Kansas Agricultural Experiment Station Research Reports

Volume 4
Issue 7 *Kansas Field Research*

Article 10

2018

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Recommended Citation

Kumar, V.; Stahlman, P. W.; and Boyer, G. (2018) "Palmer Amaranth Populations from Kansas with Multiple Resistance to Glyphosate, Chlorsulfuron, Mesotrione, and Atrazine," *Kansas Agricultural Experiment Station Research Reports*: Vol. 4: Iss. 7. https://doi.org/10.4148/2378-5977.7611

Palmer Amaranth Populations from Kansas with Multiple Resistance to Glyphosate, Chlorsulfuron, Mesotrione, and Atrazine

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Summary

Multiple herbicide-resistant (MHR) Palmer amaranth poses a serious management concern for growers across the United States. Since 2014, several Palmer amaranth populations with suspected resistance to most commonly used herbicides were collected in random field surveys across Kansas. This study aimed to characterize the resistance levels to glyphosate (EPSPS inhibitor), mesotrione (HPPD inhibitor), chlorsulfuron (ALS inhibitor), and atrazine (PS II inhibitor) in three suspected MHR Palmer amaranth populations (KW2, PR8, and BT12) compared to a known herbicide-susceptible (SUS) population. Dose-response studies revealed that PR8 and BT12 populations had 7- to 14-fold level resistance to glyphosate, and up to 12-fold level of resistance to chlorsulfuron (Glean herbicide) on the basis of visible control (LD_{50} values) and shoot dry weight response (GR_{50} values). The KW2, PR8, and BT12 populations also showed 2- to 4-fold resistance to mesotrione (Callisto herbicide) relative to SUS population. Based on plant dry weight response (GR_{50} values), the KW2 and BT12 populations showed 5- and 16-fold resistance to atrazine (AAtrex 4L herbicide), respectively, compared with the SUS population. These results confirm the first report on the evolution of a Palmer amaranth population (BT12) with multiple resistance to glyphosate (12 to 14 fold), chlorsulfuron (11 fold), mesotrione (2 to 4 fold), and atrazine (16 fold) in Kansas. Further studies are in progress to investigate the response of these MHR populations to fomesafen (PPO inhibitor); 2,4-D; and dicamba (synthetic auxins) herbicides.

Introduction

Palmer amaranth (*Amaranthus palmeri*) is the most troublesome broadleaf weed species in the United States, including Kansas (Wychen, 2017). Palmer amaranth possesses several unique traits, including extended period of emergence, fast growth habit, and high seed production potential. A single female plant of Palmer amaranth can produce up to 600,000 seeds and can cause heavy infestation in field crops (Burke et al., 2007; Keeley et al., 1987). Season-long competition from Palmer amaranth at densities of 10 plants m^{-1} row of soybean was found to cause up to 68% yield reduction (Klingaman and Oliver, 1994).

In recent years, multiple herbicide-resistant (MHR) Palmer amaranth populations have become an increasing management concern for Kansas growers. Palmer amaranth's resistance to sulfonylurea (ALS inhibitors) and atrazine (PS II inhibitor) has been reported in Kansas since the early and mid-90s, respectively (Heap, 2018). Glyphosate-resistant (GR) Palmer amaranth was confirmed in Kansas in 2011. A Palmer amaranth population resistant to multiple herbicide modes of action, including mesotrione (HPPD inhibitor), atrazine (PS II inhibitor), and chlorsulfuron (ALS inhibitor) has previously been reported in Kansas (Nakka, 2016). Since 2014, state-wide random field surveys have monitored the distribution, frequency, and resistance levels of MHR Palmer amaranth populations across the Kansas cropping systems. The main objective of this research was to investigate suspected MHR Palmer amaranth populations for resistance to multiple herbicide modes of action (glyphosate, chlorsulfuron, mesotrione, and atrazine) relative to a known susceptible population.

