CHARACTERIZATION OF A NOVEL FIVE-WAY-RESISTANT POPULATION OF WATERHEMP
(*AMARANTHUS TUBERCULATUS*)

BY

CODY MATTHEW EVANS

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2016

Urbana, Illinois

Master's Committee:

>   Associate Professor Aaron G. Hager, Chair
>   Professor Patrick J. Tranel
>   Professor Dean E. Riechers
>   Associate Professor Adam S. Davis

ABSTRACT

Waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] is a dioecious summer annual species native to much of the Midwest. This species exhibits many intriguing characteristics including, but not limited to, obligate outcrossing that results in a high level of genetic diversity, prolific seed production, prolonged seedling emergence, and seed dormancy, that help it thrive in contemporary agricultural fields. Waterhemp has become one of the most significant challenges growers face with respect to weed control. In the last decade, the frequency of waterhemp populations resistant to herbicides encompassing multiple sites of action has increased, adding to the difficulty and cost of controlling this species. Waterhemp has been reported to be resistant to herbicides having the following sites of action: acetolactate synthase (ALS), 5-enolpyruvyl-shikimate-3-phosphate (EPSPS), protoporphyrinogen oxidase (PPO), TIR1 auxin receptor, Photosystem II (PSII), and 4-hydroxyphenylpyruvate dioxygenase (HPPD).

 In 2012, a grower reported the failure to control a waterhemp population in conventional white corn with topramezone, an HPPD inhibitor. Records indicate the field had been planted with conventional corn hybrids and glyphosate-resistant soybean varieties in an annual rotation. Additionally, the grower used a rotation of different herbicides including HPPD, PPO, ALS, and PSII inhibitors for weed control. Preliminary investigation and screening suggested that the population might be resistant to herbicides from five site-of-action groups.

Chapter 1 of this thesis includes a literature review of HPPD, PPO, ALS, PSII, and auxin herbicides, known resistance mechanisms in weeds, and a section on waterhemp biology. Chapter 2 discusses the original greenhouse screenings of progeny created from seed collected from the grower's field (designated Champaign County Resistant (CHR)). To quantify the

magnitude of resistance, foliar dose response experiments were conducted with mesotrione, atrazine, and 2,4-D. Molecular assays were conducted to confirm PPO inhibitor and ALS inhibitor resistance. Chapter 3 contains information about field research conducted to determine foliar herbicide options to manage CHR in corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). Results from these experiments indicate control of CHR exceeding 80% 21 days after treatment (DAT) was achieved with four foliar-applied herbicides: glyphosate, glufosinate, dicamba, and paraquat. Additional field research is presented in Chapter 4 describing CHR's response to soil-applied herbicides used in corn and soybean. Results from this research demonstrate that few options provided residual control of CHR greater than 80% 28 DAT. Chapter 4 also includes a summary of experiments, as well as future implications of this research.

iv

to the U of I Herbicide Evaluation Program for picking up the funding for my project where another company left off, I am eternally grateful.

I would like to thank my parents Patrick and Susie Evans and the rest of my family and friends for providing the support and encouragement that allows a person to pursue their goals to fruition. Finally, I want to express my gratitude to my beautiful and loving wife Nikki Evans. She has had to put up with muddy clothes smelling of an assortment of herbicides, occasional indentured servitude on weekends unlucky enough to harbor a spray date and/or data collection, time away from family and friends, and a seemingly endless stream of fun facts about weed identification and crop health during any windshield time we share together. I must admit, I got lucky with her.

So to reiterate, a great deal of what I have done has been influenced by the great people that I have met during my time at the University of Illinois and I am forever grateful for their help and support.

TABLE OF CONTENTS

CHAPTER 1

LITERATURE REVIEW

1.1 Target site and mode-of-action of acetolactate synthase inhibitors

Acetolactate synthase (ALS), also referred to as acetohydroxyacid synthase (AHAS), is the first common enzyme in the biosynthetic pathway of the branched-chain amino acids, valine, leucine, and isoleucine (Durner et al. 1991). This pathway employs the use of parallel steps involving four enzymes (anabolic AHAS, ketol-acid reductoisomerase, hydroxyacid dehydratase, and a transaminase) to produce valine, leucine, and isoleucine. The common precursor for these amino acids is pyruvate. In addition to pyruvate, isoleucine also requires a second precursor, 2-ketobutyrate (Duggleby and Pang 2000).

