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# Basis of Atrazine and Mesotrione Synergism for Controlling Atrazine- and HPPD Inhibitor-Resistant Palmer Amaranth

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## ABSTRACT

Palmer amaranth (*Amaranthus palmeri* S. Watson) resistant to atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine2,4-diamine] and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides was confirmed in a seed corn (*Zea mays* L.) production field in Nebraska, in 2014. Neither atrazine nor HPPD inhibitors (mesotrione [2-(4-mesyl2-nitrobenzoyl)-3-hydroxycylohex-2-enone], tembotrione {2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy) methyl]benzoyl]-1,3-cyclohexanedione}, or topramezone {[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone}) applied post-emergence were able to control resistant Palmer amaranth even at greater than label rates. However, their tank mixtures even at lower than the label rate provided more than 90% control under greenhouse and field conditions. The objectives of this study were to investigate the effect of atrazine on mesotrione absorption and translocation when tank mixed or vice versa in atrazine- and HPPD inhibitor-resistant Palmer amaranth from Nebraska. Tank mixing commercial formulation of atrazine at 560 g $ha^{-1}$ increased $^{14}$C-mesotrione absorption to 51% compared to 39% with $^{14}$C-mesotrione alone. However, $^{14}$C-atrazine absorption or translocation was not affected by mesotrione at 26 g $ha^{-1}$ in the tank mixture. Similarly, mesotrione did not affect the metabolism of $^{14}$C-atrazine in resistant or susceptible plants when tank mixed compared to $^{14}$C-atrazine applied alone. Increased absorption of mesotrione when tank mixed with atrazine could be one of the reasons of atrazine and mesotrione synergism besides their biochemical interaction in the atrazine- and HPPD inhibitor-resistant Palmer amaranth biotype from Nebraska.


## Core Ideas

- Atrazine applied in tank-mixture increased mesotrione absorption.
- Mesotrione applied in tank mixture did not affect atrazine absorption and translocation.
- Atrazine metabolism was not affected by mesotrione applied in tank mixture.

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Herbicides belonging to the photosystem II (PS II)- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor site of action are some of the most commonly used herbicides for weed control in field corn, seed corn, and popcorn (Bollman et al., 2008; Chahal et al., 2015; Fleming et al., 1988; Mitchell et al., 2001; Swanton et al., 2007). Repeated use of PS II- and HPPD-inhibiting herbicides, specifically in corn-based cropping system, has resulted in the evolution of 26 and two resistant weed species, respectively, in the United States (Heap, 2019).

A Palmer amaranth biotype resistant to PS II {atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine2,4-diamine]} and HPPD inhibitors {mesotrione [2-(4-mesyl2-nitrobenzoyl)-3-hydroxycylohex-2-enone]}, tembotrione {2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy) methyl] benzoyl]-1,3-cyclohexanedione}, or topramezone {[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone}) was reported in a seed corn production field with continuous or repeated use of the above-mentioned herbicide products in south-central Nebraska (Jhala et al., 2014). Jhala et al. (2014) reported improved control of atrazine- and HPPD inhibitor-resistant Palmer amaranth with POST application of mesotrione at 106 g $ha^{-1}$ or topramezone at 25 g $ha^{-1}$ tank mixed with atrazine at 560 g $ha^{-1}$.

Abendroth et al. (2006) reported 60% control of susceptible Palmer amaranth with mesotrione at 35 g $ha^{-1}$ tank mixed with atrazine at 280 g $ha^{-1}$ compared to 15 to 27% control when applied individually. Similarly, Sutton et al. (2002) reported 90% control of atrazine-resistant redroot pigweed (*A. retroflexus* L.) with mesotrione at 2.7 g $ha^{-1}$ tank mixed with atrazine at 60 g $ha^{-1}$ compared to 30% control with mesotrione or no control with atrazine applied alone. While Palmer amaranth in Nebraska has been confirmed resistant to both atrazine and HPPD inhibitors, synergistic interaction was observed with their tank mixture applied POST (Jhala et al., 2014). Besides the biochemical and physiological overlap of atrazine and mesotrione due to their complementary site of action when applied in a tank mixture (Hess, 2000; Pallett et al., 1998),

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**Abbreviations:** AIC, Akaike's information criterion; ATL, above the treated leaf; DAT, days after treatment; HAT, hours after treatment; HPLC, high-performance liquid chromatography; HPPD, 4-hydroxyphenylpyruvate dioxygenase; LSS, liquid scintillation spectrometry; TL, treated leaf.

mechanism of synergism might also involve other biochemical and physiological effects including increased herbicide uptake or translocation, reduced herbicide detoxification, and/or increased susceptibility of different plant parts to a herbicide action (Putnam and Ries, 1967; Salzman et al., 1992; Shaw and Wesley, 1993), resulting in improved control of atrazine- and HPPD inhibitor-resistant Palmer amaranth. Therefore, the main objectives of this study were to (i) determine the response of atrazine- and HPPD inhibitor-resistant Palmer amaranth to different rates of mesotrione or atrazine applied POST alone or in tank mixture, (ii) quantify the absorption and translocation of $^{14}$C-atrazine or $^{14}$C-mesotrione applied alone or tank mixed with commercial formulations of mesotrione or atrazine, respectively, and (iii) determine the metabolism of $^{14}$C-atrazine applied alone or tank mixed with a commercial formulation of mesotrione in atrazine- and HPPD inhibitor-resistant and susceptible Palmer amaranth from Nebraska.

