KANSAS SOYBEAN COMMISSION- FINAL REPORT (FY 2018-2019)

Title: Investigation of Herbicide Resistance in Waterhemp and Palmer amaranth in Kansas: Survey, Mechanism, and Management.

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Objective # 1. Determine the baseline sensitivity of waterhemp and Palmer amaranth populations from Kansas fields to glyphosate, 2,4-D, dicamba, and lactofen.

Materials and Methods:

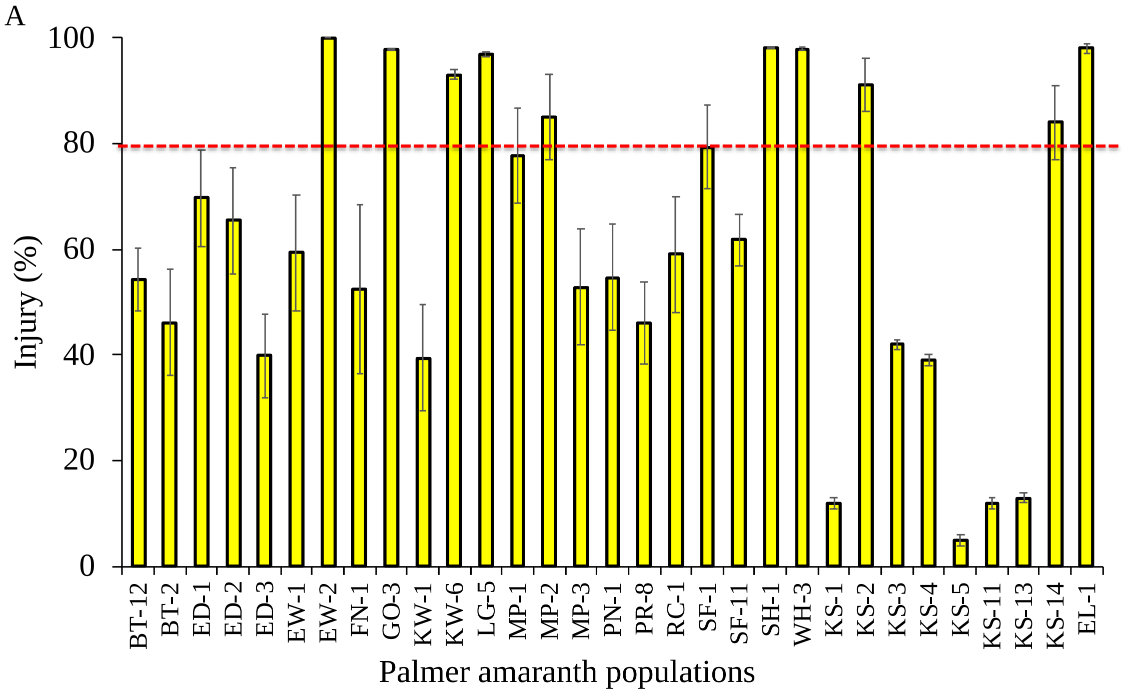
a) ***Discriminate-Dose Experiments.*** Greenhouse experiments were initiated at the Kansas State University Agricultural Research Center (KSU-ARC) near Hays, KS to investigate the baseline sensitivity of Palmer amaranth populations to glyphosate (Roundup PowerMax®), 2,4-D (Defy® LV-6), dicamba (Clarity®), and lactofen (Cobra®) herbicides. Separate discriminate-dose experiments were conducted for confirming resistance to each herbicide. Seeds of each population were sown on the surface of commercial potting mixture in germination flat trays. After emergence, seedlings of each Palmer amaranth population were transplanted into 10- by 10-cm plastic pots containing the same commercial potting mixture. Experiments were conducted in randomized complete block designs, with 12 replications (1 plant/pot). A discriminate dose of each herbicide (Roundup PowerMax at 32 fl oz/a; Defy at 18.4 fl oz/a; Clarity at 16 fl oz/a; Cobra at 12.5 fl oz/a) was separately applied to 8- to 10-cm tall Palmer amaranth plants inside a spray chamber. Data on visible injury (on a scale of 0 to 100, 0 being no injury and 100 being complete plant death) were assessed at 7, 14, and 21 days after treatment (DAT).