ALS-inhibiting herbicides are categorized into five structural families. In the mid 1970s, DuPont discovered that sulfonylurea derivatives could be developed as potent herbicides (Duggleby and Pang 2000). Around that same time American Cyanamid developed the imidazolinone herbicides, which also are inhibitors of ALS (Shaner et al. 1984). ALS-inhibiting herbicides were found to be very effective at controlling weeds in agronomic crops and many possessed good crop selectivity. The crop selectivity of the ALS herbicides is based on the crop's ability to rapidly metabolize the herbicide to nontoxic metabolites, however uptake and translocation also can impact selectivity. ALS-inhibiting herbicides are commercialized for use in soybean, corn, wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) (Lovell et al. 1996).

Early successful commercialization of the sulfonylurea and imidazolinone herbicides spurred other companies to discover several other families of ALS-inhibiting herbicides, including triazolopyrimidines (Gerwick et al. 1990), pyrimidinyl(thio or oxy)benzoates (Stidham 1991), and sulfonylamino-carbonyl-triazolinones (Scoggan et al. 1999). Injury to plants by ALS-inhibiting herbicides is characterized by chlorosis and necrosis of the apical meristems, stunted growth, stacked internodes and purpling along the midrib following foliar applications (Lovell et al. 1996). Sulfonylurea and imidazolinone herbicides have been the most widely adopted families of ALS inhibitors in Illinois compared with the other three families.

1.1.1 Sulfonylurea herbicides

The basic structure of this herbicide family is X-SO$_2$-NH-CO-NH-Y, where X is usually a substituted phenyl group and Y is a substituted pyrimidine or triazine ring (Duggleby and Pang 2000). Sulfonylurea (SU) herbicides have extremely high biological activity, with field application rates ranging from 10–100 grams per hectare. Although being extremely effective at low rates, their toxicity to animals is extremely low as evidenced by very high LD$_{50}$ values (e.g., chlorosulfuron LD$_{50}$ in rats is approximately 6 grams per kilogram body weight). In the soil, these herbicides are rapidly degraded by a combination of non-enzymatic hydrolysis and microbial degradation (Brown and Kearney 1991). Many of the commercially available sulfonylurea herbicides possess excellent crop selectivity. Selectivity is not due to a non-sensitive ALS, which is equally susceptible to inhibition in both sensitive and tolerant plants (Ray 1986). Selectivity results from a plant species' ability to convert the herbicide to non-toxic derivatives via hydroxylation, conjugation, hydrolytic, and cleavage reactions (Brown 1990).

The sulfonylurea herbicides are potent inhibitors of root and shoot growth in target weed species. Visible symptoms usually are slow to appear, and include vein reddening, leaf chlorosis, terminal bud death, and necrosis 4 to 10 days after treatment (Brown 1990). The original discovery of the biochemical site-of-action of chlorsulfuron resulted from a study showing that the inhibition of bacterial growth by a sulfonylurea on a medium containing valine could be reversed by the addition of isoleucine. These observations helped researchers determine that sulfonylureas inhibit the enzyme acetolactate synthase, which catalyzes the first step in the biosynthesis of valine, leucine, and isoleucine (Larossa and Schloss 1984).

1.1.2 Imidazolinone herbicides

The imidazolinone (IMI) herbicide family consists of a 4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl moiety linked at the 2-position to an aromatic ring system (Duggleby and Pang 2000). Application rates range from 100–1000 grams per hectare, which indicates they are approximately 10-fold less potent than the sulfonylurea family. IMI herbicides share the same low mammalian toxicity characteristics as the SU family. Certain plant species are tolerant to IMI herbicides due to their ability to metabolize the herbicide to non-toxic derivatives (Duggleby and Pang 2000).

IMI herbicides are absorbed by the target species through the leaves and roots and subsequently translocated via the xylem and phloem. The herbicide accumulates in the active growing regions of the plant. This results in stunting, chlorosis, and necrosis as the plant is slowly deprived of key amino acids.

Soil persistence is affected by soil texture, organic matter content, and moisture. High clay and organic matter content coupled with pH values lower than 6.0 can lead to prolonged persistence. Conditions favoring soil microbial activity will enhance herbicide degradation (Kraemer et al. 2009).