## MATERIALS AND METHODS

### Plant Materials

Seeds were collected from multiple Palmer amaranth plants that survived the layby field application of labeled rate of atrazine 2240 g a.i. ha$^{-1}$ and mesotrione 105 g a.i. ha$^{-1}$ POST in 2015 in a grower's field in Fillmore County, Nebraska (40.46° N, 97.80° E) under continuous seed corn production and were confirmed resistant to atrazine and HPPD inhibitors (Jhala et al., 2014). The level of atrazine resistance in Palmer amaranth 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 a susceptible Palmer amaranth biotype (Jhala et al., 2014). Seeds of susceptible Palmer amaranth were collected near a grower's field in Buffalo County, Nebraska (40.68° N, 99.06° E) with no history of atrazine or HPPD inhibitors application. Seeds were stored in separate paper bags in a refrigerator at 4°C in the dark until used in this study. A preliminary study was conducted under greenhouse conditions in Lincoln, NE, in 2015 to determine the level of segregation for atrazine and mesotrione resistance in resistant Palmer amaranth. Seeds from resistant and susceptible biotypes were planted in separate germination flats containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, QC, Canada), and emerged plants were later thinned to maintain 50 plants per flat. The plants were supplied with water and nutrients and kept in a greenhouse maintained at a 30/20°C day/night temperature regime with a 16-h photoperiod supplemented with 600 mmol m$^{-2}$ s$^{-1}$ photosynthetic active radiation provided with sodium vapor lamps. The treatments were laid out in a factorial arrangement using a randomized complete block design with three replications. The three factors were: (i) two herbicides (atrazine and mesotrione), (ii) two different rates of herbicides (two and four times the label rate of mesotrione 105 g a.i. ha$^{-1}$ and atrazine 2240 g a.i. ha$^{-1}$), and (iii) two Palmer amaranth biotypes (atrazine- and HPPD inhibitor-resistant and susceptible). Palmer amaranth plants at the six- to seven-leaf stage (10 to 12 cm tall) were treated with two or four times the label rate of atrazine (Aatrex, Syngenta Crop Protection, Inc., Greensboro, NC) or mesotrione (Callisto, Syngenta Crop Protection). Each herbicide treatment was prepared in distilled water and mixed with crop oil concentrate (COC, Induce, Helena Chemical Co., Collierville, TN) at 1% volume/volume (v/v) and ammonium sulfate (34% N PAK AMS, Winfield Solutions, LLC, St. Paul, MN) at 2.5% v/v. Herbicide treatments were applied using a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN 56045) fitted with an 8001E nozzle (TeeJet, Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L ha$^{-1}$ carrier volume at 207 kPa. The results indicated 100% survival of resistant Palmer amaranth plants at 21 d after application of atrazine or mesotrione at two or four times the label rate, suggesting that the field collected biotype was homogeneous for atrazine and mesotrione resistance (results not shown). On the other hand, complete control of susceptible plants was achieved with atrazine or mesotrione at two or four times the label rate; therefore, field collected resistant and susceptible biotype seeds were used for the $^{14}$C-herbicide absorption and translocation studies in 2016.

### Palmer Amaranth Response to Atrazine and Mesotrione Tank Mixture

A greenhouse study was conducted at the University of Nebraska-Lincoln in 2016 to determine the response of atrazine and HPPD inhibitor-resistant and susceptible Palmer amaranth to a tank mixture of atrazine and mesotrione in various rate combinations (Table 1). Seeds from both biotypes were planted in germination trays containing potting mix, and germinated seedlings were transplanted into square plastic pots (10 by 10 by 12 cm) containing a 2:2:2:4 soil/sand/vermiculite/peat moss mixture under greenhouse conditions as described above. The treatments were laid out in a factorial arrangement using a randomized complete block design with six replications. The two factors were: (i) tank mixtures of different rate combinations of atrazine and mesotrione and (ii) two Palmer amaranth biotypes (atrazine- and HPPD inhibitor-resistant and susceptible). The experiment was repeated under the growing conditions mentioned above. A single Palmer amaranth plant per pot was considered as an experimental unit. Palmer amaranth plants at the six- to seven-leaf stage (10–12 cm tall) were treated with atrazine or mesotrione alone or tank mixed at different rate combinations (Table 1).

Palmer amaranth control/injury was visually assessed 21 d after treatment (DAT) using a scale ranging from 0% (no control) to 100% (complete control). Control ratings were recorded based on symptoms such as chlorosis, necrosis, stand loss, and stunting compared with nontreated control plants. Aboveground biomass of each Palmer amaranth biotype was harvested at 21 DAT, oven-dried for 4 d at 65°C, and the biomass was determined. The biomass data were converted into percent biomass reduction compared to the nontreated control using the equation (Eq. [1]; Ganie et al., 2017a)

$$\text{Biomass reduction}(\%) = \frac{(\overline{C} - B)}{\overline{C}} \times 100 \qquad [1]$$

where is the mean biomass of the nontreated control and B is the biomass of an individual treated experimental unit. To determine the interactions of mesotrione tank mixed with atrazine applied POST, Colby's equation (Eq. [2]; Colby, 1967) was used to calculate the expected Palmer amaranth control or biomass reduction achieved with tank mixtures compared to a nontreated control:

Table 1. Observed and expected (determined by Colby's equation) percent control and biomass reduction of atrazine- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-resistant and susceptible Palmer amaranth by tank mixing mesotrione and atrazine at various rate combinations at 21 DAT in a greenhouse study conducted at the University of Nebraska-Lincoln in 2016.†

| Herbicide | | Observed control‡§ | | Expected control¶ | | Observed biomass reduction‡§ | | Expected biomass reduction¶ | |
|---|---|---|---|---|---|---|---|---|---|
| Mesotrione | Atrazine | Resistant | Susceptible | Resistantno. | Susceptible | Resistant | Susceptible | Resistant | Susceptible |
| g a.i. ha$^{-1}$ | | % | | | | | | | |
| 26 | 0 | 18d | 56d | – | – | 28d | 58c | – | – |
| 52 | 0 | 23d | 74c | – | – | 45c | 61c | – | – |
| 105 | 0 | 31c | 84bc | – | – | 43c | 77b | – | – |
| 210 | 0 | 56b | 88ab | – | – | 77b | 78b | – | – |
| 0 | 560 | 15d | 77bc | – | – | 34cd | 39d | – | – |
| 0 | 1120 | 15d | 77bc | – | – | 37cd | 77b | – | – |
| 0 | 2240 | 18d | 96a | – | – | 37cd | 97a | – | – |
| 0 | 4480 | 15d | 93a | – | – | 42c | 93a | – | – |
| 26 | 560 | 85a | 100a | 30 | 90 | 95a | 100a | 52 | 74# |
| 26 | 1120 | 96a | 100a | 30 | 90 | 97a | 100a | 55 | 90 |
| 26 | 2240 | 91a | 100a | 33 | 98 | 98a | 100a | 55 | 99 |
| 26 | 4480 | 100a | 100a | 30 | 97 | 100a | 100a | 58 | 97 |
| 52 | 560 | 94a | 100a | 35 | 94 | 96a | 100a | 64 | 76# |
| 52 | 1120 | 92a | 100a | 35 | 94 | 98a | 100a | 65 | 91 |
| 52 | 2240 | 93a | 100a | 37 | 99 | 98a | 100a | 65 | 99 |
| 52 | 4480 | 94a | 100a | 35 | 98 | 95a | 100a | 68 | 97 |
| 105 | 560 | 100a | 100a | 41 | 96 | 100a | 100a | 62 | 86# |
| 105 | 1120 | 96a | 100a | 41 | 96 | 100a | 100a | 64 | 95 |
| 105 | 2240 | 100a | 100a | 43 | 99 | 100a | 100a | 64 | 99 |
| 105 | 4480 | 100a | 100a | 41 | 99 | 100a | 100a | 67 | 98 |
| 210 | 560 | 95a | 100a | 63 | 97 | 99a | 100a | 84 | 86# |
| 210 | 1120 | 100a | 100a | 63 | 97 | 100a | 100a | 86 | 94 |
| 210 | 2240 | 98a | 100a | 64 | 99 | 100a | 100a | 86 | 99 |
| 210 | 4480 | 100a | 100a | 63 | 99 | 99a | 100a | 87 | 98 |

† Abbreviations: a.i., active ingredient; DAT, days after treatment.