Procedures

Fully-matured seeds of suspected MHR Palmer amaranth populations (40 to 50 plants per field) were originally collected from Barton (BT12), Kiowa (KW2), and Pratt (PR8) counties in Kansas. Seeds of a known herbicide-susceptible (SUS) Palmer amaranth population were collected from the Kansas State University Ashland Bottoms research fields in Riley County, KS, and were used previously in various greenhouse and laboratory studies. Dose-response experiments were conducted by sowing seeds of each MHR and SUS population on the surface of germination trays filled with commercial potting mix. Later on, seedlings of each Palmer amaranth population were transplanted in 4 × 4-inch plastic pots containing commercial potting mixture under greenhouse conditions at the Kansas State University Agricultural Research Center near Hays, KS. For each herbicide tested, the study was set up in a randomized complete block design (blocked by population) with 6 to 8 replications. Actively growing seedlings (3- to 4-inch tall) from each population were sprayed with Roundup PowerMax (glyphosate) at doses of 0, 2, 4, 8, 16, 32, 64, 128, 256, and 512 fluid oz/a. Ammonium sulfate at 2% (wt/v) was included with all glyphosate treatments. Doses for Callisto (mesotrione) herbicide along with 1% v/v of crop oil concentrate (COC) and 2.5% v/v of urea ammonium nitrate (UAN, 28%) included: 0, 0.187, 0.375, 0.75, 1.5, 3, 6, 12, 24, and 48 oz/a. Doses for Glean (chlorsulfuron) herbicide along with 0.25% v/v of nonionic surfactant (NIS) included: 0, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 oz/a. Doses for AAtrex (atrazine) herbicide along with 1% v/v of Agri-Dex crop oil concentrate (COC) included: 0, 2, 4, 8, 16, 32, 64, 128, 256, and 512 fl oz/a. Herbicide applications were made using a cabinet spray chamber equipped with an even flat-fan nozzle tip (TeeJet 8001EXR) calibrated to deliver 10 gallons per acre of spray solution at 40 psi. Herbicide-treated plants were returned to the greenhouse and watered and fertilized as needed. Data on percent visible control (0 = no control, 100 = dead plant) were visually assessed at 7, 14, and 21 days after treatment (DAT) of each herbicide. Shoot dry weights were also determined at 21 DAT. For each dose-response experiment, data were analyzed using a 3-parameter log-logistic model in *R* software using the following equation (Ritz et al. 2015):

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

where y refers to the response variable (% visible control or shoot dry weight), d is the upper limit, b is the slope of each curve, *e* is the herbicide dose required to cause 50% control or 50% shoot dry weight reduction (referred to as LD_{50} or GR_{50}), and x is the herbicide dose. Nonlinear regression parameter estimates and standard errors for each population were determined using the *drc* package in R software. Resistance level (referred as R/S ratio) to a particular herbicide was estimated by dividing the LD_{50} or GR_{50} value of each MHR population by the LD_{50} or GR_{50} value of the susceptible population.

Results

Resistance to Glyphosate

Based on the dose-response curves for visual control (%) data, the LD_{50} (effective dose of Roundup PowerMax required to obtain 50% control) values of PR8 and BT12 Palmer amaranth populations were 59 and 79 fl oz/a, respectively, and were greater than the 5.7 fl oz/a value obtained for the SUS population (Table 1). Based on the LD_{50} values, the PR8, and BT12 populations exhibited 10- and 14-fold level resistance to glyphosate (Table 1; Figure 1A). Similarly, the PR8 and BT12 populations had GR_{50} values (effective dose of Roundup PowerMax required to cause 50% reduction in shoot dry weights) of 39 and 66 fl oz/a, respectively, which were higher than the 5.5 fl oz/a rate for the SUS population (Table 1). Based on the shoot dry weight response, the PR8 and BT12 populations showed 7- and 12-fold level of resistance to glyphosate (Table 1; Figure 1B). Visual control and shoot dry weight response of the KW2 population to various doses of Roundup PowerMax herbicide were more or less similar to the SUS population (Table 1; Figure 1). Thus, both the visual and dry weight assessments indicated the PR8 and BT12 populations were highly resistant to glyphosate.

