

Interaction of PS II- and HPPD-Inhibiting Herbicides for Control of Palmer Amaranth Resistant to Both Herbicide Sites of Action

Parminder S. Chahal and Amit J. Jhala*

ABSTRACT

Palmer amaranth (*Amaranthus palmeri* S. Watson) resistant to post-emergence (POST) applications of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides has been confirmed in a continuous corn (*Zea mays* L.) seed production field in Nebraska. Field studies were conducted in 2014 through 2016 near Shickley in Fillmore County, Nebraska, to determine the response of Palmer amaranth to PS II- and HPPD-inhibiting herbicides applied pre-emergence (PRE) and to their tank-mixtures applied PRE or POST. Results from the dose-response study indicated that atrazine at 3757 and 17,920 g a.i. ha⁻¹ applied PRE provided 25 and 45% Palmer amaranth control, respectively, at 28 d after treatment (DAT). Mesotrione and isoxaflutole doses required for 90% control were 1030 and 1140 g ha⁻¹, respectively. To determine the interactions of mesotrione, tembotrione, or topramezone tank-mixed with atrazine applied POST, Colby's equation was used to calculate the expected Palmer amaranth control achieved with those tank-mixtures compared to a nontreated control. Based on Colby's equation, additive interactions occurred for Palmer amaranth control, density reduction, and biomass reduction when atrazine tank-mixed with mesotrione or isoxaflutole was applied PRE at all rate combinations. However, synergistic interactions occurred when mesotrione or topramezone tank-mixed with atrazine was applied POST. The results indicated that Palmer amaranth from Nebraska is resistant to both PS II- and HPPD-inhibiting herbicides applied PRE or POST alone; however, they can be applied POST in a tank-mixture with different site of action herbicides for effective control and to reduce the risk of herbicide resistance evolution.

Core Ideas

- Additive interactions with photosystem II- and HPPD-inhibitors tank-mixtures applied pre-emergence.
- Synergistic interactions with photosystem II- and HPPD-inhibitors tank-mixtures applied post-emergence.
- Palmer amaranth resistant to photosystem II- and HPPD-inhibitors applied pre-emergence or post-emergence.

PALMER AMARANTH is the most problematic and difficult to control weed in agronomic cropping systems in the United States (Chahal et al., 2015, 2017; Kohrt and Sprague, 2017). Palmer amaranth biotypes have evolved resistance to single as well as to multiple site of action herbicides in several states in the United States (Heap, 2017). Palmer amaranth has been reported to be resistant to microtubule-, acetolactate synthase (ALS)-, PS II-, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)-, HPPD-, and protoporphyrinogen oxidase (PPO)-inhibitor in the United States (Heap, 2017).

Weed control in corn depends widely on the use of PS II- and HPPD-inhibitors due to their broad-spectrum weed control, tank-mixing compatibility with other site of action herbicides, PRE and POST activity, and crop safety (Bollman et al., 2008; Fleming et al., 1988; Mitchell et al., 2001; Swanton et al., 2007). The continuous and repeated use of these herbicides, specifically in corn-based cropping systems, has resulted in the evolution of 26 weed species resistant to PS II-inhibitor (atrazine [6-chloro-N-ethyl-N²-(1-methylethyl)-1,3,5-triazine-2,4-diamine]) and 2 weed species resistant to HPPD-inhibitor (isoxaflutole [(5-cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(trifluoromethyl)-phenyl]methanone}, mesotrione [2-(4-mesylyl-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone], tembotrione [2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione}, and topramezone {[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone}) in the United States (Heap, 2017). A Palmer amaranth biotype resistant to both PS II- (atrazine) and HPPD-inhibitor (mesotrione, tembotrione, and topramezone) was recently reported in a continuous corn seed production field near Shickley in Fillmore County, south-central Nebraska (Jhala et al., 2014). The field had been under corn seed production for the previous 8 yr, with continual use of PS II- and HPPD-inhibiting herbicides for weed control. The level of Palmer amaranth resistance to atrazine applied POST was 9- to 14-fold, while the level of resistance to mesotrione, tembotrione, and topramezone applied POST was 4-, 4- to 6-, and 14- to 23-fold, respectively, compared to the two susceptible Palmer amaranth populations from Nebraska (Jhala et al., 2014). The PS II- and HPPD-inhibiting herbicides can be applied PRE as well as POST in field corn, seed corn, and popcorn (Mitchell et al., 2001; Swanton et al., 2007). Previous

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Dep. of Agronomy and Horticulture, Univ. of Nebraska-Lincoln, Lincoln, NE 68583. Received 11 Dec. 2017. Accepted 25 June 2018.
*Corresponding author (Amit.Jhala@unl.edu).

Abbreviations: DAT, days after treatment; HPPD, 4-hydroxyphenylpyruvate dioxygenase; POST, post-emergence; PRE, pre-emergence; PS, photosystem.

studies have reported a difference in the level of resistance to atrazine applied PRE compared to POST in PS II-inhibitor-resistant tall waterhemp [*Amaranthus rudis* Sauer] (Hausman et al., 2011; Ma et al., 2016). Therefore, the response of PS II- and HPPD-inhibitor-resistant Palmer amaranth to PS II- and HPPD-inhibiting herbicides applied PRE needs to be quantified.

Several studies have demonstrated that tank-mixing PS II-inhibitor with HPPD-inhibitor provided a synergistic effect for controlling giant ragweed (*Ambrosia trifida* L.), common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.), common waterhemp (*Amaranthus rudis* Sauer), and redroot pigweed (*Amaranthus retroflexus* L.) (Abendroth et al., 2006; Hugi et al., 2008; Sutton et al., 2002; Woodyard et al., 2009a, 2009b). In addition, synergistic interaction has been reported for PS II- and HPPD-inhibiting herbicide tank-mixtures applied POST for control of susceptible as well as resistant Palmer amaranth (Abendroth et al., 2006; Jhala et al., 2014). Jhala et al. (2014) reported 58 to 73% control of PS II- and HPPD-inhibitor-resistant Palmer amaranth with mesotrione or topramezone applied POST compared to 87 to 99% control when tank mixed with atrazine at 21 DAT. However, when the PS II- and HPPD-inhibitor tank-mixture is applied PRE, the synergistic interaction varies among weed species (Bollman et al., 2006). No information is available regarding the synergistic interactions of PS II- and HPPD-inhibitor applied PRE or POST in tank-mixture for control of PS II- and HPPD-inhibitor-resistant Palmer amaranth. The objectives of this study were to evaluate the response of PS II- and HPPD-inhibitor-resistant Palmer amaranth to different rates of PS II- and HPPD-inhibiting herbicides applied PRE and to their tank mixtures applied PRE or POST.

