

## Article

# Investigating the Efficacy of Selected Very-Long-Chain Fatty Acid-Inhibiting Herbicides on Iowa Waterhemp (*Amaranthus tuberculatus*) Populations with Evolved Multiple Herbicide Resistances

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**Abstract:** Very long chain fatty acid (VLCFA)-inhibiting herbicides (Herbicide group (HG) 15) have been applied to corn and soybean fields in Iowa since the 1960s. The VLCFA-inhibiting herbicides are now applied more frequently to control multiple herbicide-resistant (MHR) waterhemp (*Amaranthus tuberculatus* Moq. J.D. Sauer) populations that are ubiquitous across the Midwest United States as resistance to the VLCFA-inhibiting herbicides is not widespread. Waterhemp has evolved multiple resistances to herbicides from seven sites of action (HG 2, 4, 5, 9, 14, 15, and 27), and six-way herbicide-resistant populations have been confirmed. Thus, the objective of this study was to determine if selected Iowa waterhemp populations are less sensitive to VLCFA-inhibiting herbicides when additional herbicide resistance traits have evolved within the selected population. Dose–response assays were conducted in a germination chamber to determine the efficacy of three selected VLCFA-inhibiting herbicides (acetochlor, S-metolachlor, and flufenacet) on selected Iowa MHR waterhemp populations. An herbicide-susceptible, three-way, four-way, and five-way herbicide-resistant waterhemp population responded to the herbicide treatments differently; however, several of the four-way and five-way herbicide-resistant populations exhibited resistance ratios greater than 1 when treated with acetochlor and S-metolachlor. Selected four-way herbicide-resistant waterhemp populations from Iowa were subjected to a dose–response assay in the field using the same VLCFA-inhibiting herbicides, and all herbicides achieved control greater than 80% at the maximum labeled rate. The results of the experiments provide evidence that some MHR waterhemp populations may exhibit decreased susceptibility the VLCFA-inhibiting herbicides, but generally, these herbicides remain efficacious on Iowa MHR waterhemp populations.

**Keywords:** cross-resistance; herbicide resistance; residual herbicides; weed management

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## 1. Introduction

Very-long-chain fatty acids (VLCFA) inhibiting herbicides (herbicide group (HG) 15) interfere with the elongation of C18 fatty acid chains [1,2]. Very long chain fatty acid-inhibiting herbicides are generally considered more efficacious on monocotyledonous weeds, but they are also active on some small-seeded dicotyledonous weeds [3,4]. The VLCFA-inhibiting herbicides affect several elongases and mutations to the elongases that would provide resistance to the VLCFA-inhibiting herbicides can be lethal; thus, resistance to these herbicides is extremely rare [5,6]. Prior to 2019, resistance to VLCFA-inhibiting herbicides had been confirmed in five grass species globally: blackgrass (*Alopecurus myosuroides* Huds.), wild oat (*Avena fatua* L.), barnyardgrass (*Echinochloa crus-galli* L. P. Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and rigid ryegrass (*Lolium rigidum* Gaud.) [7]. However, resistance to the VLCFA-inhibiting herbicides evolved in two broadleaf species, waterhemp (*Amaranthus tuberculatus* Moq. J.D. Sauer) and Palmer amaranth (*Amaranthus palmeri* S. Watson) [8,9].

Waterhemp is an important weed in the Midwest United States and has rapidly evolved resistance to most herbicides used in corn and soybean production [9–12]. VLCFA-inhibiting herbicides are generally efficacious on herbicide-resistant waterhemp populations [13,14] despite evolved non-target-site resistance (NTSR) to herbicides that are present in the VLCFA-inhibiting herbicide-resistant waterhemp populations [9,15,16]. Cross-resistance due to NTSR may occur to other unrelated herbicides [17] and has been reported in multiple ( $\geq 3$ ) herbicide-resistant (MHR) rigid ryegrass, wild oat, and black grass populations [18,19]. We suggest that Iowa MHR waterhemp populations possess similar NTSR enzymes that efficiently detoxifies herbicides [20,21]. Thus, resistance to the VLCFA-inhibiting herbicides in waterhemp could evolve without mutations to the target site, like the VLCFA-inhibiting herbicide-resistant waterhemp population recently confirmed in Illinois [9].