b) ***Whole-Plant Dose-Response Experiments*.** Dose-response experiments were conducted in a greenhouse at KSU-ARC near Hays, KS to characterize the level of resistance in confirmed multiple herbicide-resistant (MHR) Palmer amaranth populations compared to a known susceptible population. Seedlings of four MHR populations (SH1, PR8, BT12, and KW2) and one susceptible (SUS) population were separately grown in 10-cm diam plastic pots containing commercial potting mixture as previously described. Experiments were performed in a randomized complete block design with 12 replications (one plant/pot for each herbicide dose per population). Doses ranging from 1/16- to 16-times the field-use rates of each herbicide (RoundUp PowerMax, Glean, Aatrex, Callisto, and Defy) were tested when Palmer amaranth seedlings were 8- to 10-cm tall. Data on percent visible control (symptoms including chlorosis, stunting, leaf curling, cupping, twisting, and necrosis) on a scale of 0 to 100, 0 being no injury/control and 100 being complete control/plant death were recorded at 7, 14, and 21 DAT. Shoot dry weights were determined at 21 DAT. Data on percent visible control and shoot dry weights from each dose-response experiments were regressed over doses of each tested herbicide using a 3-parameter log-logistic model. Nonlinear regression parameter estimates were determined using R software. Based on estimated LD_{50} or GR_{50} values (effective dose of each herbicide providing 50% control or 50% dry weight reduction), the resistance index (referred as R/S ratio) for each confirmed MHR Palmer amaranth population were estimated by dividing the LD_{50} or GR_{50} value of a resistant population by the LD_{50} or GR_{50} value of the susceptible population.

Results and Discussion:

a) *Discriminate-Dose Experiments.* Using a cutoff value of ≤ 80% visible injury, about 21, 5, and 0 Palmer amaranth populations out of 31 total tested populations exhibited resistance to Roundup PowerMax, Defy, and Clarity herbicides at 21 DAT, respectively (Figures 1A-B, 2A). In those resistant populations, the percent visible injury with RoundUp PowerMax and Defy herbicides ranged from 5 to 78% and 67 to 80%, respectively, at 21 DAT. In contrast, Clarity herbicide was effective on all tested populations, with visible injury ranging from 87 to 100% at 21 DAT (Figure 2A). In addition, Cobra herbicide was also effective on most of the tested Palmer amaranth populations. only 2 out of 23 total tested Palmer amaranth populations had ≤ 80% visible injury with Cobra herbicide at 21 DAT (Figure 2B). These results suggest that resistance to glyphosate is fairly common among Kansas Palmer amaranth populations and there are few populations that showed unacceptable control with 2,4-D herbicide.

b) *Dose-Response Experiments.* Based on shoot dry weight response (GR_{50} values), the PR8 and BT12 populations showed 7- and 12-fold level of resistance to glyphosate (RoundUp PowerMax) compared to the SUS population (Figure 3A). Shoot dry weight response of the KW2 population to various doses of Roundup PowerMax was more or less similar to the SUS population (Figure 3A). Three MHR populations viz., KW2, PR8, and BT12 showed 2-, 12-, and 11-fold resistance to chlorsulfuron (Glean XP) herbicide, respectively, compared to the SUS population (Figure 3B). The PR8 and BT12 populations also exhibited 2- and 4-fold resistance to Callisto herbicide (Figure 3C). In addition, the KW2 population had at least 3-fold resistance to Callisto herbicide.