Resistance to HPPD Inhibitors

The confirmed glyphosate-resistant Palmer amaranth populations (PR8 and BT12) also had at least 2- and 4-fold resistance to mesotrione (Callisto) herbicide on the basis of visible control data (LD_{50} values) (Table 2; Figure 2). In addition, the KW2 population also exhibited at least 3-fold resistance to mesotrione based on LD_{50} values. Based on shoot dry weight response (GR_{50} values), the KW2 and BT12 populations exhibited 3- and 2-fold level of resistance to mesotrione herbicide (Table 2; Figure 2). However, the shoot dry weight response of the PR8 population with various doses of mesotrione did not differ from SUS population (Table 2). These results indicated that all tested MHR populations in this study had evolved low level of resistance to mesotrione.

Resistance to ALS Inhibitors

A whole plant dose-response study indicated that all three suspected MHR Palmer amaranth populations viz., KW2, PR8, and BT12 had at least 2-, 12-, and 11-fold resistance to chlorsulfuron (Glean XP) herbicide, respectively, compared to the SUS population on the basis of percent visible control rating (LD_{50} values) (Table 3; Figure 3). The shoot dry weight response of these populations to chlorsulfuron also showed similar results (data not shown). In comparison to a previous report on ALS-resistant Palmer amaranth from Kansas (Nakka, 2016), the selected MHR populations in this study showed both low and high level of resistance to chlorsulfuron.

Resistance to PS II Inhibitors

Based on plant dry weight response (GR_{50} values), the two tested MHR Palmer amaranth populations viz., KW2 and BT12 showed at least 5- and 16-fold resistance to atrazine (AAtrex 4L herbicide) compared to the SUS population. These results indicated that both populations had developed moderate to high level resistance to atrazine (Table 4; Figure 4).

Conclusions and Implications

This research confirms the first case of a Palmer amaranth population (BT12) with multiple resistance to glyphosate (EPSPS inhibitor), mesotrione (HPPD inhibitor), chlorsulfuron (ALS inhibitor), and atrazine (PS II inhibitor) in Kansas. Increasing reports of MHR Palmer amaranth populations are of great concern as these herbicide chemistries are commonly used in Kansas cropping systems. Growers should adopt integrated weed management programs by incorporating effective and alternate herbicide modes of action and nonchemical based approaches (such as diversifying crop rotations, growing cover crops, tillage, cuttings, mowing, etc.) together on their production fields. Future studies will determine the sensitivity of these confirmed MHR Palmer amaranth populations to other herbicide modes of action, including synthetic auxins (2,4-D and dicamba), PPO inhibitors (Flexstar and Cobra herbicides) and will investigate the underlying mechanism(s) of these multiple herbicide resistance traits.

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Table 1. Regression parameter (equation 1) estimates for whole plant dose response of Palmer amaranth populations from Kansas treated with Roundup PowerMax (glyphosate) herbicide

Population[a]	d (± SE)	b (± SE)	LD_{50} or GR_{50} (± SE)	R/S[b]
Based on % visible control				
SUS	99.2 (1.9)	-5.1 (0.7)	5.7 (0.3)	-
KW2	99.1 (1.9)	-3.4 (0.4)	8.4 (0.4)	1
PR8	82.2 (2.9)	-2.1 (0.3)	59.1 (4.5)	10
BT12	82.1 (2.6)	-3.5 (0.7)	79.1 (4.4)	14
Based on shoot dry weight				
SUS	94.4 (5.1)	5.6 (2.1)	5.5 (0.6)	-
KW2	96.2 (4.7)	7.1 (3.1)	6.1 (0.7)	1
PR8	103.1 (5.1)	1.9 (0.3)	39.2 (4.5)	7
BT12	94.3 (4.6)	2.5 (0.8)	66.0 (8.3)	12

[a]Abbreviations: d, upper limit; b, slope of each curve; SUS, herbicide susceptible population from Ashland Bottoms research fields, Riley, KS; KW2, suspected multiple herbicide-resistant (MHR) population from Kiowa County, KS; PR8, suspected MHR population from Pratt County, KS; BT12, suspected MHR population from Barton County, KS; LD_{50} and GR_{50} are effective doses (fl oz/a) of Roundup PowerMax required for 50% control and shoot dry weight reduction, respectively.

