Nebraska, it was reported that Palmer amaranth sprayed when 10 cm tall was most resistant to topramezone (14- to 23-fold), followed by tembotrione (4- to 6-fold), and mesotrione (4-fold) (Jhala et al. 2014).

**Efficacy of Herbicide Programs on HPPD-RW.** Error variance among data sets was constant. Treatment-by-year interaction was not significant for the three field experiments; therefore, data were combined.

Table 4. List of PRE followed by POST herbicides used for control of HPPD-inhibiting herbicide-resistant waterhemp in field experiments conducted in 2013 and 2014 near Columbus, Platte County, NE.

Treatment	Herbicide <sup>a</sup>	Trade name	Timing	Rate	Manufacturer	Adjuvant <sup>b</sup>
1	Acetochlor/atrazine fb glyphosate + topramezone + atrazine	Harness Xtra® fb Roundup PowerMax® + Impact® + AAtrex®	PRE fb POST	g ai(ac) ha <sup>-1</sup> 2,700 fb + 1,540 + 25 + 560	Monsanto Company fb Monsanto Company + AMVAC, Commerce, CA 90040 + Syngenta Crop Protection	MSO + AMS
2	Acetochlor/atrazine fb topramezone + diflufenzopyr/dicamba + atrazine	Harness Xtra® fb Impact® + Status® + AAtrex®	PRE fb POST	2,700 fb 25 + 196 + 560	Monsanto Company fb + AMVAC + BASF Corporation + Syngenta Crop Protection	MSO + AMS
3	Acetochlor/flumetsulam/clopyralid fb glyphosate	SureStart® fb Durango®	PRE fb POST	1,490 fb 1,170	Dow Agrosciences fb Dow Agrosciences	AMS
4	Mesotrione/atrazine/S-metolachlor + atrazine + acetochlor fb  diflufenzopyr/dicamba + atrazine	Lumaz EZ® + AAtrex® + Harness® fb  Status® + AAtrex®	PRE fb  POST	2,780 + 1,080 + 1,470 fb  196 + 450	Syngenta Crop Protection + Syngenta Crop Protection + Monsanto Company fb BASF Corporation + Syngenta Crop Protection	AMS  COC + AMS
5	Mesotrione/atrazine/S-metolachlor + atrazine fb diflufenzopyr/dicamba + glyphosate	Lumaz EZ® + AAtrex® fb  Status® + Touchdown Total®	PRE fb POST	2,780 + 1,080 fb  196 + 1170	Syngenta Crop Protection + Syngenta Crop Protection fb BASF Corporation + Syngenta Crop Protection	AMS
6	Mesotrione/atrazine/S-metolachlor + atrazine fb glyphosate	Lumaz EZ® + AAtrex® fb  Touchdown Total®	PRE fb POST	2,780 + 1,080 fb  1,170	Syngenta Crop Protection + Syngenta Crop Protection fb Syngenta Crop Protection	AMS
7	Mesotrione/atrazine/S-metolachlor + atrazine fb atrazine + S-metolachlor + glufosinate	Lumaz EZ® + AAtrex® fb AAtrex® + Dual II Magnum® + Liberty 280®	PRE fb POST	2,780 + 1,080 fb 450 + 1,070 + 595	Syngenta Crop Protection + Syngenta Crop Protection fb Syngenta Crop Protection + Syngenta Crop Protection + Bayer Crop Science	AMS
8	Mesotrione/atrazine/S-metolachlor fb atrazine + glyphosate/S-metolachlor/ mesotrione	Lumaz EZ® fb AAtrex® + Halex® GT	PRE fb POST	1,550 fb 1,080 + 2,220	Syngenta Crop Protection fb Syngenta Crop Protection + Syngenta Crop Protection	NIS + AMS
9	Mesotrione/atrazine/S-metolachlor fb glyphosate	Lumaz EZ® fb Touchdown Total®	PRE fb POST	2,780 fb 1,170	Syngenta Crop Protection fb Syngenta Crop Protection	AMS
10	Mesotrione/atrazine/S-metolachlor fb diflufenzopyr/dicamba	Lumaz EZ® fb Status®	PRE fb POST	2,780 fb 98	Syngenta Crop Protection fb BASF Corporation	NIS
11	Mesotrione/atrazine/S-metolachlor fb diflufenzopyr/dicamba	Lumaz EZ® fb Status®	PRE fb POST	2,780 fb 196	Syngenta Crop Protection fb BASF Corporation	NIS
12	Pyroxasulfone/fluthiacet-methyl/atrazine fb pyroxasulfone/fluthiacet-methyl	Anthem ATZ® fb Anthem®	PRE fb POST	1,260 fb 94	FMC Corporation fb FMC Corporation	NIS
13	Rimsulfuron + S-metolachlor/atrazine fb  glyphosate	Resolve DF® + Bicep II Magnum® fb Abundit Extra®	PRE fb POST	18 + 3,240  840	DuPont, Wilmington, DE 19898 + Syngenta Crop Protection fb DuPont	AMS
14	Saflufenacil/dimethenamid- <i>P</i> + dimethenamid- <i>P</i> fb	Verdict® + Outlook® fb	PRE fb	730 + 263 fb	BASF Corporation	
15	topramezone + diflufenzopyr/dicamba Thiencarbazone-methyl/isoxaflutole + atrazine fb diflufenzopyr/dicamba	Impact® + Status® Corvus® + AAtrex® fb  Status®	POST PRE fb POST	18 + 196 129 + 1,120 fb 196	+ BASF Corporation fb AMVAC + BASF Corporation Bayer Crop Science + Syngenta Crop Protection fb BASF Corporation	NIS  NIS

