**INVESTIGATIONS OF 2,4-D-RESISTANT WATERHEMP AND SEED TREATMENT
AND HERBICIDE INTERACTIONS IN SOYBEAN**

A Thesis

Presented to

The Faculty of the Graduate School

At the University of Missouri-Columbia

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

BLAKE REMINGTON BARLOW

Dr. Kevin Bradley, Thesis Supervisor

July 2017

The undersigned, appointed by the dean of the Graduate School, have examined the Thesis
entitled

**INVESTIGATIONS OF 2,4-D-RESISTANT WATERHEMP AND SEED TREATMENT
AND HERBICIDE INTERACTIONS IN SOYBEAN**

Presented by Blake Remington Barlow,

A candidate for the degree of

Master of Science

And hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Kevin W. Bradley

Dr. Raymond E. Massey

Mr. Gregory A. Luce

ACKNOWLEDGEMENTS

There are several individuals who I cannot thank enough for making this journey of getting a master's degree in weed science possible. First, I would like to thank my advisor Dr. Kevin Bradley for taking me on as a graduate student. I have learned more than I ever anticipated in the past two and a half years under your guidance. It is has been an honor to have studied under someone who is as hard working and dedicated to his profession as you are. You have served as a great role model both professionally and personally. I could not have asked for a better advisor throughout this endeavor. Thank you!

I would also like to thank Dr. Mandy Bish for everything you have done for me. If there was ever a time that I had a question, I always knew that Mandy would be there and would do anything to help me; I cannot thank you enough. I apologize for the countless hours that you spent in the greenhouse thinning thousands of waterhemp plants from each flat. I would also like to thank Meghan Biggs for all of the countless hours that were spent in the greenhouse and field supporting my research. Alex Long and David Kleinsorge, thank you guys for always guiding me in the right direction and your willingness to assist with any task. I would like to thank fellow graduate students Jaime Farmer, Zach Trower, Eric Oseland, Derek Whalen, Shea Farrell, and Brian Dintelmann for always being willing to jump in and help whenever it was needed. To the undergraduate student workers Austin Straatmann, Drake Gleason, Wyatt Coffman, Blake Kasten, and Jesse Kleinheider, I know it wasn't always fun, but thank you all for

everything that you did to help me on my projects. Thank you to my committee members Dr. Raymond Massey and Gregory Luce for taking the time out of your busy schedules to assist me in this process.

Most importantly I thank all of my friends and family for motivation, support, and love. Mom and Dad, words cannot describe how much I appreciate the love and support that you two have given me, not just through this process, but since the beginning. To my sisters Ashley and Amber thank you for always talking me through things whenever I would be stressed to the max and call you guys. You guys have always been there and encouraged and supported me, and for that I am thankful. Lastly, I would like to thank my soon-to-be wife Beca. Thank you for always encouraging me and being patient with how stubborn I am. Your love and support has kept me motivated and thankful for all I have. I can only hope that I can show you the same in the chapters of our life to come. There is no way that I could have completed this amazing chapter of my life without all of you in it. Thank you all very much!

TABLE OF CONTENTS

Acknowledgements	ii
List of Tables	v
List of Figures.....	vi
CHAPTER I: Literature Review	1
Waterhemp.....	1
Resistance	2
Herbicide Resistant Weed Management Strategies	5
New Technologies	6
Soybean Seed Treatments	7
Pre-Emergence Residual Herbicides.....	9
Interaction Between Seed Treatment and PRE Herbicides.....	10
Summary and Objectives	10
Literature Cited	12
CHAPTER II: Investigations of the Potential Interactions Between Pre-emergence Residual Herbicides and Seed Treatment in Soybean.....	21
Abstract	21
Introduction.....	23
Materials and Methods.....	25
Results and Discussion	28
Literature Cited	34
CHAPTER III: Investigations of 2,4-D Resistance in a Missouri Waterhemp Population	49
Abstract	49
Introduction.....	50
Materials and Methods.....	52
Results and Discussion	55
Literature Cited	62

LIST OF TABLES

Table	Page
Chapter II:	
2.1 Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr average from April through October in 2015 and 2016 at the Boone and Carroll county research locations.	38
2.2 Source of materials used in the experiments.	39
2.3 Analysis of variance for visual soybean injury, leaf area, stand, height, biomass, and yield 30 days after soybean emergence (DAE) in 2015 and 2016.	41
2.4 Influence of varieties, herbicides, and seed treatments on soybean visual injury, stand, and leaf area 30 days after soybean emergence in 2015 and 2016.	42
2.5 Influence of varieties, herbicides, and seed treatments on soybean height, biomass, and yield 30 days after soybean emergence in 2015 and 2016.	46
Chapter III:	
3.1 Herbicide rates and adjuvants used in field experiments in 2015 and 2016 at the suspected resistant waterhemp site.	67
3.2 Sources of materials used in the experiments.	69
3.3 Herbicide rates and adjuvants used in greenhouse dose-response experiments conducted in 2015 and 2016.	70
3.4 Visual injury and biomass reduction in response to different herbicide chemistries and doses at the Renick, Missouri field site in 2015 and 2016.	72
3.5 Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr averages from May through October in 2015 and 2016 at the Renick, Missouri field site.	74
3.6 Estimates of the herbicide dose resulting in 50% reduction in survival and visual control for the MO-Ren and MO-S waterhemp populations 21 DAA.	75

LIST OF FIGURES

Figure		Page
	Chapter III:	
3.1	Survival of a suspected multiple-resistant (MO-Ren) and susceptible (MO-S) waterhemp population 21 DAA in response to 2,4-D, fomesafen, atrazine, glyphosate, mesotrione, chlorimuron, and dicamba.	76
3.2	Visual control of a suspected multiple-resistant (MO-Ren) and susceptible (MO-S) waterhemp population 21 DAA in response to 2,4-D, fomesafen, atrazine, glyphosate, mesotrione, chlorimuron, and dicamba.	77

Chapter 1

Literature Review

Waterhemp

Amaranthus species are the most troublesome weed species currently found in United States production agriculture (Bradley et al. 2007; Legleiter and Bradley 2008; Rosenbaum and Bradley 2013; Sellers et al. 2003; Webster et al. 2005). There are ten *Amaranthus* species that are common in the United States, including the dioecious species: common waterhemp (*Amaranthus rudis*), tall waterhemp (*Amaranthus tuberculatus*), Palmer amaranth (*Amaranthus palmeri*), and sandhills amaranth (*Amaranthus arenicola*) and the monoecious species, smooth pigweed (*Amaranthus hybridus*), redroot pigweed (*Amaranthus retroflexus*), Powell amaranth (*Amaranthus powellii*), tumble pigweed (*Amaranthus albus*), prostrate pigweed (*Amaranthus blitoides*), and spiny amaranth (*Amaranthus spinosus*) (Gleason and Cronquist 1963; Hager et al. 2002). Of these *Amaranthus* species, common waterhemp and tall waterhemp are the most common and problematic weeds that farmers throughout the Midwest must contend with when growing corn (*Zea mays* L.) or soybean (*Glycine max* L.) (Norbdy et al. 2007). Since common waterhemp and tall waterhemp are genetically similar and a great deal of hybridization has occurred between the two, most botanists now group the two species into one collective “waterhemp” species (*Amaranthus tuberculatus*) (Norbdy et al. 2007; Pratt and Clark 2001). Waterhemp thrive and are found predominantly in wet areas of agriculture fields, but can adapt to a variety of conditions (Rosenbaum and Bradley 2013). *Amaranthus* species, and specifically waterhemp, are very prolific seed producers. A single female plant is able to produce 250,000 seeds in a single growing season, with some plants able to produce as many as 1,000,000 seeds

(Hager et al. 1997; Patzoldt et al. 2002; Sellers et al. 2003). Waterhemp also has an extremely aggressive growth habit, and will germinate early in the growing season, within 350 growing degree days, but also longer into the growing season than most summer annual weeds (Hager et al. 2002; Sellers et al. 2003).

Amaranthus species are troublesome due to their ability to compete for water, nutrients, sunlight and other resources. It was shown in a 3 year study that soybean yield was reduced by 55% because of competition with smooth pigweed (Moolani et al. 1964). Redroot pigweed, Palmer amaranth, and waterhemp at a density of 8 plants m⁻¹ of row also reduced soybean yield by 38, 79, and 56%, respectively in soybean (Bensch et al. 2003). Hager et al. (2002) observed that waterhemp at densities ranging from 89 to 362 plants per m² resulted in a 43% reduction in soybean yield. Waterhemp with densities ranging from 82 to 445 plants per m² reduced corn yields from 10% to 36% (Cordes et al. 2004).

Resistance

Herbicide resistance is defined as the inherited ability of a plant to survive and reproduce following exposure to a dose of a herbicide that would normally be lethal to the wild type (Prather et al. 2000). For herbicides, factors that affect how rapidly weed populations shift to predominantly resistant individuals include (1) the intensity of the selection (which is a function of herbicide dose, frequency, and timing of application), (2) mutation rate and the initial frequency of resistant individuals in the population exposed to the herbicide, (3) the genetic basis of the resistance (mode of inheritance, dominance), (4) life-history characteristics of the weed species (such as annual vs. perennial life cycle, self-fertilization vs. cross-pollination, fecundity, extent of seed dormancy), and (5) the rate of reproduction and potential for recruitment of

susceptible or resistant individuals from outside the population (e.g., from the soil seedbank or by immigration) (Norsworthy et al. 2012). The first incidences of evolved herbicide resistance were reported in wild carrot (*Daucus carota* L.), which evolved resistance to the auxin analog class of herbicides after several seasons of consecutive use of 2,4-D (Switzer 1957; Whitehead and Switzer 1963), and common groundsel (*Senecio vulgaris* L.), which evolved resistance to simazine and atrazine after receiving either one or two annual applications of these herbicides for 10 consecutive years (Ryan 1970).

Glyphosate is a broad spectrum herbicide that inhibits 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme found only in plants and certain bacteria (Dill 2005). Prior to the introduction of glyphosate resistant crops, glyphosate was traditionally used in non-cropping situations and orchards (Dill 2005). In 1996, soybean was the first glyphosate-resistant crop that was introduced (Dill 2005). Since 1996, glyphosate-resistant crops have been rapidly adopted (Fernandez-Cornejo and McBride 2002). The percentage of herbicide tolerant soybeans went from 17 percent in 1997 to 68 percent in 2001 to 94 percent in 2016, and the large majority of these varieties were glyphosate resistant (USDA 2016). Most producers rely on herbicides as the predominant method to achieve weed control (Young 2006). One practice that can lead to resistance in a weed biotype is the continuous use of a single active ingredient or mechanism of action (MOA) over a long period of time, resulting in the selection of resistant plants (Al-Khatib et al. 1998; Jasieniuk et al. 1996). This occurred soon after the introduction of glyphosate-resistant crops. Producers went from controlling their weed with tillage, crop rotation, and switching between herbicides with different mechanisms of actions to an almost complete dependence on glyphosate alone for weed control. The first glyphosate-resistant waterhemp biotype was identified in Platte County, Missouri in 2005 (Bradley et al. 2006; Heap 2016).

There are now glyphosate-resistant waterhemp biotypes found in 18 states, and one case in Ontario, Canada (Heap 2016). In a more recent survey of waterhemp populations in Missouri, Schultz et al. (2015a) found that 99% were resistant to ALS-inhibitors, 29% exhibited resistance to glyphosate, 30% were resistant to PSII-inhibitors, 5% were resistant to PPO inhibitors, and 2% were resistant to HPPD inhibitors.

Waterhemp is a dioecious plant, so gene mutation is more likely than with monocious plants because genes can be transferred between male and females via pollen (Steckel 2007; Tranel et al. 2010). Waterhemp has been reported to have resistance to herbicides from one or more of the following sites of action: 5-Enolpyruvylshikimate-3-phosphate synthase inhibitor (EPSPS), acetoacetate synthase inhibitor (ALS), protoporphyrinogen oxidase inhibitor (PPO), photosystem II inhibitor (PSII), 4-hydroxyphenylpyruvate dioxygenase inhibitor (HPPD), and TIR1 auxin receptor (growth regulator) (Heap 2016). There have been several mechanisms discovered in waterhemp that explain herbicide resistance (Schultz et al. 2015a). Resistance to glyphosate has been conferred through 1) a non-target mechanism, 2) through the amplification of the EPSPS gene, and 3) a Pro¹⁰⁶ Ser substitution in the EPSPS enzyme (Bell et al. 2013; Heap 2016; Legleiter and Bradley 2008; Nandula et al. 2013). One mechanism of resistance to ALS inhibiting herbicides is through a non-target mechanism that appears to be metabolism-based, (Guo et al. 2015), but the most common resistance mechanism is a Trp⁵⁷⁴ Leu amino acid substitution (Patzoldt and Tranel 2007). PPO-inhibiting herbicide resistance in all PPO-resistant waterhemp populations reported thus far is conferred by a codon deletion at amino acid 210 in the *PPX2* gene, (Heap 2016; Patzoldt et al. 2006) while resistance to PSII-inhibiting herbicides most commonly occurs through a Ser²⁶⁴ Gly amino acid substitution in the *psbA* enzyme (Foes et al. 1998). Resistance to HPPD inhibiting herbicides was first discovered in 2009 and the

mechanism responsible for resistance has been determined to be enhanced oxidative metabolism (Heap 2016; Ma et al. 2013). The first confirmed population of 2,4-D-resistant waterhemp was discovered in 2009 in a warm season native-grass seed production field in Nebraska (Bernards et al. 2012). This biotype of waterhemp exhibited 10-fold resistance to 2,4-D, and reduced sensitivity to dicamba as a result of continual use of low rates of 2,4-D to control broadleaf weeds in this setting (Bernards et al. 2012). A second population resistant to 2,4-D has since been discovered in Illinois in a row crop setting (Sabate et al. 2016). The mechanism responsible for the resistance to 2,4-D in these waterhemp populations is currently unknown, but there is current research being done to determine the mechanism responsible for resistance in the Illinois biotype (Bernards et al. 2012; Heap 2016; Sabate et al. 2016).

