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Characterization of MCPA resistance in Palmer amaranth (*Amaranthus palmeri*)

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Research Article

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Abstract

Phenoxy herbicides (2,4-D and MCPA) are widely used to manage broadleaf weeds including Palmer amaranth (*Amaranthus palmeri* S. Watson), one of the most troublesome weeds in U.S. cropping systems. Previously, we documented resistance to 2,4-D and MCPA in an *A. palmeri* population (KCTR) from Kansas. Our recent research suggested rapid metabolism of 2,4-D bestows resistance in KCTR *A. palmeri*; nonetheless, the mechanism of MCPA resistance in this population is still unknown. The objectives of this research were to (1) evaluate the level of resistance to MCPA in KCTR compared with two known susceptible populations of *A. palmeri*, MSS and KSS; (2) study the absorption and translocation of [^{14}C]MCPA in KCTR and MSS plants: (3) investigate the metabolic profile of [^{14}C]MCPA in KCTR and MSS and compare those with MCPA-tolerant wheat (*Triticum aestivum* L.) plants; and (4) assess the possible role of cytochrome P450 enzymes (P450s) in MCPA metabolism. Experiments were conducted to assess the level of resistance in KCTR. Using [^{14}C]MCPA, the absorption, translocation, and metabolic profiles were assessed in *A. palmeri*. Involvement of P450s was confirmed using malathion, a known P450 inhibitor. Regression analyses indicate that KCTR population exhibits an ∼3-fold resistance to MCPA. No difference in absorption of [^{14}C]MCPA was found between MSS and KCTR. However, the KCTR plants translocated less [^{14}C]MCPA at 48 h after treatment (HAT) and metabolized MCPA more rapidly than MSS plants at 12 and 24 HAT. MCPA resistance in KCTR was reversed upon treatment with malathion, indicating the involvement of P450s in metabolism of this herbicide. This is the first report of characterization of MCPA resistance in *A. palmeri*.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Watson, family Amaranthaceae) is a summer annual dicot weed, native to the deserts of the southwestern United States and northwestern Mexico (Sauer 1957). *Amaranthus palmeri* is one of the most economically damaging weeds in U.S. cropping systems (Van Wychen 2020, 2022). If uncontrolled *A. palmeri* infestation can cause economic damage of up to 11% to 91% in several crops (Klingaman and Oliver 1994; MacRae et al. 2008; Massinga et al. 2001; Morgan et al. 2001; Rowland et al. 1999; Smith et al. 2000). Several biological characteristics of this species, such as its high rate of photosynthesis, prolific seed production, and dioecious nature, make it highly competitive, able to rapidly adapt to new ecological conditions, and prone to evolve resistance to herbicides (Briscoe Runquist et al. 2019; Keeley et al. 1987). *Amaranthus palmeri* has already evolved resistance to herbicide chemical classes over nine sites of action (Heap 2023), including synthetic auxin herbicides (SAH), and such populations pose a serious threat to its management.

Phenoxy herbicides, a subgroup of SAH have been in use for weed control for more than eight decades. Both 2,4-D and MCPA are widely used to selectively control dicot weeds in cereal crops such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and sorghum [*Sorghum bicolor* (L.) Moench] and in turf (Peterson et al. 2016). Selectivity is most often based on the ability of tolerant species to metabolically degrade these herbicides faster than susceptible species (Mithila et al. 2011). In dicots, treatment with SAH results in a series of rapid physiological and biochemical reactions leading to abnormal growth and, ultimately, production of abscisic acid and ethylene (Mithila et al. 2011), which further inhibits photosynthesis and produces H_2O_2 and reactive oxygen species (Grossmann 2010), leading to lipid peroxidation and plant death. Extensive use of SAH resulted in selection pressure, and as a result, 42 weed species have been reported to have evolved resistance to SAH, and 30 of those species were found to be resistant to phenoxy herbicides (Heap 2023). Examples include wild radish (*Raphanus raphanistrum* L.) (Jugulam et al. 2013), corn poppy (*Papaver rhoeas* L.) (Taberner et al. 1992), prickly lettuce (*Lactuca serriola* L.) (Riar et al. 2011), Powell's amaranth (*Amaranthus powellii* S. Watson) (Aicklen et al. 2022), and *A. palmeri* (Shyam et al. 2021). In 1979, the first case of MCPA resistance was reported in Canada thistle [*Cirsium arvense* (L.) Scop.] from Sweden (Fogelfors

1979), followed by 16 other weed species (Heap 2023). Reduced translocation and enhanced metabolism have been reported as accounting for MCPA resistance in common hemp-nettle (*Galeopsis tetrahit* L.) (Weinberg et al. 2006), while increased translocation to belowground parts was reported in an MCPA-resistant *R. raphanistrum* (Jugulam et al. 2013).

An *A. palmeri* population (KCTR) from a long-term conservation-tillage experimental field (Department of Agronomy, Kansas State University) grown with continuous sorghum for 45 yr was found resistant to six herbicide mode of action groups, including the herbicides 2,4-D and MCPA (Shyam et al. 2021). This *A. palmeri* exhibits 11-fold resistance to 2,4-D via enhanced metabolism (Shyam et al. 2022). However, the mechanism of resistance to MCPA in this *A. palmeri* population is still unknown. We hypothesize that KCTR plants may exhibit metabolic resistance to MCPA, similar to 2,4-D resistance. The objectives of this research were to (1) evaluate the level of resistance to MCPA in KCTR *A. palmeri* compared with two susceptible populations, MSS and KSS; 2) study the absorption and translocation of [^{14}C]MCPA in KCTR and MSS plants; (3) investigate the metabolic profile of [^{14}C]MCPA in KCTR and MSS and compare those with MCPA-tolerant wheat plants; and (4) assess the possible role of cytochrome P450 enzymes (P450s) in MCPA metabolism.