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

§ The nontreated control data were not included in the statistical analysis. Experimental run × treatment interaction for Palmer amaranth control and biomass reduction was not significant at 21 DAT; therefore, data were combined over two experimental runs.

¶ Expected value determined by Colby's equation: $E = (X + Y) - (XY/100)$, where $E$ is the expected percent control with two herbicides, and $X$ and $Y$ are the observed percent control with both herbicides applied individually.

# Expected biomass reduction of susceptible biotype is significantly lower than its observed biomass reduction ($P \leq 0.05$) as determined by the $t$ test, indicating synergistic interactions of two herbicides applied in tank-mixtures.

$$E = (X + Y) - \frac{(X \times Y)}{100} \quad [2]$$

where $E$ is Palmer amaranth control or biomass reduction expected with the application of two herbicides in a tank mixture compared to a nontreated control. Expected values were calculated using the observed Palmer amaranth control or biomass reduction $X$ and $Y$ achieved with the individual application of two herbicides. The expected and observed control or biomass reduction achieved with herbicides in tank-mixture were subjected to $t$ test 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.

### $^{14}$C-Atrazine and $^{14}$C-Mesotrione Absorption and Translocation Studies

Seeds of resistant and susceptible Palmer amaranth biotypes used in the greenhouse study at the University of Nebraska-Lincoln were also used at the Kansas State University in 2016. Seeds were planted in separate germination trays containing potting mix and uniform-sized plants were transplanted at the two-leaf stage into square plastic pots (8 by 8 by 10 cm) containing potting mix under greenhouse conditions. The greenhouse was maintained at 30/22°C day/night temperature regime with a 16-h photoperiod supplemented with 250 mmol m$^{-2}$ s$^{-1}$ photosynthetic active radiation provided by sodium vapor lamps. Seven days after transplanting, plants were transferred into a growth chamber maintained at 30/22°C day/night temperatures, 75% (±4%) relative humidity, and a 16 h photoperiod. Fluorescent bulbs were used to provide 550 mmol m$^{-2}$ s$^{-1}$ photosynthetic active radiation in the growth chamber. To evaluate the effect of atrazine or mesotrione on the absorption/translocation of mesotrione or atrazine applied in tank mixture, respectively, two separate studies were conducted with $^{14}$C-atrazine (Syngenta Crop Protection) applied alone or tank mixed with formulated mesotrione in one study and $^{14}$C-mesotrione (Institute of Isotopes Co., Ltd., Budapest, Hungary) applied alone or tank mixed with formulated atrazine in another study under laboratory conditions.

Based on the results from greenhouse tank-mixture studies conducted in Nebraska, the label rate of atrazine at 560 g

ha$^{-1}$ tank mixed with the lowest rate of mesotrione at 26 g ha$^{-1}$ caused synergistic interactions for control of atrazine- and HPPD inhibitor-resistant Palmer amaranth, similar to other mesotrione and atrazine tank-mixed combinations (Table 1). Therefore, 2240 g ha$^{-1}$ atrazine tank mixed with 26 g ha$^{-1}$ mesotrione and 560 g ha$^{-1}$ atrazine tank mixed with 105 g ha$^{-1}$ mesotrione were used for the $^{14}$C-herbicide absorption and translocation studies. One milliliter of $^{14}$C-mesotrione working solution equivalent to 105 g of mesotrione in a carrier volume of 187 L ha$^{-1}$ was prepared by mixing 45.5 µL of $^{14}$C-mesotrione water solution (specific activity of 4255 kBq mg$^{-1}$), 1 µL of Callisto herbicide, 25 µL AMS, 10 µL COC, and 918.5 µL of water. Similarly, one mL of $^{14}$C-mesotrione and formulated atrazine tank-mixed working solution equivalent to 105 g of mesotrione and 560 g of atrazine tank mixed in a carrier volume of 187 L ha$^{-1}$ was prepared by mixing 45.5 µL of $^{14}$C-mesotrione water solution (specific activity of 4255 kBq mg$^{-1}$), 1 µL of Callisto herbicide, 6.3 µL Aatrex herbicide, 25 µL AMS, 10 µL COC, and 914.9 µL of water. In the $^{14}$C-atrazine study, 1 mL of $^{14}$C-atrazine working solution equivalent to 2240 g of atrazine in a carrier volume of 187 L ha$^{-1}$ was prepared by mixing 90 µL of $^{14}$C-atrazine water solution (specific activity of 27,448 kBq mg$^{-1}$), 25 µL of Aatrex herbicide, 25 µL AMS, 10 µL COC, and 850 µL of water. Similarly, 1 mL of $^{14}$C-atrazine and formulated mesotrione tank-mixed working solution equivalent to 2240 g of atrazine and 26 g of mesotrione tank mixed in a carrier volume of 187 L ha$^{-1}$ was prepared by mixing 90 µL of $^{14}$C-atrazine water solution (specific activity of 27,448 kBq mg$^{-1}$), 25 µL of Aatrex herbicide, 0.29 µL of Callisto herbicide, 25 µL AMS, 10 µL COC, and 849.71 µL of water. The treatments within each study were laid out in a factorial arrangement with two herbicide treatments ($^{14}$C-herbicide applied alone or tank mixed with commercial formulation of tank-mixed partner) and five harvest time points (4, 8, 24, 48, and 72 h after treatment (HAT)) using a randomized complete block design with six replications. The experiment was repeated using the same procedure under similar growing conditions.