[b] R/S is calculated as a ratio of LD_{50} or GR_{50} of an MHR population to LD_{50} or GR_{50} of the SUS population.

Table 2. Regression parameter (equation 1) estimates for whole plant dose-response of Palmer amaranth populations from Kansas treated with Callisto (mesotrione) herbicide

Population[a]	d (± SE)	b (± SE)	LD_{50} or GR_{50} (± SE)	R/S[b]
Based on % visible control				
SUS	99.3 (3.2)	-1.9 (0.3)	0.4 (0.03)	-
KW2	89.4 (3.3)	-1.7 (0.3)	1.5 (0.15)	3
PR8	98.1 (2.1)	-4.4 (1.7)	0.8 (0.03)	2
BT12	92.3 (3.8)	-1.4 (0.2)	1.9 (0.13)	4
Based on shoot dry weight				
SUS	100.1 (5.1)	3.2 (0.9)	0.22 (0.01)	-
KW2	99.9 (5.3)	1.3 (0.3)	0.65 (0.03)	3
PR8	100.1 (4.9)	0.7 (0.2)	0.25 (0.01)	1
BT12	99.9 (4.3)	1.1 (0.2)	0.58 (0.04)	2

[a]Abbreviations: d, upper limit; b, slope of each curve; SUS, herbicide susceptible population from Ashland Bottoms research fields in Riley County, KS; KW2, suspected multiple herbicide-resistant (MHR) population from Kiowa County, KS; PR8, suspected MHR population from Pratt County, KS; BT12, suspected MHR population from Barton County, KS; LD_{50} and GR_{50} are effective doses (fl oz/a) of Callisto herbicide required for 50% control and shoot dry weight reduction, respectively.

[b] R/S is calculated as a ratio of LD_{50} or GR_{50} of an MHR population to LD_{50} or GR_{50} of the SUS population.

Table 3. Regression parameter (equation 1) estimates for whole plant dose response of Palmer amaranth populations from Kansas treated with Glean XP (chlorsulfuron) herbicide

Population[a]	d (± SE)	b (± SE)	LD_{50} (± SE)	R/S[b]
Based on % visible injury				
SUS	99.9 (3.3)	-1.2 (0.5)	0.03 (0.001)	-
KW2	86.9 (1.7)	-6.9 (2.2)	0.06 (0.001)	2
PR8	98.8 (4.8)	-0.6 (0.1)	0.38 (0.01)	12
BT12	57.4 (2.4)	-1.6 (0.4)	0.34 (0.04)	11

[a]Abbreviations: d, upper limit; b, slope of each curve; SUS, herbicide susceptible population from Ashland Bottoms research fields in Riley County, KS; KW2, suspected multiple herbicide-resistant (MHR) population from Kiowa County, KS; PR8, suspected MHR population from Pratt County, KS; BT12, suspected MHR population from Barton County, KS; LD_{50} are effective doses (oz/a) of Glean herbicide required for 50% control of each population, respectively.
[b]R/S is calculated as a ratio of LD_{50} of an MHR population to LD_{50} of the SUS population.

Table 4. Regression parameter (equation 1) estimates for whole plant dose response of Palmer amaranth populations from Kansas treated with AAtrex 4L (atrazine) herbicide

Population [a]	d (± SE)	b (± SE)	GR_{50} (± SE)	R/S[b]
Based on shoot dry weights				
SUS	100.1 (5.5)	0.7 (0.05)	0.31 (0.06)	-
KW2	100.6 (4.6)	0.4 (0.07)	1.74 (0.08)	5
BT12	101.8 (5.4)	1.0 (0.16)	5.2 (0.09)	16

[a]Abbreviations: d, upper limit; b, slope of each curve; SUS, herbicide susceptible population from Ashland Bottoms research fields in Riley County, KS; KW2, suspected multiple herbicide-resistant (MHR) population from Kiowa County, KS; BT12, suspected MHR population from Barton County, KS; GR_{50} are effective doses (fl oz/a) of AAtrex 4L herbicide required for 50% shoot dry weight reduction of each population, respectively.
[b]R/S is calculated as a ratio of GR_{50} of an MHR population to GR_{50} of the SUS population.