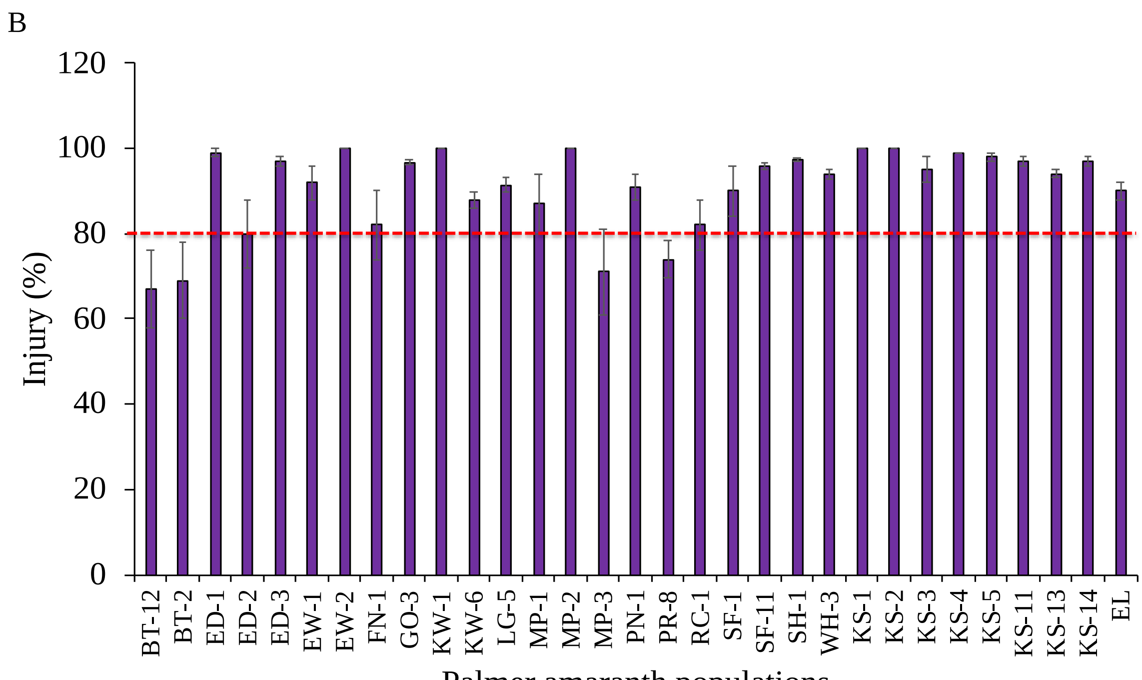


Figure 1. Visible injury (%) response of Palmer amaranth populations to RoundUp PowerMax (A) and Defy (B) herbicides in discriminate-dose experiments at 21 DAT.