Palmer amaranth plants at 10 to 12 cm height were treated with $^{14}$C-herbicide solution and a total of 10 ML solution containing 3.3 kBq $^{14}$C-herbicide was applied per plant with 10 1-ML droplets on the upper surface of the fully expanded fourth-youngest leaf using a Wiretrol (10 ML, Drummond Scientific Co., Broomall, PA) (Nakka et al., 2017a). The treated plants were moved to the same growth chamber within 30 min after treatment. At 4, 8, 24, 48, and 72 HAT, plants were dissected into treated leaf (TL), leaves above the treated leaf (ATL), leaves below the treated leaf (BTL), and the roots. Treated leaves were rinsed twice in a 20-mL scintillation vial containing 5 mL of wash solution (10% v/v ethanol aqueous solution with 0.5% of Tween-20) for 1 min to remove the unabsorbed herbicide from the surface of the treated leaf (Ganie et al., 2017b; Godar et al., 2015; Ou et al., 2018). The leaf rinse was mixed with 15 mL of scintillation cocktail [Ecolite-(*R*), MP Biomedicals, LLC, Santa Ana, CA], and radioactivity was determined by using liquid scintillation spectrometry (LSS) (Beckman Coulter LS6500 Multipurpose Scintillation Counter, Beckman Coulter, Inc., Brea, CA). All plant parts were wrapped in a single layer of tissue paper and oven dried at 60°C for 48 h. Plant parts were then combusted for 3 min using a biological oxidizer (OX-501, RJ Harvey Instrument) to recover the radiolabeled herbicide in a proprietary $^{14}$C-trapping scintillation cocktail, and radioactivity was determined using LSS. Herbicide absorption and translocation were calculated using the equations in Ganie et al. (2017b), and Godar et al. (2015).

$$\%\,Absorption = \left(\frac{Total\ radioactivity\ applied - radioacivity\ recovered\ in\ wash\ solution}{Total\ radioactivity\ applied}\times 100\right) \quad [3]$$

$$\%\text{Radioactivity in treated leaf} = \left(\frac{Radioactivity\ recovered\ in\ treated\ leaf}{Total\ radioactivity\ applied}\times 100\right) \quad [4]$$

$$\%\text{Radioactivity in abovetreated leaf} = \left(\frac{Radioactivity\ recovered\ in\ abovetreated\ leaf}{Total\ radioactivity\ applied}\times 100\right) \quad [5]$$

$$\%\text{Radioactivity in belowtreated leaf} = \left(\frac{Radioactivity\ recovered\ in\ belowtreated\ leaf}{Total\ radioactivity\ applied}\times 100\right) \quad [6]$$

$$\%\text{Translocation} = \left(\frac{Total\ radioactivity\ recovered\ in\ abovetreated + belowtreated\ leaves + roots}{Total\ radioactivity\ applied}\times 100\right) \quad [7]$$

In this study, plants were not oversprayed with herbicide solution, since in some cases overspraying with commercial formulations of herbicide might show differences in absorption compared to non-oversprayed plants (Kniss et al., 2011; Shaner, 2009). Therefore, a preliminary study was conducted to compare the absorption and translocation pattern of $^{14}$C-atrazine or $^{14}$C-mesotrione using the oversprayed vs. non-oversprayed methods. In the oversprayed method, a fully expanded fourth-youngest leaf was covered with plastic wrap (Saran Premium Wrap, Racine, WI) and plants were sprayed with commercial formulation of mesotrione at 105 g ha$^{-1}$ or atrazine at 2240 g ha$^{-1}$ alone or tank mixed with atrazine at 560 g ha$^{-1}$ or mesotrione at 26 g ha$^{-1}$, respectively, using a single-tip chamber sprayer. At 1 HAT, the plastic wrap was removed, and the covered leaf was marked and applied with 10 1-ML droplets of respective radiolabeled solutions using a Wiretrol. In the non-oversprayed method, leaves were not covered, and plants were not sprayed with herbicides in the spray chamber and only 10 µL solution was applied per plant with 10 1-µL droplets on the upper surface of the fully expanded fourth-youngest leaf using a Wiretrol. No significant difference was observed in absorption and translocation of $^{14}$C-atrazine or $^{14}$C-mesotrione between the oversprayed and non-oversprayed methods based on ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute, 2011) (data not shown). Therefore, all absorption and translocation experiments were conducted using the non-oversprayed method.

### $^{14}$C-Atrazine Metabolism Study

Atrazine- and HPPD inhibitor-resistant and susceptible Palmer amaranth biotypes were grown in the greenhouse and moved to the growth chamber as described above in the absorption and translocation study. Ten to 12 cm tall plants were treated with 20 ML (10 ML each on the fourth and fifth youngest leaves) of 6.7 kBq $^{14}$C-atrazine solution which is equivalent to 2240 g atrazine ha$^{-1}$ applied alone or tank mixed with commercial formulation of mesotrione at 26 g ha$^{-1}$ (Nakka et al., 2017b). Plants were then returned to the same growth chamber. At 4, 8, 24, and 48 HAT, TL were dissected and rinsed twice in a 20-mL scintillation vial containing 5 mL of wash solution for 1 min to remove unabsorbed radiolabeled herbicide from the surface of the TL. Rinsed treated leaves were grounded in liquid N with a pre-chilled mortar and pestle.

Parent $^{14}$C-atrazine and its metabolites were extracted by incubating in 15 mL of 90% acetone at 4°C for 16 h and then centrifuged at 6500 rpm for 10 min (Godar et al., 2015; Nakka et al., 2017b). The collected supernatant was concentrated by evaporating at 50°C for 2 to 3 h using a rotary evaporator (Centrivap, Labconco, Kansas City, MO) until a final volume of 100 to 600 ML was achieved. The concentrated supernatant was transferred to a 1.5 mL centrifuge tube and centrifuged at 13,000 rpm for 10 min at room temperature. The total extractable radioactivity in each sample was measured by LSS and normalized to 3000 dpm 50 μL$^{-1}$ using 50% acetonitrile (high-performance liquid chromatography [HPLC] grade, ThermoFisher Scientific) (Godar et al., 2015; Nakka et al., 2017b). A reverse-phase HPLC (System Gold, Beckman Coulter, Pasadena, CA) was used to resolve the total extractable radioactivity in 50 μL of the samples into parent $^{14}$C-atrazine and its conjugated metabolites. Reverse phase HPLC was performed with a Zorbax SB-C18 Column (4.6 by 250 mm, 5 mm particle size; Agilent Technologies, Santa Clara, CA) at a flow rate of 1 mL min$^{-1}$ with eluent A (water with 0.1% trifluoroacetic acid) and eluent B (acetonitrile with 0.1% trifluoroacetic acid) (Godar et al., 2015; Nakka et al., 2017b). Radiolabeled compounds were detected with a radioflow detector (EG&G Berthold, LB 509) and Ultima-Flo M cocktail (PerkinElmer). Parent $^{14}$C-atrazine remaining in each sample was determined as a percentage of total extractable radioactivity recorded by the peak areas. The experiment was repeated under the growing conditions mentioned above.