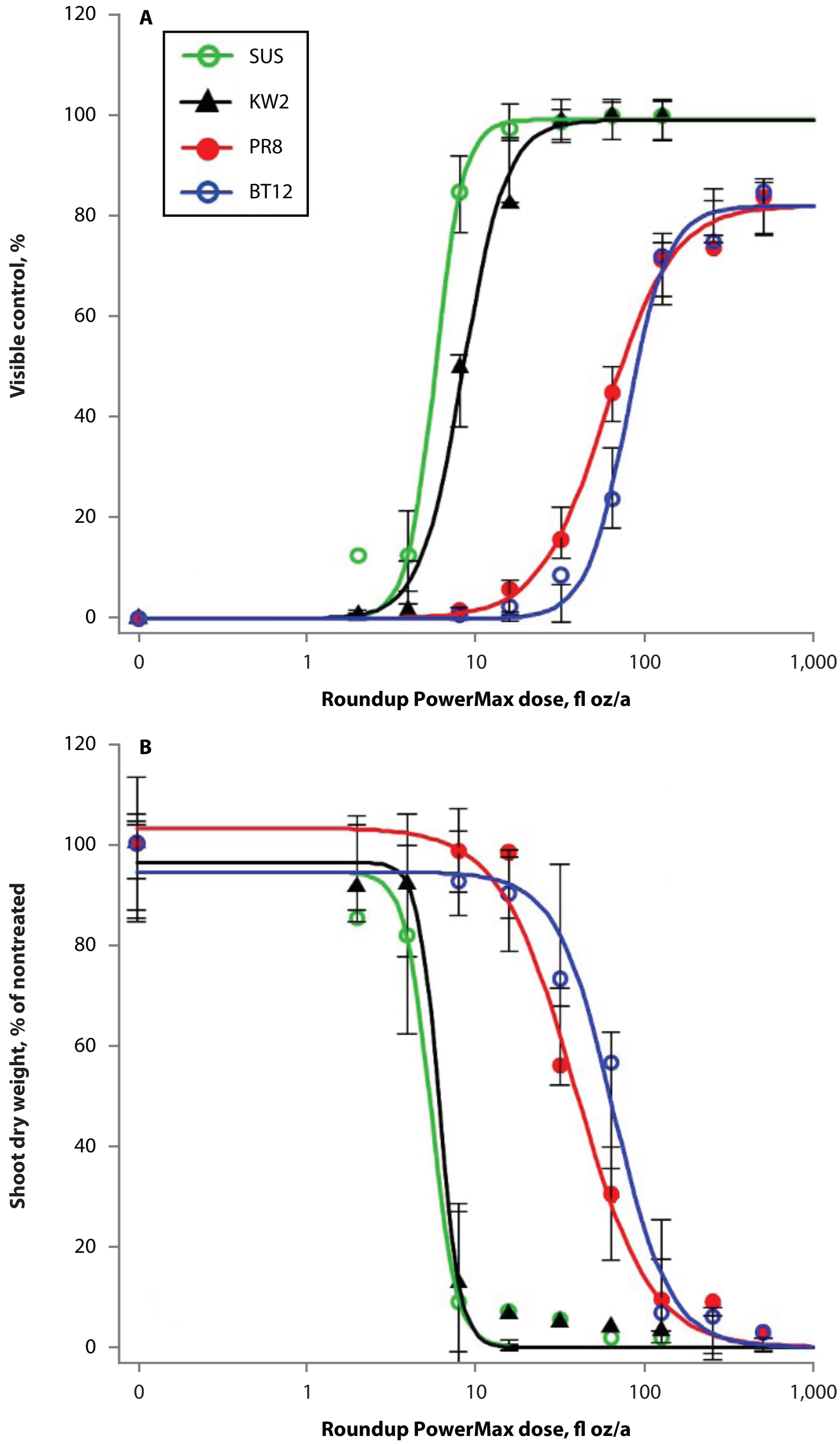