Research describing the selected VLCFA-inhibiting herbicides efficacy on Iowa waterhemp populations is important as MHR waterhemp populations are pervasive; these herbicides are extensively and intensively applied to control the MHR waterhemp, and resistance to these herbicides has evolved in areas of relatively proximity [8,9,22]. If the control of the VLCFA-inhibiting herbicides is reduced on MHR waterhemp, the utility of this herbicide group as an effective weed management tool could be lost. Thus, the objectives of the study were to determine whether Iowa MHR waterhemp possessing NTSR respond differently to VLCFA-inhibiting herbicides, and if additional evolved herbicide resistances in a population influence control. The hypothesis of the study was different MHR profiles possessing NTSR in selected Iowa waterhemp populations will cause a differential response to the VLCFA-inhibiting herbicides, and control will decrease when the waterhemp populations possess more herbicide resistance traits.

## 2. Materials and Methods

### 2.1. Germination Chamber Dose–Response Assay

A dose–response assay was conducted in a germination chamber (Percival Scientific, Inc., Perry, IA, USA) at Iowa State University to assess the susceptibility of an herbicide-susceptible and MHR waterhemp population to select VLCFA-inhibiting herbicides. The herbicides used in the germination chamber dose–response assay included acetochlor (Harness<sup>®</sup>, 2.7 kg active ingredient (ai) ha<sup>-1</sup> (maximum labeled rate), Bayer CropScience, Chesterfield, MO, USA), S-metolachlor (Dual II Magnum<sup>®</sup>, 2.1 kg ai ha<sup>-1</sup>, BASF, Research Triangle Park, NC, USA), and flufenacet (Cadou<sup>®</sup>, 0.8 kg ai ha<sup>-1</sup>, Bayer CropScience, Chesterfield, MO, USA). The three herbicides were chosen as acetochlor is the most efficacious VLCFA-inhibiting herbicide on *Amaranthus* spp. followed by S-metolachlor then flufenacet [8,9,22,23]. Any segregating response(s) to these herbicides by the Iowa MHR waterhemp populations could provide evidence of decreased or increased control. The field rates were converted from 1.12 ai kg ha<sup>-1</sup> to parts per million (ppm). The experimental design was completely randomized with two replications. The experiment was conducted three times.

The tested MHR waterhemp populations were selected from populations collected across Iowa in 2011 [11]. The collected waterhemp populations were subjected to a dose–response assay which included the following herbicides: acetolactate synthase (EC 2.2.1.6)-inhibiting (ALS, HG 2) (imazethapyr, 105 g ai ha<sup>-1</sup>), photosystem II (EC 1.10.3.9)-inhibiting (PSII, HG 5) (atrazine, 2225 g ai ha<sup>-1</sup>), 5-enolpyruvylshikimate-3-phosphate synthase (EC 2.5.1.19)-inhibiting (glyphosate, 1264 g ai ha<sup>-1</sup> (HG 9)), protoporphyrinogen oxidase (EC 1.3.3.4)-inhibiting (PPO, HG 14) (lactofen, 220 g ai ha<sup>-1</sup>), and 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)-inhibiting (HPPD, HG 27) (mesotrione, 105 g ai ha<sup>-1</sup>) herbicides. The herbicide resistance profiles of the selected MHR waterhemp populations included 3-way herbicide resistance (HG 2, 5, and 27), 4-way herbicide resistance (HG 2, 5, 9, and 27), and 5-way herbicide resistance (HG 2, 5, 9, 14, and 27) (Table 1). Two populations of each MHR phenotype were selected to assess if populations expressed similar susceptibility to the herbicides. The waterhemp population resistance profiles were confirmed in

the greenhouse and >75% of the individuals of the population expressed resistance to 4x of the maximum-labeled rates of respective herbicides, which is accepted to be classified as herbicide-resistant. The herbicide-susceptible population was collected along the edge of a wooded area adjacent to an agricultural field with minimal herbicide and adjacent pollen exposure at the Iowa State University Curtiss Farm, Ames, Iowa, USA (42.00° N, 93.66° W) in 2006. The herbicide-susceptible population underwent the herbicide screen to confirm the susceptibility of the population. The seeds of each waterhemp population were kept in cold storage at 6 °C for one year. Seeds from each population were then placed in a petri dish with a small amount of water and kept at 6 °C for two weeks to break dormancy. The petri dishes were then placed into a dryer without lids for 48 h at 45 °C. Seeds were then kept in cold storage at 6 °C until needed.