### Statistical Analysis

Palmer amaranth control estimates and aboveground biomass reduction from the greenhouse study, $^{14}$C-mesotrione or $^{14}$C-atrazine absorption or translocation, and data for parent $^{14}$C-atrazine or its metabolites were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute, 2011). Experimental runs were considered fixed effects, whereas treatment replications were considered a random effect in the model. Data were combined when there was no experimental run-by-herbicide treatment interaction. Before analysis, data were tested for normality and homogeneity of variance using Shapiro–Wilks goodness-of-fit and Levene's test in SAS. Log scale transformation was applied to the data when normality and homogeneity of variance assumptions were not met. The nontreated control was not included in the data analysis for control estimates and aboveground biomass reduction from the greenhouse study. Where the ANOVA indicated herbicide 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.

Regression analysis was performed for the $^{14}$C-mesotrione and $^{14}$C-atrazine absorption/translocation and parent $^{14}$C-atrazine and its metabolites present in TL over time using R software (R version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria). Nonlinear regression models used in previous absorption/metabolism studies such as a two-parameter rectangular hyperbolic function, a two parameter asymptotic regression function, or a three-parameter log-logistic function (Bukun et al., 2009; Frihauf et al., 2010; Thomas et al., 2007) were fitted to data using "drm" function in the "drc" package (drc 2.3, Ritz and Strebig, 2016; R 3.1.1) in R software, and linear regression model was fitted using "lm" function in STATS package in R software. The above-fitted models were compared using Akaike's information criterion (AIC) approach in R software, and the models with the lowest AIC values were selected. Using the AIC approach, a two-parameter rectangular hyperbolic function was fitted to $^{14}$C-mesotrione absorption and translocation, $^{14}$C-mesotrione present in ATL and TL, $^{14}$C-atrazine absorption, and $^{14}$C-atrazine present in TL:

$$\mathrm{RH} = \frac{\left(A_{\max} \times t\right)}{\left(10/\alpha\right) \times t_{\alpha} + t} \quad [8]$$

where RH describes the $^{14}$C-mesotrione absorption and translocation, $^{14}$C-mesotrione present in ATL or TL, and $^{14}$C-atrazine absorption, and $^{14}$C-atrazine present in TL; $A_{\max}$ is the maximum percentage of applied herbicide dose that was absorbed, translocated, or present in ATL or TL at time ($t$); is the arbitrary percentage (90% in this study) of $A_{\max}$; and $t_a$ represents the number of HAT required to reach 90% of the $A_{\max}$.

## RESULTS

### Response of Palmer Amaranth to Tank Mixtures of Atrazine and Mesotrione

Mesotrione applied POST alone at 210 g ha$^{-1}$ provided greater control of atrazine- and HPPD inhibitor-resistant Palmer amaranth compared with mesotrione applied at 26 to 105 g ha$^{-1}$ (Table 1). However, comparatively greater control of susceptible Palmer amaranth was achieved with mesotrione at 105 or 210 g ha$^{-1}$ (84–88%) compared to resistant Palmer amaranth (31–56%). Atrazine applied at labeled rate of 2240 g ha$^{-1}$ provided greater (96%) control of susceptible Palmer amaranth compared to only 15% control of resistant plants at 560 and 4480 g ha$^{-1}$ (Table 1). However, tank mixing mesotrione at 26 to 210 g ha$^{-1}$ with atrazine at 560 to 4480 g ha$^{-1}$ improved control of resistant plants (85–100%) along with complete control of susceptible plants at 21 DAT. Similar results were observed for Palmer amaranth biomass reduction with atrazine or mesotrione applied alone or tank mixed.

The expected control/biomass reduction of atrazine- and HPPD inhibitor-resistant Palmer amaranth calculated using Colby's equation (Eq. [2]) was significantly lower than the observed control/biomass reduction with tank mixtures, implying that synergistic interactions occurred by tank mixing atrazine with mesotrione (Table 1). However, additive interactions

Table 2. $^{14}$C-atrazine and $^{14}$C-mesotrione recovered from the harvested plant parts of atrazine- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-resistant and susceptible Palmer amaranth at different hours after $^{14}$C-herbicide application in the absorption and translocation studies conducted at Kansas State University, Manhattan, in 2016.†

| h after treatment | Herbicide recovered | |
|---|---|---|
| | $^{14}$C-atrazine‡ | $^{14}$C-mesotrione‡ |
| | % of applied | |
| 4 | 83a | 94a |
| 8 | 82a | 92ab |
| 24 | 81a | 89b |
| 48 | 81a | 89b |
| 72 | 71b | 88b |
| LSD, $P \leq 0.05$ | 4.5 | 1.5 |

† Experimental run × treatment interaction and main effects for $^{14}$C-atrazine and $^{14}$C-mesotrione recovered were not significant; therefore, data were combined over two experimental runs and herbicide treatments. LSD = Least square difference.

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

occurred with most of atrazine and mesotrione tank mixtures for control/biomass reduction of susceptible Palmer amaranth, partly because lower than 0.25 times the label rates of atrazine or mesotrione were not used in this study and even the lowest rates applied provided 56 to 77% susceptible Palmer amaranth control. Abendroth et al. (2006) reported synergistic interactions for biomass reduction of atrazine- and HPPD inhibitor-susceptible Palmer amaranth with atrazine at 280 g ha$^{-1}$ tank mixed with mesotrione at 17 to 35 g ha$^{-1}$ and additive interaction occurred when mesotrione was tank mixed at a greater rate of 52.5 g ha$^{-1}$. In our study, atrazine at the label rate of 2240 g ha$^{-1}$ tank mixed with mesotrione at 26 g ha$^{-1}$ and, similarly, mesotrione at the label rate 105 g ha$^{-1}$ tank mixed with atrazine at 560 g ha$^{-1}$ provided >90% control of resistant and susceptible Palmer amaranth, which was similar to other tank-mixed rate combinations. Therefore, these rates were used for the absorption and translocation study.