Herbicide Resistant Weed Management Strategies

Integrated weed management (IWM) is defined as the application of multiple alternative weed control measures, which include cultural, genetic, mechanical, biological, and chemical means of weed control (Regnier and Janke 1990; Shaw 1982; Walker and Buchanan 1982). Although none of these practices on their own can be expected to achieve acceptable levels of weed control, if multiple components are utilized, significant advances in weed control can be made (Swanton and Weise 1991). Integrating an understanding of weed biology into current weed-management systems, while reducing the weed seeds in the soil seedbank, plays a critical role in developing a sustainable weed management program (Bhowmik 1997; Walsh and Powles 2007). To have effective herbicide-resistant weed management there is a need to implement chemical and nonchemical management tactics to diversify selection pressure on weed populations and minimize the spread of resistance genes (Norsworthy et al. 2012). The following twelve best management practices for herbicide-resistant weed management have been adopted

by the Weed Science Society of America: 1) understand the biology of the weeds present; 2) use a diversified approach to weed management focused on reducing weed seed production and the number of weed seeds in the soil seedbank; 3) plant into weed-free fields and keep fields as weed free as possible; 4) plant weed-free crop seed; 5) scout fields routinely; 6) use multiple, effective mechanisms of action against the most troublesome weeds and those prone to herbicide resistance; 7) apply the labeled herbicide rate at recommended weed sizes; 8) emphasize cultural management techniques that suppress weeds by using crop competitiveness; 9) use mechanical and biological management practice where appropriate; 10) prevent field to field and within field movement of weed seed or vegetative propagules; 11) manage weed seed at harvest and postharvest to prevent a buildup of the weed seedbank; and 12) prevent an influx of weeds into the field by managing field borders (Norsworthy et al. 2012).

New Technologies

In the early 1940's, academic and industrial laboratories discovered and synthesized an array of derivatives of IAA, a hormone involved in plant growth and development called auxin. The products that were tested consisted of 1-naphthalene acetic acid (1-NAA) and the phenoxyacetic acids 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-dichlorophenoxy acetic acid (2,4-D) (Grossmann 2003; Grossmann 2009; Peterson 1967; Sterling and Hal 1997). These herbicides changed the way that growers controlled broadleaf weeds in their cereal crops (Peterson 1967). These newly discovered herbicides made it possible for the first time to apply a selective herbicide post-emergence (POST) to a cereal crop that would not injure the crop, but instead would kill the problematic broadleaf weeds. Since this initial discovery, a variety of other classes of auxin herbicides have been synthesized, including the phenoxyacetic acids, benzoic acids, pyridinecarboxylic acids, aromatic carboxymethyl

derivatives and quinolinecarboxylic acids (Grossmann 2009). Historically, the use of these herbicides was restricted to cereal crops and pastures, however there are currently soybean and cotton varieties being developed with a trait that confers resistance to 2,4-D and a separate trait that confers resistance to dicamba. The transgene that is responsible for conferring 2,4-D resistance is from a soil bacterium of the aryloxyalkanoate dioxygenase enzyme (AAD), and can effectively degrade pyridyloxyacetate auxin herbicides such as triclopyr, fluroxypyr, and 2,4-D to herbicidally-inactive compounds (Wright et al. 2010). The transgene that is responsible for conferring resistance to dicamba is from the genetically engineered bacterial gene dicamba monooxygenase (DMO). This gene encodes a Rieske non-heme monooxygenase enzyme capable of metabolizing dicamba when expressed from either the nuclear genome or chloroplast genome of transgenic plants (Behrens et al. 2007). These technologies should provide growers with new options for the control of broadleaf weeds that have evolved resistance to glyphosate, like waterhemp (Craigmyle et al. 2013). However, as previously mentioned, a 2,4-D-resistant waterhemp population has already been discovered after repeated use of 2,4-D in a native-grass seed production field in Nebraska (Bernards et al. 2012). A controlled greenhouse experiment was also reported in Arkansas where dicamba resistance was selected for in a Palmer amaranth population after three generations of sub-lethal doses of dicamba (Tehranchian et al. 2016).

Soybean Seed Treatments

The treating of seeds prior to sowing dates back to primitive peoples that coated crop seed with the blood and ashes of sacrificed people and animals in the belief it would increase productivity (Buttress and Dennis 1947). Other ideas included the soaking of crop seed in water, urine, wine, and/or manure. These ideas originated independently of any attempt at the prevention of diseases and damage from insects, but rather were solely an attempt to increase

crop yields. (Buttress and Dennis 1947). The first idea of treating the seed to prevent diseases was brining seed wheat in salt water to prevent smut in London in 1660 (Tull 1733). The first commercial dust seed treater was invented by Ben Gustafson in 1930 and was used to apply a copper carbonate dust to crop seed (Anonymous 2016). The first liquid seed coating occurred in 1946 and the advantages of this technique were to make the application process more uniform, improve seed flowability, heighten variety differentiation and visual identification through coloring, and protect operators from dust (Anonymous 2016). In the mid 1900's, several fungicides were discovered that offered some activity against a wide range of seed and seedling diseases, and these were used extensively until the 1990's (Knake 2013). In 1972 researchers at Shell Development Company's Biological Research Center discovered nithiazine, which is a lead structure for neonicotinoids, a commonly used insecticide (Kollmeyer et al. 1999; Maienfisch et al. 2001). Thirteen years after the discovery, Bayer synthesized the insecticide imidacloprid, and published a patent on this new invention in 1985 (Soloway et al. 1978). Shortly after imidacloprid was released into the marketplace, nitenpyram, and acetamiprid were also launched (Minamida et al. 1993; Takahashi et al. 1992). All three of these products are considered the first generation neonicotinoids (Maienfisch et al. 1999). There are several terms used for seed treatment including seed priming, seed treatment, and osmo priming (Hoseini et al. 2013). In 1991, imidacloprid was made available as an on-seed insecticide (Anonymous 2016). Since then, products that have been introduced into seed treatments include fungicides, insecticides and nematode control products that all protect crops from many types of pests (Knake 2013). The use of seed treatments has risen dramatically, and continues to increase. In 1997, global seed treatment sales were estimated at \$700 million. By 2010, seed treatment sales increased to \$2.25 billion, and by 2016 estimates reached \$3.43 billion (Knake 2013). Many

attribute this to increasing farm sizes, increases in conservation and no-tillage planting which are likely to increase disease and insect problems, and earlier planting in cool wet conditions (Houghton 2004; Knake 2013).

Pre-Emergence Residual Herbicides

Herbicide sequence is defined as two or more applications of herbicides with different sites of action within one crop, and herbicide rotation is the application of herbicides with different sites of action to multiple crops over multiple growing seasons in a field (Beckie 2006). Both herbicide sequence and herbicide rotation have the greatest effect in delaying resistance (Beckie et al. 2001; Wrubel and Gressel 1994). The use of a pre-emergence (PRE) residual herbicide followed by a post-emergence (POST) herbicide with residual activity has been shown to reduce weed densities (Bradley 2013; Legleiter et al. 2009; Schultz et al. 2015b). The use of PRE herbicides has been increasing in recent years. For example, in 1996 the dinitroaniline herbicides were used on slightly more than 28 million acres but by 2012, there were approximately 56 million acres treated with these same herbicides (USDA 2016). Flumioxazin and sulfentrazone are soil-applied PRE herbicides that are protoporphyrinogen oxidase inhibitors (PPO) and are commonly used in soybean production (Niekamp et al. 1999). These herbicides have the potential to injure the soybean crop in cool, wet conditions (Niekamp et al. 1999). Soybeans are typically able to metabolize these herbicides but in cool wet conditions the plants metabolism slows and injury can occur (Wise et al. 2015). These symptoms can also appear following a heavy rain event; droplets of concentrated herbicide that were applied to the soil can splash up onto the soybean leaf causing necrotic lesions (Wise et al. 2015). Previous research suggests that different soybean cultivars respond differently to sulfentrazone (Dayan et al. 1997; Hulting et al. 1997; Swantek et al. 1998; Zhaohu et al. 1997). Dyan et al. found that the

metabolic degradation of sulfentrazone in soybean is a major factor for imparting tolerance, as well as the differential intrinsic ability of each cultivar to overcome herbicide-induced peroxidative stress. According to Hulting et al. (1997), the greatest indicator of soybean intolerance to sulfentrazone is soybean height, with some cultivars having up to a 71% height reduction when treated with 280 g ai/ha PRE. However, very few seed companies rate their soybean varieties to tell consumers whether the variety is tolerant or susceptible to PPO-inhibiting herbicides.

Interaction Between Seed Treatment and PRE Herbicides

Fluopyram fungicide sold by Bayer CropScience as ILeVO is a seed treatment currently on the market to manage sudden death syndrome (Wise et al. 2015). It is known that this seed treatment can cause a “halo effect” on soybean, which manifests itself as a discoloration and necrosis on the tips of the cotyledons. This injury happens because the fungicide is systemic within the plant and accumulates in the roots and cotyledons and causes phytotoxicity (Wise et al. 2015). These conditions resemble the damage that can occur when PRE herbicides are applied in cool wet conditions.

Summary and Objectives

Amaranthus species are currently the most troublesome weed species in production agriculture in the United States. The rapid acceptance of glyphosate resistant crops since their introduction in 1996, and the heavy reliance on glyphosate for weed control since then, has selected for glyphosate resistant weed species, and predominantly glyphosate resistant pigweeds. One of the best management practices for herbicide resistant weeds established by the Weed Science Society of America (WSSA) is to start with a weed-free field. One common method of

achieving this in soybean production is to apply a PRE herbicide. Certain PRE herbicides are known to cause injury to soybean in cool wet conditions. Producers are planting earlier in the spring in these cool, wet conditions for a variety of reasons. In order to protect their seedlings in these conditions, soybean seed treatments have increased in popularity. The objectives of this portion of this research are to: 1) determine if there are any interactions that exist between PRE emergence residual herbicides and seed treatments in soybean and 2) determine if any potential interactions lead to stand loss, height and/or biomass reduction, and yield loss.

Several agrochemical and seed companies are proposing another method to manage glyphosate-resistant weeds is through new herbicide-resistance traits in soybean that allow the synthetic auxin herbicides 2,4-D or dicamba to be sprayed post-emergence to these new crops. For many years, these herbicides could only be used as part of a pre-plant burndown program in soybean. In 2014 a grower in central Missouri claimed that he was unable to control waterhemp with 2,4-D after routinely applying the herbicide as part of his preplant herbicide program for several years consecutively. The objectives of this portion of the research are to: 1) determine if this waterhemp population is resistant to 2,4-D and 2) to determine if this biotype exhibits resistance or multiple resistance to any other herbicide family.

Literature Cited

- Al-Khatib K, Baumgartner JR, Peterson DE, Currie RS (1998) Imazethapyr resistance in common sunflower (*Helianthus annuus*). *Weed Sci* 46:403-407
- Anonymous (2016) Innovation in practice since 1926.
<http://www.seedgrowth.bayer.com/~media/SeedGrowth/Bayer100YearsFlash/index.aspx?iframe=true&width=995&height=700>. Accessed: 10/21/2016, 2016
- Beckie H, Hall L, Tardif F (2001) Impact and management of herbicide-resistant weeds in Canada. Pages 747-754 in *Proceedings of the British Crop Protection Council Conference*. Brighton, UK
- Beckie HJ (2006) Herbicide-resistant weeds: Management tactics and practices. *Weed Technol* 20:793-814
- Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: Enlarging and preserving biotechnology-based weed management strategies. *Science* 316:1185-1188
- Bell MS, Hager AG, Tranel PJ (2013) Multiple resistance to herbicides from four site-of-action groups in waterhemp (*Amaranthus tuberculatus*). *Weed Sci* 61:460-468
- Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Sci* 51:37-43
- Bernards ML, Crespo RJ, Kruger GR, Gaussoin R, Tranel PJ (2012) A waterhemp (*Amaranthus tuberculatus*) population resistant to 2,4-D. *Weed Sci* 60:379-384
- Bhowmik PC (1997) Weed Biology: Importance to weed management. *Weed Sci* 45:349-356

- Bradley K (2013) Herbicide-resistance in the Midwest: current status and impacts. *Weed Sci. Soc Am Abstr* 271
- Bradley K, Legleiter T, Hunter L, Nichols C, Foresman C (2007) The status of glyphosate-resistant waterhemp in Missouri. *N Cent Weed Sci. Soc Absrt* 192
- Bradley K, Li J, Monnig N (2006) Greenhouse investigations of suspected glyphosate-resistant common waterhemp populations from Missouri. *Weed Sci Soc Am* 46
- Buttress F, Dennis R (1947) The early history of cereal seed treatment in england. *Agricultural History* 21:93-103
- Cordes JC, Johnson WG, Scharf P, Smeda RJ (2004) Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. *Weed Technol* 18:999-1005
- Craigmyle BD, Ellis JM, Bradley KW (2013) Influence of herbicide programs on weed management in soybean with resistance to glufosinate and 2,4-D. *Weed Technol* 27:78-84
- Dayan FE, Weete JD, Duke SO, Hancock HG (1997) soybean (*Glycine max*) cultivar differences in response to sulfentrazone. *Weed Sci* 45:634-641
- Dill GM (2005) Glyphosate-resistant crops: history, status and future. *Pest Management Science* 61:219-224
- Fernandez-Cornejo J, McBride WD (2002) Adoption of bioengineered crops. ERS Agriculture Economic Report No. AER810. <http://www.ers.usda.gov/publications/aer810/>
- Foes MJ, Tranel PJ, Wax LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. *Weed Sci* 46:514-520
- Gleason H, Cronquist A (1963) Manual of vascular plants of northeastern United States and adjacent Canada. Van Nostrand Reinhold, New York