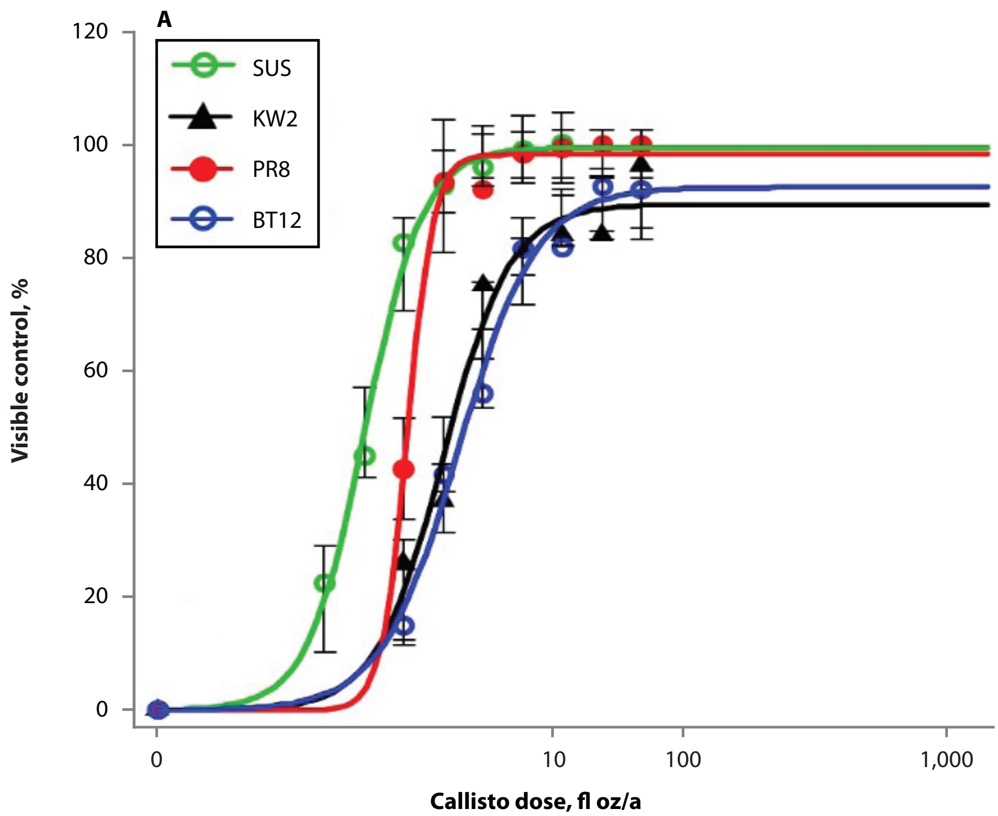