<sup>a</sup>Abbreviations: Herbicide premix (/); herbicide tankmix (+); fb, followed by.

<sup>b</sup>AMS, ammonium sulfate (20.5 g L<sup>-1</sup>; DSM Chemicals North America Inc., Augusta, GA 30901); COC, crop oil concentrate (1% v/v; Agridex®, Helena Chemical Co., Collierville, TN 38017); NIS, nonionic surfactant (0.25% v/v; Induce®, Helena Chemical Co., Collierville, TN 38017), MSO, methylated seed oil (1% v/v; Noble®, Winfield Solutions, Shoreview, MN 55126).

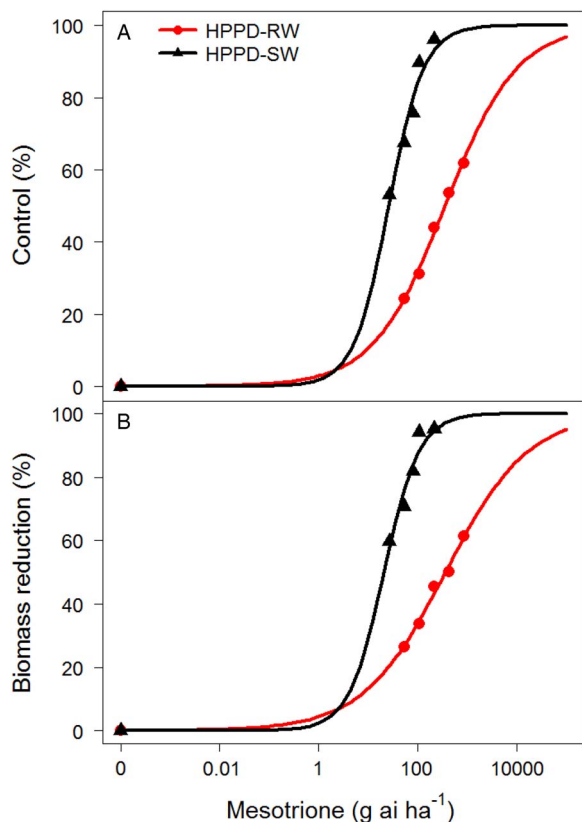


Figure 1. Control of (A) and biomass reduction (B) of 8- to 10-cm tall HPPD-inhibiting herbicide-resistant (HPPD-RW) and susceptible (HPPD-SW) waterhemp biotype at 21 d after treatment with POST-applied mesotrione in dose-response studies under greenhouse conditions.