- Grossmann K (2003) Mediation of herbicide effects by hormone interactions. *Journal of Plant Growth Regulation* 22:109-122
- Grossmann K (2009) Auxin herbicides: current status of mechanism and mode of action. *Pest Management Science* 66:113-120
- Guo J, Riggins CW, Hausman NE, Hager AG, Riechers DE, Davis AS, Tranel PJ (2015) Nontarget-site resistance to ALS inhibitors in waterhemp (*Amaranthus tuberculatus*). *Weed Sci* 63:399-407
- Hager A, Wax L, Simmons F, Stoller E (1997) Waterhemp management in agronomic crops. Urbana, IL: University of Illinois 12 p.
- Hager AG, Wax LM, Stoller EW, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. *Weed Sci* 50:607-610
- Heap I (2016) The international survey of herbicide resistant weeds.
www.weedscience.com/summary/home.aspx. Accessed August 22, 2016,
- Hoseini M, Feqenabi F, Tajbakhsh M, Babazadeh-Igdir H (2013) Introduction of seed treatment techniques (seed priming) *International Journal of Biosciences* 3:1-12
- Houghton D (2004) Smart seed coatings.
http://media.corporate-ir.net/media_files/NSD/LNDC/furrowjan2004.pdf. Accessed: November 23, 2016
- Hulting A, Wax L, Stoller E (1997) Tolerance levels of soybean cultivars to sulfentrazone. *Proc. North Cent Weed Sci Soc* 53, 76
- Jasieniuk M, Brûlé-Babel AL, Morrison IN (1996) The evolution and genetics of herbicide resistance in weeds. *Weed Sci* 44:176-193

Knake R (2013) The role of seed treatment in modern U.S. Crop Production.

http://www.isafarmnet.com/2013OFNConf/RayKnake_Seed_Treatments.pdf. Accessed:

November 23, 2016

Kollmeyer WD, Flattum RF, Foster JP, Powell JE, Schroeder ME, Soloway SB (1999)

Discovery of the nitromethylene heterocycle insecticides. In *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, ed. Yamamoto, JE casida, 71-89 Tokyo: Springer-Verlag

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. *Weed Sci* 56:582-587

Legleiter TR, Bradley KW, Massey RE (2009) Glyphosate-resistant waterhemp (*Amaranthus rudis*) control and economic returns with herbicide programs in soybean. *Weed Technol* 23:54-61

Ma R, Kaundun SS, Tranel PJ, Riggins CW, McGinness DL, Hager AG, Hawkes T, McIndoe E, Riechers DE (2013) Distinct detoxification mechanisms confer resistance to mesotrione and atrazine in a population of waterhemp. *Plant Physiology* 163:363-377

Maienfisch P, Brandl F, Kobel W, Rindlisbacher A, Senn R (1999) CGA 293'343: A novel, broad-spectrum neonicotinoid insecticide. Pages 177-209 in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*. New York: Springer Japan

Maienfisch P, Huerlimann H, Rindlisbacher A, Gsell L, Dettwiler H, Haettenschwiler J, Sieger E, Walti M (2001) The discovery of thiamethoxam: a second generation neonicotinoid. *Pest Management Science* 57:165-176

Minamida I, Iwanaga K, Tabuchi T, Aoki I, Fusaka T, Ishizuka H, Okauchi T (1993) Synthesis and insecticidal activity of acyclic nitroethene compounds containing a

- heteroarylmethylamino group studies on acyclic nitroethene compounds (Part 2). Journal of Pesticide Science 18:41-48
- Moolani MK, Knake EL, Slife FW (1964) Competition of smooth pigweed with corn and soybeans. Weeds 12:126-128
- Nandula VK, Ray JD, Ribeiro DN, Pan Z, Reddy KN (2013) Glyphosate resistance in tall waterhemp (*Amaranthus tuberculatus*) from Mississippi is due to both altered target-site and nontarget-site mechanisms. Weed Sci 61:374-383
- Niekamp JW, Johnson WG, Smeda RJ (1999) Broadleaf weed control with sulfentrazone and flumioxazin in no-tillage soybean (*Glycine max*). Weed technol 13:233-238
- Norbdy D, Hartzler B, Bradley K (2007) Biology and management of waterhemp. West Lafayette, IN Purdue Extension. Glyphosate, Weeds, and Crop Series Publication GWC-13:1-12
- Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci 60:31-62
- Patzoldt WL, Hager AG, McCormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. Proc. of the National Academy of Sci 103:12329-12334
- Patzoldt WL, Tranel PJ (2007) Multiple ALS mutations confer herbicide resistance in waterhemp (*Amaranthus tuberculatus*). Weed Sci 55:421-428

- Patzoldt WL, Tranel PJ, Hager AG (2002) Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. *Crop Protection* 21:707-712
- Peterson GE (1967) The discovery and development of 2,4-D. *Agricultural History* 41:243-254
- Prather T, Ditomaso J, Holt J (2000) Herbicide resistance: Definition and management strategies. University of California ANR Publication 8012: 1-14
- Pratt DB, Clark LG (2001) *Amaranthus rudis* and *A. tuberculatus*, One species or two? *Torrey Botanical Society* 128:282-296
- Regnier EE, Janke RR (1990) evolving strategies for managing weeds. Pages 174-202 in Edwards CA, Lal R, Madden P, Miller RH, and House G, eds. *Sustainable Agricultural Systems*, Soil and Water Conservation Society, Ankeny, IA: St. Louis Press
- Rosenbaum KK, Bradley KW (2013) A survey of glyphosate-resistant waterhemp (*Amaranthus rudis*) in Missouri soybean fields and prediction of glyphosate resistance in future waterhemp populations based on in-field observations and management practices. *Weed Technol* 27:656-663
- Ryan G (1970) resistance of common groundsel to simazine and atrazine. *Weed Sci* 18:614-616
- Sabate S, Tranel PJ, Hager AG (2016) Genetics of 2,4-D resistance in an Illinois waterhemp population. *North Cent Weed Sci Soc*, Des Moines, Iowa
- Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015a) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. *Weed Sci* 63:336-345

- Schultz JL, Myers DB, Bradley KW (2015b) Influence of soybean seeding rate, row spacing, and herbicide programs on the control of resistant waterhemp in glufosinate-resistant soybean. *Weed Technol* 29:169-176
- Sellers BA, Smeda RJ, Johnson WG, A. KJ, Ellersieck MR (2003) Comparative growth of six *Amaranthus* species in Missouri. *Weed Sci* 51:329-333
- Shaw W (1982) Integrated weed management systems technology for pest management. *Weed Sci* 30:2-12
- Soloway S, Henry A, Kollmeyer W, Padgett W, Powell J, Roman S, Tieman C, Corey R, Horne C, Geisbühler H (1978) Nitromethylene heterocycles as insecticides. Pages 153-58 in Shankland DL, Hollingworth RM, Smyth Jr. T, eds. *Pesticide and Venomm Neurotoxicity*. New York: Plenum
- Steckel LE (2007) The dioecious *Amaranthus* spp.: Here to stay. *Weed Technol* 21:567-570
- Sterling TM, Hal J (1997) Mechanism of action of natural auxins and the auxinic herbicides. Pages 111-41 in Roe MR, Kuhr RJ, Burotn JD, eds. *Herbicide Activity: Toxicology, Biochemistry and Molecular Biology*. Amsterdam: IOP Press
- Swantek JM, Sneller CH, Oliver LR (1998) Evaluation of soybean injury from sulfentrazone and inheritance of tolerance. *Weed Sci* 46:271-277
- Swanton CJ, Weise SF (1991) Integrated seed management: The rationale and approach. *Weed Technol* 5:657-663
- Switzer C (1957) The existence of 2, 4-D-resistant strains of wild carrot. Pages 315-318 in *Proc. of the 11th Northeast Weed Control Conference*, Columbia, MO: NEWSS

- Takahashi H, Mitsui J, Takakusa N, Matsuda M, Yoneda H, Suzuki J, Ishimitsu K, Kishimoto T (1992) NI-25, a new type of systemic and broad spectrum insecticide. Pages 89-96 in Proc. of Brighton Crop Protection Conference, Pest and Diseases, Brighton, UK
- Tehranchian P, Norsworthy JK, Powles SB (2016) To what extent does repeated use of dicamba select for resistance in Palmer amaranth? Weed Sci Soc America, San Juan, Puerto Rico
- Tranel PJ, Riggins CW, Bell MS, Hager AG (2010) Herbicide resistances in *Amaranthus tuberculatus*: A call for new options. Journal of Agricultural and Food Chemistry 59:5808-5812
- Tull J (1733) The horse-hoing husbandry. London: Printed for the author
- USDA (2016) Adoption of genetically engineered crops in the U.S. Accessed August 23, 2016, 2016
- USDA UERSa (2016) Agriculture resource management survey, 1996-2012 in USDA ERSa ed.: National Agriculture Statistics Service
- Walker RH, Buchanan GA (1982) Crop manipulation in integrated weed management systems. Weed Sci 30:17-24
- Walsh MJ, Powles SB (2007) Management strategies for herbicide-resistant weed populations in australian dryland crop production systems. Weed Technol 21:332-338
- Webster TM (2005) Weed survey-southern states, 2001: Broadleaf crops subsection (cotton, peanut, soybean, tobacco, and forestry). Pages 291-306 in Proc. of 58th S. Weed Sci. Soc
- Whitehead C, Switzer C (1963) The differential response of strains of wild carrot to 2, 4-D and related herbicides. Canadian Journal of Plant Science 43:255-262

- Wise K, Mueller DS, Kandel Y, Young B, Johnson B, Legleiter T (2015) Soybean seedling damage: Is there an interaction between the ILeVO seed treatment and pre-emergence herbicides? Iowa State University. Agriculture and Natural Resources
- Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z, Hammock BD (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. *Proc. of the National Academy of Sciences of the United States of America* 107:20240-20245
- Wrubel RP, Gressel J (1994) Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. *Weed Technol* 8:635-648
- Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. *Weed Technol* 20:301-307
- Zhaohu L, Walker R, Wehtje G (1997) Laboratory studies for predicting response of soybean cultivars to sulfentrazone. Pages 177-178 *in* Proc. of S. Weed Sci. Soc. Houston, TX

Chapter II
Investigations of the Potential Interactions Between Pre-emergence Residual Herbicides
and Seed Treatment in Soybean

Blake R. Barlow and Kevin W. Bradley

ABSTRACT

Field experiments were conducted in 2015 and 2016 in Missouri to determine whether interactions exist between PRE herbicides and seed treatments. The experiments were conducted in a randomized complete block design with factorial arrangements of varieties, seed treatments, and herbicides. Two genetically similar varieties of soybean were chosen, one with known tolerance to PPO-inhibiting herbicides and one with known sensitivity. Each soybean variety was treated with imidacloprid, pasteuria nishizawae + thiamethoxam, and fluopyram + imidacloprid. Chlorimuron-ethyl + flumioxazin + pyroxasulfone, chlorimuron-ethyl + flumioxazin + metribuzin, and chlorimuron-ethyl + sulfentrazone were applied PRE to each variety and seed treatment combination at twice the labeled use rate. Chlorimuron-ethyl + sulfentrazone resulted in 70 and 19% injury to the sensitive variety in 2015 and 2016, respectively, which was the highest visual injury observed from any herbicide treatment in either year. In 2015, chlorimuron-ethyl + sulfentrazone and chlorimuron-ethyl + flumioxazin + pyroxasulfone resulted in the greatest height reductions in both varieties but this reduction was more severe in the sensitive (40 to 47%) compared to the tolerant (23 to 28%) variety. Overall, the tolerant variety yielded 23%, or 855 kg ha⁻¹ more than the sensitive variety, and for both varieties the sulfentrazone-containing treatments resulted in the highest yield losses. As was the

case with visual injury, stand, leaf area and height, the severity of soybean yield loss was greater in the sensitive compare to the tolerant variety. The results from this research indicate that there is a larger interaction between PPO-inhibiting herbicides and varieties than there is between herbicides and seed treatments, or seed treatments and varieties.

Nomenclature: Soybean, *Glycine max* (L.) Merr; sulfentrazone; flumioxazin; imidacloprid; *pasteuria nishizawae*; thiamethoxam; fluopyram

Key Words: PRE herbicides, seed treatment, ILeVO interaction; yield

The rapid adoption of glyphosate resistant crops since their introduction in 1996, and the heavy reliance on glyphosate for weed control since that time, has selected for glyphosate-resistant weed species, and predominantly glyphosate resistant *Amaranthus* species, in U.S. corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and cotton (*Gossypium hirsutum* L.) production. Two of the best management practices for herbicide resistant weed management are to start with a weed-free field, and to use multiple herbicide mechanisms of action (Norsworthy et al. 2012). Both of these practices can be achieved in soybean through the use of a pre-emergence (PRE) residual herbicide. The use of PRE residual herbicides have been shown to reduce the densities of glyphosate-resistant *Amaranthus* species in soybean (Bradley 2013; Legleiter et al. 2009; Schultz et al. 2015), and their use has been increasing in recent years. For example, in 1996 the dinitroaniline herbicides were used on slightly more than 28 million acres but by 2012, there were approximately 56 million acres treated with these same herbicides (USDA 2016a). In 2006, there were 70,000 lbs of sulfentrazone applied in the US, while nine years later sulfentrazone use had risen to 2.4 million lbs and was the second most used herbicide in soybean (USDA 2016b; USDA 2006).