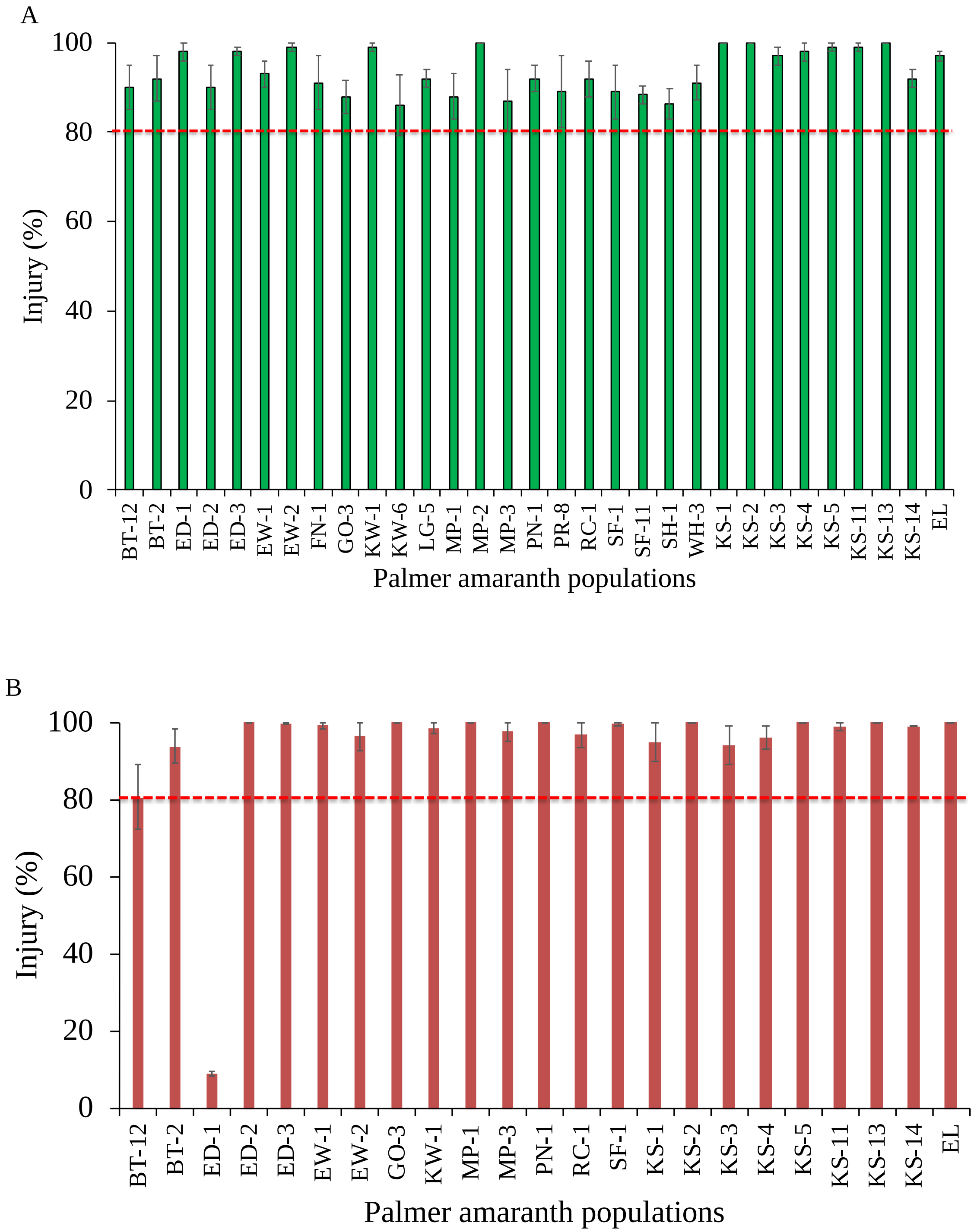
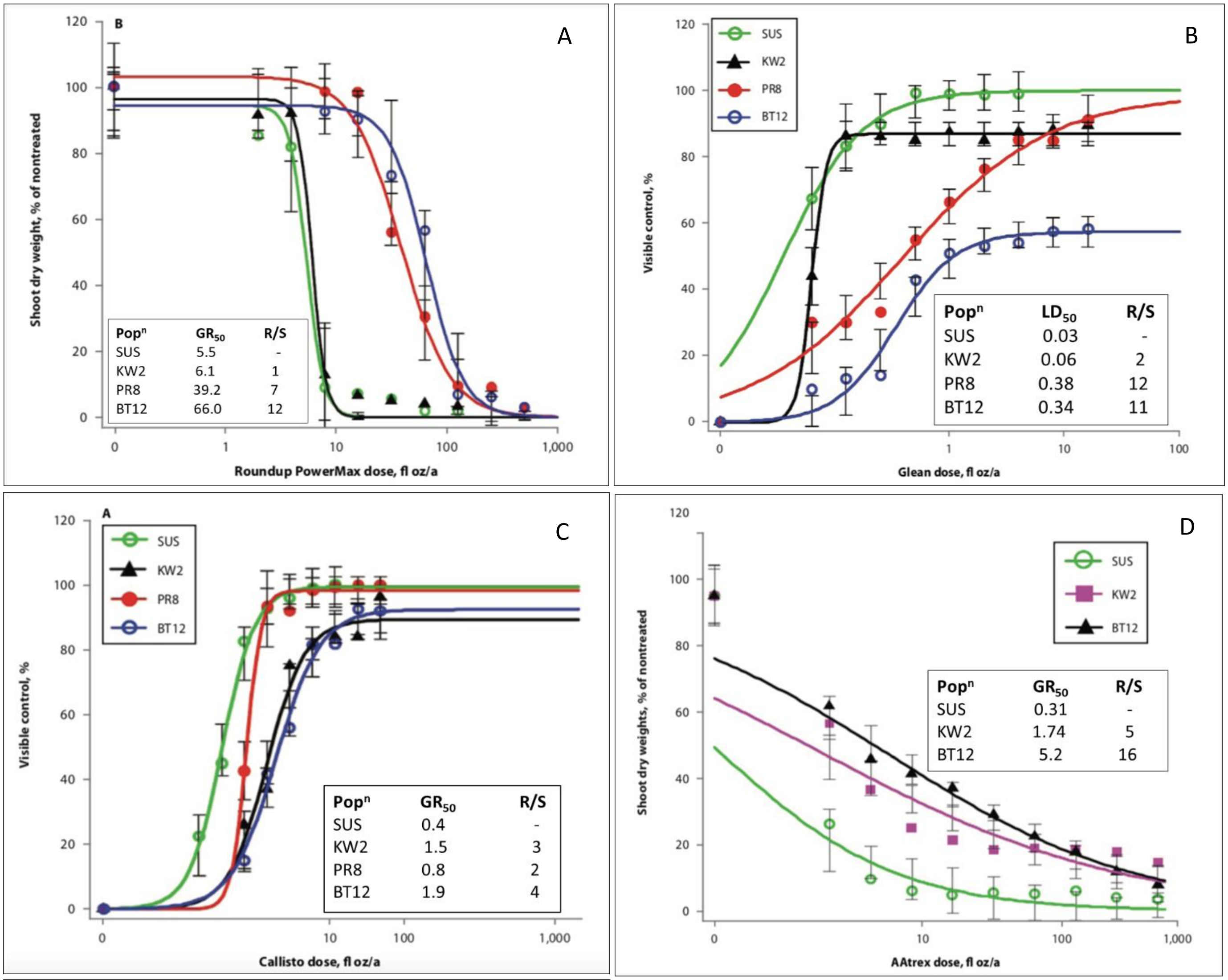