MATERIALS AND METHODS

Site Description and Experimental Design

A field study was conducted in 2014 and 2015 to evaluate the response of Palmer amaranth to different rates of PS II- (atrazine) and HPPD-inhibitors (isoxaflutole and mesotrione) applied PRE. Field experiments were performed in a grower's field confirmed with the presence of PS II- and HPPD-inhibitor-resistant Palmer amaranth near Shickley in Fillmore County, Nebraska (40.46° N, 97.80° E). An additional field study was conducted in 2014, 2015, and 2016 at the same location to evaluate the response of Palmer amaranth to PS II- (atrazine) and HPPD-inhibitors (isoxaflutole, mesotrione, tembotrione, and topramezone) applied PRE or POST in tank mixture. Soil texture at the research site was a Crete silt loam (fine, smectitic, mesic Pachic Udertic Argiustoll) with a pH of 6.5, 26% sand, 57% silt, 17% clay, and 3.5% organic matter. A glyphosate [N-(phosphonomethyl)glycine]-resistant corn hybrid (Mycogen 2D351, Dow AgroSciences, Indianapolis, IN) was seeded at 87,500 seeds ha⁻¹ in rows spaced 76 cm apart on 3 June 2014, 30 May 2015, and 1 June 2016 in the PRE or POST tank-mixture study and no crop was planted in the PRE dose response study. The treatments in both studies were arranged in a randomized complete block design with four replications and the experimental plots were 3 m wide and 9 m long. The experimental site was under a center-pivot irrigation system and monthly mean air temperatures, along with total precipitation during the 2014, 2015, and 2016 growing seasons and the 30-yr

average for the research site are provided in Table 1. During 2014 and 2015, 130 to 280 mm of rainfall was received within 2 d after PRE, while 7 mm rainfall was received at 14 d after PRE application at the experimental site in 2016.

Herbicide Application

The dose response study included seven rates (0, 0.5×, 1.0×, 2.0×, 4.0×, 8.0×, and 16×) of atrazine (1× = 1120 g a.i. ha⁻¹), mesotrione (1× = 105 g a.i. ha⁻¹), and isoxaflutole (1× = 105 g a.i. ha⁻¹) applied PRE. In a tank-mixture study applied PRE, mesotrione or isoxaflutole at 105 or 210 g ha⁻¹ were applied alone or tank mixed with atrazine at 1120 or 2240 g ha⁻¹ and applied within 3 d after planting corn. In a tank-mixture study applied POST, mesotrione (105 g ha⁻¹), tembotrione (72 g ha⁻¹), or topramezone (22 g ha⁻¹) were applied alone or tank mixed with atrazine at 1120 or 2240 g ha⁻¹. Based on the label recommendations, herbicides were applied when Palmer amaranth was 12 to 15 cm tall. Herbicide treatments were applied with a CO₂-pressurized backpack sprayer calibrated to deliver 140 L ha⁻¹ at 276 kPa, consisting of a four-nozzle boom fitted with AIXR 110015 flat-fan nozzles.

Data Collection

Palmer amaranth control or injury was assessed visually at 14 and 28 DAT based on a 0 to 100% scale, with 0% corresponding to no control and 100% corresponding to plant death. Palmer amaranth density was assessed from two randomly selected 0.25 m² quadrats per plot at 28 DAT and aboveground biomass was harvested from the same quadrant areas, oven-dried at 65°C for 3 d, and weighed. In the dose response study, Palmer amaranth density was not collected and aboveground biomass was harvested from two randomly selected 0.25 m² quadrats per plot at 28 DAT, oven-dried at 65°C for 3 d, and weighed. Palmer amaranth density and biomass data were converted into percent density or biomass reduction compared with the non-treated control (Ganie et al., 2017; Sarangi et al., 2017):

$$\text{Biomass / Density reduction (\%)} = \frac{(\bar{C} - B)}{\bar{C}} \times 100 \quad [1]$$

where *C* is the biomass or density of the nontreated control plot and *B* is the biomass or density collected from the experimental plot. At maturity, corn was harvested from the middle two rows of each plot with a plot combine, and the weight and moisture content were measured. Corn yields were adjusted to 15.5% moisture content (Ganie et al., 2017).

Statistical Analysis

A three-parameter log-logistic function was fitted for the dose response study to determine the effective rates of atrazine, isoxaflutole, or mesotrione required for Palmer amaranth control or biomass reduction by 25% (ED₂₅), 50% (ED₅₀), and 90% (ED₉₀) using the drc package c (drc 2.3, Christian Ritz and Jens Streibig, R 3.1.0, <https://cran.r-project.org/web/packages/drc/index.html>) in software R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al., 2007):

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

Table 1. Monthly mean air temperature and total precipitation during the 2014, 2015, and 2016 growing seasons and the 30-yr average at Shickley, NE.†

Month	Mean temperature				Total precipitation			
	2014	2015	2016	30-yr average	2014	2015	2016	30-yr average
	°C				mm			
March	5	7	9	5	2	12	14	48
April	11	12	12	11	94	42	99	68
May	18	17	16	17	57	108	200	124
June	23	23	25	22	154	264	7	117
July	23	24	25	25	56	124	55	86
August	23	22	23	24	154	69	147	88
September	19	22	20	19	76	104	52	86
October	14	14	15	12	47	22	64	59
Annual	11	12	13	11	664	908	726	763

† Mean air temperature and total precipitation data were obtained from the NWS-COOP (2017).

where Y is the percent control or percent aboveground biomass reduction, x is the herbicide rate, d is the upper limit, e is the ED_{25} , ED_{50} , or ED_{90} values, and b represents the relative slope around the parameter e .

Palmer amaranth control estimates, density reduction, aboveground biomass reduction, and corn yield for PRE and POST tank-mixture study were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC 27513). Herbicide treatments and experimental years were considered fixed effects, whereas replications were considered a random effect in the model. Data were combined over years when there was no year \times treatment interaction. The nontreated control was not included in the data analysis for control estimates and density and biomass reduction. Before analysis, data were tested for normality and homogeneity of variance using Shapiro-Wilks goodness-of-fit and Levene's test in SAS. The normality and homogeneity of variance assumptions were met; therefore, no data transformation was required. Where the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ with the Tukey-Kramer's pairwise comparison test to reduce type I error for series of comparisons. To determine the interactions of mesotrione, tembotrione, or topramezone tank mixed with atrazine applied POST, Colby's equation (Colby, 1967) was used to calculate the expected Palmer amaranth control, density reduction, or biomass reduction achieved with those tank-mixtures compared to a nontreated control.

$$E = (X + Y) - (XY/100) \quad [3]$$

where E is Palmer amaranth control, density reduction, or biomass reduction which is expected with the application of herbicides A and B in a tank mixture compared to a nontreated control. Expected values were calculated using the observed Palmer amaranth control, density reduction, or biomass reduction X and Y achieved with the individual application of herbicides A and B in the study, respectively. The expected and observed control, density reduction, or biomass reduction achieved with herbicide A and B tank mixture were subjected to t tests in SAS to determine whether the means were different. Herbicide tank mixture was considered "synergistic" if the expected mean was significantly lower than the observed mean; and "additive" if there was no difference between the expected and observed means.