Flumioxazin and sulfentrazone are both PRE residual herbicides that are protoporphyrinogen oxidase inhibitors (PPO) and are commonly used in soybean production, but have the potential to injure soybean especially in cool, wet conditions. Soybeans are typically able to metabolize these herbicides but in cool and wet conditions the plant's metabolism slows and injury can occur (Wise et al. 2015). Injury is often greatest following a heavy rain event; droplets of concentrated herbicide that were applied to the soil can splash up onto the soybean leaf causing necrotic lesions (Wise et al. 2015). Previous research suggests that soybean cultivars respond differently to sulfentrazone (Dayan et al. 1997; Hulting et al. 2001; Swantek et

al. 1998; Zhao et al. 1997). Dayan et al. (1997) found that the metabolic degradation of sulfentrazone in soybean is a major factor for imparting tolerance, as well as the differential intrinsic ability of each cultivar to overcome herbicide-induced peroxidative stress. According to Hulting et al. (2001), the greatest indicator of soybean intolerance to sulfentrazone is soybean height, with some cultivars having height reductions up to 71% when treated with 280 g ai/ha PRE. However, very few seed companies rate their soybean varieties to tell consumers whether the variety is tolerant or susceptible to PPO-inhibiting herbicides.

The concept of treating crop seeds prior to planting dates back to 1837 with primitive people soaking the seed in blood, water, urine, wine, and/or manure in an attempt to improve crop productivity by increasing yield (Buttress and Dennis 1947). It was not until 1660 that it was considered that treating seeds might help with disease prevention (Tull 1733). Tull found that brining seed wheat in salt water prior to sowing reduced smut in wheat production. Some of the first commercially available seed treatments were in a dust form, but these were hazardous to the applicator and often did not adequately coat the entire seed (Anonymous 2016b). In 1946, a liquid form of seed treatment was available, which increased uniform seed coverage, improved seed flow ability, and allowed for visual identification through coloring (Anonymous 2016b). In 1985, Bayer synthesized the insecticide imidacloprid, one of the first generation neonicotinoids, and soon thereafter it was made available as an insecticide seed treatment in 1991 (Maienfisch et al. 1999). Since that time, many other insecticides, fungicides, and nematicides have been introduced into the seed treatment market in order to protect crops from a variety of pests (Anonymous 2016b). The use of seed treatments has risen dramatically, and is currently the fastest growing agriculture chemical sector (Anonymous 2013). In 1997, global seed treatment sales were estimated at \$700 million. By 2011, the seed treatment market was valued at \$2.43

billion with fungicides accounting for 35% and insecticides accounting for 52%. By 2018, the global seed treatment market is expected to reach \$5.6 billion (Anonymous 2013). Many attribute this increase to increases in farm sizes and conservation and no-tillage planting which are likely to increase disease and insect problems, and earlier planting in cool wet conditions (Houghton 2004; Anonymous 2013). Fluopyram is a fungicide seed treatment currently on the market for the management of sudden death syndrome in soybean (Wise et al. 2015). It is known that this seed treatment can cause a “halo effect” on soybean, which manifests itself as a discoloration and necrosis on the tips of the cotyledons. This injury happens because the fungicide is systemic within the plant and accumulates in the roots and cotyledons and causes phytotoxicity (Wise et al. 2015). These conditions resemble the damage that can occur when PRE PPO herbicides are applied in cool wet conditions.

The objectives of this research were to: 1) determine if there are any interactions that exist between PRE residual herbicides, varieties and seed treatments in soybean, and 2) determine if any potential interactions lead to stand loss, height and/or biomass reduction, and yield loss.

Materials and Methods

Site Description. A field experiment was conducted in 2015 in Boone County, Missouri (38.90°N, -92.21°W) and was repeated in 2016 at a site in Carroll County, Missouri (39.57°N, -93.33°W). Both sites had been in a corn-soybean rotation for several years and the previous year's crop was corn. Glyphosate-resistant soybean seed were planted at a density of 370,000 seeds ha⁻¹ in rows spaced 76-cm apart at the Boone and Carroll County sites on May 4 and May 6 in 2015 and 2016, respectively. The soil at the Boone County site was a Mexico silt loam with

2.2% organic matter, and a pH of 6.5. The soil at the Carroll County site was a Grundy silt loam and a Lagonda silty clay loam with organic matter of 3.2% and a pH of 5.9. Monthly rainfall totals and average monthly temperature as well as the 30 yr average for each site are presented in Table 2.1. Source of all herbicides used in the experiments are presented in Table 2.2.

The experiment was conducted as a randomized complete block design with a factorial arrangement of two varieties, four seed treatments, and four herbicides. The experiment was conducted with five replications in 2015, and six replications in 2016. Individual plots measured 2 by 9 m in size. Two varieties of soybean were planted: 1) Pioneer P34T07R2, a variety of soybean that is described as sensitive to protoporphyrinogen oxidase- (PPO) inhibiting herbicides, and 2) Pioneer P35T58R, a variety of soybean described as tolerant to PPO-inhibiting herbicides (Anonymous 2016a). Each variety of seed was commercially treated with three separate seed treatment mixtures: 1) imidacloprid + prothioconazol + penflufen + metalaxyl, 2) *pasteuria nishizawae* + thiamethoxam + prothioconazol + penflufen + metalaxyl, and 3) fluopyram + imidacloprid + prothioconazol + penflufen + metalaxyl. Non-treated seed of each variety were included for comparison. Each variety and seed treatment combination received treatment with PRE herbicide applications of: 1) 0.043 kg ai/ha chlorimuron-ethyl + 0.016 kg ai/ha flumioxazin + 0.196 kg ai/ha pyroxasulfone, 2) 0.44 kg ai/ha chlorimuron-ethyl + 0.143 kg ai/ha flumioxazin + 0.5 kg ai/ha metribuzin, and 3) 0.09 kg ai/ha chlorimuron-ethyl + 0.7 kg ai/ha sulfentrazone. These represent twice the labeled use rate of these herbicides. A non-treated control was also included for comparison and these plots were maintained weed-free with a PRE application of 1.71 kg ai/ha s-metolachlor (1/2 labeled rate) and hand weeding. All herbicide treatments were applied with a CO₂-pressurized backpack sprayer calibrated to deliver 140 L ha⁻¹ with XR8002 flat fan nozzles at 117 kPa at a constant speed of 5 km ha⁻¹. All PRE herbicide

treatments were applied at or just prior to planting. The trial was maintained weed-free through applications of glyphosate (0.95 kg ai/ha) following measurements taken 10 and 30 days after emergence (DAE), and escapes were hand removed throughout the growing season.

Treatment Evaluation and Data Collection. Visual crop injury evaluations, stand counts, height measurements, biomass readings, and leaf area were taken 10 and 30 days after emergence (DAE) in the two innermost rows of each plot. Yield was determined by harvesting the two innermost soybean rows within each plot with a small plot combine (Kincaid®, Haven, KS) and moisture was adjusted to 13%. Visual crop injury evaluations were assessed on a scale of 0 to 100 percent, where 0 represented no plant death or crop injury and 100 was equivalent to complete plant death. Stand counts were assessed by counting the number of living plants in 2, 1-m length of the two innermost treated rows in each plot. Soybean plant height was recorded by measuring five representative plants from each plot from the soil surface to the top of the uppermost fully expanded trifoliolate. These same five plants were harvested at the soil surface and biomass readings were taken by drying plants for five days at 50°C in a commercial dryer. Leaf area was determined with a Li-Cor 3000 Portable Area Meter (Li-Cor, Lincoln, NE).

Statistical Analysis. All data were all analyzed using the PROC GLMMIX procedure in SAS (SAS 9.4, SAS® Institute Inc. Cary, NC). Replications, seed treatments, PRE herbicides, and soybean varieties were considered fixed effects. Significant interactions were present between years; therefore all data are presented separately by year (Table 2.3). Individual treatment differences were separated using Fisher's protected LSD at $P \leq 0.05$. Significant interactions were present between varieties, seed treatments, and herbicides. There were also significant variety by herbicide and variety by seed treatment interactions. However, there was not a significant variety by seed treatment by herbicide interaction present in either year.

RESULTS AND DISCUSSION

Visual Injury. There was more visual soybean injury observed in 2015 than in 2016 (Table 2.4). When averaged across all herbicide and seed treatments, the tolerant variety was injured 25% while the sensitive variety was injured 37% in 2015. In 2016 only 11 and 13% injury occurred to the tolerant and sensitive varieties, respectively. This is likely due to the fact that Boone County received 23.7 and 44.7 cm more rainfall in April and May of 2015, respectively, than Carroll County in 2016 (Table 2.1). Herbicides such as metribuzin have been shown to injure soybean, and that injury can be enhanced with excessive moisture (Coble and Schrader 1973). When averaged across all seed treatments and varieties, chlorimuron-ethyl + sulfentrazone and chlorimuron-ethyl + flumioxazin + pyroxasulfone resulted in the highest visual soybean injury in both years. Chlorimuron-ethyl + flumioxazin + metribuzin resulted in less visual injury than the other herbicide treatments in both years, and in 2016 injury from this treatment was not different than the non-treated control. When averaged across all varieties and herbicides, the non-treated seed resulted in more injury than any other seed treatment in 2016 (Tables 2.3 and 2.4). This is likely a result of conditions that were ideal for seedlings diseases to form, and the non-treated seed suffered more injury than the treated seed. Wise et al. (2015) reported that systemic seed treatments such as fluopyram can cause discoloration on soybean cotyledons that can resemble disease or other abiotic stress such as herbicide injury. Chlorimuron-ethyl + sulfentrazone resulted in 70 and 19% injury to the sensitive variety in 2015 and 2016, respectively, which were the highest visual injury observed in both years. The sensitive variety treated with imidacloprid, *pasteuria nishizawe* + thiamethoxam and fluopyram + imidacloprid exhibited the most visual

injury in 2015. However in 2016, the no seed treatment control resulted in the highest visual injury for both varieties. This may be due to conditions that were ideal for seedling diseases.

Stand Counts. There were differences in stand counts between the tolerant and sensitive varieties in 2015, with the tolerant variety having higher populations (Table 2.4). However, in 2016, there was no difference in stand counts between the two varieties (Table 2.3). Similarly, there were no differences between herbicides in 2016, however in 2015 there was a difference between all four herbicides tested. All three herbicide treatments resulted in a 22 to 40% reduction in soybean stand, with chlorimuron-ethyl + sulfentrazone resulting in the greatest reductions. Reiling et al. (2006) reported that soybean cultivars that are known to be sensitive to sulfentrazone can have up to 20% stand reduction when sulfentrazone is applied PRE at twice the labeled use rate. When the same rate of sulfentrazone was applied to a cultivar that is tolerant to sulfentrazone, the stand was only reduced by 12%. Burnside (1972) has also shown that herbicides sprayed at higher than labeled rates have the potential to reduce soybean stand (Burnside 1972; Swantek et al. 1998).

There were differences in stand counts between seed treatments in both years but there was only a variety by seed treatment interaction in 2015 (Tables 2.3 and 2.4). In 2015, when averaged across varieties and herbicides, *pasteuria nishizawae* + thiamethoxam resulted in a higher soybean stand than either of the other seed treatments or the non-treated control. Bradley et al. (2001) reported that seed treated with fungicide protectants resulted in a 6% increase in soybean stand when compared to non-treated seed in a no-till situation. When comparing the seed treatments across each variety, soybean stands were highest with the tolerant variety treated with *pasteuria nishizawae* + thiamethoxam than with any other variety-seed treatment combination. Soybean stands were 12 to 19% lower with all other variety-seed treatment

combinations. Bradley (2008) showed that soybean seed treatments that contain a fungicide can increase soybean stand by up to 69% compared to non-treated seed in cool wet conditions. In warmer, dryer conditions however, Bradley (2008) showed that seed treatments containing a fungicide can decrease soybean stand by almost 27% when compared to non-treated seed.

Leaf Area. There were no differences between any of the factors on soybean leaf area in 2016 (Table 2.3). In 2015, however, there were differences in soybean leaf area between varieties and herbicides (Table 2.4). The tolerant variety had more leaf area than the sensitive variety across all herbicide and seed treatments (Table 2.4). Since the sensitive variety displayed more visual injury, it was expected that there would be less leaf area with the sensitive variety than with the tolerant. Salzman and Renner (1992) also showed that herbicides such as metribuzin, when applied at 420 g ha^{-1} , can reduce soybean leaf area by as much as 44%. Chlorimuron-ethyl + flumioxazin + pyroxasulfone and chlorimuron-ethyl + sulfentrazone caused the greatest reductions in leaf area in both varieties, but leaf area reductions were most severe (55 to 67%) in the sensitive variety.

Height. There was more soybean height reduction in the sensitive variety than the tolerant variety in both years with a 12% and 6% difference between these varieties in 2015 and 2016, respectively (Table 2.5). In 2015, chlorimuron-ethyl + sulfentrazone and chlorimuron-ethyl + flumioxazin + pyroxasulfone resulted in the greatest height reductions in both varieties but this reduction was more severe in the sensitive (40 to 47%) compared to the tolerant (23 to 28%) variety. Dayan et al (1997) reported that soybean cultivars respond differently to sulfentrazone, and height can be reduced by up to 58 and 77% when exposed to 2X and 3X rates respectively. Soybean cultivars respond differently to sulfentrazone, and soybean height is a better indication of injury than biomass (Swantek et al. 1998; Hulting et al. 2001). Swantek et al. (1998) showed

that soybean height was reduced by up to 48% in a susceptible variety and only 22% in a tolerant variety with 0.56 kg ai ha⁻¹ sulfentrazone.