Figure 2. Visible injury (%) response of Palmer amaranth populations to Clarity (A) and Cobra (B) herbicides in discriminate-dose experiments at 21 DAT.



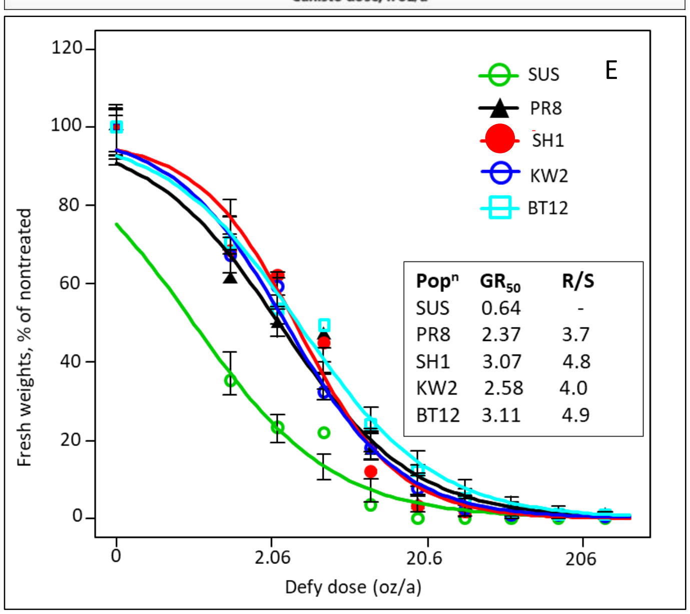


Figure 3. Response of selected multiple herbicide-resistant (MHR) and susceptible (SUS) Palmer amaranth populations from Kansas, when treated with various doses of RoundUp PowerMax (A), Glean (B), Callisto (C), Aatrex (D) and Defy (E) herbicides at 21 DAT.

Based on shoot dry weights, two MHR Palmer amaranth populations viz., KW2 and BT12 exhibited 5- and 16-fold resistance to atrazine (Aatrex 4L) (Figure 3D). Based on fresh weight response, all four MHR populations had 3.7- to 4.9-fold resistance to 2,4-D (Defy) in comparison to the SUS population (Figure 3E). These results confirmed the first case of a Palmer amaranth population from Kansas with multiple resistance to RoundUp PowerMax, Glean, Aatrex, Callisto, and Defy herbicides.

Note: We had also collected 31 populations of common waterhemp through random soybean field surveys from southeast Kansas in fall 2018. However, herbicide resistance screening work was not initiated due to high seed dormancy exhibited by all these populations.

Objective # 2. <u>Investigate the mechanism of 2,4-D resistance in Palmer amaranth.</u>

Preliminary screening: Vegetative clones of ~10 Palmer amaranth plants (KCTR) that survived repeated application of 2,4-D in a long-term conservation tillage experimental trial were generated in the greenhouse at Manhattan, KS. When these clones were at 10-12 cm tall, they were sprayed with 2,4-D (2,4-D Amine 4L) at 1120 and 2240 g ae ha-1 (2- and 4-fold of the recommended field rate, respectively). In response to 2,4-D treatment, 8 plants (out of 10) survived the 2,4-D application with varying injury. Seeds were collected from 2 of these plants, that showed high level of resistance to 2,4-D and these seeds were used in further studies.