Materials and Methods

Plant Materials and Growing Conditions

The KCTR *A. palmeri* population reported to have evolved resistance to 2,4-D and MCPA (Shyam et al. 2021) was used in this study. Two known MCPA-susceptible populations MSS (Mississippi) and KSS (Kansas) were also used for comparison. All experiments were conducted in the greenhouse and/or controlled-environment growth-chamber facilities. The greenhouse was maintained at 32/23 ± 2 C day/night temperatures, 60 ± 10% relative humidity, and 15/9-h day/night photoperiod provided with sodium vapor lamps delivering 500 µmol m^{-2} s^{-1} illumination at canopy level, while the growth chambers were maintained at 32/22 C day/night temperatures and 60 ± 10% relative humidity, with a 16/8 day/night photoperiod, provided by incandescent and fluorescent bulbs delivering 600 µmol m^{-2} s^{-1} illumination at plant canopy level. All three populations were raised in the greenhouse from seeds in trays (21 by 6 by 4 cm). The MSS, KSS, and KCTR *A. palmeri* seeds were planted, and upon germination, seedlings at the cotyledon stage were transplanted to individual pots (6 by 6 by 6.5 cm) containing commercial pre-mix (Pro-Mix® premium potting mix, Premier Tech Home and Garden, Mississauga, ON, Canada). The seedlings (6- to 8-cm height) were transferred to the growth chambers to acclimatize.

MCPA Dose–Response Experiments

Amaranthus palmeri plants (MSS, KSS, and KCTR) at 10- to 12-cm height (2- to 3-wk old), were treated with MCPA (MCPA-ester 4®, Albaugh, Iowa, USA) at 0 (nontreated [NT]), 70, 140, 280, 560 (label recommended, i.e., 1× dose), 1,120, and 2,240 g ae ha^{-1}, using a bench-type sprayer (Research Track Sprayer, De Vries Manufacturing, Hollandale, MN, USA) fitted with a single flat-fan nozzle (8002 TeeJet® tip, Spraying Systems, Wheaton, IL, USA) that delivers 187 L ha^{-1} in a single pass of 4.85 km h^{-1} at 207 kPa pressure. Treated seedlings were transferred back to the growth chamber after 45 min of MCPA application. *Amaranthus palmeri* plants were watered daily as required. Visual injury ratings (VIR; 0 to 100) were recorded every week, up to 3 wk after treatment (WAT), with 0 (no herbicide injury) and 100 (dead plant) compared with NT plants. At 3 WAT, aboveground plant parts were harvested separately, placed in brown paper bags, and dried in an oven at 65 C for 72 h. Individual plant dry biomass was weighed. The relative dry biomass was determined as a percent of NT using Equation 1:

$$\text{RDW} = \left(\frac{\text{DW}}{\text{ADW}}\right) \times 100 \quad [1]$$

In Equation 1, RDW is the relative dry weight as percentage of NT, DW is the dry weight of the individual plant (in grams), and ADW is the average dry weight of NT (in grams).

Absorption and Translocation of [^{14}C]MCPA in KCTR and MSS *Amaranthus palmeri*

There was no statistical difference between MSS and KSS Palmer amaranth regarding their response to the 1× dose of MCPA. However, MSS plants exhibited more injury than KSS plants at the 1× dose of MCPA (Figure 1); MSS plants were therefore used for subsequent experiments for comparison with KCTR plants.

MSS and KCTR *A. palmeri* seedlings were grown as described earlier, and 10- to 12-cm- height plants were used. A stock solution containing ^{14}C-radiolabeled MCPA (Moravek, Brea, CA, USA) mixed with commercial MCPA was prepared to obtain the equivalent to the field recommended dose (560 g ha^{-1}) in a carrier volume of 187 L ha^{-1}. Each plant was treated with 10 µl of stock solution of [^{14}C]MCPA, with total radioactivity of 0.083 kBq µl^{-1} (5,000 dpm µl^{-1}), on the adaxial surface of the third or fourth fully opened leaf in the form of small 1-µl droplets with the help of a

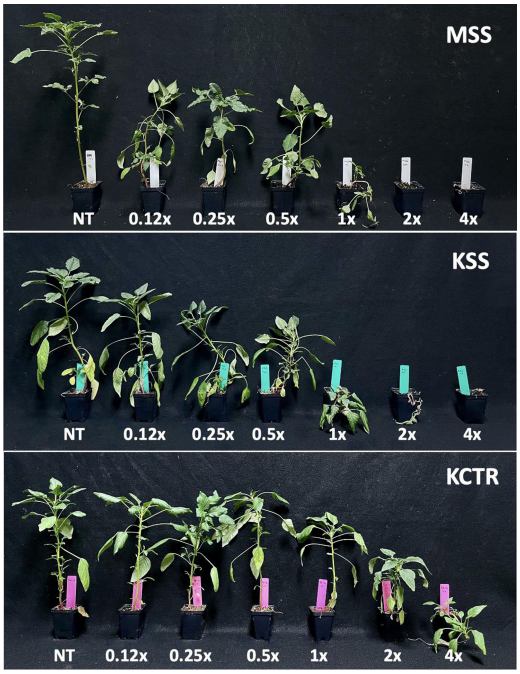

Figure 1. Response of MSS, KSS, and KCTR *Amaranthus palmeri* to seven doses of MCPA at 3 wk after treatment (WAT). 1× is the field recommended dose of MCPA (560 g ha^{-1}). MSS, MCPA-susceptible population from Mississippi; KSS, MCPA-susceptible population from Kansas; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