1.1.3 ALS inhibitor resistance in weed species

ALS inhibitors are very effective systemic herbicides with low use rates and low mammalian toxicity. The widespread use of these herbicides lead to the evolution of resistance in many weed species. 158 weed species have evolved resistance to ALS inhibitors globally (Heap 2016). The number of weed species that have evolved resistance to ALS inhibitors surpasses that of all other herbicide groups (Heap 2016). Weeds achieve resistance through either a point mutation in the ALS gene or enhanced metabolism that renders the herbicide non-toxic. Enhanced metabolism generally has resulted in lower magnitudes of resistance (< 10-fold), whereas target-site resistance often is 100-fold or more (Burnet et al. 1994).

In most of the reported cases, resistance to ALS inhibitors is due to single point mutations in the ALS gene. These mutations decrease the sensitivity of the encoded enzyme to inhibition by the herbicide (Tranel and Wright 2002). In waterhemp, ALS gene mutations resulting in Trp574Leu, Ser653Asn, and Ser653Thr amino acid substitutions have been reported. The Trp574Leu substitution confers high-level resistance broadly across all families of ALS inhibitors, whereas the Ser653Asn substitution confers resistance primarily to IMI herbicides (Patzoldt and Tranel 2007).

Another method whereby plants overcome ALS inhibition is enhanced metabolism, which rapidly detoxifies the herbicide after uptake. Metabolic detoxification mimics the process that confers selectivity in crop plants to ALS inhibitors (Yu and Powles 2014). Metabolism-based resistance to ALS inhibitors has been reported in only a few species, including rigid ryegrass (*Lolium rigidum* Gaud.), blackgrass (*Alopecurus myosuroides* Huds.), rigid brome (*Bromus rigidum* Roth), wild oat (*Avena fatua* L.), late watergrass [*Echinochloa phyllopogon* (Stapf.) Koss.], and wild mustard (*Sinapis arvensis* L.) (Yu and Powles 2014). Some weed populations have been reported to express both types of resistance to ALS-inhibiting herbicides (Ma et al. 2013).

1.2 Target site and mode-of-action of protoporphyrinogen oxidase inhibitors

Protoporphyrinogen oxidase (PPO) is a key enzyme in the tetrapyrrole biosynthetic pathway that synthesizes chlorophyll and heme in photosynthetic organisms (Hess 2000). The first committed precursor of this pathway is 5-aminolevulinic acid (ALA), which is produced from glutamate and converted by a series of reactions into protoporphyrin IX (Jacobs et al. 1991). PPO, the last enzyme in the tetrapyrrole pathway before the pathway branches toward chlorophyll and heme synthesis, catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX (Li and Nicholl 2005). This enzyme is the target of many structural classes of PPO inhibitors including diphenyl ethers, cyclic imides, oxadiazoles, phenylphthalimides, triazolinones, and thiadiazolidines (Li et al. 2004; Matsumoto 2002)

The inhibition of PPO prevents the synthesis of chlorophylls, hemes, and cytochromes in the chloroplast (Duke et al. 1991). As a result, excess protoporphyrinogen (Protogen) IX is

thought to move into the cytoplasm where it is converted to protoporphyrin (Proto) IX via

extraplastidic oxidation (Lee and Duke 1994). This conversion to Proto IX in the cytoplasm

occurs away from antioxidant and enzymatic protective mechanisms. Once the Proto IX is

exposed to light, cytosolic Proto molecules interact with oxygen to form singlet oxygen and

oxygen radicals. These forms of oxygen peroxidize the unsaturated fatty acids of cell

membranes. The peroxidation of lipids induces a rapid loss of membrane function and integrity,

bleaching of chloroplast pigments, necrosis of tissue, growth inhibition, and plant death

(Grossmann et al. 2011).

PPO-inhibiting herbicides can be applied to plant foliage or soil. Some PPO inhibitors have

been used for more than forty years. Many PPO-inhibiting herbicides are very poorly

translocated following plant uptake, which can cause a reduction in efficacy (Matsumoto 2002).

Crop selectivity can be attributed to rapid metabolic detoxification (mostly by GST enzymes)

and moderate leaf absorption (Grossmann and Schiffer 1999).

1.2.1 PPO resistance in weed species

Currently, nine species worldwide have been reported resistant to PPO-inhibiting

herbicides (Heap 2016). The first case of resistance was discovered in a population of common

waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) in 2001 (Dayan et al. 2014). Plants

from this population were reported to survive acifluorfen up to 32 times the recommended

rate (Li et al. 2004).