**PRE-Only Herbicide Program.** PRE herbicides evaluated in this study provided 51% to 96% control and density reduction of the HPPD-RW (Table 7). Pyroxasulfone applied alone at 270 g ha<sup>-1</sup> and several other PRE-applied herbicide mixtures with different SOA (Treatments 1, 2, 6, 7, 8, and 9) provided ≥93% HPPD-RW control at 30 and 41 DAT. At 56 DAT, mesotrione/atrazine/*S*-metolachlor, pyroxasulfone (180 and 270 g h<sup>-1</sup>), and pyroxasulfone + saflufenacil + atrazine provided 95% to 98% control without difference among them. Moreover, at 56 DAT, there was no difference between higher pyroxasulfone rates (180 and 270 g ha<sup>-1</sup>) and pyroxasulfone applied in tank-mixtures with other herbicides (Treatments 6 and 7). Similarly, other studies have shown ≥90% control of pigweed species with pyroxasulfone applied alone or in tank-mixtures (Knezevic et al. 2009; Mahoney et al. 2014; Nurse et al. 2010).

Table 5. Estimated ED<sub>50</sub> and ED<sub>90</sub> values based on control (%) in 8- to 10-cm HPPD-inhibiting herbicide-resistant (HPPD-R) and susceptible (HPPD-S) waterhemp biotype at 21 d after treatment in a dose-response study with mesotrione, tembotrione, and topramezone conducted under greenhouse conditions at the University of Nebraska–Lincoln.

Biotype <sup>a</sup>	HPPD-inhibiting herbicides <sup>b</sup>		<i>P</i> -value <sup>c</sup>	Resistance level <sup>d</sup>
	ED <sub>50</sub> (± SE)	ED <sub>90</sub> (± SE)		
	g ai ha <sup>-1</sup>			
	Mesotrione			
HPPD-SW	26 (2)	152 (17)		-
HPPD-RW	342 (28)	-	***	13
	Tembotrione			
HPPD-SW	11 (2)	57 (6)		-
HPPD-RW	61 (4)	673 (99)	***	6
	Topramezone			
HPPD-SW	3 (0.5)	15 (2)		-
HPPD-RW	8 (1)	72 (10)	***	3

<sup>a</sup>Abbreviations: HPPD-SW, 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide-susceptible waterhemp biotype collected from a field in Clay County, NE in 2014; HPPD-RW, HPPD-inhibiting herbicide-resistant waterhemp biotype from a field in Platte County, NE in 2013.

<sup>b</sup>ED<sub>50</sub>, effective dose required to control 50% population; ED<sub>90</sub>, effective dose required to control 90% population.

<sup>c</sup>HPPD-RW vs HPPD-SW *t*-statistics comparison of ED<sub>50</sub>, \*\*\*  $\alpha < 0.01$ .

<sup>d</sup>Resistance level was calculated by dividing ED<sub>50</sub> value of HPPD-RW by HPPD-SW for each herbicide.

The HPPD-RW is neither acetolactate synthase nor triazine resistant, and results of PRE-only herbicide programs suggest that PRE herbicide options are available for effective control of the HPPD-RW in corn.