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pipette. The treated plants were transferred back to the growth chamber 20 min after the treatment. Plant tissue was harvested separately as treated leaf (TL), above treated leaf (ATL), and below treated leaf (BTL) at 24 and 48 h after treatment (HAT). The TL was washed twice with 5 ml of wash solution (10% v/v ethanol with 0.5% v/v Tween-20; BFC Chemicals, Wilmington, DE, USA) for 1 min in 20-ml scintillation vials. The rinsate was mixed with 10 ml of scintillation cocktail (EcoLite(+)™, MP Biomedicals, Solon, OH, USA) and the total radioactivity was recorded using a liquid scintillation counter (LSC; LS6500 Liquid Scintillation Counter, Beckman Coulter, Brea, CA, USA). ATL, TL, and BTL plant tissues were wrapped separately in wipes (Kimwipes®, Kimberley-Clark, Roswell, GA, USA), oven-dried at 60 C for 72 h, and then combusted using a biological oxidizer (OX-501, RJ Harvey Instruments, Tappan, NY, USA). Evolved CO_2 was trapped in a ^{14}C trapping cocktail (Carbon14 (C-14) cocktail, Z Scientific, New City, NY, USA). The radioactivity was recorded with the help of LSC. LSC data were converted to percent herbicide absorbed and translocated using following equations (Equations 2 to 6):

$$\text{Abs.}(\%) = \left[\left(R_{\text{Applied}} - R_{\text{Rinsate}}\right)/R_{\text{Applied}}\right] \times 100 \quad [2]$$

$$\text{Trans.}(\%) = 100 - \left[R_{\text{TL}}/\left(R_{\text{Applied}} - R_{\text{Rinsate}}\right) \times 100\right] \quad [3]$$

$$\text{TL}(\%) = R_{\text{TL}}/\left(R_{\text{Applied}} - R_{\text{Rinsate}}\right) \times 100 \quad [4]$$

$$\text{ATL}(\%) = R_{\text{ATL}}/\left(R_{\text{Applied}} - R_{\text{Rinsate}}\right) \times 100 \quad [5]$$

$$\text{BTL}(\%) = R_{\text{BTL}}/\left(R_{\text{Applied}} - R_{\text{Rinsate}}\right) \times 100 \quad [6]$$

In these equations, R_{Applied} is the amount of radioactivity applied (in disintegrations per minute [dpm]), R_{Rinsate} is the radioactivity of wash solution (in dpm), Abs. (%) is the percentage absorbed, Trans. (%) is the percentage translocated, TL (%) is the percentage of radioactivity recovered from treated leaf, ATL (%) is the percentage of radioactivity recovered from plant parts above the treated leaf, BTL (%) is the percentage of radioactivity recovered from plant parts below the treated leaf.

Metabolism of [^{14}C]MCPA in KCTR and MSS Amaranthus palmeri

Along with KCTR and MSS *A. palmeri*, winter wheat 'KS Western Star' (WS) plants were also used as a positive control, because of wheat's ability to metabolize MCPA naturally (Cole and Loughman 1983). KCTR and MSS seedlings were grown as described earlier. WS seeds of wheat were germinated on filter paper and later transplanted to individual pots filled with pre-mix. Each plant was treated with 10 µl of stock solution of [^{14}C]MCPA, with total radioactivity of 0.13 kBq µl^{-1} (8,000 dpm µl^{-1}), on the adaxial surface of the third or fourth fully opened leaf for *A. palmeri* and the second fully opened leaf for wheat plants in the form of small droplets (1 µl) with the help of a pipette. The treated plants were transferred back to the growth chamber 20 min after the treatment. Aboveground plant tissue of KCTR, MSS *A. palmeri*, and wheat plants was harvested at 12 HAT, and additionally for KCTR and MSS plants at 24 HAT. The TL was harvested separately and washed as described earlier. The whole plant tissue (aboveground plant parts plus the TL) was wrapped in aluminum foil and immediately frozen in liquid nitrogen. Each sample was then homogenized with a pestle in a mortar and transferred to 15 ml of 90% (v/v) acetone in 50-ml centrifuge tubes to extract the parent [^{14}C]MCPA and its metabolites. This solution was kept at 4 C for at least 16 h and then centrifuged at 5,000 × *g* for 10 min. The supernatant was transferred to a new centrifuge tube and concentrated to a volume of 500 to 1,000 µl with the help of a rotary evaporator (Centrivap, Labconco, Kansas City, MO, USA) at 45 C for 90 min. The supernatant was transferred to 2-ml microcentrifuge tubes and centrifuged at 10,000 × *g* for 10 min. A 90-µl injection of the final supernatant was run through a reverse-phase high-performance liquid chromatograph (1260 Infinity II LC System, Agilent, Santa Clara, CA, USA) to analyze the parent MCPA and the associated metabolites.

Assessment of the Possible Role of P450 in Metabolism of MCPA in KCTR Amaranthus palmeri

Malathion (Spectracide® Malathion Insect Spray Concentrate, United Industries, St Louis, MO, USA), a known P450 inhibitor was used to assess the ability of P450 enzymes to metabolize MCPA in *A. palmeri*. Malathion can help minimize herbicide metabolism mediated by P450 activity (Siminszky et al. 2006). Malathion was sprayed at 1,500 g ai ha^{-1}, 30 min before MCPA application, followed by soil drenching with 50 ml of a 5 mM solution of malathion at 24 h following MCPA application. All spray applications were done as described earlier for the dose–response experiments. The VIR and RDW were recorded at 3 WAT as described earlier for the dose–response experiments.