**Table 1.** Descriptions of multiple herbicide-resistant waterhemp populations collected in 2011 and used in the germination chamber very long chain fatty acid-inhibiting herbicide dose–response experiment.

Classification	Abbreviation	Herbicide Group [HG]	Resistance Profile	Location
Herbicide-Susceptible	C		Susceptible	Story County, Iowa, USA
3-Way Resistant	3A		2, 5, 27	Henry County, Iowa, USA
3-Way Resistant	3B		2, 5, 27	Cherokee County, Iowa, USA
4-Way Resistant	4A		2, 5, 9, 27	Monona County, Iowa, USA
4-Way Resistant	4B		2, 5, 9, 27	Plymouth County, Iowa, USA
5-Way Resistant	5A		2, 5, 9, 14, 27	Not Recorded
5-Way Resistant	5B		2, 5, 9, 14, 27	Woodbury County, Iowa, USA

Dose–response assays were conducted by adding 6 mL of different herbicide concentrations to petri dishes containing blue blotter paper. Herbicide concentrations were established based on a 3.16 log scale from 0.1 to 10.0 ai ppm along with a non-treated control. Twenty stratified seeds were placed on the treated blue blotter paper inside of the petri dishes and placed in the germination chamber. The germination chamber was adjusted to 14 h photoperiods with 25/20 °C diurnal temperature. Light was supplemented by mercury halide light providing 600–1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density.

Waterhemp control was determined by visually counting the number of treated seeds that germinated and the number of germinated non-treated seeds 2 days after treatment (DAT). Once the number of germinated seeds was recorded, the number of germinated treated seeds was divided by the number of germinated non-treated seeds to obtain a quotient to represent the percent of control. The quotient was then multiplied by 100 to obtain the percent of waterhemp control, where 0% equaled no control and 100% equaled complete control. The evaluation time was selected as approximately 90% germination had occurred in the non-treated controls after 2 days: radicals were visible, and the lack of edaphic conditions in the petri dish allowed for a better seed–herbicide interaction to exacerbate the time to incur phytotoxicity.

## 2.2. Field Dose–Response Assay

The susceptibility of MHR waterhemp to the VLCFA-inhibiting was further characterized in the field with natural waterhemp population infestations. The VLCFA-inhibiting herbicide dose–response assays were conducted in Grundy County, Iowa (42.22° N, 92.35° W) in 2016 and Story County, Iowa USA (42.00° N, 93.25° W) in 2017. The soil types for the Grundy County site were a Tama silty clay loam (fine-silty, mixed, superactive, mesic typic argiudoll) and Sawmill–Garwin complex (fine-silty, mixed, superactive, mesic cumulic and typic endoaquoll) with a pH of 7.1 and 4.0% organic matter. The soil types for the Story County site were a Clarion loam (fine-loamy, mixed, superactive, mesic typic hapludoll) and Nicolett loam (fine-loamy, mixed, superactive, mesic aquic hapludoll) with a pH of 6.8 and 3.0% organic matter. Both waterhemp populations expressed 4-way resistance (Grundy, HG 2, 5, 9 and 27; Story County, HG 2, 5, 14, and 27). Evidence of herbicide resistance was anecdotally determined by seeing death and survival of treated waterhemp