There were some slight differences in soybean height due to seed treatments in 2015, but in 2016 seed treatments did not have a significant effect on soybean height (Tables 2.3 and 2.5). Height of soybean treated with fluopyram + imidacloprid was 5 to 7% less than soybean treated with *pasteuria nishizawae* + thiamethoxam and non-treated seed, but was not different than imidacloprid alone. Wise et al. (2015) indicated that seed treatments containing fluopyram are known to cause early season injury to soybean, and one of those injury symptoms could be height reduction.

Biomass. There were differences in soybean biomass between varieties and herbicides in 2016, but there were no differences in soybean biomass between any of the factors measured in 2015 (Table 2.3). In 2016 across all herbicide and seed treatments, plant biomass was reduced 9% more in the sensitive compared to the tolerant soybean variety (Table 2.5). All herbicide treatments also reduced soybean biomass compared to the non-treated control. Both of these observations are consistent with the other measurements taken in this research.

Yield. Even though there was some visual injury observed and reduction in soybean stand and biomass in 2016, none of this resulted in differences in soybean yield (Table 2.3). In 2015, however, there were differences in soybean yield due to varieties and herbicide treatments (Tables 2.3 and 2.5). Overall, the tolerant variety yielded 23%, or 855 kg ha⁻¹ higher, than the sensitive variety and for both varieties the sulfentrazone-containing treatments resulted in the highest yield losses (Table 2.5). As was the case with visual injury, stand, leaf area and height, the severity of the yield loss was greater in the sensitive compare to the tolerant variety. For

example, the sulfentrazone-containing treatments reduced yield of the tolerant variety by 28 to 29%, but reduced yield of the sensitive variety by 33 to 75%. Studies have shown that soybean cultivars respond differently to sulfentrazone applied PRE to soybean, and that it can lead to significant yield reductions (Belfry et al. 2016; Reiling et al. 2006; Swantek et al. 1998) Belfry et al. (2016) reported yield losses of 32% in one cultivar of soybean, and only 5% in another when 840 g ha⁻¹ of sulfentrazone was applied PRE. Swantek et al. (1998) also showed that different soybean cultivars react differently to sulfentrazone, and that yield can be reduced by as much as 28% when 560 g ha⁻¹ of sulfentrazone is applied PRE.

The results from this research suggest that there is a larger interaction between PPO-inhibiting herbicides and varieties than there is between herbicides and seed treatments, or seed treatments and varieties. Visual injury, stand, leaf area, height and yield reductions were greater in the sensitive compared to the tolerant variety. Oliver et al. (1997) found that soybean cultivars varied in their tolerance to sulfentrazone, and showed that 34% of 248 soybean cultivars tested were sensitive to sulfentrazone. In this research, there was not a seed treatment that performed better or worse consistently, and seed treatments did not have an effect on yield in either year. Even though it is known that fluopyram can cause early-season soybean seedling damage commonly referred to as the “halo effect” (Wise et al. 2015), these results indicate that this damage does not correlate with yield loss. There were also no interactions between the seed treatments and herbicides evaluated in this research that led to yield loss, even when soybeans are planted in cool, wet, conditions and encounter 2X rates of these herbicides. The use of a pre-emergence (PRE) residual herbicide followed by a post-emergence (POST) herbicide with residual activity has been shown to reduce densities of some of the most troublesome herbicide resistant weeds in the U.S. like the *Amaranthus* species (Bradley, 2013; Legleiter et al., 2009;

Schultz et al., 2015). So even though PRE residual herbicides like flumioxazin and sulfentrazone are capable of causing early-season soybean injury (Belfry et al. 2016; Hulting et al. 2001; Niekamp and Johnson 2001; Reiling et al. 2006; Swantek et al. 1998; Taylor-Lovell et al. 2001), the results of this research indicate that this injury does not necessarily translate into yield loss, and that there is no deleterious interaction between these herbicides and several common soybean seed treatments. Ultimately the results of this research indicate it is more important for a grower to know the tolerance of their soybean variety to PPO-inhibiting herbicides like sulfentrazone, but unfortunately very few seed companies provide these kind of rankings.

Literature Cited

Anonymous (2013) The role of seed treatment in modern U.S. crop production.

<https://docs.google.com/viewerng/viewer?url=http://bloximages.chicago2.vip.townnews.com/minnesotafarmguide.com/content/tncms/assets/v3/editorial/8/5a/85a4a3a6-5ebb-11e3-be43-001a4bcf887a/52a23e6c9d650.pdf.pdf>. Accessed: November 23, 2016

Anonymous (2016a) Herbicide x variety guide.

<https://www.pioneer.com/CMRoot/Pioneer/US/products/stewardship/2016-soybean-herbicide-guide-ProdList.pdf>. Accessed: 4/4/2017

Anonymous (2016b) Innovation in practice since 1926.

<http://www.seedgrowth.bayer.com/~media/SeedGrowth/Bayer100YearsFlash/index.ashx?iframe=true&width=995&height=700>. Accessed: 10/21/2016

Belfry KD, Shropshire C, Sikkema PH (2016) Identity-preserved soybean tolerance to protoporphyrinogen oxidase-inhibiting herbicides. *Weed Technol* 30:137-147

Bradley CA, Wax LM, Ebelhar SA, Bollero GA, Pedersen WL (2001) The effect of fungicide seed protectants, seeding rates, and reduced rates of herbicides on no-till soybean. *Crop Protection* 20:615-622

Bradley CA (2008) Effect of fungicide seed treatments on stand establishment, seedling disease, and yield of soybean in North Dakota. *Plant Disease*. 92:120-125

Bradley K (2013) Herbicide-resistance in the Midwest: Current status and impacts. *Weed Sci. Soc Am Abstr* 271

Burnside OC (1972) Tolerance of soybean cultivars to weed competition and herbicides. *Weed Sci.* 20:294-297

- Buttress F, Dennis R (1947) The early history of cereal seed treatment in England. *Agricultural History* 21:93-103
- Coble HD, Schrader JW (1973) Soybean tolerance to metribuzin. *Weed Sci.* 308-309
- Dayan FE, Weete JD, Duke SO, Hancock HG (1997) Soybean (*Glycine max*) cultivar differences in response to sulfentrazone. *Weed Sci* 45:634-641
- Houghton D (2004) Smart seed coatings.
http://media.corporate-ir.net/media_files/NSD/LNDC/furrowjan2004.pdf. Accessed: November 23, 2016
- Hulting A, Wax LM, Nelson RL, Simmons FW (2001) Soybean (*Glycine max* (L.) Merr.) cultivar tolerance to sulfentrazone. *Crop Protection* 20:679-683
- Legleiter TR, Bradley KW, Massey RE (2009) Glyphosate-resistant waterhemp (*Amaranthus rudis*) control and economic returns with herbicide programs in soybean. *Weed Technol* 23:54-61
- Maienfish P, Brandl F, Kobel W, Rindlisbacher A, Senn R (1999) CGA 293'343: A novel, broad-spectrum neonicotinoid insecticide. Pages 177-209 in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*. New York: Springer Japan
- Niekamp JW, Johnson WG (2001) Weed management with sulfentrazone and flumioxazin in no-tillage soybean (*Glycine max*). *Crop Protection* 20:215-220
- Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. *Weed Sci* 60:31-62

- Oliver D, Swantek J, Castillo T (1997) Soybean cultivar susceptibility to sulfentrazone. Arkansas Crop Prot Assoc. Abstr. Res. Conf. Vol I, Fayetteville, AR
- Reiling KL, Simmons FW, Riechers DE, Stechel, LE (2006) Application timing and soil factor affect sulfentrazone phytotoxicity to two soybean (*Glycine max* (L.) Merr.) cultivars. Crop Protection 25:230-234
- Salzman FP, Renner KA (1992) Responses of soybean to combinations of clomazone, metribuzin, linuron, alachlor, and atrazine. Weed Technol 6:922-929
- Schultz JL, Myers DB, Bradley KW (2015) Influence of soybean seeding rate, row spacing, and herbicide programs on the control of resistant waterhemp in glufosinate-resistant soybean. Weed Technol 29:169-176
- Swantek JM, Sneller CH, Oliver LR (1998) Evaluation of soybean injury from sulfentrazone and inheritance of tolerance. Weed Sci 46:271-277
- Taylor-Lovel S, Wax LM, Nelson R (2001) Phytotoxic response and yield of soybean (*Glycine max*) varieties treated with sulfentrazone or flumioxazin. Weed Technol 15:95-102
- Tull J (1733) The horse-hoing husbandry. London: Printed for the author
- [USDA] US Department of Agriculture UERSa (2016a) Agriculture resource management survey, 1996-2012 in USDA ERSa ed.: National agricultural statistics service
- [USDA] US Department of Agriculture (2016b) 2015 Agricultural chemical use survey soybeans: National Agricultural Statistics Service
- [USDA] US Department of Agriculture (2006) 2006 Agriculture chemical use survey soybeans: National Agricultural Statistics Service

- Wise K, Mueller DS, Kandel Y, Young B, Johnson B, Legleiter T (2015) Soybean seedling damage: Is there an interaction between the ILeVO seed treatment and pre-emergence herbicides? Iowa State University. Agriculture and Natural Resources
- Zhaohu L, Walker R, Wehtje G (1997) Laboratory studies for predicting response of soybean cultivars to sulfentrazone. Pages 177-178 *in* Proc. of S. Weed Sci. Soc. Houston, TX

Table 2.1 Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr average from April through October in 2015 and 2016 at the Boone and Carroll county research locations.

Month	Rainfall				Temperature			
	Boone County		Carroll County		Boone County		Carroll County	
	2015	30 yr avg.	2016	30 yr avg.	2015	30 yr avg.	2016	30 yr avg.
	mm				C			
April	83.6	114.1	59.9	98.0	14.4	12.8	13.1	12.2
May	144.3	126.0	99.6	135.9	18.7	17.8	17.0	17.8
June	192.0	114.1	74.4	138.9	23.8	22.8	25.1	23.3
July	213.4	111.0	214.4	112.0	25.3	25.0	25.2	25.6
August	79.8	111.0	363.2	108.0	23.4	24.4	24.5	24.4
September	29.7	98.0	117.1	117.1	22.3	20.0	21.9	19.4
October	29.2	84.1	48.5	93.0	14.9	13.3	16.1	13.3

^a30 year averages obtained from National Climatic Data Center (2017).

Table 2.2. Sources of materials used in the experiments.

Herbicide	Trade Name	Formulation	Manufacturer	Address
Chlorimuron + sulfentrazone	Authority XL	70 DG	FMC	Philadelphia, PA
Chlorimuron + flumioxazin + metribuzin	Trivence	61.3 WDG	DuPont	Wilmington, DE
Chlorimuron + flumioxazin + pyroxasulfone	Fierce XLT	62.41 WDG	Valent	Walnut Creek, CA
<i>S</i> -metolachlor	Dual II Magnum	82.4 EC	Syngenta	Greensboro, NC
Glyphosate	Roundup Powermax	4.5 SC	Monsanto Company	St. Louis, MO
Ammonium sulfate	N-Pak AMS	3.4 L	Winfield Solutions	St. Paul, MN

^a Abbreviations: DG, dispersible granule; WDG, water dispersible granule; EC, emulsifiable concentrate; SC, soluble concentrate; L, liquid.

Table 2.3. Analysis of variance for visual soybean injury, leaf area, stand, height, biomass, and yield 30 days after soybean emergence (DAE) in 2015 and 2016.

	Visual		Stand		Leaf Area		Height		Biomass		Yield	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
ANOVA	P > F											
Variety (V)	<.0001	0.0303	0.0366	NS	0.0103	NS	<.0001	0.0489	NS	0.0416	<.0001	NS
Herbicide (H)	<.0001	<.0001	<.0001	NS	<.0001	NS	<.0001	NS	NS	0.0010	<.0001	NS
Seed Treatment (ST)	NS	<.0001	0.0064	<.0001	NS	NS	0.0342	NS	NS	NS	NS	NS
V by H	<.0001	0.0004	<.0001	0.0276	0.0137	NS	0.0006	NS	NS	NS	<.0001	NS
V by ST	0.0282	0.0425	0.0113	NS	NS	NS	NS	NS	NS	NS	NS	NS
H by ST	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V by H by ST	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^a Abbreviations. DAE, days after emergence

^b NS, not significant at the $\alpha = 0.05$ level.

Table 2.4. Influence of varieties, herbicides, and seed treatments on soybean visual injury, stand, and leaf area 30 days after soybean emergence in 2015 and 2016.