## $^{14}$C-Atrazine and $^{14}$C-Mesotrione Recovered

$^{14}$C-atrazine and $^{14}$C-mesotrione recovered at 4 to 72 HAT did not vary across experimental runs and herbicide treatments ($^{14}$C-mesotrione or $^{14}$C-atrazine applied alone or tank mixed with a commercial formulation of atrazine or mesotrione, respectively) based on ANOVA. Therefore, $^{14}$C- herbicide percent recovered data at 4 to 72 HAT were combined over experimental runs and each herbicide treatment (Table 2). At 4 and 8 HAT, 92 to 94% of the applied $^{14}$C-mesotrione was recovered compared to 88 to 89% at 24, 48, and 72 HAT in the $^{14}$C-mesotrione absorption and translocation study (Table 2). In the $^{14}$C-atrazine absorption and translocation study, 81 to 83% of the applied $^{14}$C-atrazine was recovered between 4 and 48 HAT compared to 71% at 72 HAT. An herbicide recovery of <80% in the absorption and translocation studies might not yield accurate results due to potential issues with the experimental techniques (Kniss et al., 2011). In this study, on average 90% of applied $^{14}$C-mesotrione and 81% of applied $^{14}$C-atrazine were recovered (Table 2), indicating the reliability of the experimental techniques employed.

## $^{14}$C-Mesotrione Absorption and Translocation

The ANOVA suggested no effect of experimental run or its interaction with herbicide treatments or biotype for $^{14}$C-mesotrione absorption and translocation or $^{14}$C-mesotrione recovered in ATL and TL samples. Therefore, data were combined over two experimental runs (data not shown). No significant Palmer amaranth biotype × HAT interaction occurred; however, significant interaction of herbicide treatment and HAT was observed for $^{14}$C-mesotrione absorption and translocation, and $^{14}$C-mesotrione recovered in ATL and TL samples. Therefore, data were combined over two biotypes and regression analysis was performed. The $^{14}$C-mesotrione amount recovered from BTL and roots (<2% of applied) was not affected by herbicide treatments, biotypes, HAT, or their interactions; therefore, regression analysis was not performed. The two-parameter rectangular hyperbolic model suggested that maximum absorption of $^{14}$C-mesotrione was greater (51% of applied) when tank mixed with the commercial formulation of atrazine compared to $^{14}$C-mesotrione applied alone (39% of applied) (Table 3, Fig. 1A). The time required for 90% of the maximum $^{14}$C-mesotrione absorption to occur was similar (27–40 HAT) when $^{14}$C-mesotrione was tank mixed with atrazine compared with applied alone

Table 3. Parameter estimates and test of lack-of-fit at 95% level for the two-parameter rectangular hyperbolic function† fitted to $^{14}$C-mesotrione absorption and translocation, and $^{14}$C-mesotrione compounds present in above-treated leaf (ATL) and treated leaf (TL) of atrazine- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-resistant and susceptible Palmer amaranth in a laboratory study conducted at Kansas State University, Manhattan, in 2016.

| Herbicide treatment | $^{14}$C-mesotrione absorption‡ | | | $^{14}$C-mesotrione translocation‡ | | | $^{14}$C-mesotrione present in ATL‡ | | | $^{14}$C-mesotrione present in TL‡ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $A_{max}$§ | $t_{90}$§ | Lack-of-fit¶ | $A_{max}$§ | $t_{90}$§ | Lack-of-fit¶ | $A_{max}$§ | $t_{90}$§ | Lack-of-fit¶ | $A_{max}$§ | $t_{90}$§ | Lack-of-fit¶ |
| | % of applied | | | | | | | | | | | |
| $^{14}$C-mesotrione | 39 ± 1.8a | 27 ± 6.8a | 0.6 | 4.8 ± 0.6a | 88 ± 6a | 0.4 | 3.6 ± 1.9a | 104 ± 16a | 1.0 | 24 ± 1.2b | 21 ± 6.6a | 0.6 |
| $^{14}$C-mesotrione + atrazine | 51 ± 1.9b | 40 ± 7.1a | 0.4 | 5.7 ± 0.9a | 82 ± 9a | 0.9 | 3.4 ± 1.1a | 87 ± 14a | 0.4 | 33 ± 1.3a | 34 ± 7.2a | 0.1 |

† RH = $(A_{max} \times t)/[10/a) \times t_a + t]$, where RH describes $^{14}$C-mesotrione absorption or translocation, $^{14}$C-mesotrione present in above-treated, below-treated, or treated leaf, $A_{max}$ is the maximum percentage of applied herbicide dose that will be absorbed or translocated at time ($t$), is the arbitrary percentage (90%) of $A_{max}$, and $t_a$ represents the number of hours after treatment required to reach 90% of the $A_{max}$. $^{14}$C-mesotrione translocation represents the total amount of $^{14}$C-herbicide recovered from ATL, BTL, and roots as percent of applied $^{14}$C-herbicide.

‡ $A_{max}$ and $t_{90}$ values are mean ± standard error of the mean (SEM). See Eq. [3], [4], [5], and [7] for % $^{14}$C-mesotrione absorption, % $^{14}$C-mesotrione present in above the treated leaf (ATL), % $^{14}$C-mesotrione present in treated leaf (TL), and % $^{14}$C-mesotrione translocation, respectively.

§ The predicted parameters were compared among herbicide treatments using the $t$ test and means within columns with no common letter(s) are significantly different at $P \leq 0.05$.