plants with the respective herbicide at the maximum labeled rate. The experimental design was a randomized complete block replicated three times. Plots were 3.0 by 7.6 m, and herbicides were applied one day after crop planting using 140 L ha<sup>-1</sup> carrier volume through a CO<sub>2</sub>-powered backpack sprayer with TeeJet AI110015 nozzles (Spraying Systems Co., Wheaton, IL, USA). Experiments were conducted with crop competition (corn) and no crop competition (fallow) in 2016 and 2017, respectively. Acetochlor (2.7 kg ai ha<sup>-1</sup>), S-metolachlor (2.1 kg ai ha<sup>-1</sup>), and flufenacet (0.8 kg ai ha<sup>-1</sup>) were applied at 0.25x, 0.5x, 1x, 2x, and 4x, where the 1x amount was based on the maximum-labeled rate for each herbicide. A non-treated control was included in the experiments as well. Both experiment sites received approximately 2.5 cm of natural rain within 7 days after treatment to facilitate herbicide activity. Waterhemp control was visually evaluated, and ratings were conducted at 28 days after treatment (DAT) using a scale from 0% to 100%, where 0% equaled no control and 100% equaled complete control.

### 2.3. Statistical Analysis

All statistical analyses were performed using Statistical Analysis Software, SAS 9.3 (Statistical Analysis Systems version 9.3, SAS Institute, Cary, NC, USA). Dose–response data from germination chamber and field assays were subjected to an analysis of variance using the GLIMMIX procedure in SAS. Means and interactions of significant effects were separated using Fisher’s protected LSD test at  $p \leq 0.05$ .

Dose–response curves for the germination chamber assay were created using a logarithmic curve Equation (1):

$$Y = Y_0 + A \times \ln(X) \quad (1)$$

where Y = percent of waterhemp control, Y<sub>0</sub> and A are nonlinear constants, and X = herbicide concentration. Deviations from the curve are described by  $r^2$ . The two-parameter logarithmic model estimated the lethal rate to control 50% (LD<sub>50</sub>) and 90% (LD<sub>90</sub>) of the population for each herbicide and waterhemp population.

Dose–response curves for the field assay were created using a sigmoidal curve Equation (2):

$$Y = Y_0 + A / (1 + \exp(-(X - X_0)/B)) \quad (2)$$

where Y = percent of waterhemp control, Y<sub>0</sub> = maximum waterhemp control, X<sub>0</sub> = maximum rate, and A and B are nonlinear constants. Deviations from the curve are described by  $r^2$ . The 4-parameter sigmoidal model estimated the herbicide rate that caused a LD<sub>50</sub> and a LD<sub>90</sub> for each herbicide and waterhemp population.

The data points from both experiments were used to derive the model equations on SigmaPlot 12.5 (Systat Software, Inc. version 12.5, San Jose, CA, USA). Resistance ratios (R/S) were calculated by dividing the LD<sub>50</sub> of the resistant (R) populations by the LD<sub>50</sub> of the susceptible (S) population, respectively [24].

## 3. Results and Discussion

### 3.1. Responses of MHR Waterhemp Populations to VLCFA-Inhibiting Herbicides under Germination Chamber Conditions

Herbicide, rate, and waterhemp population were significant effects for efficacy ( $p < 0.001$ ). Significant interactions were detected; thus, data were separately analyzed across all main effects. Acetochlor and S-metolachlor provided the highest efficacy across all concentrations followed by flufenacet (Tables 2 and 3). The calculated resistance for acetochlor were all under 2 (Table 2). The low resistance ratios from this experiment provide evidence that the tested MHR waterhemp populations are still susceptible to acetochlor (Table 2). Other research found that acetochlor was the most efficacious VLCFA-inhibiting herbicide on MHR *Amaranthus* spp. [8,9]. However, the doses of acetochlor to achieve an LD<sub>90</sub> on all waterhemp populations were higher than the maximum labeled rate (2.7 ppm) (Table 2). Although the resistance ratios were under 2, the 3A, 4A, and 5B populations expressed resistance ratios greater than 1 (Table 2). While very high resistance ratios are indicative of a target-site mutation, NTSR usually does

incur a high resistance ratio [9,24,25]. This result could be foreshadowing that Iowa MHR waterhemp populations could shift from acetochlor-susceptible to -resistant under recurrent selection pressure [24,26].