Factor	Visual Injury		Stand		Leaf Area
	2015	2016	2015	2016	2015
	——% injury ^a ——		————soybean ha ⁻¹ ————		—% relative to control—
Variety					
Tolerant	25 b	11 b	242,594 a	—— ^b	77 a
Sensitive	37 a	13 a	230,732 b	——	63 b
Herbicide					
Non-treated	0 c	8 c	287,325 a	——	105 a
Chlorimuron-ethyl + flumioxazin + pyroxasulfone	48 a	13 ab	225,048 c	——	52 c

Chlorimuron-ethyl + flumioxazin + metribuzin	26 b	11 bc	261,953 b	—	70 b
Chlorimuron-ethyl + sulfentrazone	51 a	15 a	172,326 d	—	54 c
Seed Treatment					
Non-treated seed	—	18 a	227,519 b	226,805 b	—
Imidacloprid	—	10 b	232,132 b	312,134 a	—
<i>Pasteuria nishizawae</i> + thiamethoxam	—	9 b	253,715 a	323,735 a	—
Fluopyram + imidacloprid	—	9 b	233,285 b	331,081 a	—
Variety by herbicide					
Tolerant by non-treated	0 f	9 cd	295,562 a	279,512 ab	99 ab

Tolerant by chlorimuron-ethyl + flumioxazin + pyroxasulfone	45 c	13 b	206,925 e	279,252 bc	58 cd
Tolerant by chlorimuron-ethyl + flumioxazin + metribuzin	23 e	9 bcd	253,715 cd	311,104 ab	79 bc
Tolerant by chlorimuron-ethyl + sulfentrazone	32 d	11 bcd	214,174 e	307,534 ab	74 c
Sensitive by non-treated	0 f	7 d	279,087 ab	308,221 ab	111 a
Sensitive by chlorimuron-ethyl + flumioxazin + pyroxasulfone	52 b	12 bc	243,171 d	308,358 ab	45 de
Sensitive by chlorimuron-ethyl + flumioxazin + metribuzin	29 de	12 bc	270,190 bc	316,871 a	62 cd
Sensitive by chlorimuron-ethyl + sulfentrazone	70 a	19 a	130,479 f	258,657 c	33 e

Variety by seed treatment

Tolerant by non-treated	26 d	19 a	221,094 b	—	—
Tolerant by imidacloprid	27 d	8 bc	241,194 b	—	—

Tolerant by <i>pasteuria nishizawae</i> + thiamethoxam	20 e	7 c	273,156 a	—	—
Tolerant by fluopyram + imidacloprid	29 cd	8 bc	234,933 b	—	—
Sensitive by non-treated	33 bc	17 a	233,944 b	—	—
Sensitive by imidacloprid	37 ab	12 b	223,071 b	—	—
Sensitive by <i>pasteuria nishizawae</i> + thiamethoxam	39 a	11 b	234,274 b	—	—
Sensitive by fluopyram + imidacloprid	40 a	11 b	231,638 b	—	—

^a Means followed by the same letter are not different, $P \geq 0.05$.

^b Values represented by — are not different, $P \geq 0.05$

Table 2.5. Influence of varieties, herbicides, and seed treatments on soybean height, biomass, and yield 30 days after soybean emergence in 2015 and 2016.

Factor	Height		Biomass	Yield
	2015	2016	2016	2015
	-% relative to control- ^a		-% relative to control-	-kg ha ⁻¹ -
Variety				
Tolerant	82 a	109 a	95 a	3,776 a
Sensitive	70 b	103 b	86 b	2,921 b
Herbicide				
Non-treated control	93 a	— ^b	106 a	4,417 a
Chlorimuron-ethyl + flumioxazin + pyroxasulfone	66 c	—	83 b	3,046 c

Chlorimuron-ethyl + flumioxazin + metribuzin	80 b	—	91 b	3,732 b
Chlorimuron-ethyl + sulfentrazone	65 c	—	82 b	2168 d
Seed Treatment				
Non-treated seed	79 a	—	—	—
Imidacloprid	76 ab	—	—	—
<i>Pasteuria nishizawae</i> + thiamethoxam	77 a	—	—	—
Fluopyram + imidacloprid	72 b	—	—	—
Variety by herbicide				
Tolerant by non-treated control	93 a	—	—	4,575 a

Tolerant by chlorimuron-ethyl + flumioxazin + pyroxasulfone	72 c	—	—	3,246 cd
Tolerant by chlorimuron-ethyl + flumioxazin + metribuzin	85 b	—	—	4,012 b
Tolerant by chlorimuron-ethyl + sulfentrazone	77 c	—	—	3,273 c
Sensitive by non-treated herbicide	93 ab	—	—	4,260 ab
Sensitive by chlorimuron-ethyl + flumioxazin + pyroxasulfone	60 d	—	—	2,846 d
Sensitive by chlorimuron-ethyl + flumioxazin + metribuzin	75 c	—	—	3,515 c
Sensitive by chlorimuron-ethyl + sulfentrazone	53 d	—	—	1,063 e

^a Means with the same letter are not different $P \geq 0.05$.

^b Values represented by — are not different $P \geq 0.05$.

Chapter III

Investigations of 2,4-D and Multiple Herbicide Resistance in a Missouri Waterhemp Population

Blake R. Barlow and Kevin W. Bradley

Field and greenhouse dose-response experiments were conducted in 2015 and 2016 to investigate the potential for 2,4-D and multiple herbicide resistance in a waterhemp biotype from Missouri (designated MO-Ren). In the field, visual control of the MO-Ren biotype with 0.56 to 4.48 kg 2,4-D ha⁻¹ ranged from 26 to 77% in 2015 and from 15 to 55% in 2016. Estimates of the 2,4-D dose required to provide 50% visual control of the MO-Ren biotype were 1,440 g ha⁻¹ compared to only 465 g 2,4-D ha⁻¹ for the susceptible biotype. Based on comparisons to a susceptible biotype in dose-response experiments, the MO-Ren biotype was approximately 3-fold resistant to 2,4-D, and approximately 12-, 19-, 68-, 8-, and 284-fold resistant to fomesafen, atrazine, glyphosate, mesotrione, and chlorimuron, respectively. The only two herbicides that provided effective control of the MO-Ren biotype in these experiments were dicamba and glufosinate. Results from these experiments confirm that the MO-Ren waterhemp population is resistant to 2,4-D, atrazine, chlorimuron, fomesafen, glyphosate, and mesotrione, making this population the third 2,4-D-resistant waterhemp population identified in the U.S, and the first population resistant to six different herbicidal modes of action.

Nomenclature: Waterhemp, *Amaranthus rudis*; 2,4-D; glyphosate; dicamba; fomesafen, atrazine; chlorimuron; glufosinate, mesotrione.

Key words: Multiple herbicide resistance, auxinic herbicides, growth regulator herbicides.

Amaranthus species are the most troublesome weed species currently found in United States production agriculture (Legleiter and Bradley 2008; Rosenbaum and Bradley 2013; Sellers et al. 2003; Webster 2005). Of the *Amaranthus* species, common waterhemp (*Amaranthus rudis*) and tall waterhemp (*Amaranthus tuberculatus*) are the most common and problematic weeds that farmers throughout the Midwest must contend with when growing corn (*Zea mays L.*) or soybean (*Glycine max L.*) (Norbdy et al. 2007). Since common and tall waterhemp are genetically similar and a great deal of hybridization has occurred between the two, most botanists now group the two species into one collective “waterhemp” species (Norbdy et al. 2007; Pratt and Clark 2001). *Amaranthus* species, and specifically waterhemp, are very prolific seed producers. A single female plant is able to produce at least 250,000 seeds in a single growing season, with some plants able to produce as many as 1,000,000 seeds (Hager et al. 1997; Patzoldt et al. 2002; Sellers et al. 2003). Waterhemp also has an extremely aggressive growth habit and a discontinuous emergence pattern; it can germinate early in the growing season (as soon as 350 growing degree days), but can also germinate later in the growing season than most other summer annual weed species (Hager et al. 2002; Sellers et al. 2003).

Amaranthus species are troublesome due to their ability to compete for water, nutrients, sunlight and other resources. Redroot pigweed, Palmer amaranth, and waterhemp reduced soybean yield by 38, 79, and 56%, respectively, at a density of 8 plants m⁻¹ of row (Bensch et al. 2003). Hager et al. (2002) observed that waterhemp densities of 89 to 362 plants per m² resulted in a 43% reduction in soybean yield. Waterhemp with densities ranging from 82 to 445 plants per m² reduced corn yields from 10% to 36% (Cordes et al. 2004).

Herbicide resistance is defined as the inherited ability of a plant to survive and reproduce following exposure to a dose of a herbicide that would normally be lethal to the wild type

(Prather et al. 2000). The first incidences of evolved herbicide resistance were reported in wild carrot (*Daucus carota* L.), which evolved resistance to the auxin analog class of herbicides after several seasons of consecutive use of 2,4-D (Switzer 1957; Whitehead and Switzer 1963), and common groundsel (*Senecio vulgaris* L.), which evolved resistance to simazine and atrazine after receiving either one or two annual applications of these herbicides for 10 consecutive years (Ryan 1970). One practice that can lead to resistance in a weed biotype is the continuous use of a single active ingredient or mechanism of action (MOA) over a long period of time, resulting in the selection of resistant plants (Al-Khatib et al. 1998; Jasieniuk et al. 1996). This phenomenon has occurred across a wide geography in the U.S. since the introduction of glyphosate-resistant crops; producers went from controlling their weeds with tillage, crop rotation, and switching between herbicides with different mechanisms of action to an almost complete dependence on glyphosate alone for weed control. The first glyphosate-resistant waterhemp biotype was identified in Platte County, Missouri in 2005 (Bradley et al. 2006; Heap 2017). There are now glyphosate-resistant waterhemp biotypes found in 19 states, and 3 cases in Ontario, Canada (Heap 2017). Waterhemp has also been reported to have resistance to herbicides from one or more of the following sites of action: 5-Enolpyruvylshikimate-3-phosphate synthase inhibitor (EPSPS), acetoacetate synthase inhibitor (ALS), protoporphyrinogen oxidase inhibitor (PPO), photosystem II inhibitor (PSII), 4-hydroxyphenylpyruvate dioxygenase inhibitor (HPPD), and TIR1 auxin receptor (growth regulator) (Heap 2017).

The first confirmed population of 2,4-D-resistant waterhemp was discovered in 2009 in a warm season native-grass seed production field in Nebraska (Bernards et al. 2012). This biotype exhibited 10-fold resistance to 2,4-D, and reduced sensitivity to dicamba as a result of continual use of low rates of 2,4-D to control broadleaf weeds in this setting (Bernards et al. 2012). A

second population resistant to 2,4-D has since been discovered in Illinois in a row crop setting, and is resistant to five different herbicidal modes of action (Heap 2017; Sabate et al. 2016). The mechanism responsible for the resistance to 2,4-D in these waterhemp populations is currently unknown (Bernards et al. 2012; Heap 2017; Sabate et al. 2016). Historically, the use of synthetic auxin herbicides was restricted to cereal crops and pastures, however there are now soybean and cotton varieties with a trait that confers resistance to 2,4-D and a separate trait that confers resistance to dicamba. These technologies should provide growers with new options for the control of broadleaf weeds like waterhemp that have evolved resistance to glyphosate in cotton and soybean production systems (Craigmyle et al. 2013). However, as previously mentioned, at least two 2,4-D-resistant waterhemp populations have already been discovered. A controlled greenhouse experiment was also reported in Arkansas where dicamba resistance was selected for in a Palmer amaranth population after three generations of sub-lethal doses of dicamba (Tehranchian et al. 2016).

In 2014, a grower in central Missouri claimed that he was unable to control waterhemp with 2,4-D after routinely applying the herbicide as part of his pre-plant herbicide program for several years consecutively. The objectives of this research were to determine the level of 2,4-D and multiple herbicide resistances in this waterhemp population through field and greenhouse dose-response experiments.

MATERIALS AND METHODS

Field Experiments. An experiment was conducted during the summer of 2015 and repeated in 2016 in the location where the suspected 2,4-D resistant waterhemp was reported in Randolph County, Missouri (39.32°N, -92.39°W). The field had been in continuous soybean production

and had a history of repeated glyphosate, PPO, and 2,4-D use as a pre-plant burndown. The soil type in this location was a Putnam silt loam with a pH of 5.9 and organic matter content of 1.9%. Individual plots measured 3 by 8 meters in size and treatments were arranged in a randomized complete block design with four replications. All treatments were applied at a constant speed of 5 km h⁻¹ with a hand-held CO₂-pressurized research backpack sprayer that delivered 140 L ha⁻¹, and were sprayed on 8/7 and 6/6 in 2015 and 2016, respectively. All treatments that contained a synthetic auxin herbicide were applied using TTI11002 nozzles. All other treatments that did not contain a group 4 herbicide were applied using 8002 flat-fan nozzles (TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton IL. 60187). The treatments included in the experiment are shown in Table 3.1, while the source of all materials is presented in Table 3.2. Because of the diversity of herbicide treatments evaluated, the experiment was conducted as a bare ground trial in the absence of any crop. All treatments were applied when waterhemp reached an average height of 10 cm. Visual ratings were taken 7, 14, 28, and 42 days after herbicide application (DAA). At the 42 DAA timing, surviving waterhemp plants were counted and harvested by clipping them at the soil surface in 2, 1-m² quadrats per plot. Biomass was determined by drying the harvested waterhemp plants for five days at 50°C in a commercial dryer. All data were analyzed using the PROC GLIMMIX procedure in SAS (SAS 9.4, SAS® Institute Inc. Cary, NC). Individual treatment means were separated using Fisher's Protected LSD at P≤0.05. Significant interactions were present between years; therefore all data are presented separately by year.

Greenhouse Dose-Response. Seed were collected from the suspected resistant population designated MO-Ren in the fall of 2014 and treated with a 50:50 water bleach solution for 5 minutes, then rinsed with water and suspended in a 0.1% agarose solution and stored at a

constant 4°C prior to the start of each experiment. The susceptible biotype of waterhemp (designated MO-S) were from a population that was confirmed to be susceptible to multiple herbicide classes through a preliminary screening done prior to the experiments. In each experiment, 0.15 g seed from the MO-S population were weighed and broadcast into 25-by-55-cm greenhouse flats containing a mixture of 2/3 commercial potting medium (Premier Tech Horticulture, Quakertown, PA), and 1/3 soil. Simultaneously, approximately 75 seeds from the MO-Ren population were pipetted from the agarose solution onto flats containing this same potting medium and soil mix. After emergence, plants were thinned so that each greenhouse flat contained 20 to 25 plants. Plants were maintained in a greenhouse at 25 to 30°C, watered and fertilized as needed, and provided with artificial lighting from metal halide lamps (600 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$) simulating a 16-h-photoperiod day.