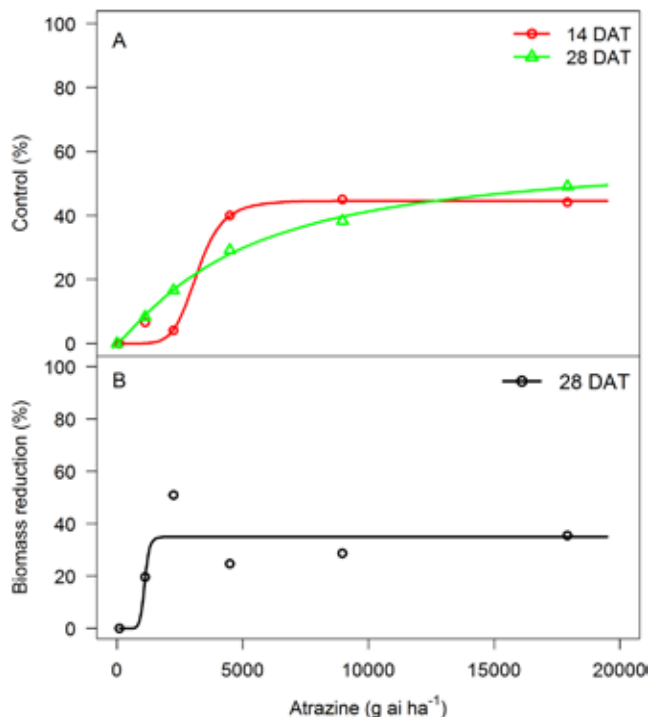


Fig. 1. Dose response of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth biotype to atrazine applied pre-emergence. (A) Control at 14 and 28 d after treatment (DAT); and (B) Percent biomass reduction at 28 DAT in a field dose-response study conducted at Shickley, NE, in 2014 and 2015. Percent biomass reduction was calculated using the equation:

$$\text{Biomass reduction (\%)} = \frac{(\bar{C} - B)}{\bar{C}} \times 100, \text{ where } \bar{C} \text{ is the mean biomass of nontreated control replicate, and } B \text{ is the biomass of an individual treated experimental unit.}$$

RESULTS

Dose-Response Study

Palmer amaranth was controlled <10% at 14 and 28 DAT with the labeled rate of atrazine (1120 g ha^{-1}) applied PRE (Fig. 1A). Atrazine at 3757 g ha^{-1} was required to provide 25% control (ED_{25}) at 28 DAT, 3.4 times the labeled rate (Table 2). The highest rate ($17,920 \text{ g ha}^{-1}$) of atrazine provided only 40 to 45% control at 14 and 28 DAT; therefore, ED_{50} and ED_{90} values were not calculated. Similar results were observed with Palmer amaranth biomass reduction at 28 DAT (Fig. 1B). To

Table 2. Effective herbicide dose required for 25, 50, and 90% control of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth and test of lack-of-fit at 95% level for three-parameter log-logistic function fitted to Palmer amaranth control estimates at 14 and 28 d after pre-emergence application in a field dose-response study conducted at Shickley, NE, in 2014 and 2015. †

Herbicide	Control, %							
	14 DAT†				28 DAT†			
	ED ₂₅ (± SE)	ED ₅₀ (± SE)	ED ₉₀ (± SE)	Lack-of-fit‡	ED ₂₅ (± SE)	ED ₅₀ (± SE)	ED ₉₀ (± SE)	Lack-of-fit‡
	g a.i. ha ⁻¹				g a.i. ha ⁻¹			
Atrazine	3337 (390)	na	na	0.38	3757 (237)	na	na	0.98
Mesotrione	6 (3)	32 (10)	773 (186)	0.40	10 (4)	48 (14)	1030 (312)	0.20
Isoxaflutole	16 (3)	46 (4)	378 (48)	0.96	16 (6)	68 (21)	1141 (432)	0.50

† DAT, days after treatment; ED₂₅, effective herbicide dose (g a.i. ha⁻¹) required to control 25% population on the basis of control estimates; ED₅₀, effective dose (g a.i. ha⁻¹) required to control 50% population on the basis of control estimates; ED₉₀, effective dose required to control 90% population on the basis of control estimates in a field dose-response study conducted at Shickley, NE; SE, standard error; na, not applicable to calculate ED₅₀ or ED₉₀ as ≥ 50% control was not achieved with all herbicide rates applied.

‡ A test of lack of fit at the 95% level was not significant for any of the dose response curves tested for the herbicides, indicating that the fitted model was correct.

Table 3. Effective herbicide dose required for 25, 50, and 90% biomass reduction of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth and test of lack-of-fit at the 95% level for three-parameter log-logistic function fitted to the percent biomass reduction at 28 d after pre-emergence application in a field dose-response study conducted at Shickley, NE, in 2014 and 2015. †

Herbicide	Biomass reduction, %‡			
	ED ₂₅ (± SE)	ED ₅₀ (± SE)	ED ₉₀ (± SE)	Lack-of-fit§
	g a.i. ha ⁻¹			
Atrazine	1191 (343)	na	na	0.52
Mesotrione	60 (10.7)	156 (15)	1115 (225)	0.70
Isoxaflutole	35 (11.65)	217 (44)	na	0.53

† ED₂₅, effective herbicide dose required to reduce biomass by 25%; ED₅₀, effective herbicide dose required to reduce biomass by 50%; ED₉₀, effective herbicide dose required to reduce biomass by 90% at 28 d after pre-emergence herbicide application in a field dose-response study conducted at Shickley, NE; SE, standard error; na, not applicable to calculate ED₅₀ or ED₉₀ as ≥ 50% biomass reduction was not achieved with all herbicide rates applied.

‡ Palmer amaranth biomass data were converted into percent biomass reduction compared with the nontreated control plots using the

formula: $\text{Biomass reduction}(\%) = \frac{(\bar{C} - B)}{\bar{C}} \times 100$, where C is the

biomass of the nontreated control plot and B is the biomass collected from the experimental plot.

§ A test of lack of fit at 95% level was not significant for any of the dose response curves tested for the herbicides, indicating that the fitted model was correct.

achieve 25% biomass reduction, atrazine at 1191 g ha⁻¹ was required, and atrazine applied at the highest rate provided only 35% biomass reduction; therefore, ED₅₀ and ED₉₀ values for biomass reduction were not calculated (Table 3).

Mesotrione at the labeled rate (105 g ha⁻¹) applied PRE provided 65 to 70% Palmer amaranth control at 14 and 28 DAT (Fig. 2A). Mesotrione at 48 and 1030 g ha⁻¹ was required to achieve 50% (ED₅₀) and 90% (ED₉₀) Palmer amaranth control at 28 DAT, 0.5 and 10 times the labeled rate, respectively (Table 2). Similarly, Palmer amaranth biomass was reduced by 50 and 90% with mesotrione at 156 and 1115 g ha⁻¹, respectively (Table 3). Isoxaflutole applied at the labeled rate (105 g ha⁻¹) provided 59 to 70% control and the highest rate (1640 g ha⁻¹) provided 95 to 98% control at 14 and 28 DAT (Fig. 3A). Palmer amaranth was controlled 50 and 90% with isoxaflutole at 46 and 378 g ha⁻¹ at 14 DAT, and isoxaflutole at 68 and 1141 g ha⁻¹ at 28 DAT, respectively (Table 2). Palmer amaranth

biomass was reduced by 50% with isoxaflutole at 217 g ha⁻¹; however, >80% biomass reduction was never achieved with any of the isoxaflutole rates tested (Table 3).