Experimental Designs and Data Analysis

The MCPA dose–response and uptake, translocation, and metabolism experiments were conducted in a randomized complete block design with four replications of each treatment. The P450-inhibitor experiment was conducted according to a factorial design with two *A. palmeri* populations (KCTR and MSS), three doses of MCPA (0, 560, and 1,120 g ae ha^{-1}), two doses of malathion (0 and 1,500 g ai ha^{-1}), and five replications of each treatment. All experiments were repeated once. Data were analyzed in RStudio using Levene's test for homogeneity of two runs, and data that were not significantly different were pooled from the two runs of experiments. Pooled data for relative dry weight (as % NT) were analyzed using a three-parameter log-logistic regression mode with the DRC package in RStudio (Knezevic et al. 2007; Ritz et al. 2015). The following three-parameter regression model was fit (Equation 7):

$$Y = d + \exp[b(\log x - e)] \quad [7]$$

In the equation, Y is the response variable (i.e., relative dry weight); x is the applied MCPA dose; d is the upper limit; b is the relative slope around e; and e is GR_{50}, which is the dose of herbicide required for 50% biomass reduction.

For the uptake and translocation experiments, the total percentage of absorbed MCPA was determined as percentage applied, whereas the percentage of MCPA translocation was presented as percentage of parent herbicide absorbed (Equations 2 to 6). High-performance liquid chromatography (HPLC) chromatograms represent the concentration of parent MCPA and its metabolites within the plant system. Means were separated using Tukey's honest significant difference (HSD) test at a significance level of $\propto = 0.05$.

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Results and Discussion

MCPA Dose–Response Assay

Dose–response results confirmed that KCTR is resistant to MCPA (Table 1). Data analyses estimated GR_{50} values of 733.3 g ae ha^{-1} MCPA for KCTR, 224.4 g ae ha^{-1} for KSS, and 261.8 g ae ha^{-1} for MSS (Table 1). This implies that KCTR has 2.8- fold resistance over KSS and 3.3-fold resistance over MSS. (Figure 2).The level of visible injury of MCPA on MSS, KSS, and KCTR plants is distinctive (Figure 1). The Tukey's HSD test found no significant difference in the estimated GR_{50} values for KSS and MSS (Table 1).

While resistance to MCPA is modest (2.8- to 3.3-fold) in KCTR *A. palmeri* when compared with other weed species, such as *R. raphanistrum* (10-fold) (Jugulam et al. 2013) and oriental mustard (*Sisymbrium orientale* L.) (20-fold) (Preston et al. 2013), it is similar to that of *G. tetrahit* (3-fold) (Weinberg et al. 2006). In addition, the *A. palmeri* KCTR population was found to exhibit a variable level of cross-resistance to other SAH with an 11-fold resistance to 2,4-D (Shyam et al. 2022) and a 14-fold resistance to dicamba (Foster and Steckel 2022).

Table 1. GR_{50} values estimated from regression model using Equation 2.

Amaranthus palmeri population[a]	GR_{50}[b]	SE	RI[c]
		g ae ha^{-1}	
KSS	224.4b	48	
MSS	261.8b	80	
KCTR	733.3a	298	3.3 (compared with KSS); 2.8 (compared with MSS)

[a]KSS, MCPA-susceptible population from Kansas; MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.
[b]GR_{50}, herbicide required for 50% dry weight reduction. Letters represent significant differences identified by separation of means using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$).
[c]RI (resistance index) is the ratio of GR_{50} values of resistant and susceptible populations.

Absorption and Translocation of [^{14}C]MCPA in KCTR and MSS *Amaranthus palmeri*

There was no significant difference in absorption of MCPA between the KCTR and MSS plants at 12, 24, and 48 HAT. More than 90% of the herbicide was absorbed by the plants within 12 HAT with no significant increase at later time points (Figure 3). At 24 HAT, KCTR and MSS plants did not show any difference in the amount of [^{14}C]MCPA translocated to either ATL or BTL. Moreover, ~85% of the MCPA absorbed remained in the TL in both resistant and susceptible populations. However, at 48 HAT, KCTR plants showed significantly reduced translocation of herbicide to BTL compared with MSS (Figure 4). Importantly, the MSS plants translocated MCPA three times faster than KCTR at 48 HAT (Figure 5).

The SAH are systemic in nature and thus translocate via both xylem and phloem in plants (Mithila et al. 2011). Reduced translocation has been reported to account for MCPA resistance in *G. tetrahit* (Weinberg et al. 2006) and 2,4-D resistance in *R. raphanistrum* (Goggin et al. 2016) and *P. rhoeas* (Rey-Caballero et al. 2016). However, more MCPA was found to translocate to belowground parts in an MCPA-resistant *R. raphanistrum* compared with its susceptible counterpart (Jugulam et al. 2013).

Metabolism of [^{14}C]MCPA in KCTR and MSS *Amaranthus palmeri*

Parent [^{14}C]MCPA and its metabolites were characterized using reverse-phase HPLC in MSS and KCTR *A. palmeri* along with wheat. The peak retention time of the parent compound of MCPA was found at 13.9 min, while the MCPA metabolites appeared before this retention time in both *A. palmeri* and wheat. Five major metabolites were identified in MSS and KCTR plants and four metabolites were found in wheat (Figure 6). At 12 HAT, MSS plants retained ~45% of parent [^{14}C]MCPA, while KCTR and wheat plants rapidly metabolized 91% and 87% of the parent [^{14}C]