**Table 2.** Waterhemp population control with acetochlor under germination chamber conditions.

Population <sup>a</sup>	LD <sub>50</sub> <sup>b</sup>	LD <sub>90</sub> <sup>b</sup>	R/S <sup>c</sup>	Efficacy Curve
C	1.2	NA <sup>d</sup>		$Y = 45.8 + 18.6 \times \ln(X), r^2 = 0.81$
3A	1.1	6.1	1.1	$Y = 49.6 + 22.4 \times \ln(X), r^2 = 0.75$
3B	0.5	5.6	0.4	$Y = 62.3 + 16.2 \times \ln(X), r^2 = 0.78$
4A	1.5	NA	1.3	$Y = 41.1 + 20.6 \times \ln(X), r^2 = 0.86$
4B	0.9	6.2	0.8	$Y = 52.9 + 20.3 \times \ln(X), r^2 = 0.94$
5A	0.8	6.3	0.7	$Y = 55.2 + 18.9 \times \ln(X), r^2 = 0.77$
5B	1.4	10.0	1.2	$Y = 44.0 + 20.0 \times \ln(X), r^2 = 0.77$

<sup>a</sup> 3A,B = 3-way herbicide-resistant; 4A,B = 4-way herbicide-resistant; 5A,B = 5-way herbicide-resistant, C = herbicide susceptible. <sup>b</sup> LD<sub>50,90</sub> = The herbicide rate that provided control for 50% and 90% of the waterhemp plants. <sup>c</sup> Resistance ratio = R/S = LD<sub>50</sub> (multiple herbicide-resistant waterhemp population) LD<sub>50</sub> (herbicide-susceptible waterhemp population)<sup>-1</sup>. <sup>d</sup> NA = not achieved.

**Table 3.** Waterhemp population control with S-metolachlor under germination chamber conditions.

Population <sup>a</sup>	LD <sub>50</sub> <sup>b</sup>	LD <sub>90</sub> <sup>b</sup>	R/S <sup>c</sup>	Efficacy Curve
C	1.2	NA <sup>d</sup>		$Y = 41.2 + 14.6 \times \ln(X), r^2 = 0.84$
3A	0.4	4.5	0.3	$Y = 66.3 + 15.7 \times \ln(X), r^2 = 0.96$
3B	0.7	8.3	0.6	$Y = 55.5 + 16.2 \times \ln(X), r^2 = 0.91$
4A	2.6	NA	2.2	$Y = 37.5 + 13.1 \times \ln(X), r^2 = 0.83$
4B	3.2	NA	2.7	$Y = 37.5 + 13.1 \times \ln(X), r^2 = 0.69$
5A	1.4	NA	1.2	$Y = 43.5 + 18.3 \times \ln(X), r^2 = 0.72$
5B	2.1	NA	1.8	$Y = 39.7 + 14.2 \times \ln(X), r^2 = 0.66$

<sup>a</sup> 3A,B = 3-way herbicide-resistant; 4A,B = 4-way herbicide-resistant; 5A,B = 5-way herbicide-resistant, C = herbicide susceptible. <sup>b</sup> LD<sub>50,90</sub> = The herbicide rate that provided control for 50% and 90% of the waterhemp plants. <sup>c</sup> Resistance ratio = R/S = LD<sub>50</sub> (multiple herbicide-resistant waterhemp population) LD<sub>50</sub> (herbicide-susceptible waterhemp population)<sup>-1</sup>. <sup>d</sup> NA = not achieved.

Resistance ratios for S-metolachlor was under 2 for all MHR populations except for the four-way herbicide-resistant waterhemp populations (4A, R/S = 2.2; 4B, R/S = 2.7) (Table 3). An LD<sub>90</sub> for S-metolachlor was only achieved on the three-way herbicide-resistant populations, which was above the maximum labeled rate (2.1 ppm) (Table 3). The higher resistance ratio achieved by the four-way herbicide-resistant waterhemp population when treated with S-metolachlor is parallel with other studies confirming resistance to the VLCFA-inhibiting herbicides (8; 9). The five-way herbicide-resistant waterhemp populations exhibited resistance ratios greater than 1, which suggests decreased susceptibility [24] (Table 3). Again, this could suggest that Iowa MHR waterhemp populations could evolve from S-metolachlor-susceptible to -resistant under recurrent selection pressure [24,26].