Tank Mixtures of Photosystem II- and HPPD-Inhibitor Applied Pre-Emergence

Year × treatment interaction for Palmer amaranth control at 14 DAT, density reduction, and corn yield was significant; therefore, data were presented separately for 3 yr (Tables 4, 5, and 6). Year × treatment interaction for Palmer amaranth control at 28 DAT and biomass reduction was significant, with no difference between 2015 and 2016; therefore, data were combined, but are presented separately for 2014 (Tables 4 and 5). Atrazine applied at 1120 or 2240 g ha⁻¹ provided 10 to 18% Palmer amaranth control at 28 DAT in 2014 and 2015–2016 (Table 4). Mesotrione at 105 or 210 g ha⁻¹ applied PRE alone, or tank mixed with atrazine at 1120 or 2240 g ha⁻¹ provided similar control of 35 to 70% in 2014 and 16 to 29% control in 2015–2016 at 28 DAT. Similar results were observed with Palmer amaranth density and biomass reduction at 28 DAT (Table 5). Palmer amaranth density was reduced by 48 to 86% in 2014, 6 to 40% in 2015, and 15 to 54% in 2016 with mesotrione or atrazine applied alone or tank mixed. Similarly, Palmer amaranth biomass was reduced by 28 to 64% in 2014 and 29 to 41% in 2015–2016 with mesotrione or atrazine applied alone or tank mixed (Table 5). No differences were observed between expected control values calculated using Colby's equation and observed control during experimental years, implying that additive but not synergistic interactions occurred for Palmer amaranth control by tank mixing atrazine with mesotrione. Similarly, additive interactions for density and biomass reduction were observed with mesotrione tank mixed with atrazine at all rate combinations (Table 5). Isoxaflutole at 105 or 210 g ha⁻¹ applied alone or tank mixed with atrazine at 1120 or 2240 g ha⁻¹ provided 47 to 68% control in 2014 and 10 to 34% control in 2015–2016 at 28 DAT (Table 4). Similarly, Palmer amaranth density was reduced by 44 to 67%, 9 to 31%, and 48 to 87% in 2014, 2015, and 2016, respectively, with isoxaflutole applied alone or tank mixed with atrazine at all rate combinations (Table 5). Palmer amaranth biomass reduction of 38 to 59% was achieved in 2014 and 20 to 37% in 2015–2016 with isoxaflutole applied alone or tank mixed with atrazine (Table 5). Additive interactions were observed for Palmer amaranth control, density

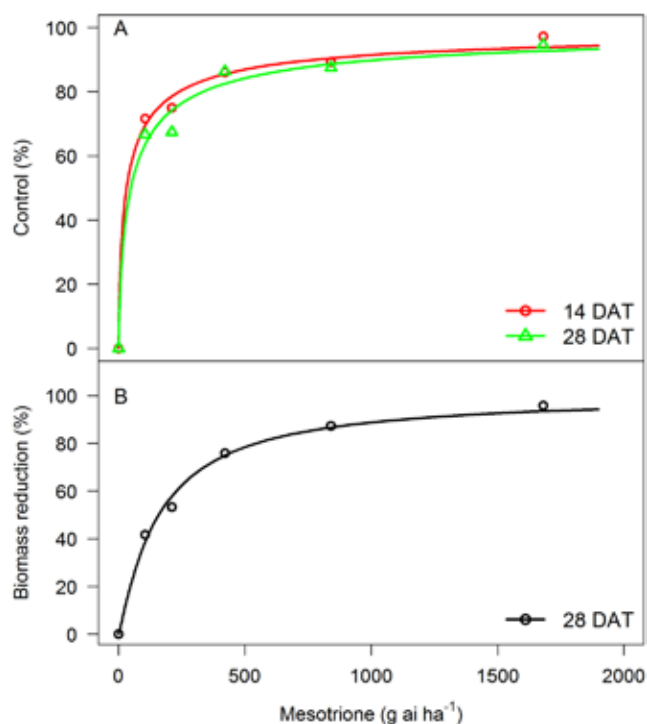


Fig. 2. Dose response of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth biotype to mesotrione applied pre-emergence. (A) Control at 14 and 28 d after treatment (DAT); and (B) Percent biomass reduction at 28 DAT in a field dose-response study conducted at Shickley, NE, in 2014 and 2015. Percent biomass reduction was calculated using the equation:

$$\text{Biomass reduction (\%)} = \frac{(\bar{C} - B)}{\bar{C}} \times 100, \text{ where } \bar{C} \text{ is the biomass of nontreated control replicate, and } B \text{ is the biomass of an individual treated experimental unit.}$$

reduction, and biomass reduction with all tank-mixed combinations of isoxaflutole and atrazine applied PRE.

Corn Yield

Mesotrione or isoxaflutole applied alone, or tank mixed with atrazine at all rate combinations provided 7193 to 12,627 kg ha⁻¹ corn yield in 2014 (Table 6). Though statistically similar to other treatments, both atrazine applied alone and the nontreated control provided 4061 to 8486 kg ha⁻¹ in 2014. In 2015, atrazine applied alone, the nontreated control, as well as mesotrione or isoxaflutole applied alone or tank mixed with atrazine at all rate combinations provided 9675 to 13,695 kg ha⁻¹ yield. In 2016, the nontreated control and atrazine, mesotrione, or isoxaflutole applied alone as well as mesotrione tank mixed with atrazine resulted in 991 to 3887 kg ha⁻¹ yield and isoxaflutole tank mixed with atrazine provided 3840 to 8754 kg ha⁻¹ yield.

Tank Mixtures of Photosystem II and HPPD Inhibitor Applied Post-Emergence

Year × treatment interaction for Palmer amaranth control at 14 DAT, and density and biomass reduction was not significant; therefore, data were combined over 3 yr (Tables 7 and 8). Year × treatment interaction for Palmer amaranth control at 28 DAT was significant, with no difference between 2015 and 2016; therefore, data were combined, but are presented separately

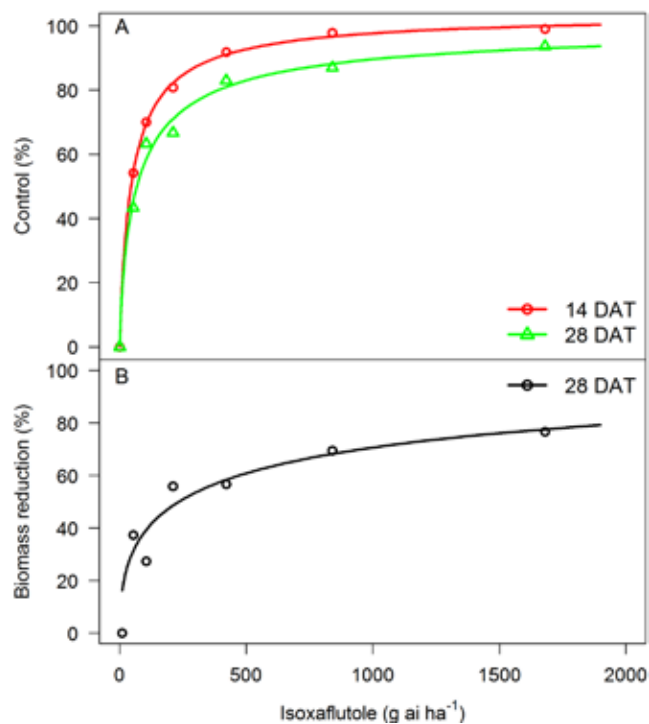


Fig. 3. Dose response of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth biotype to isoxaflutole applied pre-emergence. (A) Control at 14 and 28 d after treatment (DAT); and (B) Percent biomass reduction at 28 DAT in a field dose-response study conducted at Shickley, NE, in 2014 and 2015. Percent biomass reduction was calculated using the equation:

$$\text{Biomass reduction (\%)} = \frac{(\bar{C} - B)}{\bar{C}} \times 100, \text{ where } \bar{C} \text{ is the biomass of nontreated control replicate, and } B \text{ is the biomass of an individual treated experimental unit.}$$

for 2014 (Table 7). Year × treatment interaction for corn yield was significant, with no difference between 2014 and 2016; therefore, data were combined, but are presented separately for 2015 (Table 8). Atrazine at 1120 or 2240 g ha⁻¹ applied POST provided 0 to 30% control at 14 and 28 DAT (Table 7). Mesotrione at 105 g ha⁻¹ tank mixed with atrazine at 1120 or 2240 g ha⁻¹ provided 69 to 78% control compared to 41% control with mesotrione applied alone at 14 DAT. Similarly, at 28 DAT, Palmer amaranth was controlled 23 to 53% with mesotrione tank mixed with atrazine at all rate combinations compared to 14% control with mesotrione applied alone in 2015–2016; however, tank mixing atrazine to mesotrione did not improve control (74–90%) compared to mesotrione applied alone (62%) in 2014. Similar results were observed with Palmer amaranth density and biomass reduction during experimental years. Palmer amaranth density was reduced 82 to 85% with mesotrione tank mixed with atrazine compared to 15 to 40% control with their applications alone (Table 8). Likewise, Palmer amaranth biomass was reduced by 68 to 71% with mesotrione and atrazine tank mixtures compared to 18 to 34% reduction with atrazine or mesotrione applied alone (Table 8). Synergistic interactions were observed for Palmer amaranth control, density reduction, and biomass reduction with mesotrione at 105 g ha⁻¹ tank mixed with atrazine at 1120 ha⁻¹ (Tables 7 and 8).

Table 4. Effect of pre-emergence herbicide treatments on observed and expected control of photosystem (PS) II- and 4-hydroxyphenyl-pyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth in a field study conducted at Shickley, NE, in 2014, 2015, and 2016.

Herbicide treatment	Rate g a.i. ha ⁻¹	Control†									
		14 DAT‡						28 DAT‡			
		2014		2015		2016		2014		2015–2016	
Observed	Expected§	Observed	Expected§	Observed	Expected§	Observed	Expected§	Observed	Expected§		
		%									
Mesotrione	105	75ab	–	50a	–	45cd	–	35abc	–	18a	–
Mesotrione	210	88a	–	32a	–	33d	–	58ab	–	16a	–
Atrazine	1120	3c	–	35a	–	44cd	–	13c	–	15a	–
Atrazine	2240	48b	–	17a	–	48bcd	–	18bc	–	10a	–
Isoxaflutole	105	82a	–	7a	–	64abcd	–	47ab	–	10a	–
Isoxaflutole	210	88a	–	47a	–	76abc	–	52ab	–	34a	–
Mesotrione + atrazine	105 + 1120	86a	76a	38a	66a	64abcd	66a	70a	45a	19a	33ab
Mesotrione + atrazine	105 + 2240	91a	88a	40a	59ab	54abcd	68a	60ab	46a	29a	29ab
Mesotrione + atrazine	210 + 1120	93a	88a	42a	48abc	55cd	61a	67ab	64a	19a	32ab
Mesotrione + atrazine	210 + 2240	99a	66a	45a	11c	63abcd	54a	70a	58a	24a	13b
Isoxaflutole + atrazine	105 + 1120	87a	83a	14a	39abc	85ab	81a	48ab	54a	24a	27ab
Isoxaflutole + atrazine	105 + 2240	90a	90a	19a	23bc	93a	82a	55ab	53a	27a	21b
Isoxaflutole + atrazine	210 + 1120	98a	89a	30a	44abc	88a	86a	68a	57a	33a	47a
Isoxaflutole + atrazine	210 + 2240	96a	87a	30a	29abc	88a	78a	62ab	63a	23a	13b

† Means within columns with no common letter(s) are significantly different according to Fisher's Protected LSD test where $P \leq 0.05$. The nontreated control data were not included in the statistical analysis. Year-by-treatment interaction for Palmer amaranth control was significant at 14 DAT; therefore, data were presented separately for 3 yr. Year \times treatment interaction for Palmer amaranth control was significant at 28 DAT, with no difference between 2015 and 2016; therefore, data were combined, but are presented separately for 2014.

‡ DAT, days after treatment.

§ Expected value determined by the Colby's equation: $E = (X + Y) - (XY/100)$, where E is expected percent control with herbicide A + B; and X and Y is observed percent control with herbicide A and B, respectively. Expected value was not significantly different from the observed value ($P \geq 0.05$) as determined by *t* test, indicating additive interactions of herbicides A and B applied in tank mixture.

Table 5. Effect of pre-emergence herbicide treatments on density and biomass reduction of photosystem (PS) II- and 4-hydroxyphenyl-pyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth at 28 d after treatment in a field study conducted at Shickley, NE, in 2014, 2015, and 2016.

Herbicide treatment	Rate g a.i. ha ⁻¹	Density reduction†‡						Biomass reduction†‡			
		2014		2015		2016		2014		2015–2016	
		Observed	Expected§	Observed	Expected§	Observed	Expected§	Observed	Expected§	Observed	Expected§
		%									
Mesotrione	105	35c	–	32a	–	32d	–	43a	–	33ab	–
Mesotrione	210	70abc	–	13a	–	15d	–	53a	–	37a	–
Atrazine	1120	48abc	–	13a	–	44bcd	–	39a	–	29ab	–
Atrazine	2240	60abc	–	6a	–	27d	–	28a	–	16b	–
Isoxaflutole	105	67abc	–	9a	–	48bcd	–	53a	–	20b	–
Isoxaflutole	210	44bc	–	23a	–	55abcd	–	38a	–	24ab	–
Mesotrione + atrazine	105 + 1120	61abc	41c	19a	33a	40cd	50a	63a	70a	41a	39a
Mesotrione + atrazine	105 + 2240	86a	47bc	17a	41a	39cd	44a	60a	56a	33ab	46a
Mesotrione + atrazine	210 + 1120	70abc	73ab	22a	35a	36cd	52a	62a	73a	37a	46a
Mesotrione + atrazine	210 + 2240	84ab	79a	40a	37a	54bcd	49a	64a	66a	34ab	38a
Isoxaflutole + atrazine	105 + 1120	57abc	68abc	16a	27a	75ab	67a	59a	68a	30ab	43a
Isoxaflutole + atrazine	105 + 2240	60abc	75a	18a	32a	87a	68a	51a	65a	29ab	34a
Isoxaflutole + atrazine	210 + 1120	58abc	53abc	31a	40a	84ab	76a	53a	62a	37a	49a
Isoxaflutole + atrazine	210 + 2240	54abc	55abc	24a	45a	82ab	70a	42a	55a	34ab	46a

† Means within columns with no common letter(s) are significantly different according to Fisher's Protected LSD test where $P \leq 0.05$. The nontreated control data were not included in the statistical analysis. Palmer amaranth density and biomass data were converted into percent density reduction

and biomass reduction compared with the nontreated control plots using the formula: $\text{Biomass / Density reduction}(\%) = \frac{(\bar{C} - B)}{C} \times 100$, where C is the density or biomass of the nontreated control plot and B is the density or biomass collected from the experimental plot.