**POST-Only Herbicide Program.** Four POST herbicide programs provided ≥90% control of the HPPD-RW at 21 DAT with ≥84% density reduction at 35 DAT (Table 8). For example, glyphosate (Treatment 1) provided ≥93% HPPD-RW control and density reduction. Thus, the HPPD-RW was very sensitive to glyphosate due to the fact that the experimental site had been under seed corn production at least for last five years with no use of glyphosate. Mesotrione/atrazine + diflufenzopyr/dicamba (Treatment 2), mesotrione/atrazine + glufosinate (Treatment 3), and mesotrione/atrazine + metribuzin (Treatment 4) also provided 92% control

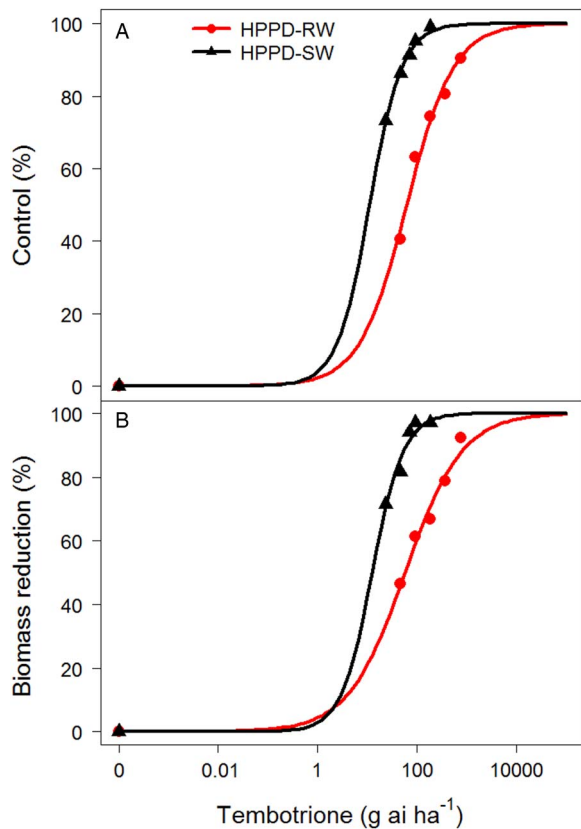


Figure 2. Control of (A) and biomass reduction (B) of 8- to 10-cm tall HPPD-inhibiting herbicide resistant (HPPD-RW) and susceptible (HPPD-SW) waterhemp biotype at 21 d after treatment with POST-applied tembotrione in dose-response studies under greenhouse conditions.

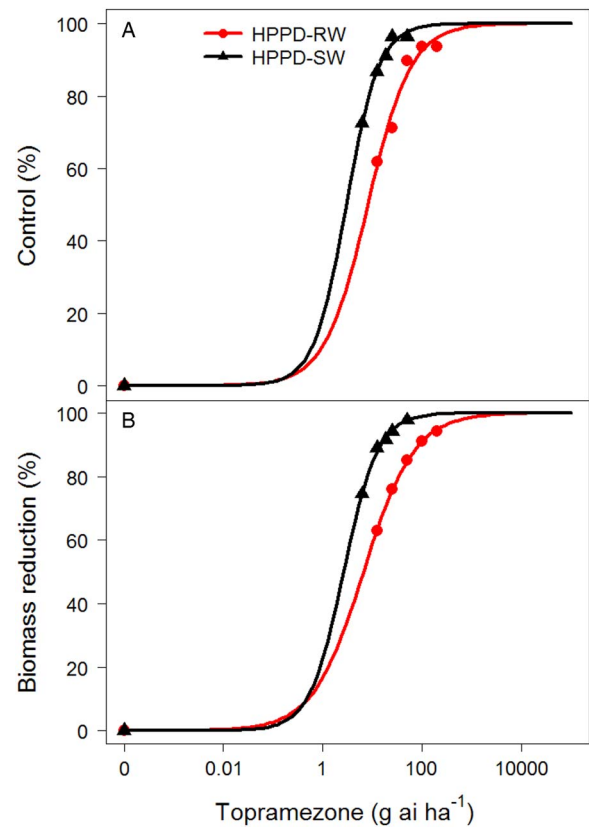


Figure 3. Control of (A) and biomass reduction (B) of 8- to 10-cm tall HPPD-inhibiting herbicide resistant (HPPD-RW) and susceptible (HPPD-SW) waterhemp biotype at 21 d after treatment with POST-applied topramezone in dose-response studies under greenhouse conditions.