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

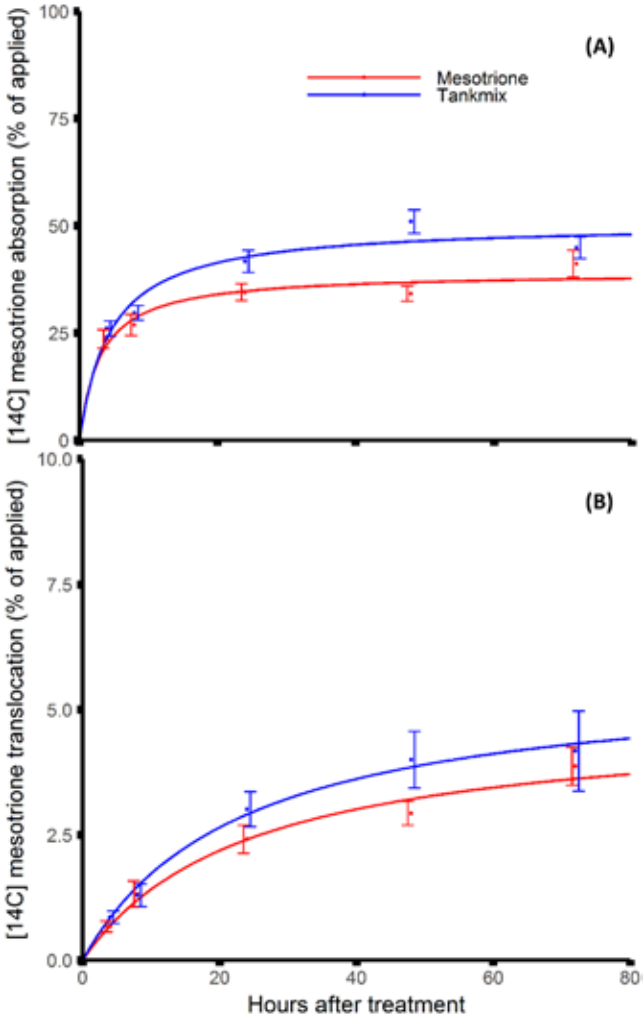


Fig. 1. Response of atrazine- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-resistant and susceptible Palmer amaranth to $^{14}$C-mesotrione applied alone or tank mixed with commercial formulation of atrazine in terms of (A) $^{14}$C-mesotrione absorption (% of applied), and (B) $^{14}$C-mesotrione translocation (% of applied) at different hours after treatment in a laboratory study conducted at Kansas State University, Manhattan, in 2016. The bars represent standard error.

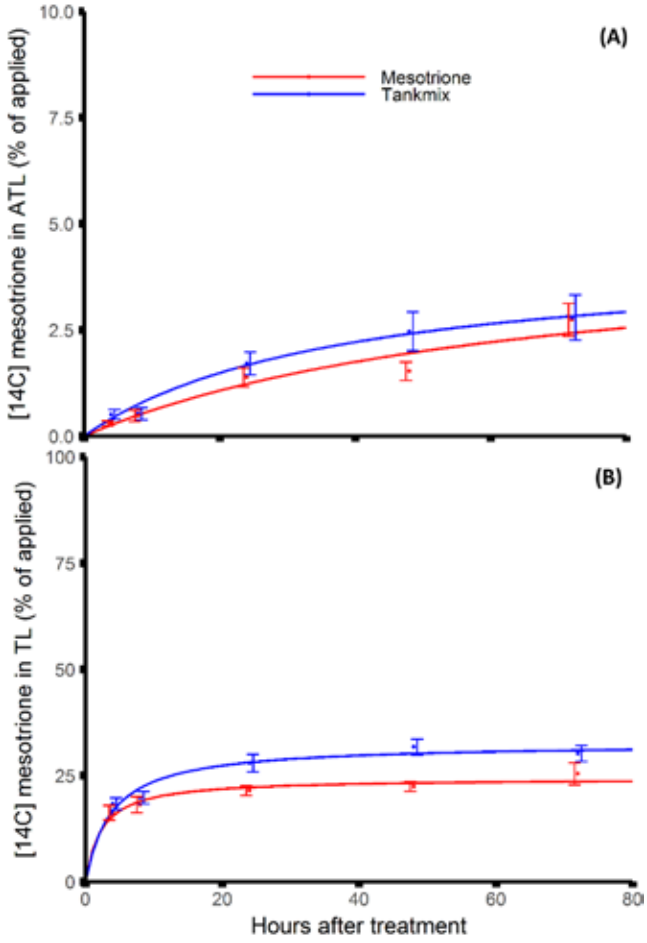


Fig. 2. Response of atrazine- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-resistant and susceptible Palmer amaranth to $^{14}$C-mesotrione applied alone or tank mixed with commercial formulation of atrazine in terms of (A) $^{14}$C-mesotrione present in above-treated leaf (ATL) (% of applied) and (B) $^{14}$C-mesotrione present in treated leaf (TL) (% of applied) at different hours after treatment in a laboratory study conducted at Kansas State University, Manhattan, in 2016. The bars represent standard error.

(Table 3). Maximum $^{14}$C-mesotrione recovered in ATL was similar (3.4–3.6% of applied) when applied alone or tank mixed with atrazine (Table 3, Fig. 2A). Similarly, the time taken for 90% of the maximum $^{14}$C-mesotrione to recover in ATL was similar (87–104 HAT) when $^{14}$C-mesotrione was applied alone or tank mixed with atrazine. Similar to the absorption, $^{14}$C-mesotrione recovered in TL was greater (33% of applied) when tank mixed with commercial formulation of atrazine compared to when applied alone (24% of applied) (Table 3, Fig. 2B). The time required between 90% of the maximum $^{14}$C-mesotrione to recover in the TL was similar (21–34 HAT) between herbicide treatments (Table 3). The model suggested that the maximum $^{14}$C-mesotrione translocation was similar (4.8–5.7% of applied) between $^{14}$C-mesotrione applied alone or tank mixed with commercial formulation of atrazine (Fig. 1B).

## $^{14}$C-Atrazine Absorption and Translocation

Experimental run × $^{14}$C-atrazine treatment interaction for $^{14}$C-atrazine absorption and translocation, and $^{14}$C-atrazine recovered in ATL, TL, and BTL was not significant; therefore, data were combined over two experimental runs. The maximum $^{14}$C-atrazine absorption was similar (84–85% of applied) when the commercial formulation of mesotrione was tank mixed compared with $^{14}$C-atrazine alone (Fig. 3A). Similarly, $t_{90}$ was similar (12–13 HAT) among tank mixture and $^{14}$C-atrazine alone. $^{14}$C-atrazine present in TL did not vary among $^{14}$C-atrazine tank mixed with mesotrione and $^{14}$C-atrazine alone (Fig. 3B). Overall, absorption and translocation of $^{14}$C-atrazine were not affected when tank mixed with commercial formulation of mesotrione. Therefore, increased $^{14}$C-atrazine absorption or translocation was ruled out as the basis for atrazine and mesotrione synergism to control resistant Palmer amaranth.