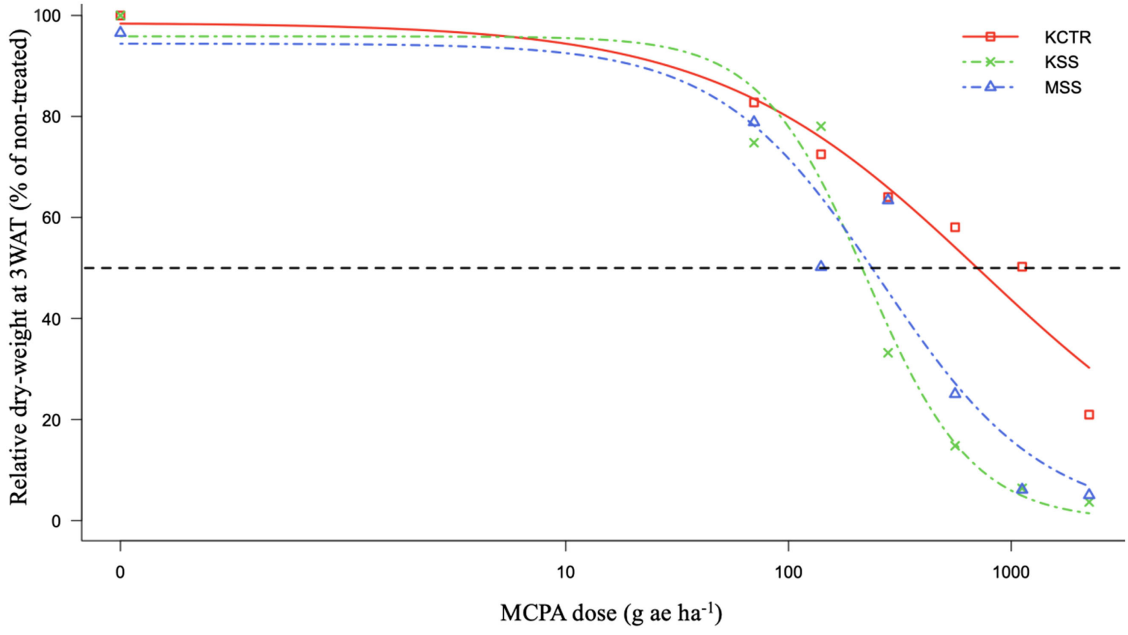

Figure 2. MCPA dose–response curves for MSS, KSS, and KCTR *Amaranthus palmeri* at 3 wk after treatment (WAT). The horizontal dashed line in the plot represents 50% of relative dry weight. MSS, MCPA-susceptible population from Mississippi; KSS, MCPA-susceptible population from Kansas; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

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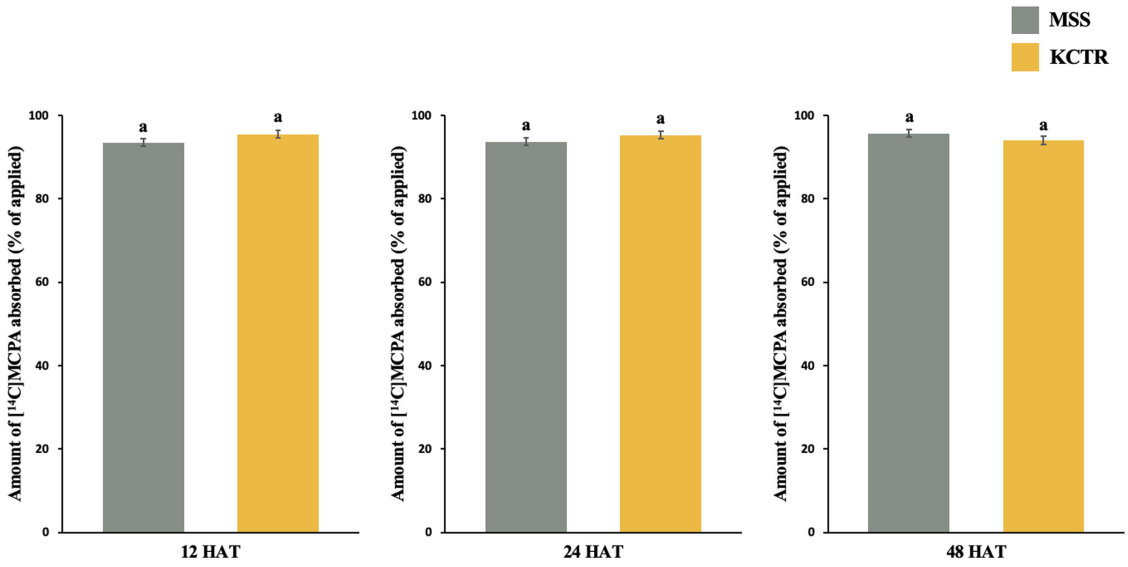

Figure 3. Bar graphs representing relative absorption of [^{14}C]MCPA in MSS and KCTR *Amaranthus palmeri* as percentage applied at 12, 24, and 48 h after treatment (HAT). Error bars represent standard error of the mean, and lowercase letters represent significant differences identified by separation of means using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