of HPPD-RW at 21 DAT. This is due to synergistic effect of HPPD-inhibiting herbicides and photosystem II-inhibiting herbicides (e.g., atrazine and metribuzin). Previous studies have confirmed improved control of *Amaranthus* species in corn by tank-mixing HPPD- and photosystem II-inhibiting herbicides (Abendroth et al. 2006; Woodyard et al. 2009).

There was no difference in HPPD-RW control (21 DAT) between glyphosate and mesotrione/atrazine in tank mixtures with diflufenzopyr/dicamba, glufosinate, or metribuzin. All of these treatments resulted in  $\geq 92\%$  HPPD-RW control. Mesotrione/atrazine + metribuzin caused 15% temporary stunting in corn at 10 DAT (data not shown). Fluthiacet-methyl + mesotrione showed poor (53%) control of the HPPD-RW at 21 DAT (Table 8). Similar results were obtained by Jhala et al. (2014), who reported that fluthiacet-methyl used alone was not effective in controlling *Amaranthus* species.

The results of POST-only herbicide programs indicated that glyphosate, and premix of mesotrione/atrazine tank mixed with synthetic auxins glufosinate and metribuzin, are effective herbicide programs for control of HPPD-RW in corn.

**PRE fb POST Herbicide Programs.** Most PRE fb POST herbicide programs provided  $\geq 83\%$  control and density reduction of HPPD-RW at 32 DAPOST (Table 9). The HPPD-RW was  $\geq 86\%$  controlled with PRE herbicide 30 DAPRE. The HPPD-RW control (%) in PRE was higher when treated with  $2,780 \text{ g ha}^{-1}$  (Treatments 9, 10, and 11) than  $1,550 \text{ g ha}^{-1}$  (Treatment 8) of mesotrione/*S*-metolachlor/atrazine. Furthermore, adding atrazine ( $1,080 \text{ g ha}^{-1}$ ) to mesotrione/atrazine/*S*-metolachlor ( $2,780 \text{ g ha}^{-1}$ ) did not improve the HPPD-RW control (%). The mesotrione/atrazine/*S*-metolachlor ( $2,780 \text{ g ha}^{-1}$ ) provided nearly complete or complete control of HPPD-RW. This mixture, with



Table 6. Estimated ED<sub>50</sub> and ED<sub>90</sub> values based on biomass reduction (%) in 8- to 10-cm HPPD-inhibiting herbicide-resistant (HPPD-R) and susceptible (HPPD-S) waterhemp biotype 21 d after treatment in a dose-response study with mesotrione, tembotrione, and topramezone, conducted under greenhouse conditions at the University of Nebraska–Lincoln.

Biotype <sup>a</sup>	HPPD-inhibiting herbicides <sup>b</sup>		<i>P</i> -value <sup>c</sup>	Resistance level <sup>d</sup>
	ED <sub>50</sub> (± SE)	ED <sub>90</sub> (± SE)		
	g ai ha <sup>-1</sup>			
	Mesotrione			
HPPD-SW	20 (1)	123 (10)		
HPPD-RW	355 (27)	-	***	18
	Tembotrione			
HPPD-SW	12 (1)	61 (4)		
HPPD-RW	56 (4)	-	***	5
	Topramezone			
HPPD-SW	3 (0.4)	15 (1)		
HPPD-RW	7 (1)	86 (13)	***	2

<sup>a</sup>Abbreviations: HPPD-SW, 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide-susceptible waterhemp biotype collected from a field in Clay County, NE in 2014; HPPD-RW, HPPD-inhibiting herbicide-resistant waterhemp biotype from a field in Platte County, NE in 2013.