Figure 1. Visible control (%) (A) and shoot dry weight (B) response of three MHR Palmer amaranth populations (KW2, PR8, and BT12) and an herbicide-susceptible population (SUS) in a whole plant dose–response experiment with Roundup PowerMax (glyphosate) herbicide.



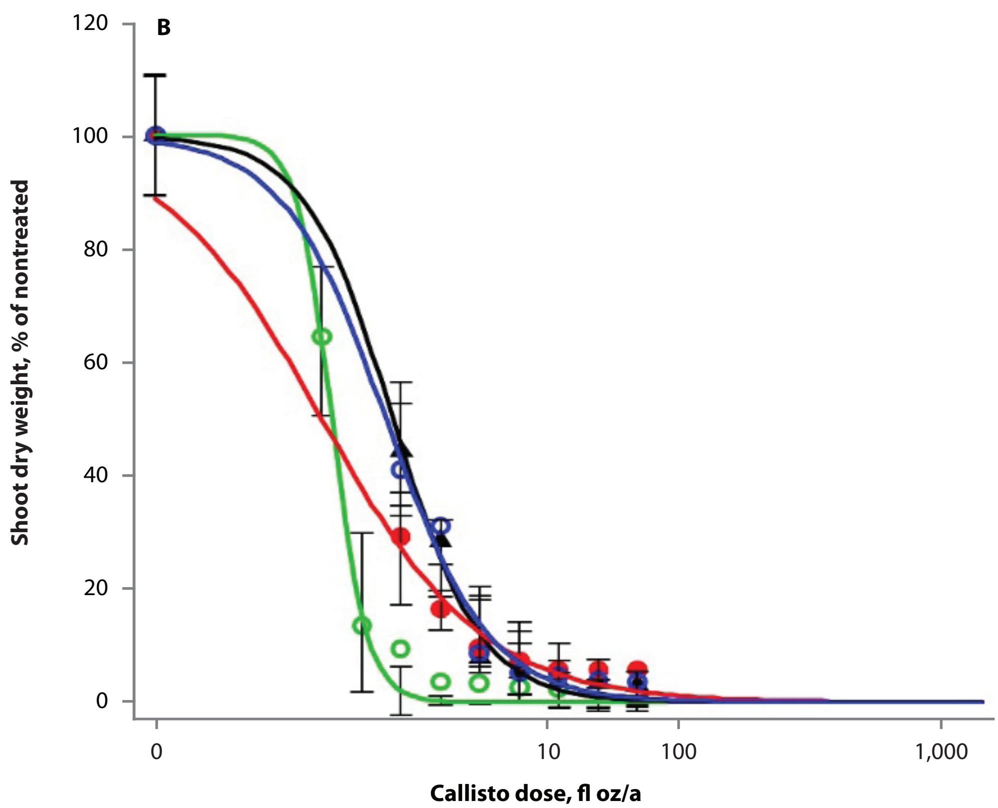


Figure 2. Visible control (%) (A) and shoot dry weight (B) response of three MHR Palmer amaranth populations (KW2, PR8, and BT12) and an herbicide-susceptible population (SUS) in a whole plant dose–response experiment with Callisto (mesotrione) herbicide.