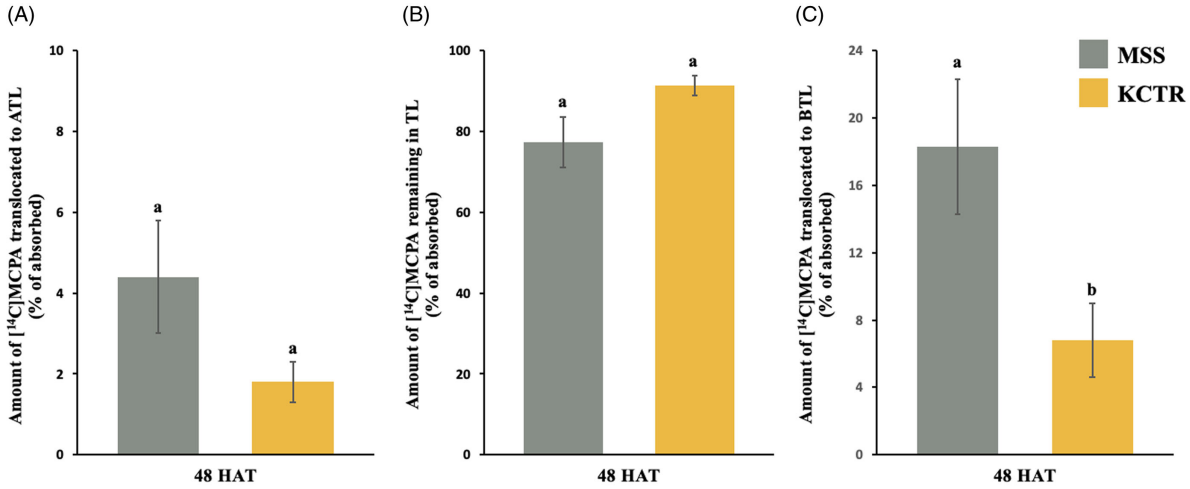

Figure 4. Bar graphs representing parent [^{14}C]MCPA translocated by MSS and KCTR *Amaranthus palmeri* to (A) above treated leaf (ATL), (B) on the treated leaf (TL), and (C) below the treated leaf (BTL) at 48 h after treatment (HAT). Error bars represent standard error of the mean, and lowercase letters represent significant differences identified by separation of means using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

MCPA, respectively (Figure 7). Importantly, at 24 HAT, KCTR plants metabolized ~ 95% to 98% of the MCPA, while MSS plants still had up to 30% to 35% of the parent molecule (Figure 7). While the rate of metabolism is similar in both wheat and KCTR, the pattern of metabolites appears different (Figure 6). Peak 1 in KCTR is quite important and likely a conjugate, owing to its high polarity. In addition, peak 4 appears quite prominent in wheat when compared with KCTR.

Rapid metabolism of 2,4-D was reported to account for resistance in KCTR *A. palmeri* (Shyam et al. 2022) and its related species, waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Figueiredo et al. 2018). Reduced translocation to the apical meristem and enhanced metabolism in the root zone has been reported to impart MCPA resistance in *G. tetrahit* (Weinberg et al. 2006), where MCPA is metabolized via methyl hydroxylation followed by glycosylation to yield the *O*-glycoside of MCPA. The resistant plants of KCTR metabolized MCPA at a rate similar to that of wheat and could detoxify ~95% to 100 % of parent MCPA within 24 HAT, possibly producing glycoside of phenoxy acetic acid (4-chloro-2-hydroxymethylphenoxyacetic acid) as major terminal residue (~49%) via methyl hydroxylation (Cole and Loughman 1983). Other metabolites may include sugar esters, aglycones, and ether-soluble conjugates in relatively low concentrations. Phase I hydroxylation of MCPA or 2,4-D followed by phase II conjugation is common in wheat (Bristol et al. 1977). The MCPA metabolites are more polar than parent MCPA and, if conjugated, are typically less phloem-mobile; therefore, the reduced translocation of MCPA found in KCTR at 48 HAT is possibly because of the formation of the metabolites of MCPA (Bristol et al. 1977).

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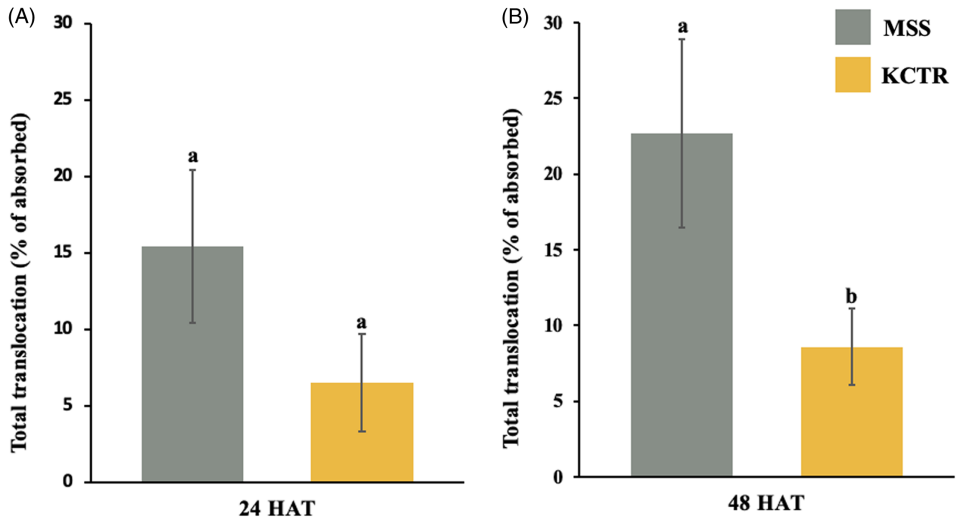

Figure 5. Bar graphs representing the total translocation of [^{14}C]MCPA in MSS and KCTR *Amaranthus palmeri* at (A) 24 and (B) 48 h after treatment (HAT). Error bars represent standard error of the mean, and lowercase letters represent significant differences identified by separation of means using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