Herbicide treatments were applied when the plants reached 10-cm in height using a compressed air laboratory spray chamber equipped with an 8001EVS nozzle (Teejet Spraying Systems, Wheatland, IL) delivering 140 L ha⁻¹ at 234 kPa. The herbicides and rates evaluated are listed in Table 3.3. The herbicide rates ranged from one tenth the standard labeled rate (0.1X) up to twenty times the standard labeled rate (20X), depending on the herbicide and likelihood of low or high level resistance. A non-treated control was included from both populations in each experiment for comparison. Dose-response experiments were replicated twice for atrazine, chlorimuron, fomesafen, and dicamba, three times for 2,4-D, and have only been conducted once for mesotrione and glyphosate. The MO-S population is not susceptible to glyphosate, so a different susceptible population from the preliminary screening was used for comparison to the MO-Ren population in this experiment. Each herbicide screening occurred as an individual

experiment and was conducted in a randomized complete block design with a factorial arrangement of populations and herbicides rates with four replications.

Visual ratings were taken 7, 14, and 21 days after application (DAA). Visual injury evaluations were assessed on a scale of 0 to 100 percent, where 0 represented no plant death or injury, and 100 was equivalent to complete plant death. At 21 DAA, surviving plants were counted, cut at the soil surface and dried for 5 days in a forced air oven at 50° C, and then dry weights recorded. Dose response curves for each herbicide were created in Sigma Plot 12.3 statistical software (Systat Software, San Jose, CA) using a non-linear, log-logistic regression model: $Y = c + \{(d - c) / [1 + (x / g)^b]\}$, where Y is the measurement taken, c and d are the coefficients corresponding to the lower and upper asymptotes, b is the slope of the line, g is the herbicide rate at the point of inflection halfway between the upper and lower asymptotes, and x (independent variable) is the herbicide dose (Fernández et al. 2016).

RESULTS AND DISCUSSION

Field Experiment. Results from the field experiments indicate that this waterhemp biotype is resistant to six of the eight herbicides tested in the field, including 2,4-D. Visual control of the suspected resistant waterhemp biotype with 0.56 to 4.48 kg ha⁻¹ 2,4-D ranged from 26 to 77% and 15 to 55% 21 DAA in 2015 and 2016, respectively (Table 3.4). By 42 DAA, the visual control was similar or lower than that observed 21 DAA in both years. The lower visual control of MO-Ren in 2016 compared to 2015 may be a result of the waterhemp population becoming less susceptible to 2,4-D after repeated exposure over time. The continual use of the same herbicide or herbicides with the same mechanism of action will inevitably select for tolerant

species or for resistant biotypes (Shaner 2014). For example, Tehranchian et al. (2016) reported that a population of dicamba-susceptible Palmer amaranth became resistant in only three generations in a controlled greenhouse experiment. Biomass reduction of the waterhemp plants treated with 2,4-D followed similar trends as the visual control ratings; plant biomass was reduced by 34 to 87% in 2015 and by 48 to 58% in 2016 in response to the range of 2,4-D rates evaluated. The 4.48 kg ha⁻¹ rate of 2,4-D represents four times the labeled rate for 10- to 15-cm waterhemp, and yet this rate did not provide acceptable levels of control or biomass reduction in the field. Bernards et al. (2012) discovered the first 2,4-D-resistant waterhemp in a warm season grass-production field, and 3,584 kg ha⁻¹ of 2,4-D only resulted in a 71% control 7 DAA. Only one other biotype of waterhemp has been identified as 2,4-D resistant, and this is first biotype to evolve resistance in a row crop setting in Illinois in 2016 (Heap 2017; Sabate et al. 2016).

The biotype tested in this experiment also exhibited resistance to atrazine (PSII inhibitor), chlorimuron (ALS inhibitor), fomesafen (PPO inhibitor), glyphosate (EPSPS inhibitor), and mesotrione (HPPD inhibitor). This population exhibited very high levels of resistance to atrazine, with visual injury never exceeding 27% in either year, and the highest level of biomass reduction was 22% with 4.48 kg ha⁻¹ atrazine in 2016 (Table 3.4). The first waterhemp population discovered with resistance to PSII-inhibiting herbicides was identified in 1994 in Missouri (Heap 2017). Since that time, Schultz et al. (2015) determined that approximately 30% of the waterhemp populations across Missouri are resistant atrazine. Foes et al. (1998) reported that an Illinois waterhemp population required more than 20 kg ha⁻¹ atrazine to achieve 50% control.

Similarly, this waterhemp population was highly resistant to chlorimuron, with visual injury never exceeding 7% either year. Biomass was only reduced by as much as 14% either year

(Table 3.4). These results were expected, since the majority of the waterhemp population across the Midwest are now resistant to ALS-inhibiting herbicides (Heap 2017). For example, of the 187 populations of waterhemp collected by Schultz et al. (2015) in a survey of Missouri waterhemp populations, 186 of the populations were resistant to ALS-inhibiting herbicides.

Visual control of this waterhemp biotype was higher in 2015 (22 to 47%) than 2016 (2 to 9%) in response to fomesafen (Table 3.4). Biomass reduction followed similar trends as the visual control, with an 11 to 44% and 3 to 26% biomass reduction in response to increasing fomesafen rates in 2015 and 2016, respectively. Schultz et al. (2015) reported that approximately 17% of the waterhemp populations remaining in Missouri soybean fields at harvest are resistant to PPO herbicides, however the percentage of fields with PPO-resistant waterhemp continues to increase in Missouri due to widespread post-emergence use of these herbicides as ‘rescue’ treatments (Kevin Bradley, personal communication).

Glyphosate provided only 6 to 24% and 2 to 21% visual control of the MO-Ren biotype in 2015 and 2016, respectively (Table 3.4). Biomass reduction was greater in 2016 (18 to 33%) than in 2015 (6 to 11%). This may be a result of the field site receiving 30 mm more precipitation in June 2016 than August 2015 (Table 3.5). Skelton et al. (2016) reported that the efficacy of herbicides such as glyphosate is reduced when waterhemp is under drought stress and that waterhemp dry matter was reduced by 2% when receiving 10 ml water day⁻¹, but dry matter of plants that received 40 ml day⁻¹ was reduced by 56%. Nevertheless, these results clearly indicate glyphosate resistance in the MO-Ren population as well. Schultz et al. (2015) reported that 29% of Missouri waterhemp populations were resistant to glyphosate, and glyphosate-resistant waterhemp now also occurs in 19 U.S. states and one province in Canada (Heap 2017).

Mesotrione resulted in 34 to 73% and 38 to 88% visual control of the waterhemp population by 21 DAA in 2015 and 2016, respectively (Table 3.4). By 42 DAA in both years, visual control was very similar to that observed 21 DAA. Waterhemp biomass was also reduced by 18 to 78% and 27 to 77% in 2015 and 2016, respectively. These results indicate that this population is also resistant to mesotrione. In other investigations of HPPD resistance in waterhemp, Hausman et al. (2011) observed from 13 to 27% visual injury in response to the labeled rate of mesotrione in a biotype from Illinois, while McMullan and Green (2011) reported only 41% injury in response to a 2X rate of mesotrione on a waterhemp biotype from Iowa.

The only two herbicides that controlled the MO-Ren waterhemp adequately in the field were dicamba and glufosinate at the highest rates (Table 3.4). By 42 DAA, 2.24 and 4.48 kg ha⁻¹ dicamba provided 93-99% control of this waterhemp biotype, and 95 to 100% biomass reduction. Even though 2,4-D and dicamba are both synthetic auxin, group 8 herbicides, they are different chemical families, which can lead to different plant responses to each. These results are similar to those reported by Bernards et al. (2012) in that both populations exhibit resistance to 2,4-D, but are both susceptible to dicamba. Both waterhemp biotypes demonstrate some tendency towards reduced sensitivity to dicamba at labeled rates, but both biotypes are susceptible to the herbicide.

Control of the MO-Ren biotype was also variable in response to glufosinate and was dependent on rate. By 21 DAA in 2015, visual control ranged from 56 to 90%, and was very similar at that observed 42 DAA. In 2016 however, visual control ranged from 47 to 89% 21 DAA, but by 42 DAA had fallen to 28 to 71%. Since glufosinate is primarily a contact herbicide, control was highest after the initial application, but waterhemp plants that were at least partially defoliated put on new growth after all of the initial leaves had abscised. Coetzer et al. (2002)

also reported that a single application of glufosinate was not as effective as two applications because of plant regrowth and the contact nature of glufosinate.

Greenhouse Dose Response Experiments. The doses required to achieve 50% control and survival of the MO-Ren biotype were higher than that for the susceptible biotype for 2,4-D, fomesafen, atrazine, glyphosate, mesotrione, and chlorimuron (Table 3.6; Figures 3.1 and 3.2). Results from the dose-response experiments confirmed that the MO-Ren waterhemp population is resistant to 2,4-D (Table 3.6; Figures 3.1 and 3.2), making this population the third 2,4-D-resistant waterhemp population identified in the U.S. MO-Ren was approximately 3-fold more resistant to 2,4-D than the susceptible population based on survival and visual control data (Table 3.6). Estimates of the 2,4-D dose required to achieve 50% visual control were 1,440 g ha⁻¹ for the MO-Ren biotype while only 465 g ha⁻¹ was required to achieve the same level of control of the susceptible biotype. Bernards et al. (2012) reported similar results in that 1,864 g 2,4-D ha⁻¹ was required to achieve 50% visual control of the Nebraska biotype.

Similar to the field results described previously, resistance to atrazine, glyphosate, and HPPD- ALS- and PPO-inhibiting herbicides was also confirmed in the MO-Ren biotype. The MO-Ren biotype exhibited high level resistances to atrazine and chlorimuron, which is a common response of *Amaranthus* species to these herbicides in many other locations throughout the U.S. (Bell et al. 2013; Foes et al. 1998; Lovell et al. 1996; McMullan and Green 2011; Patzoldt et al. 2003; Patzoldt et al. 2005; Sprague et al. 1997a; Sprague et al. 1997b). For example, Sprague et al. (1997a) reported a waterhemp population with 1,920-fold resistance that required rates higher than 1000 g ai ha⁻¹ to achieve 50% control. A majority of the waterhemp populations across the Midwest are now resistant to ALS-inhibiting herbicides, and many of them exhibit ultra high levels of resistance (Heap, 2017; Schultz et al. 2015). The MO-Ren

biotype exhibited approximately 20-fold resistance to glyphosate, which is similar to the 19-fold resistance reported in the first glyphosate resistant waterhemp population discovered (Legleiter and Bradley 2008). The mesotrione dose required to achieve 50% visual control of the MO-Ren biotype was 406 g ha⁻¹, which resulted in an approximate 8-fold level of resistance (Table 3.6). However, the control of mesotrione in both populations was inconsistent over each greenhouse screening therefore another run of this experiment is being conducted in order to better understand the true level of resistance to this herbicide.

Similar to the field study, one of the only herbicides that provided effective control of the MO-Ren biotype was dicamba. Both the MO-Ren and MO-S biotypes were effectively controlled with dicamba, and the dose required to provide 50% survival was similar (613 and 628 g ha⁻¹, respectively). As mentioned previously, Bernards et al. (2012) also reported that the Nebraska 2,4-D resistant population exhibited reduced sensitivity to dicamba, requiring approximately 3 times more dicamba to achieve 50% visual control than the susceptible populations.

The results of this research indicate that multiple resistances across six sites of herbicidal action are present in the MO-Ren waterhemp, and that this biotype is resistant to 2,4-D. This population exhibited 3-fold resistance to 2,4-D and there are currently only two other known waterhemp populations that have been reported with resistance to 2,4-D; one in Nebraska discovered in a warm season grass production field, and one in Illinois in a corn and soybean production field (Bernards et al. 2012; Heap 2017). Technologies such as 2,4-D resistant corn and soybean have been developed and are awaiting approval to be commercialized. Dicamba-resistant corn, cotton, and soybean, and 2,4-D-resistant cotton technologies have already been developed and have been recently released for commercial use. The fact that there are already

three waterhemp populations with resistance to 2,4-D and that at least one of these populations has demonstrated a reduced sensitivity to dicamba should be emphasized in order to ensure that proper stewardship of these new technologies is followed (Bernards et al. 2012; Heap 2017; Tehranchian et al. 2016). There are currently no other reported waterhemp biotypes with resistance to six different herbicide classes (Heap 2017), which also underlines the need to use a diversified approach towards weed management that includes any of the appropriate cultural, mechanical, and biological control tactics available in a given production system rather than relying on any one method alone (Norsworthy et al. 2012).