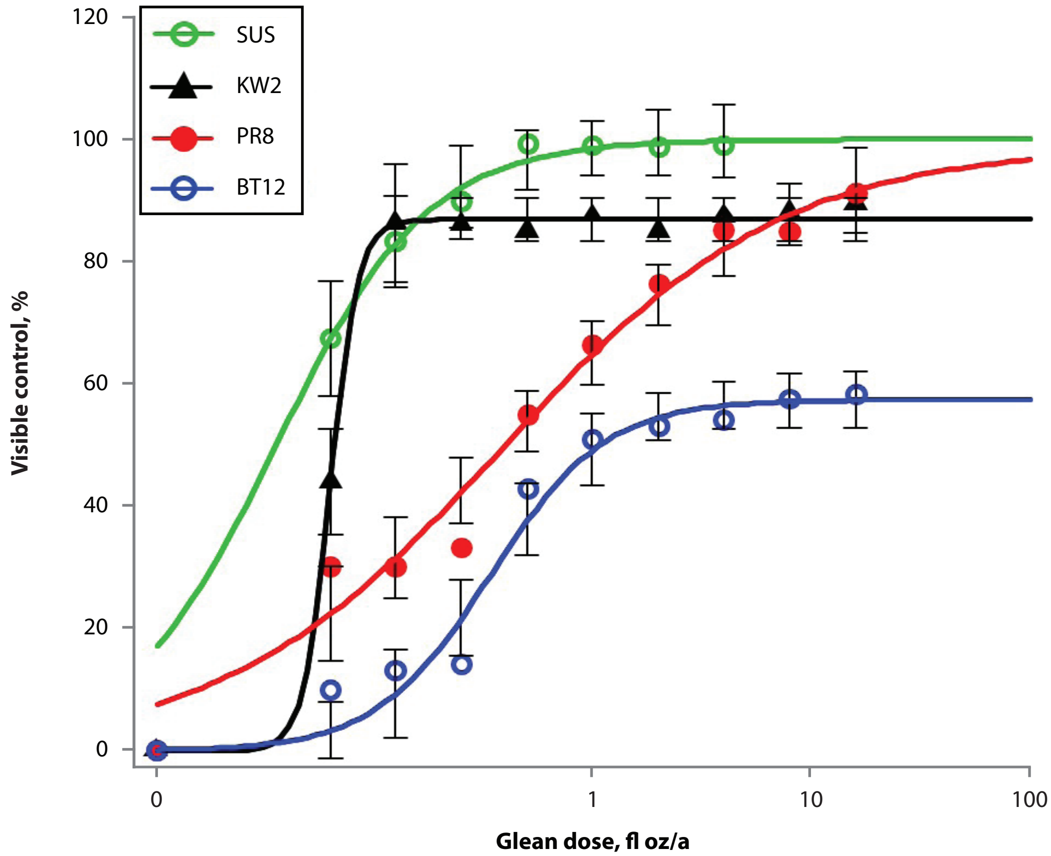


Figure 3. Visible control (%) response of three MHR Palmer amaranth populations (KW2, PR8, and BT12) and an herbicide-susceptible (SUS) population in a whole plant dose–response experiment with Glean (chlorsulfuron) herbicide.

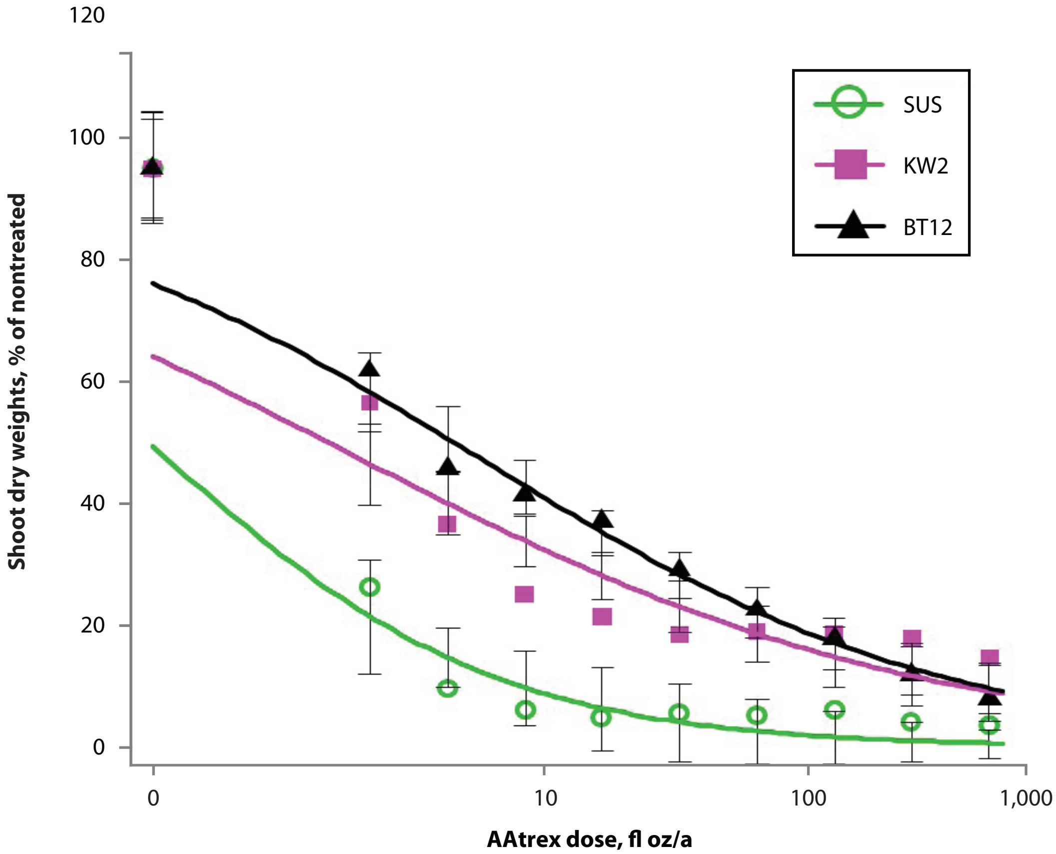


Figure 4. Shoot dry weight response of two MHR Palmer amaranth populations (KW2 and BT12) and an herbicide-susceptible population (SUS) in a whole plant dose–response experiment with AAtrex 4L (atrazine) herbicide.