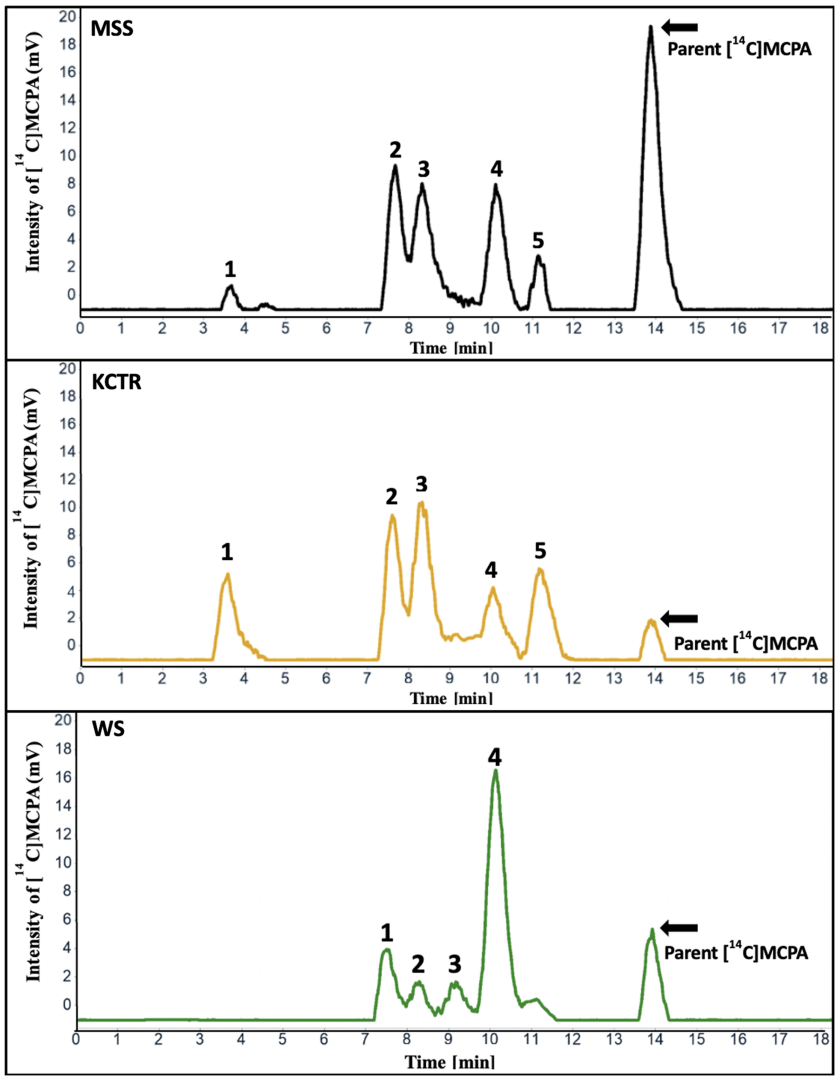

Figure 6. High-performance liquid chromatography chromatograms representing parent [^{14}C]MCPA and its metabolites in MSS and KCTR *Amaranthus palmeri* and wheat (WS) seedlings at 12 h after treatment (HAT). The peak of the parent compound was retained at 13.9 min. Numbers on the chromatograms represent metabolites of MCPA, whereas the area under the peak accounts for the relative concentration of compounds. MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

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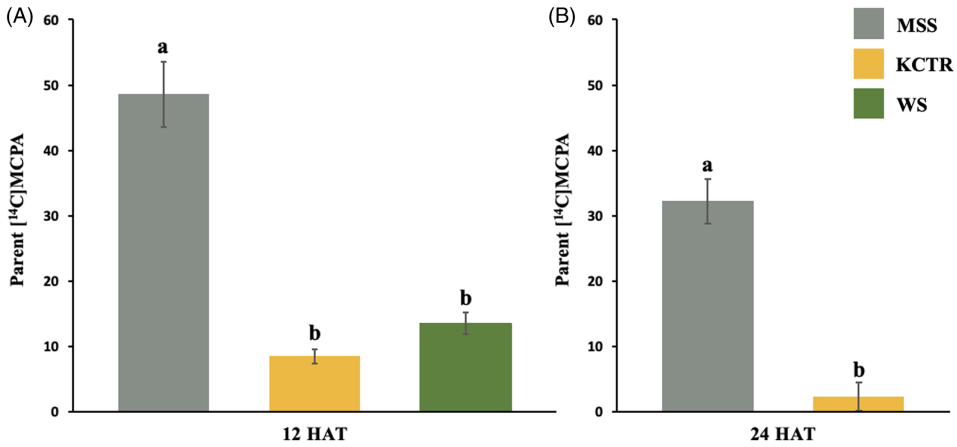

Figure 7. Parent [^{14}C]MCPA retained in MSS and KCTR *Amaranthus palmeri* and wheat (WS) seedlings at (A) 12 and (B) 24 h after treatment (HAT). Error bars represent the standard error of mean, and lowercase letters represent significant differences identified by separation of means using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

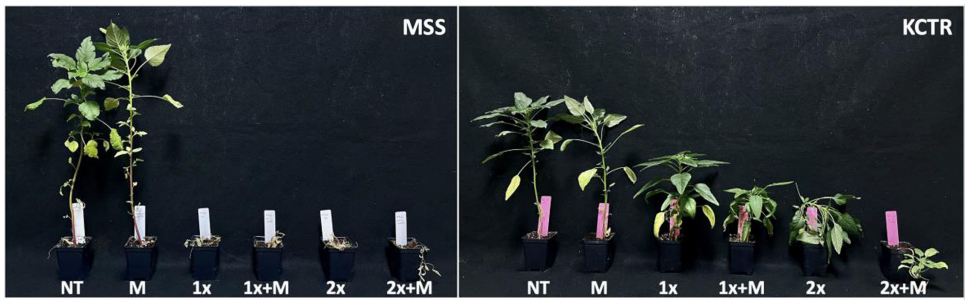

Figure 8. Visible injury of MSS and KCTR *Amaranthus palmeri* treated with MCPA alone or with a combination of malathion and MCPA at 3 wk after treatment (WAT). M, malathion (1,500 g ha^{-1} plus soil drench of 50 ml of 5 mM) treatment; x, field recommended dose of MCPA (560 g ha^{-1}). MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