Dose-response: Dose-response experiments were conducted in controlled environmental growth chambers with three Palmer amaranth populations: KCTR, and two susceptible i.e., KSS (Kansas 2,4-D susceptible Palmer amaranth) and MSS (Mississippi 2,4-D susceptible). Ten-12 cm seedlings of each of the three population were sprayed separately with 2,4-D at the rate of 0, 70, 140, 280, 560, 1120, 2240, 4480, 8960 g ae ha^{-1}. Visual injury and dry shoot biomass data were collected at 4 weeks after treatment. Dose-response analysis of visual injury data suggested that KCTR is 13.6-fold and 10.2-fold resistant compared to KSS and MSS respectively. The dry shoot biomass data indicated that KCTR is 14.6-fold and 9.3-fold resistant compared to KSS and MSS respectively.

Mechanism of 2,4-D resistance in KCTR Palmer amaranth: Experiments were conducted to investigate the mechanism of 2,4-D resistance in KCTR Palmer amaranth with KSS and MSS plants used for comparison. Plants of the three populations were grown under controlled environmental conditions, and 10-12 cm tall plants were treated with a working solution containing both 14C radiolabeled 2,4-D and commercial 2,4-D formulation. The absorption, translocation and metabolism of 14C 2,4-D will be determined at 4 harvesting time-points: 6, 24, 48 and 72 HAT (hours after treatment). Preliminary data suggest that there is no difference in maximum 14C 2,4-D absorption in these populations. However, MSS showed relatively higher time required to achieve maximum absorption compared to KCTR and KSS. Experiments are in progress to assess the 2,4-D translocation and metabolism in KCTR in comparison with MSS and KSS Palmer amaranth. In summary, KCTR is ~ 10-14-fold resistant to 2,4-D compared to MSS and KSS.

Objective # 3. Evaluation of integrated herbicide programs for managing herbicide-resistant (HR) waterhemp and Palmer amaranth in Roundup Ready 2 Xtend® soybeans.

Materials and Methods:
Two field experiments were established: One at the Kansas State University Agricultural Research Center (KSU-ARC) near Hays, KS and a second at the Kansas State University Ashland Bottoms (KSU-AB) research farm near Manhattan, KS. Soybean plots were established in no-till wheat stubble at the KSU-ARC; whereas, the study site at KSU-AB was under a conventional tillage system. Experiments at both sites were established in randomized complete block design with 4 replications. The study site at KSU-AB had a natural infestation of glyphosate-resistant (GR) Palmer amaranth; whereas, a seedbank of GR Palmer amaranth was artificially established at KSU-ARC site. A Roundup Ready 2 Xtend soybean, Asgrow "AG34X7", was planted at 156,900 seeds/ac on May 22, 2018 at KSU-ARC. Roundup Ready 2 Xtend soybean, Asgrow "AG39X7", was planted at 130,000 seeds/ac on June 4 at the KSU-AB site. The selected preemergence (PRE) herbicide programs were applied at each location immediately after soybean planting. The selected POST treatments were applied on June 22 at KSU-ARC, and July 16 at KSU-AB, respectively. Data on soybean injury and Palmer amaranth control were collected at biweekly intervals throughout the growing season after PRE herbicide treatments at both sites. Palmer amaranth biomass at soybean maturity was collected from the center of each plot at both locations. Soybean grain yield was recorded using a plot combine by harvesting the middle two rows. Palmer amaranth control (%), and soybean grain yield (bu/A) was subjected to ANOVA using PROC MIXED in SAS 9.3 software. Means were separated by Fisher's protected LSD test at $P < 0.05$.