‡ Year \times treatment interaction for Palmer amaranth density reduction was significant; therefore, data were presented separately for 3 yr. Year \times treatment interaction for Palmer amaranth biomass reduction was significant, with no difference between 2015 and 2016; therefore, data were combined, but are presented separately for 2014.

§ Expected value determined by the Colby's equation: $E = (X + Y) - (XY/100)$, where E is the expected density or biomass reduction with herbicide A + B; and X and Y is the observed density or biomass reduction with herbicide A and B, respectively. Expected value was not significantly different from the observed value ($P \geq 0.05$) as determined by *t* test, indicating additive interactions of herbicides A and B applied in tank mixtures.

Table 6. Effect of pre-emergence herbicide treatments on corn yield in a field study conducted at Shickley, NE, in 2014, 2015, and 2016.

Herbicide treatment	Rate g a.i. ha ⁻¹	Corn yield†		
		2014	2015	2016
Nontreated control	–	4061b	9675a	991d
Mesotrione	105	7193ab	10,234a	2654d
Mesotrione	210	9499ab	11,309a	1967d
Atrazine	1120	4286b	10,404a	3023d
Atrazine	2240	8486ab	9939a	2570d
Isoxaflutole	53	10,886ab	10,590a	3000d
Isoxaflutole	105	9398ab	14,619a	3532cd
Mesotrione + atrazine	105 + 1120	8269ab	9993a	3445cd
Mesotrione + atrazine	105 + 2240	10,646ab	12,211a	3316cd
Mesotrione + atrazine	210 + 1120	8690ab	11,408a	3453cd
Mesotrione + atrazine	210 + 2240	11,588a	11,329a	3887bcd
Isoxaflutole + atrazine	105 + 1120	8676ab	9798a	3840bcd
Isoxaflutole + atrazine	105 + 2240	10,587ab	11,479a	7894ab
Isoxaflutole + atrazine	210 + 1120	12,627a	13,695a	8754a
Isoxaflutole + atrazine	210 + 2240	11,562a	10,891a	7415ab

† Means within columns with no common letter(s) are significantly different according to Fisher's Protected LSD test where $P \leq 0.05$. Year \times treatment interaction for corn yield was significant; therefore, data were presented separately for 3 yr.

Tembotrione applied alone at 72 g ha⁻¹ or tank mixed with atrazine at 1120 or 2240 g ha⁻¹ provided 77 to 88% control at 14 DAT (Table 7). At 28 DAT, Palmer amaranth was controlled 90 to 95% in 2014 and 33 to 63% in 2015–2016 with tembotrione applied alone or tank-mixed with atrazine at all rate combinations. Similarly, Palmer amaranth density and biomass was reduced by 75 to 86% with tembotrione applied alone or tank mixed with atrazine (Table 8). Synergistic interactions for Palmer amaranth control were observed with tembotrione at 72 g ha⁻¹ tank mixed with atrazine at 1120 or 2240 g ha⁻¹ at 28 DAT in 2015–2016. In contrast, additive interactions were observed for Palmer amaranth control with tembotrione and atrazine tank mixtures at 28 DAT in 2014 and for density and biomass reduction in 2014 and 2015–2016 (Table 8). Topramezone at 22 g ha⁻¹ tank mixed with atrazine at 1120 or 2240 g ha⁻¹ provided 78 to 82% control compared to 45% control when applied alone at 14 DAT (Table 7). Similarly, at 28 DAT, Palmer amaranth was controlled 73 to 83% with a tank mixture of topramezone and atrazine compared to 50% control with topramezone applied alone in 2014. Palmer amaranth density and biomass was reduced by 46 to 83% and 73 to 87%, respectively, with topramezone applied alone or tank mixed with atrazine (Table 8). Synergistic interactions occurred when topramezone at 22 g ha⁻¹ tank mixed with atrazine at 1120 or 2240 g ha⁻¹ at 14 DAT and with atrazine at 1120 g ha⁻¹ at 28 DAT in 2014; however, additive interactions were observed at 28 DAT in 2015–2016. Similarly, synergistic interactions were observed for density and biomass reduction with topramezone tank mixed with atrazine at 1120 g ha⁻¹.

Corn Yield

Mesotrione, tembotrione, or topramezone applied alone or tank mixed with atrazine provided 10,648 to 15,348 kg ha⁻¹ corn yield in 2015 and 7808 to 10,919 kg ha⁻¹ in 2014 to 2016 (Table 8). Though statistically similar to other treatments, atrazine

applied alone and the nontreated control provided 5693 to 8330 kg ha⁻¹ in 2015 and 4096 to 6658 kg ha⁻¹ yield in 2014 to 2016.

DISCUSSION

Dose-Response Study

Atrazine applied PRE at the labeled rate (1120 g ha⁻¹) provided <10% control of PS II- and HPPD-inhibitor-resistant Palmer amaranth at 28 DAT. Similarly, Kohrt and Sprague (2017) reported 0 to 24% control of PS II-inhibitor-resistant Palmer amaranth in Michigan with atrazine at 1120 g ha⁻¹ applied PRE at 45 DAT. Jhala et al. (2014) reported <15% control of the same PS II- and HPPD-inhibitor-resistant Palmer amaranth from Nebraska with atrazine at 1120 g ha⁻¹ applied POST at 21 DAT. Palmer amaranth was controlled 45% with atrazine at 17,920 g ha⁻¹ applied PRE. Jhala et al. (2014) reported <20% control of the same Palmer amaranth biotype with atrazine at 13,452 g ha⁻¹ applied POST. Palmer amaranth biomass was reduced by 23 to 35% with atrazine at 1120 or 17,920 g ha⁻¹ applied PRE, similar to 12 to 30% biomass reduction of the same Palmer amaranth biotype from Nebraska with atrazine at 1120 or 13,452 g ha⁻¹ applied POST (Jhala et al., 2014). Similarly, a PS II- and HPPD-inhibitor-resistant Palmer amaranth biotype from Kansas showed 20% biomass reduction with atrazine at 1120 g ha⁻¹ applied POST; however, 70% biomass reduction was observed with atrazine at 17,920 g ha⁻¹ (Nakka et al., 2017). In contrast, previous studies have reported 80 to 100% control or biomass reduction of susceptible Palmer amaranth with atrazine at 1120 g ha⁻¹ applied either PRE or POST (Grichar et al., 2005; Jhala et al., 2014; Nakka et al., 2017), signifying a high level of atrazine resistance in Palmer amaranth from Nebraska.