Literature Cited

- Al-Khatib K, Baumgartner JR, Peterson DE, Currie RS (1998) Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Sci 46:403-407
- Bell MS, Hager AG, Tranel PJ (2013) Multiple resistance to herbicides from four site-of-action groups in waterhemp (*Amaranthus tuberculatus*). Weed Sci 61:460-468
- Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37-43
- Bernards ML, Crespo RJ, Kruger GR, Gaussoin R, Tranel PJ (2012) A waterhemp (*Amaranthus tuberculatus*) population resistant to 2,4-D. Weed Sci 60:379-384
- Bradley K, Li J, Monnig N (2006) Greenhouse investigations of suspected glyphosate-resistant common waterhemp populations from Missouri. Weed Sci Soc Am 46
- Coetzer E, Al-Khatib k, Peterson DE (2002) Glufosinate efficacy on *Amaranthus* species in glufosinate-resistant soybean (*Glycine max*). Weed Technol 16:326-331
- Cordes JC, Johnson WG, Scharf P, Smeda RJ (2004) Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. Weed Technol 18:999-1005
- Craigmyle BD, Ellis JM, Bradley KW (2013) Influence of herbicide programs on weed management in soybean with resistance to glufosinate and 2,4-D. Weed Technol 27:78-84
- Fernández P, Alcántara-de la Cruz R, Cruz-Hipólito H, Osuna MD, De Parado R (2016) Underlying resistance mechanisms in the *cynosurus echinatus* biotype to acetyl CoA carboxylase-inhibiting herbicides. Frontiers in Plant Science 7:1-10

- Foes MJ, Tranel PJ, Wax LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. *Weed Sci* 46:514-520
- Hager A, Wax L, Simmons F, Stoller E (1997) Waterhemp management in agronomic crops. Urbana, IL: University of Illinois 12 p.
- Hager AG, Wax LM, Stoller EW, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. *Weed Sci* 50:607-610
- Hausman NE, Singh S, Tranel PJ, Riechers DE, Kaundun SS, Polge ND, Thomas DA, Hager AG (2011) Resistance to HPPD-inhibiting herbicides in a population of waterhemp (*Amaranthus tuberculatus*) from Illinois, United States. *Pest Management Sci* 67:258-261
- Heap I (2017) The international survey of herbicide resistant weeds.
www.weedscience.com/summary/home.aspx. Accessed May 1, 2017
- Jasieniuk M, Brûlé-Babel AL, Morrison IN (1996) The evolution and genetics of herbicide resistance in weeds. *Weed Sci* 44:176-193
- Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. *Weed Sci* 56:582-587
- Lovell ST, Wax LM, Horak MJ, Peterson DE (1996) Imidazolinone and sulfonylurea resistance in a biotype of common waterhemp (*Amaranthus rudis*). *Weed Sci* 44:789-794
- McMullan PM, Green JM (2011) Identification of tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in Iowa. *Weed Technol* 25:514-518
- Norby D, Hartzler B, Bradley K (2007) Biology and management of waterhemp. West Lafayette, IN Purdue Extension. Glyphosate, Weeds, and Crop Series Publication GWC-13:1-12

- Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: Best management practices and recommendations. *Weed Sci* 60:31-62
- Patzoldt WL, Tranel PJ, Hager AG (2002) Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. *Crop Protection* 21:707-712
- Patzoldt WL, Dixon BS, Tranel PJ (2003) Triazine resistance in *Amaranthus tuberculatus* (Moq) sauer that is not site-of-action mediated. *Pest Management Science* 59:1134-1142
- Patzoldt WL, Tranel PJ, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. *Weed Sci* 53:30-36
- Prather T, Ditomaso J, Holt J (2000) Herbicide resistance: Definition and management strategies. University of California ANR Publication 8012: 1-14
- Pratt DB, Clark LG (2001) *Amaranthus rudis* and *A. tubercuatus*, one species or two? *Torrey Botanical Society* 128:282-296
- Rosenbaum KK, Bradley KW (2013) A survey of glyphosate-resistant waterhemp (*Amaranthus rudis*) in Missouri soybean fields and prediction of glyphosate resistance in future waterhemp populations based on in-field observations and management practices. *Weed Technol* 27:656-663
- Ryan G (1970) Resistance of common groundsel to simazine and atrazine. *Weed Sci* 18:614-616
- Sabate S, Tranel PJ, Hager AG (2016) Genetics of 2,4-D resistance in an Illinois waterhemp population. N C Weed Sci Soc Des Moines, Iowa, 2016

- Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri (*Amaranthus rudis* Sauer) populations. *Weed Sci* 63:336-345
- Sellers BA, Smeda RJ, Johnson WG, A. KJ, Ellersieck MR (2003) Comparative growth of six *Amaranthus* species in Missouri. *Weed Sci* 51:329-333
- Shaner DL (2014) Lessons learned from the history of herbicide resistance. *Weed Sci* 62:427-431
- Skelton JJ, Ma R, Riechers DE (2016) Waterhemp (*Amaranthus tuberculatus*) control under drought stress with 2,4-dichlorophenoxyacetic acid and glyphosate. *Weed Biology and Management* 16:34-41
- Sprague CL, Stoller EW, Wax LM (1997a) Response of an acetolactate synthase (ALS)-resistant biotype of *Amaranthus rudis* to selected ALS-inhibiting and alternative herbicides. *Weed Research* 37:93-101
- Sprague CL, Stoller EW, Wax LM, Horak MJ (1997b) Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. *Weed Sci* 45:192-197
- Switzer C (1957) The existence of 2, 4-D-resistant strains of wild carrot. Pages 315-318 in *Proc. of the 11th Northeast Weed Control Conference*, Columbia, MO: NEWSS
- Tehranchian P, Norsworthy JK, Powles SB (2016) To what extent does repeated use of dicamba select for resistance in palmer amaranth? *Weed Sci Soc of America* San Juan, Puerto Rico
- Webster TM (2005) Weed survey-southern states, 2001: Broadleaf crops subsection (cotton, peanut, soybean, tobacco, and forestry). Pages 291-306 in *Proc. of 58th S. Weed Sci Soc*

Whitehead C, Switzer C (1963) The differential response of strains of wild carrot to 2, 4-D and related herbicides. Can J Plant Sci 43:255-262

Table 3.1. Herbicide rates and adjuvants used in field experiments in 2015 and 2016 at the suspected resistant waterhemp site.

Herbicide	Rate	Adjuvant Used	Rate
	kg ai or ae ha ⁻¹		% v/v or kg ai ha ⁻¹
2,4-D Amine	0.56	NIS	0.25% v/v
	1.12	Drift Control Agent	0.29 kg ai ha ⁻¹
	2.24		
	4.48		
Atrazine	1.12		
	2.24		
	4.48		
Chlorimuron	0.01	NIS	0.25% v/v
	0.02	Drift Control Agent	0.29 kg ai ha ⁻¹
	0.05		
Fomesafen	0.34	COC	1% v/v
	0.68	Drift Control Agent	0.29 kg ai ha ⁻¹
	1.36		
Dicamba	0.56	NIS	0.25% v/v
	1.12	Drift Control Agent	0.29 kg ai ha ⁻¹
	2.24		
	4.48		
Glyphosate	0.84	AMS	2.86 kg ai ha ⁻¹
	1.68	Drift Control Agent	0.29 kg ai ha ⁻¹
	3.36		
Glufosinate	0.59	AMS	2.86 kg ai ha ⁻¹
	1.19	Drift Control Agent	0.29 kg ai ha ⁻¹

	2.38		
Mesotrione	0.11	COC	1% v/v
	0.21	Drift Control Agent	0.29 kg ai ha ⁻¹
	0.42		

^a Abbreviations: NIS, non-ionic Surfactant; COC, crop oil concentrate; AMS, ammonium sulfate; % v/v, percent volume to volume

Table 3.2. Sources of materials used in the experiments.

Herbicide	Trade Name	Formulation	Manufacturer	Address
2,4-D Amine	Weedar	4 L	Nufarm	Alsip, IL
Atrazine	AAtrex	4 L	Syngenta	Greensboro, NC
Chlorimuron	Classic	25 DG	DuPont	Wilmington, DE
Fomesafen	Flexstar	1.88 SL	Syngenta	Greensboro, NC
Dicamba	Clarity	4 L	BASF	Research Triangle Park, NC
Glyphosate	Roundup Powermax	4.5 SC	Monsanto Company	St. Louis, MO
Glufosinate	Liberty	280 SL	Bayer CropScience	Research Triangle Park, NC
Mesotrione	Callisto	4 SC	Syngenta	Greensboro, NC
Non-Ionic Surfactant	Astute	100 L	MFA	Columbia, MO
Crop Oil Concentrate	Relay	100 L	MFA	Columbia, MO
Ammonium Sulfate	N-Pak AMS	3.4 L	Winfield Solutions	St. Paul, MN
Drift Control Agent	InterLock	100 L	Winfield Solutions	St. Paul, MN

^a Abbreviations: L, liquid; DG dispersible granule; SL soluble liquid; SC, soluble concentrate.

Table 3.3. Herbicide rates and adjuvants used in greenhouse dose-response experiments conducted in 2015 and 2016.

Herbicide	Rate	Adjuvant Used	Rate
	kg ai or ae ha ⁻¹		% v/v or kg ai ha ⁻¹
2,4-D Amine	0.28 0.56 1.12 2.24 4.48	NIS	0.25% v/v
Atrazine	0.11 1.12 11.21 22.42	COC	1% v/v
Chlorimuron	0.001 0.01 0.13 1.31	NIS	0.25% v/v
Fomesafen	0.09 0.17 0.33 0.66 1.32	COC	1% v/v
Dicamba	0.28 0.56 1.12 2.24 4.48	NIS	0.25% v/v

Glyphosate	0.21	NIS	0.25% v/v
	0.42	AMS	2.86 kg ai ha ⁻¹
	0.84		
	1.68		
	3.36		
Mesotrione	0.03	COC	1% v/v
	0.05		
	0.11		
	0.21		
	0.42		

^a Abbreviations: NIS, non-ionic Surfactant; COC, crop oil concentrate; AMS, ammonium sulfate; % v/v, percent volume to volume

Table 3.4. Visual injury and biomass reduction in response to different herbicide chemistries and doses at the Renick, Missouri field site in 2015 and 2016.

Herbicide	Rate	Visual Control				Biomass	
	—kg ha ⁻¹ —	21 DAA		42 DAA		42 DAA	
		2015	2016	2015	2016	2015	2016
		% Control ^a				% Reduction	
2,4-D	0.56	26 hi	15 e-i	29 gh	16 hi	34 g	48 fg
	1.12	39 efg	20 e-h	48 f	19 hi	69 de	53 ef
	2.24	63 c	28 ed	56 f	32 fg	79 bcd	61 c-f
	4.48	77 b	55 b	71 e	51 e	87 abc	58 def
Atrazine	1.12	19 i	0 i	18 ij	0 j	3 hi	13 jkl
	2.24	23 i	5 hi	27 ghi	1 j	5 hi	18 h-l
	4.48	22 i	6 ghi	20 hij	2 j	11 hi	22 h-k
Chlorimuron	0.01	6 j	0 i	6 lk	0 j	0 i	14 i-l
	0.02	7 j	0 i	6 lk	0 j	3 hi	12 jkl
	0.05	6 j	0 i	6 lk	0 j	1 i	6 kl
Fomesafen	0.34	34 gh	4 i	28 ghi	2 j	11 hi	3 l
	0.68	25 hi	5 hi	22 g-j	3 j	15 hi	16 h-l
	1.36	45 ef	9 f-i	47 f	9 ij	44 fg	26 hij
Dicamba	0.56	37 fg	22 ef	52 f	42 ef	76 cd	61 c-f
	1.12	75 b	52 b	86 cd	71 cd	96 a	77 bcd
	2.24	97 a	86 a	98 ab	93 ab	100 a	95 ab
	4.48	99 a	99 a	99 a	99 a	100 a	100 a
Glyphosate	0.84	7 j	4 i	6 lk	2 j	6 hi	18 h-l
	1.68	20 i	12 f-i	15 jk	9 ij	11 hi	33 ghi
	3.36	24 i	21 efg	23 g-j	17 hi	7 hi	33 ghi

Glufosinate	0.59	56 cd	47 bc	56 f	28 gh	74 cd	33 gh
	1.19	81 b	86 a	85 cd	68 d	92 ab	67 cde
	2.38	90 a	89 a	88 bc	71 cd	93 ab	68 cde
Mesotrione	0.11	34 gh	38 cd	31 g	18 hi	18 h	27 hij
	0.21	48 ed	43 bcd	52 f	25 gh	53 ef	49 efg
	0.42	73 b	88 a	76 de	81 bc	78 bcd	77 bc

^a Means followed by the same letter are not different $P \geq 0.05$.

Table 3.5 Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr averages from May through October in 2015 and 2016 at the Renick, Missouri field site.

Month	Rainfall				Temperature			
	2015	30 years Avg.	2016	30 year Avg.	2015	30 year Avg.	2016	30 year Avg.
		mm				C		
May	118.6	131.1	168.4	131.1	18.6	17.8	21.8	17.8
June	11.78	5.12	100.3	130.1	21.7	22.2	24.6	22.2
July	223.0	121.9	136.4	121.9	24.5	25	24.9	25
August	70.6	105.9	155.7	105.9	22.7	23.9	24.3	23.9
September	20.1	110.0	125.2	110.0	22.2	19.4	21.9	19.4
October	39.6	84.1	53.1	84.1	13.7	13.3	12.8	13.3

^a30 year averages obtained from National Climatic Data Center (2017).

Table 3.6. Estimates of the herbicide dose resulting in 50% reduction in survival and visual control for the MO-Ren and MO-S waterhemp populations 21 DAA.

Herbicide	Survival Percentage		R/S Ratio	Visual Control		R/S Ratio
	MO-Ren	MO-S		MO-Ren	MO-S	
	LD ₅₀ ^a			I ₅₀ ^b		
	g ai ha ⁻¹			g ai ha ⁻¹		
2,4-D	1,368	516	2.7	1,440	465	3.1
Fomesafen	1,149	165	6.9	1,338	111	12.1
Atrazine	1,380	213	6.5	5,146	272	18.9
Glyphosate	17,034	827	20.6	50,650	750	67.5
Mesotrione	1,014	83	12.2	406	51	8.0
Chlorimuron	144,320	1,086	132.9	160,061	564	283.8
Dicamba	613	628	1.0	364	263	1.4

^a LD₅₀: herbicide dose required to achieve 50% stand reduction.

^b I₅₀: herbicide dose required to achieve 50% visual injury.

Figure 3.1. Survival of a suspected multiple-resistant (MO-Ren) and susceptible (MO-S) waterhemp population 21 DAA in response to 2,4-D, fomesafen, atrazine, glyphosate, mesotrione, chlorimuron, and dicamba.

Figure 3.2. Visual control of a suspected multiple-resistant (MO-Ren) and susceptible (MO-S) waterhemp population 21 DAA in response to 2,4-D, fomesafen, atrazine, glyphosate, mesotrione, chlorimuron, and dicamba.