Results and Discussion:
No visual soybean injury was observed with any PRE and/or POST herbicide programs tested (data not shown). A single PRE application of Fierce XLT at 5 oz/a and Panther PRO at 12 oz/a had excellent (≥ 90%) control of glyphosate-resistant (GR) Palmer amaranth at both locations (Table 1; Figure 4). Similarly, PRE-applied Zidua PRO at 6 oz/a had 93% control of GR Palmer amaranth at the Manhattan site, but slightly lower control (82%) at the Hays site. Control of GR Palmer amaranth with PRE applied Authority Elite at 28 oz/a ranged from 77 to 87% across both locations (Table 1; Figure 4). All PRE treatments followed by a sequential POST application of a Roundup plus Engenia mixture had the highest control (95 to 100%) of GR Palmer amaranth at both sites (Table 1; Figure 4). Interestingly, GR Palmer amaranth control with a single POST application of Roundup (32 fl oz/a) plus Engenia (12.8 fl oz/a) did not differ from two applications of Roundup (32 fl oz/a) plus Engenia (12.8 fl oz/a) mixture (PRE fb POST or EPOST fb LPOST) and was also comparable to the season-long hand-weeded treatment. Consistent with visual control data, PRE alone treatments of Authority Elite, Zidua Pro, and Panther PRO also had significant Palmer amaranth biomass accumulation at both locations in comparison to PRE followed by POST treatments. Soybean grain yield with majority of the herbicide programs did not differ and ranged from 18 to 22 bushels/ac at Hays; whereas, about 10% increase in grain yield was observed with PRE fb POST vs. PRE alone programs at Manhattan (Figure 5).

Based on these results, the use of two- pass herbicide programs, including PRE herbicides such as Fierce XLT, Panther PRO, Authority Elite, or Zidua PRO followed by a sequential POST application of Roundup PowerMax + Engenia are recommended for effective and season-long control of GR Palmer amaranth in Roundup Ready 2 Xtend soybean.

Objective # 4. Develop and deliver education materials for management/containment of HR waterhemp and Palmer amaranth in Kansas soybean production.

Information generated through this research project was communicated to Kansas growers through various outlets. Field plot demonstrations for objective #3 were showcased to ag clientele at the annual fall field day at KSU-ARC and through various individual field visits at the KSU-AB site. Research results on the baseline sensitivity and characterizing multiple herbicide resistance in Palmer amaranth populations from Kansas, and herbicide recommendations for managing GR Palmer amaranth in Roundup Ready 2 Xtend soybeans were presented to the Kansas growers in the annual conference of Cover Your Acres in winter 2019 in Oberlin, KS, Weed Schools across western Kansas, and Northwest and Southwest Kansas Ag Agent meetings. Research findings were also presented (oral and poster presentations) at annual meetings of the North Central Weed Science Society in Milwaukee (WI), the Weed Science Society of America in New Orleans (LA), and the Western Society of Weed Science in Denver (CO). These results have also been published in annual field research reports and one refereed journal article.

Table 1. Effect of herbicide programs on control of glyphosate–resistant Palmer amaranth, biomass accumulations and grain yield in Roundup Ready 2 Xtend Soybeans in field experiments conducted at Hays and Manhattan.

			Hays		Manhattan		Hays	Manhattan	Hays
Herbicide (s)	Timing [1, 2, 3]	Rate (oz/a)	21 DAPOST	42 DAPOST	15 DAPOST	32 DAPOST	Biomass (g/sq ft)	Biomass (g/sq ft)	Yield (bu/a)
			—— % control ——		—— % control ——				
Authority Elite	PRE	28	80 c	77 c	87 b	86 bc	5 ab	10 a	22.7 a
Zidua Pro	PRE	6	85 b	82 b	95 ab	93 ab	7.4 a	6.5 abc	18.2 ab
Fierce XLT	PRE	5	98 a	96 a	93 ab	91 b	0 d	8.5 ab	17.4 ab
Panther Pro	PRE	12	93ab	91 ab	92 ab	90 b	3.1 bc	9.8 a	20.2 ab
Authority Elite *fb* Engenia + Roundup	PRE *fb*	28	98 a	95 a	99 a	100 a	0.5 cd	0 c	17.1 ab
	POST	12.8 + 32						0 c	
Zidua PRO *fb* Engenia + Roundup	PRE *fb*	6	99 a	97 a	99 a	100 a	0 d	0 c	21.5 a
	POST	12.8 + 32							
Fierce XLT *fb* Engenia + Roundup	PRE *fb*	5	100 a	99 a	97 a	100 a	0 d	0.7 bc	20.1 ab
	POST	12.8 + 32							
Panther PRO *fb* Engenia + Roundup	PRE *fb*	12	100 a	99 a	96 a	98 a	0 d	0 c	17.1 ab
	POST	12.8 + 32							
Engenia + Roundup *fb* Engenia + Roundup	PRE *fb*	12.8 + 32	98 a	97 a	97 a	100 a	0.3 cd	0 c	21.9 a
	POST	12.8 + 32							
Engenia + Roundup *fb* Engenia + Roundup	EPOST *fb*	12.8 + 32	96 a	98 a	94 ab	99 a	0 d	0 c	17.5 ab
	LPOST	12.8 + 32							
Engenia + Roundup	POST	12.8 + 32	96 a	95 a	96 a	96 a	1.1 cd	0 c	20.8 ab
Hand-weeded	-	-	100 a	99 a	100 a	100 a	0 d	0 c	18.7 ab

[1] Abbreviation: PRE = preemergence; POST = postemergence; EPOST = early postemergence; LPOST = late postemergence; *fb* = followed by; DAPOST, days after POST.