Resistance ratios for flufenacet could not be calculated, as the herbicide concentrations used were too low to establish an LD<sub>50</sub> or LD<sub>90</sub>. While higher rates could have been examined, the tested scale encompassed the maximum labeled rate (0.8 ppm) and would have not provided useful information. This result provides further evidence that flufenacet alone will not control waterhemp but does not dismiss the evolution of resistance to this herbicide. Flufenacet is usually applied for grass control with other efficacious herbicides when waterhemp is present (Wrucke, Bayer CropScience, Minneapolis, MN, USA, personal communication 2018 [23]).

Only one herbicide-susceptible waterhemp population was included in the study given that herbicide-susceptible populations are rare and difficult to discover. Thus, we suggest that the population included in the research is an acceptable representative of the susceptibility other herbicide-susceptible waterhemp populations to the VLCFA-inhibiting herbicides [27–29]. It is worth noting that the herbicide-susceptible waterhemp population used in the experiment was not collected from a commercial agricultural field like the MHR

waterhemp populations, in part due to the challenge of finding waterhemp populations in Iowa that have not evolved resistance to at least one herbicide site of action (Owen, Iowa State University, Ames, IA, USA, personal communication 2018).

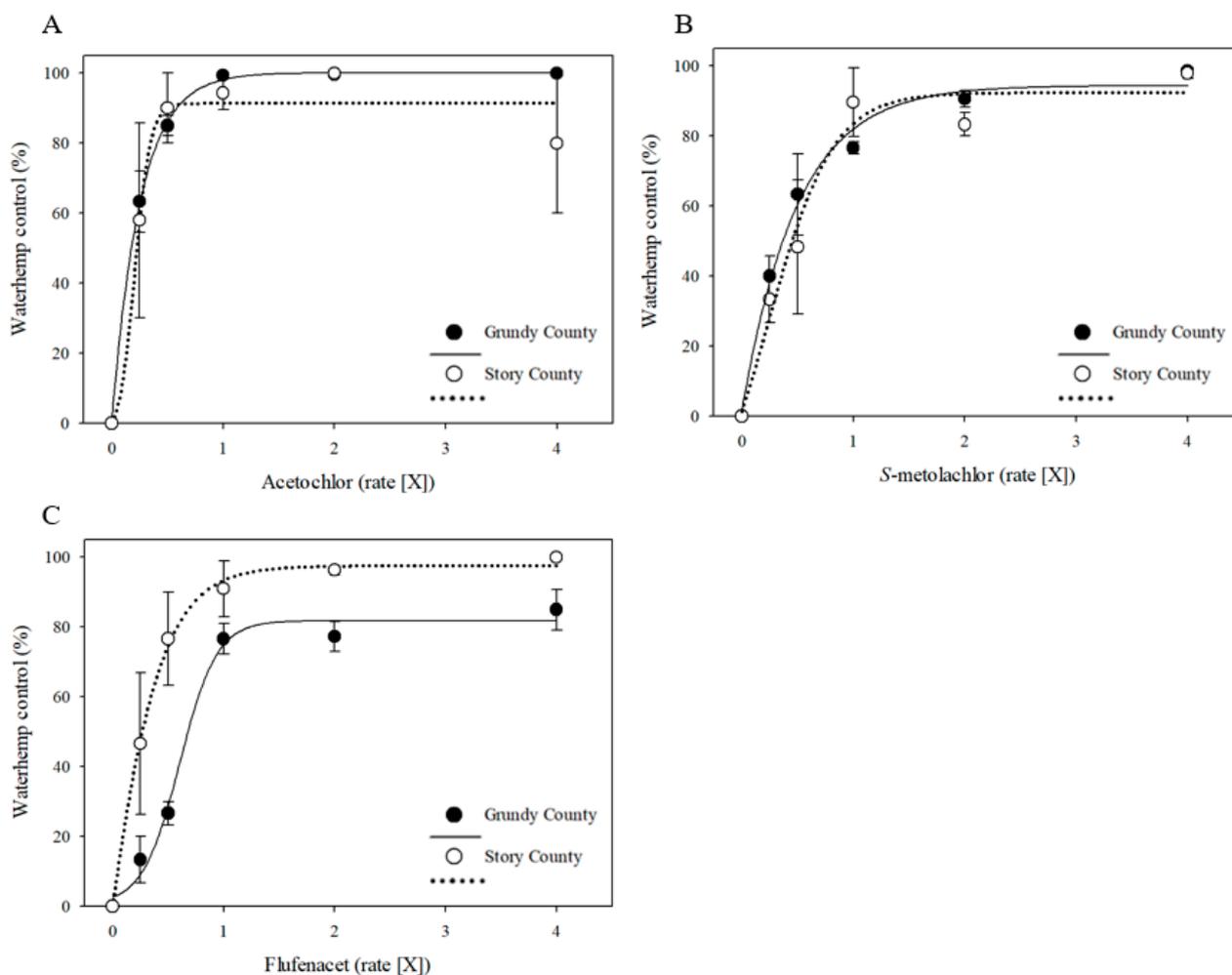
### 3.2. Responses of MHR Waterhemp Populations to VLCFA-Inhibiting Herbicides under Field Conditions