Mesotrione at 105 g ha⁻¹ applied PRE provided 70% Palmer amaranth control at 28 DAT. However, previous studies have reported 85 to 99% control of susceptible Palmer amaranth biotypes with mesotrione at 105 g ha⁻¹ applied PRE or POST (Jhala et al., 2014; Kohrt and Sprague, 2017). Mesotrione at 1030 g ha⁻¹ applied PRE provided 90% Palmer amaranth control in this study and similarly, Jhala et al. (2014) reported 90% control of the same biotype with mesotrione at 1007 g ha⁻¹ applied POST. Palmer amaranth biomass was reduced by 90% with mesotrione at 1115 g ha⁻¹ applied PRE in this study, which is similar to mesotrione at 903 g ha⁻¹ applied POST for 90% biomass reduction of the same Palmer amaranth biotype (Jhala et al., 2014). The results from this study have shown that PS II- and HPPD-inhibitor-resistant Palmer amaranth showed similar responses to different rates of atrazine or mesotrione applied PRE or POST. In contrast, Kaundun et al. (2017) reported complete control of HPPD-resistant tall waterhemp from Nebraska with mesotrione at 105 g ha⁻¹ applied PRE compared to only 37% control with POST application. Isoxaflutole applied PRE at the labeled rate (105 g ha⁻¹) provided 70% Palmer amaranth control at 28 DAT. However, previous studies have reported 86 to 99% control of susceptible Palmer amaranth with isoxaflutole at 90 or 105 g ha⁻¹ applied PRE at 28 or 56 DAT (Johnson et al., 2000; Meyer et al., 2016; Stephenson and Bond, 2012), showing a low presence of isoxaflutole resistance in a Palmer amaranth biotype from Nebraska. Mesotrione or isoxaflutole at 105 g ha⁻¹ applied PRE exhibited 70% Palmer amaranth control in this study. Kaundun et al. (2017) reported

Table 7. Effect of post-emergence herbicide treatments on observed and expected control of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxxygenase (HPPD)-inhibitor-resistant Palmer amaranth in a field study conducted at Shickley, NE, in 2014, 2015, and 2016.

Herbicide treatment	Rate g a.i. ha ⁻¹	Palmer amaranth control†					
		14 DAT‡		28 DAT‡			
		Observed	Expected§	2014		2015–2016	
				Observed	Expected§	Observed	Expected§
				%			
Mesotrione	105	41c	–	62abc	–	14def	–
Atrazine	1120	6d	–	0d	–	1 f	–
Atrazine	2240	16d	–	30cd	–	8 ef	–
Tembotrione	72	77a	–	90ab	–	33bcde	–
Topramezone	22	45bc	–	50bc	–	41bcd	–
Mesotrione + atrazine	105 + 1120	78a	43c*	90ab	62ab*	53abc	12b*
Mesotrione + atrazine	105 + 2240	69a	48c	74ab	70ab	23bc	18b
Tembotrione + atrazine	72 + 1120	83a	78ab	95a	90ab	55ab	30ab*
Tembotrione + atrazine	72 + 2240	88a	81a	90ab	94a	63ab	34ab*
Topramezone + atrazine	22 + 1120	78a	52bc*	83ab	50b*	43bcd	37ab
Topramezone + atrazine	22 + 2240	82a	51bc*	73ab	48b	76a	53a

* Expected value significantly lower than observed value ($P \leq 0.05$) as determined by *t* test, indicating synergistic interactions of herbicides A and B applied in tank mixtures.

† Means within columns with no common letter(s) are significantly different according to Fisher's Protected LSD test where $P \leq 0.05$. The nontreated control data were not included in the statistical analysis. Year-by-treatment interaction for Palmer amaranth control was not significant at 14 DAT; therefore data were combined over 3 yr. Year \times treatment interaction for Palmer amaranth control was significant at 28 DAT, with no difference between 2015 and 2016; therefore, data were combined, but are presented separately for 2014.

‡ Abbreviations: DAT, days after treatment.

§ Expected value determined by the Colby's equation: $E = (X + Y) - (XY/100)$, where *E* is the expected percent control with herbicide A + B; and *X* and *Y* are the observed percent control with herbicide A and B, respectively.

100% control of HPPD-inhibitor-resistant tall waterhemp with mesotrione or isoxaflutole at 105 g ha⁻¹ applied PRE.

Tank Mixtures of Photosystem II- and HPPD-Inhibitor Applied Pre-Emergence

Atrazine tank mixed with mesotrione or isoxaflutole did not improve Palmer amaranth control compared to mesotrione or isoxaflutole applied alone at 28 DAT in 2014 and 2015–2016 (Table 4). Similarly, Johnson et al. (2000) reported 78 to 100% control of susceptible Palmer amaranth with isoxaflutole at 80 g ha⁻¹ applied alone PRE or tank mixed with atrazine at 1680 g ha⁻¹ at 56 DAT. Likewise, tank mixing atrazine with mesotrione or isoxaflutole mostly did not improve Palmer amaranth density and biomass reduction (Table 5). However, Armel et al. (2003) reported 60 to 90% control of common lambsquarters, common ragweed (*Ambrosia artemisiifolia* L.), and smooth pigweed (*Amaranthus hybridus* L.) by tank mixing atrazine at 560 g ha⁻¹ with mesotrione at 160 g ha⁻¹ compared to 28 to 71% control with mesotrione applied alone PRE. The Colby's equation suggested additive interactions for Palmer amaranth control, density reduction, or biomass reduction by tank mixing atrazine with mesotrione or isoxaflutole at all rate combinations (Tables 4 and 5). However, previous studies have shown synergistic interactions between PS II- and HPPD-inhibitor tank-mixtures applied POST for susceptible Palmer amaranth and PS II-inhibitor-resistant redroot pigweed control (Abendroth et al., 2006; Hugie et al., 2008; Sutton et al., 2002). The synergistic interaction of PS II- and HPPD-inhibitor tank mixtures applied PRE is species specific. For instance, Bollman et al. (2006) reported a synergistic interaction with mesotrione and atrazine tank mixtures applied PRE for velvetleaf control; however, additive effects occurred for ivyleaf morningglory (*Ipomoea hederacea* Jacq.) control with most mesotrione and

atrazine tank mixtures. In addition, the interactions of two herbicides tank mixed or applied sequentially on an herbicide-resistant plant are also dependent on the underlying mechanism of herbicide resistance (Woodyard et al., 2009b). Mesotrione or isoxaflutole applied alone or tank mixed with atrazine, as well as the nontreated control, provided similar yield (9675–14,619 kg ha⁻¹) in 2015 due to poor Palmer amaranth control and density reduction of 7 to 50% with all herbicide treatments. Corn yield was lower in 2016 compared to 2014 and 2015, possibly due to higher Palmer amaranth emergence later in the season because of higher seed bank accumulation at the experimental site from the previous 2 yr of the study as well as due to low rainfall of 7 mm received after 2 wk of planting soybean [*Glycine max* (L.) Merr.] in 2016 compared to 130 to 280 mm of rainfall received within a week of planting soybean in 2014 and 2015 (Table 1).

Tank Mixtures of Photosystem II- and HPPD-Inhibitor Applied Post-Emergence

Atrazine tank mixed with mesotrione or topramezone provided 69 to 82% control at 14 DAT compared to 41 to 45% control with mesotrione or topramezone applied alone POST. Similarly, Jhala et al. (2014) reported 99% control of the same resistant Palmer amaranth biotype from Nebraska with mesotrione at 106 g ha⁻¹ or topramezone at 25 g ha⁻¹ tank mixed with atrazine at 560 g ha⁻¹ compared to 58 to 66% control with their applications alone at 21 DAT. Tembotrione applied alone provided 77% Palmer amaranth control compared to 6 to 45% control with mesotrione, topramezone, or atrazine; however, tank mixing atrazine with tembotrione did not improve control at 14 DAT (Table 7). Similarly, Kohrt and Sprague (2017) reported 91% control of 8-cm tall PS II-inhibitor-resistant Palmer amaranth with tembotrione compared to 61 to 77% control with atrazine, mesotrione, or topramezone applied POST

Table 8. Effect of post-emergence herbicide treatments on density and biomass reduction of photosystem (PS) II- and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitor-resistant Palmer amaranth at 28 d after treatment, and corn yield in a field study conducted at Shickley, NE, in 2014, 2015, and 2016.