## $^{14}$C-Atrazine Metabolism

The ANOVA indicated no significant experimental run × herbicide treatment interaction for parent $^{14}$C-atrazine or its polar metabolite recovered in TL and thus data were combined over two experimental runs. Additionally, no significant effect of $^{14}$C-atrazine treatment and $^{14}$C-atrazine treatment × HAT

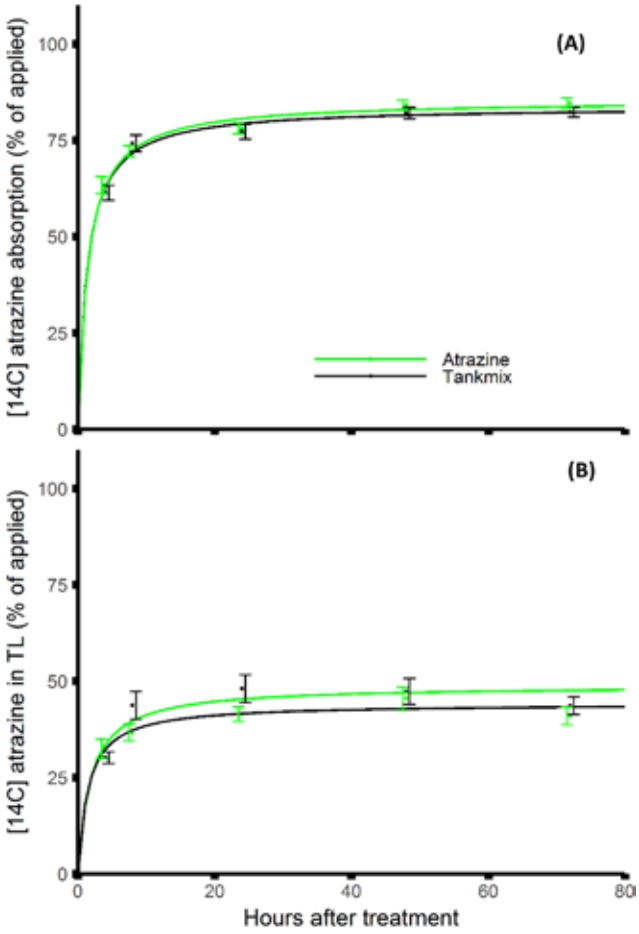


Fig. 3. Response of atrazine- and 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor-resistant and susceptible Palmer amaranth to $^{14}$C-mesotrione applied alone or tank-mixed with commercial formulation of atrazine in terms of (A) $^{14}$C-atrazine absorption (% of applied), and (B) $^{14}$C-atrazine present in treated leaf (TL) (% of applied) at different hours after treatment in a laboratory study conducted at Kansas State University, Manhattan, in 2016. The bars represent standard error.

interaction was observed for parent $^{14}$C-atrazine or its metabolite recovery in TL (data not shown). A significant biotype × HAT interaction was observed; however, these results are not discussed since this paper is focused on finding the basis of atrazine and mesotrione synergism for control of atrazine- and HPPD inhibitor-resistant Palmer amaranth. The results from $^{14}$C-atrazine metabolism study have shown that $^{14}$C-atrazine metabolism in resistant as well as susceptible Palmer amaranth was not affected when formulated mesotrione at 26 g ha$^{-1}$ was tank mixed compared to $^{14}$C-atrazine alone.

## DISCUSSION

In the greenhouse study, mesotrione at 26 to 105 g ha$^{-1}$ or atrazine at 560 to 4480 g ha$^{-1}$ provided greater control of susceptible compared with resistant Palmer amaranth. However, tank mixing mesotrione at the labeled rate of 105 g ha$^{-1}$ with even 0.25 times (560 g ha$^{-1}$) the label rate of atrazine controlled resistant Palmer amaranth >90% despite the plant's resistance to atrazine and mesotrione. Increased control of atrazine-resistant weeds such as redroot pigweed and velvetleaf (*Abutilon theophrasti* Medik.) has also been reported by tank-mixing atrazine and HPPD inhibitors (Hugie et al., 2008; Woodyard et al., 2009). The PS II inhibitor herbicides such as atrazine 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, causing damage to the cell membranes and D1 protein (Hess, 2000). The HPPD inhibitor herbicides such as mesotrione, tembotrione, or topramezone inhibit HPPD enzyme synthesis, leading to depletion of α-tocopherols and plastoquinone and reducing the competition between atrazine and plastoquinone for binding to the D1 protein (Hess, 2000; Pallett et al., 1998). In addition, plastoquinone acts as a cofactor to produce carotenoids and the inhibition of plastoquinone by HPPD inhibitors limits carotenoid synthesis (Norris et al., 1998). Carotenoids and tocopherols are responsible for protecting the chlorophyll from photooxidation by quenching the reactive oxygen species and free radicals in plants (Siefermann, 1987). Therefore, overlapping of biochemical effects of atrazine and mesotrione results in synergistic interaction when applied in tank mixture.

In this study, atrazine applied in tank mixture increased mesotrione absorption in resistant Palmer amaranth compared with mesotrione applied alone; however, atrazine absorption and translocation was not affected when mesotrione was tank mixed. A previous study reported that rapid metabolism of mesotrione confers mesotrione resistance in atrazine- and HPPD inhibitor-resistant Palmer amaranth from Nebraska (Nakka et al., 2017a), and rapid metabolism of atrazine confers atrazine resistance in this biotype (Chahal et al., 2019). A study conducted by Salzman et al. (1992) reported that clomazone {2-[(2-chlorophenyl) methyl]-4, 4-dimethyl-3-isoxazolidinone}, belonging to same mode-of-action as mesotrione, reduced metabolism of metribuzin [4-amino-6-(1,1-dimethyethyl)-3-(methylthio)-1,2,4-triazin-5 (4H)-one] or linuron [3-(3,4-dichorophenyl)-1-methoxy-1-methylurea] (PS II inhibitor) in common cocklebur (*Xanthium strumarium* L.) when tank mixed, thus providing greater control compared to metribuzin or linuron applied alone. Atrazine metabolism was not affected when mesotrione was included in tank mixture compared with atrazine applied alone; however, information on mesotrione metabolism when tank mixed with atrazine is not available. This is a preliminary study evaluating the basis of atrazine and mesotrione synergism. Further studies focusing on metabolism of mesotrione when tank mixed with atrazine need to be conducted to fully understand the mechanism of atrazine and mesotrione synergism in atrazine- and HPPD inhibitor-resistant Palmer amaranth from Nebraska.

Even though Palmer amaranth from Nebraska is resistant to atrazine and HPPD-inhibiting herbicides applied alone, these herbicides can be tank mixed with different site of action herbicides for effective Palmer amaranth control (Chahal et al., 2018). However, it is important to adopt an integrated Palmer amaranth management approach that includes the use of a PRE herbicides followed by a POST herbicide program, rotation of herbicides with distinct sites of action, crop rotation, rotation of herbicide-resistant cultivars, tillage, and harvest weed seed control methods to mitigate the evolution and spread of multiple herbicide-resistant weeds including Palmer amaranth (Chahal et al., 2018; Ganie et al., 2017a).

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