<sup>b</sup>ED<sub>50</sub>, effective dose required to control 50% population; ED<sub>90</sub>, effective dose required to control 90% population.

<sup>c</sup>HPPD-RW vs HPPD-SW *t*-statistics comparison of ED<sub>50</sub>, \*\*\*  $\alpha < 0.01$ .

<sup>d</sup>Resistance level was calculated by dividing ED<sub>50</sub> value of HPPD-RW by HPPD-SW for each herbicide.

or without atrazine, resulted in ≥97% HPPD-RW control at 30 DAPRE. Moreover, acetochlor/flumetsulam/clopyralid, pyroxasulfone/fluthiacet-methyl/atrazine, saflufenacil/dimethenamid-*P* + dimethenamid-*P*,

and thiencazone-methyl/isoxaflutole + atrazine provided ≥95% HPPD-RW control.

Treatments fb POST application of glyphosate alone (Treatments 3, 6, 9, and 13) or glyphosate +

Table 7. Effect of PRE-only herbicide programs on HPPD-inhibiting resistant waterhemp control (%) and population density reduction (%) in field experiments conducted in 2013 and 2014 near Columbus, NE.

Treatment	HPPD-inhibiting herbicide-resistant waterhemp <sup>a</sup>	Rate	Control <sup>b</sup>			Density reduction <sup>b</sup>
			DAT <sup>a</sup>			56
Herbicide <sup>c</sup>			30	41	56	56
		g ai ha <sup>-1</sup>	%			
1	Acetochlor/flumetsulam/clopyralid	1,490	93 bc	85 d	85 bc	91 bcd
2	Mesotrione/atrazine/ <i>S</i> -metolachlor + acetochlor	2,780 + 1,470	97 a	98 a	96 a	98 a
3	Pyroxasulfone	90	83 d	63 e	51 d	70 e
4	Pyroxasulfone	180	90 cd	87 cd	91 ab	97 a
5	Pyroxasulfone	270	95 abc	94 abc	92 ab	95 abc
6	Pyroxasulfone/fluthiacet-methyl/atrazine	1,260	96 ab	94 abc	92 ab	88 cd
7	Pyroxasulfone + saflufenacil + atrazine	149 + 75 + 560	96 ab	97 ab	93 ab	96 ab
8	Suflafenacil/dimethenamid- <i>P</i> + dimethenamid- <i>P</i>	730 + 263	96 ab	91 bcd	86 bc	84 d
9	Thiencazone-methyl/isoxaflutole + atrazine	129 + 1,800	90 cd	91 bcd	75 c	60 e
<i>P</i> -value <sup>d</sup>			***	***	***	***

<sup>a</sup>Abbreviations: DAT, d after treatment; HPPD, 4-hydroxyphenylpyruvate dioxygenase. The control (0%) data of non-treated experimental unit were not included in analysis. Density reduction (%) was calculated on the basis of comparison with density (plants m<sup>2</sup>) of non-treated experimental unit.

<sup>b</sup>Means presented within each column with no common letter(s) are significantly different according to Fisher's Protected LSD test where  $P \leq 0.05$ .

<sup>c</sup>Herbicide premix (/); herbicide tankmix (+).

<sup>d</sup>ANOVA, \*\*\* $\alpha < 0.01$ .

Table 8. Effect of POST-only herbicide programs on HPPD-inhibiting herbicide-resistant-waterhemp control (%) and population density reduction (%) in field experiments conducted in 2013 and 2014 near Columbus, NE.