Although the two field experiments were conducted with crop and sans crop environments, the herbicides would have controlled the emerging weeds before crop emergence making a difference of crop presence negligible. Further statistical evidence that crop presence was negligible was that the location of the experiment was not a significant effect ( $p = 0.90$ ). Herbicide, rate, and the interaction between the two main effects were significant on waterhemp control ( $p < 0.001$ ). No other significant interactions were detected. Acetochlor provided the highest control across all rates followed by *S*-metolachlor and flufenacet 28 DAT (Figure 1). No difference in control was observed between the populations when treated with acetochlor and *S*-metolachlor; control with flufenacet was higher on the Story County population compared to the Grundy County population (Figure 1). The LD<sub>50</sub> and LD<sub>90</sub> for acetochlor was below the maximum labeled rate for both populations (LD<sub>50</sub>: Grundy County = 0.18x, Story County = 0.22x; LD<sub>90</sub>: Grundy County = 0.58x; Story County = 0.50x). The LD<sub>50</sub> for *S*-metolachlor was under the maximum labeled rate for both populations (Grundy County = 0.44x; Story County = 0.37x), while the LD<sub>90</sub> was above the maximum labeled rate (Grundy County = 1.35x; Story County = 1.53x). The LD<sub>50</sub> for flufenacet on the Grundy County population was under the maximum labeled rate (0.67x), but an LD<sub>90</sub> could not be calculated with the tested rates. The LD<sub>50</sub> and LD<sub>90</sub> for flufenacet on the Story County population was below the maximum labeled rate (LD<sub>50</sub> = 0.26x; LD<sub>90</sub> = 0.82x). However, all herbicides at the maximum-labeled rate provided control above 80% at 28 DAT across both populations; acetochlor achieved the highest control (97%), followed by *S*-metolachlor (83%) and flufenacet (84%) (Figure 1). The waterhemp control with flufenacet was higher than expected, as usually this herbicide is tank-mixed with other herbicides to control waterhemp (Wrucke, Bayer CropScience, Minneapolis, MN, USA, personal communication 2018 [23]). However, the differential response between the Grundy and Story County populations to flufenacet treatments indicates that control can be variable. The Iowa MHR waterhemp populations were controlled better with the VLCFA-inhibiting herbicides compared to herbicide-resistant waterhemp populations in Illinois and Kansas [9,18]. These results suggest that Iowa MHR waterhemp populations still exhibit susceptibility to VLCFA-inhibiting herbicides under field conditions.