Herbicide treatment	Rate g a.i. ha ⁻¹	Density reduction†‡		Biomass reduction†‡		Corn yield†§	
		Observed	Expected¶¶	Observed	Expected¶¶	2015	2014–2016
		%				kg ha ⁻¹	
Nontreated control	–	–	–	–	–	5,693d	4,096b
Mesotrione	105	26c	–	34b	–	10,648abc	8,058a
Atrazine	1120	15c	–	18b	–	8,330bcd	6,658ab
Atrazine	2240	40bc	–	26b	–	8,075cd	6,019ab
Tembotrione	72	75ab	–	82a	–	13,347abc	7,956a
Topramezone	22	46abc	–	73a	–	14,360a	7,808a
Mesotrione + atrazine	105 + 1120	85a	37b*	68a	48b*	12,091abc	8,674a
Mesotrione + atrazine	105 + 2240	82a	57ab	71a	53b	11,405abc	9,530a
Tembotrione + atrazine	72 + 1120	77ab	77a	81a	87a	15,348a	10,482a
Tembotrione + atrazine	72 + 2240	79a	87a	86a	87a	13,526ab	10,919a
Topramezone + atrazine	22 + 1120	83a	53ab*	87a	78a*	15,102a	9,120a
Topramezone + atrazine	22 + 2240	78ab	66ab	80a	79a	14,060a	9,745a

* Expected value significantly lower than observed value ($P \leq 0.05$) as determined by *t* test, indicating synergistic interactions of herbicides A and B applied in tank mixtures.

† Means within columns with no common letter(s) are significantly different according to Fisher's Protected LSD test where $P \leq 0.05$.

‡ The nontreated control data were not included in the statistical analysis. Palmer amaranth density and biomass data were converted into percent density reduction and biomass reduction compared with the nontreated control plots using the formula:

Biomass / Density reduction (%) = $\frac{(C - B)}{C} \times 100$, where C is the density or biomass of the nontreated control plot and B is the density or biomass collected from experimental plot. Year \times treatment interaction for Palmer amaranth density and biomass reduction was not significant; therefore, data were combined over 3 yr.

§ Year \times treatment interaction for corn yield was significant, with no difference between 2014 and 2016; therefore, data were combined, but are presented separately for 2015.

¶¶ Expected value determined by the Colby's equation: $E = (X + Y) - (XY/100)$, where E is the expected density or biomass reduction with herbicide A + B; and X and Y are the observed density or biomass reduction with herbicide A and B, respectively.

at 21 DAT; and also reported that tank mixing atrazine at 560 g ha⁻¹ with tembotrione at 92 g ha⁻¹ did not improve control. Jhala et al. (2014) also reported similar (96–98%) control of the same Palmer amaranth biotype from Nebraska with tembotrione applied alone or tank mixed with atrazine. In contrast, Stephenson et al. (2015) and Williams et al. (2011) reported 95 to 97% control of susceptible Palmer amaranth and redroot pigweed with tembotrione tank mixed with atrazine compared to tembotrione applied alone (80–91%). Palmer amaranth control was reduced at 28 DAT due to new seedling emergence because of its extended emergence pattern; therefore, tank-mixing atrazine with topramezone or tembotrione did not improve control compared to when applied alone at 28 DAT, with the exception of tank mixing topramezone at 22 g ha⁻¹ with atrazine at 2240 g ha⁻¹. Relatively higher control was achieved in 2014 at 28 DAT possibly due to a lower density of Palmer amaranth, which ranged from 100 to 125 plants m⁻² at the experimental site in 2014 compared to 300 to 400 plants m⁻² in 2015–2016.

Tembotrione at 72 g ha⁻¹ tank-mixed with atrazine at 1120 or 2240 g ha⁻¹ mostly showed additive interactions for Palmer amaranth control, density, and biomass reduction (Tables 7 and 8). Kohrt and Sprague (2017) reported synergistic interactions for control of 8-cm tall PS II-inhibitor-resistant Palmer amaranth with lower than labeled rates of tembotrione tank mixed with atrazine, and additive interactions when tank mixing tembotrione at 92 g ha⁻¹ to atrazine at 560 g ha⁻¹. Tank mixing mesotrione or topramezone with atrazine at 1120 g ha⁻¹ resulted in synergistic interactions for Palmer amaranth control, density, and biomass reduction compared to additive

interactions when tank mixed with the higher rate of atrazine (2240 g ha⁻¹). Kohrt and Sprague (2017) reported synergistic effects for control of 8-cm tall PS II-inhibitor-resistant Palmer amaranth with mesotrione at 35 g ha⁻¹ tank mixed with atrazine at 40 to 2240 g ha⁻¹; however, additive effects occurred at higher atrazine rates (4480–35,900 g ha⁻¹). This could possibly be due to reduced absorption of mesotrione when tank mixed with the higher atrazine rate due to excess atrazine residue present on the leaf surface (Kohrt and Sprague, 2017).

The synergistic interactions of PS II- and HPPD-inhibitor tank mixtures can be related to their complementary site of action. The PS II-inhibitor such as atrazine or metribuzin compete with plastoquinone, which serves as an electron acceptor during the light reaction phase of photosynthesis, and binds at the Q_B binding site of the D1 protein, resulting in inhibition of the electron transport chain (Hess, 2000). This results in the accumulation of reactive singlet oxygen, singlet chlorophyll, and triplet chlorophyll species and damage to the cell membranes and D1 proteins (Hess, 2000). Mesotrione, tembotrione, or topramezone inhibits HPPD enzyme synthesis which leads to depletion of α -tocopherols and plastoquinone, reducing the competition between atrazine and plastoquinone for binding to the D1 protein (Hess, 1993; Pallert et al., 1998). In addition, plastoquinone acts as a co-factor to produce carotenoids and inhibition of plastoquinone by HPPD-inhibitor limits carotenoid synthesis (Norris et al., 1998). α -Tocopherols acts as an anti-oxidant in the chloroplast and reduces the photooxidation of lipids, cell walls, or D1 proteins from single oxygen species produced during photosynthesis. Both PS II- and HPPD-inhibitor block the electron

transport in PS II due to their complementary site of action and lead to the accumulation of reactive oxygen species and free radicals that damage the foliar tissue membranes (Hess, 2000).

The results of this study have confirmed that the Palmer amaranth biotype from Fillmore County, Nebraska, is resistant to PS II- and HPPD-inhibiting herbicides applied PRE as well as POST. In addition, tank mixing PS II- and HPPD-inhibitor applied POST improved Palmer amaranth control compared to when applied alone; however, tank mixtures applied PRE did not improve control. Even though Palmer amaranth from Nebraska is resistant to both PS II- and HPPD-inhibiting herbicides applied alone, they can be applied POST in a tank mixture with different site of action herbicides for effective control and to reduce the risk of herbicide-resistance evolution. However, it is crucial to adopt an integrated weed management approach that includes the use of herbicides with distinct sites of action, a PRE followed by POST herbicide program, crop rotation, the rotation of herbicide-resistant cultivars with conventional crop cultivars, tillage, and harvest weed seed control methods to mitigate the evolution and spread of multiple herbicide-resistant Palmer amaranth.

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