HPPD-inhibiting herbicide-resistant waterhemp <sup>a</sup>		Rate	Control <sup>b</sup>		Density reduction <sup>b</sup>	
			DAT <sup>a</sup>			
Treatment	Herbicide <sup>c</sup>		7	14	21	35
		g ai (ae) ha <sup>-1</sup>	%			
1	Glyphosate	1,320	98 a	98 a	94 a	93 a
2	Mesotrione/atrazine + diflufenzopyr/ dicamba	650 + 196	81 c	87 b	92 a	91 ab
3	Mesotrione/atrazine + glufosinate	650 + 595	98 a	96 a	92 a	84 b
4	Mesotrione/atrazine + metribuzin	650 + 210	94 b	91 b	92 a	97 a
5	Mesotrione + fluthiacet-methyl	105 + 7	82 c	54 c	53 b	65 c
<i>p</i> -value <sup>d</sup>			***	***	***	***

<sup>a</sup>Abbreviations: DAT, d after treatment; HPPD, 4-hydroxyphenylpyruvate dioxygenase. The control (%) data of non-treated experimental unit were not included in analyses. Density reduction (%) was calculated on the basis of comparison with density (plants m<sup>-2</sup>) of non-treated experimental unit.

<sup>b</sup>Means presented within each column with no common letter(s) are significantly different according to Fisher's Protected LSD test where  $P \leq 0.05$ .

<sup>c</sup>Herbicide premix (/); herbicide tankmix (+).

<sup>d</sup>ANOVA, \*\*\* $\alpha < 0.01$ .

topramezone + atrazine (Treatment 1), glyphosate + diflufenzopyr/dicamba (Treatment 5), and glyphosate/*S*-metolachlor/mesotrione + atrazine (Treatment 8) provided  $\geq 95\%$  HPPD-RW control and density reduction at 32 DAPOST. Diflufenzopyr/dicamba resulted in 86% to 91% control of HPPD-RW, but the control was improved to 97% when glyphosate was tank-mixed with diflufenzopyr/dicamba (Treatment 5). Non-glyphosate treatments, including topramezone + diflufenzopyr/dicamba + atrazine (Treatment 2), diflufenzopyr/dicamba + atrazine (Treatment 4), atrazine + *S*-metolachlor + glufosinate (Treatment 7) and topramezone + diflufenzopyr/dicamba (Treatment 14) resulted in  $\geq 94\%$  HPPD-RW control and density reduction at 32 DAPOST. These results suggest that many herbicide options are available to manage HPPD-RW in corn, at least in Nebraska and the upper Midwest.

Herbicide rotations and/or mixtures of active ingredients that have different SOA have been recommended by researchers as a way to prevent or delay the evolution of resistant weeds (Beckie 2006; Gressel and Segel 1990; Norsworthy et al. 2012; Wrubel and Gressel 1994). Similarly, Livingston et al. (2015) suggested that the lowest risk of evolving herbicide resistance occurred when both PRE and POST herbicide applications are part of a systematic approach to weed control. In addition, the sequential application of PRE fb POST would

also help in fields with substantial waterhemp density, which has tendency to emerge over a longer period of time (Cordes et al. 2004; Schuster and Smeda 2007).

This study confirmed the first case of HPPD-RW in Nebraska, and the third in the United States (Heap 2016b). This biotype showed the highest resistance to mesotrione, followed by tembotrione and topramezone, most likely due to the longer history of mesotrione use at the study site in a continuous seed corn production system. The results indicate that there are herbicide programs that have the potential to provide effective control of HPPD-RW in corn. Tactics for minimizing the risk of herbicide resistance should be based on the principles of integrated weed management, especially utilizing mixtures or premixes of herbicides with different SOA. Despite availability of alternative herbicides, the spread of HPPD-inhibiting herbicide resistance in *Amaranthus* spp. is increasing across other parts of Nebraska and the United States (Hausman et al. 2011; Jhala et al. 2014; McMullan and Green 2011; Thompson et al. 2012), which is of great concern because it limits the effectiveness of mesotrione, tembotrione, and topramezone on pigweed species. Future research is needed to confirm the mechanism of resistance to HPPD-inhibiting herbicides observed in this biotype from Nebraska.