The results of germination chamber dose–response assay reject the null hypothesis as four- and five-way herbicide-resistant waterhemp populations exhibited decreased susceptibility to acetochlor and *S*-metolachlor. However, in the field assay, there was a failure to reject the null hypothesis as all tested VLCFA-inhibiting herbicides applied at the maximum labeled rate provided the same control (>80%) on the MHR waterhemp populations. Thus, based on our results from both assays, the VLCFA-inhibiting herbicides will likely continue to be useful tools for aiding in MHR waterhemp control, but use of these herbicides should be with caution. Very long chain fatty acid-inhibiting herbicides are being applied more frequently to control herbicide-resistant waterhemp [22,29,30]. Recommendations include “layering” the herbicide applications, by applying VLCFA-inhibiting herbicides preemergence and again postemergence [13,30,31]. The concurrent use of any management agent will likely select for herbicide-resistant weed phenotypes [32,33]. Thus, multiple applications of VLCFA-inhibiting herbicides during a growing season increase the risk of selecting for resistance in Iowa waterhemp populations.

Future research should investigate the specific mechanisms of resistance in waterhemp populations to determine if target site, non-target site, or both exist. The hypothesis of this research was that VLCFA-inhibiting herbicides would be less efficacious in increasing the number of herbicide-resistant traits in Iowa waterhemp populations, which could be due to the ability to metabolize xenobiotics non-selectively (34) [34]. Knowledge of the herbicide resistance mechanisms in waterhemp populations would better explain if

target or non-target site resistance mechanisms exist and may help to describe why the herbicides are still efficacious and why resistance has not (yet) evolved in the selected MHR waterhemp populations. Since the MHR waterhemp populations were only sampled in Iowa, there would be value in determining if MHR waterhemp populations from other U.S. states respond similarly to the VLCFA-inhibiting herbicides. This information will provide information if there are MHR waterhemp population(s) that exhibit reduced susceptibility to the VLCFA-inhibiting herbicides, resulting in the loss of a previously efficacious tool for weed control.



**Figure 1.** Waterhemp control with acetochlor (**A**), *S*-metolachlor (**B**), and flufenacet (**C**) applied at the multiplicative of the maximum labeled rate (*X*) on 4-way herbicide-resistant waterhemp populations from Grundy (2016) and Story County (2017), Iowa 28 days after crop emergence under field conditions. Herbicide and rate were significant effects ( $p < 0.001$ ) on waterhemp control, while the population was not ( $p = 0.90$ ). No significant interactions were detected. The lethal dose to control 50% of the plants ( $LD_{50}$ ) and the lethal dose to control 90% of the plants ( $LD_{90}$ ) for acetochlor was below the maximum labeled rate for both populations ( $LD_{50}$ : Grundy County =  $0.18x$ , Story County =  $0.22x$ ;  $LD_{90}$ : Grundy County =  $0.58x$ ; Story County =  $0.50x$ ). The  $LD_{50}$  for *S*-metolachlor was under the maximum labeled rate for both populations (Grundy County =  $0.44x$ ; Story County =  $0.37x$ ), while the  $LD_{90}$  was above the maximum labeled rate (Grundy County =  $1.35x$ ; Story County =  $1.53x$ ). The  $LD_{50}$  for flufenacet on the Grundy County population was under the maximum labeled rate ( $0.67x$ ), but an  $LD_{90}$  could not be calculated with the tested rates. The  $LD_{50}$  and  $LD_{90}$  for flufenacet on the Story County population was below the maximum labeled rate ( $LD_{50} = 0.26x$ ;  $LD_{90} = 0.82x$ ). The rates needed to achieve the  $LD_{50}$  and  $LD_{90}$  were calculated from 4-parameter sigmoidal curves. Acetochlor: Grundy County =  $-13365 + 13465/(1 + \exp(-(X + 1.24)/0.25))$ ,  $r^2 = 0.99$ ; Story County =  $-4.9 + 93.6/(1 + \exp(-(X - 0.2)/0.07))$ ,  $r^2 = 0.97$ . *S*-metolachlor: Grundy County =  $7 - 9674 + 79769/(1 + \exp(-(X + 3.3)/0.5))$ ,  $r^2 = 0.98$ ; Story County =  $-38.6 + 131/(1 + \exp(-(X + 0.2)/0.3))$ ,  $r^2 = 0.93$ . Flufenacet: Grundy County =  $0.6 + 81.2/(-(X + 0.6)/0.2)$ ,  $r^2 = 0.97$ ; Story County =  $-202.8 + 300.4/(-(X + 0.2)/0.34)$ ,  $r^2 = 0.99$ .

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