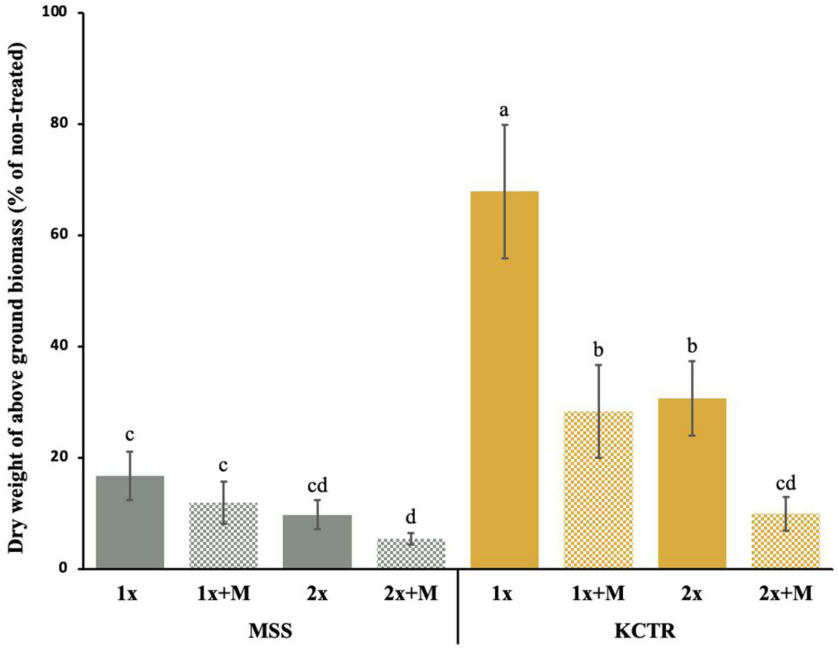

Figure 9. Bar graph representing relative dry weight of aboveground biomass as percentage of nontreated for MSS and KCTR *Amaranthus palmeri* when treated with MCPA alone and with a combination of malathion and MCPA at 3 wk after treatment (WAT). M, malathion (1,500 g ha^{-1} plus soil drench of 50 ml of 5 mM) treatment; 1x, field recommended dose of MCPA (560 g ha^{-1}). Error bars represent standard error of the mean, and lowercase letters represent significant differences identified by separation of means using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). MSS, MCPA-susceptible population from Mississippi; KCTR, 2,4-D- and MCPA-resistant population from Kansas.

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Effect of P450 Inhibitors on Metabolism of MCPA in MSS and KCTR Amaranthus palmeri

Malathion treatment itself was not responsible for any biomass reduction of KCTR or MSS plants (Figure 8). However, pretreatment with malathion followed by MCPA application increased sensitivity of KCTR to MCPA with high visible injury (Figure 8). Moreover, there was a significant biomass reduction in KCTR plants treated with malathion followed by 1× and 2× doses of MCPA compared with those treated with MCPA alone, but such a difference was not found in MSS plants in response to treatment with any dose of MCPA and malathion (Figure 9). Additionally, application of 2× MCPA along with malathion pretreatment reduced KCTR biomass similar to that of MSS plants sprayed with MCPA alone, suggesting that the metabolism of MCPA is likely mediated via P450 activity in KCTR *A. palmeri*.

al. 2007) and are primarily involved in phase I metabolism of herbicides via ring hydroxylation (Siminszky 2006). Enhanced P450-mediated metabolism is responsible for resistance to several herbicides, including acetolactate synthase inhibitors, acetyl-CoA carboxylase inhibitors, photosystem II inhibitors, 4-hydroxyphenylpyruvate dioxygenase inhibitors, and synthetic auxins in many weed species (Yu and Powles 2014; Yuan et al. 2007). Furthermore, malathion treatment has previously been reported to increase sensitivity to 2,4-D in KCTR *A. palmeri* (Shyam et al. 2022) and resistant *A. tuberculatus* (Shergill et al. 2018), indicating possible involvement of P450s in metabolism of 2,4-D.

Enhanced P450 activity could be attributed to a single-nucleotide polymorphism, alteration in gene regulation, or an increased copy number of genes coding for P450 enzymes. The P450 enzymes constitute a vast family, which makes it challenging to pin down the specific cluster involved in MCPA metabolism in *A. palmeri*. Previously, a P450 cluster (P450 81E8) was identified as metabolizing 2,4-D in a population of *A. tuberculatus* (Giacomini et al. 2020). It is important to use molecular tools to identify and characterize the role of specific P450s involved in metabolism of MCPA in KCTR *A. palmeri*. Experiments are in progress in our laboratory to identify genes involved in MCPA metabolism in KCTR *A. palmeri*.

Amaranthus palmeri is by far the most successful weed to invade new ecological environments (Sauer 1957) and the most competitive among other *Amaranthus* species (Bensch et al. 2003). The outcome of this research strongly suggests that enhanced metabolism, possibly mediated by P450s, confers resistance to MCPA in KCTR *A. palmeri*. However, the role of reduced translocation is not clear, owing to limited information about MCPA metabolites and their movement in KCTR *A. palmeri*. Evolution of resistance to SAH and other herbicides in *A. palmeri* leaves fewer options for its management and sustainable crop production. Moreover, predominance of metabolic resistance in weed species will predispose them to evolve resistance to yet to be discovered compounds. Han et al. (2021) reported involvement of a single P450 gene in metabolism of multiple herbicides in an annual ryegrass (*Lolium rigidum* Gaudin) population. Future work will focus on identification of gene(s) involved in metabolism of multiple herbicides in KCTR *A. palmeri*.

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