Table 9. Effect of PRE followed by POST herbicide programs on HPPD-inhibiting herbicide-resistant-waterhemp control (%) and population density reduction (%) in field experiments conducted in 2013 and 2014 near Columbus, NE.

HPPD-inhibiting herbicide-resistant waterhemp <sup>a</sup>				Control <sup>b</sup>		Density reduction <sup>b</sup>
				DAPRE <sup>a</sup>	DAPOST <sup>a</sup>	
Treatment	Herbicide <sup>c</sup>	Timing	Rate	30	32	32
				%		
1	Acetochlor/atrazine fb	PRE fb	g ai (ae) ha <sup>-1</sup> 2,700 fb	96 abc	96 abc	97 ab
2	Glyphosate + topramezone + atrazine	POST	1,540 + 25 + 560	86 e	97 ab	98 a
		PRE fb	2,700 fb			
3	Acetochlor/atrazine fb	POST	25 + 196 + 560	95 c	96 abc	97 ab
		PRE fb	1,490 fb			
4	Acetochlor/flumetsulam/clopyralid fb	POST	1,170	98 a	97 ab	98 a
		PRE fb	2,780 + 1,080 + 1,470 fb			
5	Mesotrione/atrazine/ <i>S</i> -metolachlor + atrazine + acetochlor fb	POST	196 + 450	97 abc	97 ab	98 ab
		PRE fb	2,780 + 1,080 fb			
6	diflufenzopyr/dicamba + atrazine fb	POST	196 + 1,170	98 a	96 abc	98 a
		PRE fb	2,780 + 1,080 fb			
7	Mesotrione/atrazine/ <i>S</i> -metolachlor + atrazine fb	POST	1,170	97 abc	98 a	98 a
		PRE fb	2,780 + 1,080 fb			
8	atrazine + <i>S</i> -metolachlor + glufosinate	POST	450 + 1,070 + 595	91 d	95 bdc	98 a
		PRE fb	1,550 fb			
9	Mesotrione/atrazine/ <i>S</i> -metolachlor fb	POST	1,080 + 2,220	98 a	97 ab	97 ab
		PRE fb	2,780 fb			
10	glyphosate	POST	1,170	97 abc	91 de	95 bc
		PRE fb	2,780 fb			
11	Mesotrione/atrazine/ <i>S</i> -metolachlor fb	POST	98	97 abc	96 abc	97 ab
		PRE fb	2,780 fb			
12	diflufenzopyr/dicamba	POST	196	96 abc	87 e	92 c
		PRE fb	1,260 fb			
13	pyroxasulfone/ fluthiacet-methyl	POST	94	88 de	97 ab	97 ab
		PRE fb	18 + 3,240 fb			
14	Rimsulfuron + <i>S</i> -metolachlor/atrazine fb	POST	840	96 abc	94 cd	97 ab
		PRE fb	730 + 263 fb			
15	Saflufenacil/dimethenamid- <i>P</i> + dimethenamid- <i>P</i>	POST	18 + 196	96 abc	86 e	83 d
		PRE fb	129 + 1,120 fb			
	glyphosate	POST	196	***	***	***
<i>P</i> -value <sup>d</sup>				***	***	***

<sup>a</sup>Abbreviations: DAPRE, d after PRE; DAPOST, d after POST; HPPD, 4-hydroxyphenylpyruvate dioxygenase. The control (%) data of non-treated experimental unit were not included in analyses. Density reduction (%) was calculated on the basis of comparison with density (plants m<sup>-2</sup>) of non-treated experimental unit.

<sup>b</sup>Means presented within each column with no common letter(s) are significantly different according to Fisher's Protected LSD test where P ≤ 0.05.

<sup>c</sup>Herbicide premix (/); herbicide tankmix (+); fb, followed by.

<sup>d</sup>ANOVA, \*\*\*α < 0.01.

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