[2] EPOST was sprayed at V3-V4 stage and LPOST was sprayed at R1 stage of soybean at both locations.

[3] Means within each column with similar alphabets are not different based on Fisher's protected LSD test ($P < 0.05$).

Figure 4. Visual response of glyphosate-resistant Palmer amaranth control in Roundup Ready 2 Xtend soybeans with PRE applied Authority Elite (A), Zidua Pro (B), Fierce XLT (C), Panther Pro (D), and Authority Elite PRE followed by Engenia + Roundup POST (E) at 32 DAPOST in Manhattan, KS.

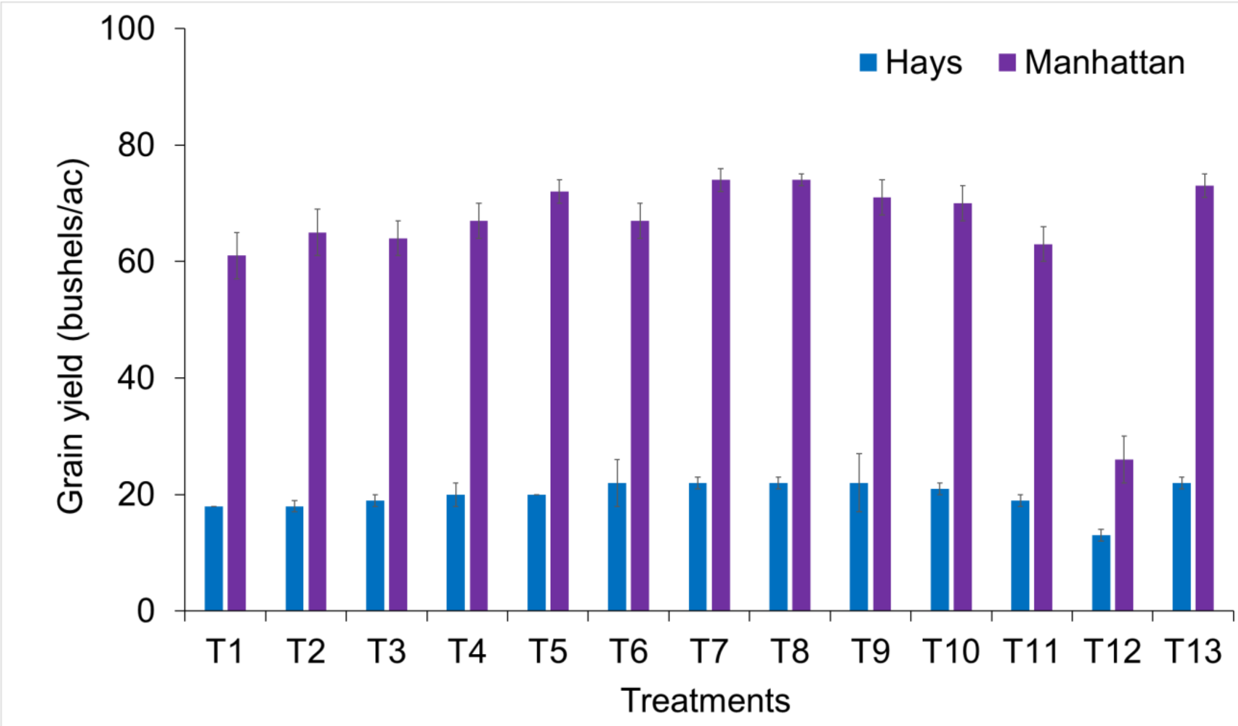


Figure 5. Effect of selected herbicide programs on Roundup Ready 2 Xtend® soybean grain yields at Kansas State University Agricultural Research Center near Hays, KS and Ashland Bottom research farm near Manhattan, KS in 2018.