The mechanism of PPO-inhibitor resistance, a unique target-site codon deletion, was first

elucidated in a waterhemp biotype from Illinois (Patzoldt et al. 2006). This mechanism involves

the loss of a glycine at position 210 in the mitochondrial isoform of the PPO enzyme. The loss of

glycine is considered to have occurred via a slippage-like mechanism within a trinucleotide

repeat of the PPX2 gene (Patzoldt et al. 2006). Specifically, the sequence motif spanning

position 210 (i.e., …TGTGGTGGA…) contains both a GTG and a TGG bi-repeat. Loss of either one

of these repeat elements results in a loss of a glycine codon (GGT) without affecting the reading

frame. The ΔG210 allele of PPX2L represents the first time a deletion mutation has been

implicated in evolved herbicide resistance (Lee et al. 2008). This codon deletion alters the

binding domain of the enzyme without negatively affecting substrate affinity; thus, overall

sensitivity to PPO-inhibiting herbicides is greatly reduced (Dayan et al. 2010).

Species tolerant to these herbicides (both crops and weeds) rapidly metabolize PPO-

inhibiting herbicides following uptake. The metabolism of acifluorfen by soybean involves a

reduction of the *p*-nitro substitution, de-esterification and conjugation (Frear et al. 1983). To

date, no cases of non-target site resistance (NTS) to PPO inhibitors have been reported.

However, this lack of NTS resistance is not an indication that this mechanism of resistance

cannot occur (Dayan et al. 2014).

1.3 Target site and mode-of-action of PSII inhibitors

The site-of-action of photosystem II (PS II) electron transport inhibiting herbicide families,

such as triazines (e.g., atrazine), phenylureas (e.g., diuron), anilides, benzimidazoles,

biscarbamates, and uracils, is within photosystem II of the photosynthesis light reaction (Duke

1990). Plants treated with these herbicides show symptoms that evolve slowly, often over

several days. Treated plants first exhibit chlorosis (yellowing), which is followed by necrosis

(tissue death). Chlorosis is due to chlorophyll destruction through photo-oxidation reactions in the chloroplast, while necrosis is due to membrane destruction through lipid peroxidation (Hess 2000).

The association of light with the mechanism of action of these herbicides is indirect, i.e., there is no direct involvement of light at the herbicide target in PS II (Hess 2000). Herbicidal inhibition of photosynthesis in PS II is achieved by binding in the Q_B pocket of the D1 protein which effectively blocks photosynthetic electron transport (Duke 1990). Once electron transport becomes blocked by the herbicide in the Q_B pocket, singlet chlorophyll cannot transfer its energy to the PS II reaction centers. As a result, singlet state chlorophyll molecules accumulate and transform into triplet chlorophyll (Hess 2000).

The overproduction of triplet chlorophyll overwhelms the carotenoid quenching system that normally dissipates the few triplet chlorophyll produced by normal photosynthesis. The excess triplet chlorophyll can initiate lipid peroxidation in two ways. The first is the direct formation of a lipid radical in unsaturated fatty acids. In a second mechanism, triplet chlorophyll reacts with oxygen to produce singlet oxygen. During normal photosynthesis, some singlet oxygen is produced but dissipated by carotenoids. Since the carotenoid quenching system has been overwhelmed, singlet oxygen initiates the destruction of lipids by forming lipid radicals in polyunsaturated fatty acids (Hess 2000).

1.3.1 Triazine resistance in weed species

Triazine herbicides have been used to control weeds since the commercialization of simazine and atrazine in 1956 and 1958, respectively (Patzoldt et al. 2003). Since then the triazine herbicides have been widely utilized in the United States for weed control in numerous crops, including corn and soybean. The first case of triazine resistance was reported in 1968. Common groundsel (*Senecio vulgaris L.*) was not controlled in a nursery where simazine or atrazine had been used once or twice annually since 1958 (Ryan 1970). There are at least 73 species reported to have evolved resistance to PS II inhibitors (Heap 2016).

The first report describing the mechanism of triazine resistance occurred in a biotype of smooth pigweed (*Amaranthus hybridus*) that had a serine-to-glycine substitution in the D1 protein at amino acid position 228 (Hirschberg and Mcintosh 1983). Later papers reported this serine-to-glycine substitution occurred at position 264 and resulted in a very high level of resistance (100-fold resistance to most symmetrical (atrazine) and asymmetrical (metribuzin) triazines) (Hess 2000; Patzoldt et al. 2003). Other weed biotypes have been identified with a mutation at amino acid 219 of the D1 protein. All resulting changes in the D1 protein binding site result in a significant reduction in herbicide efficacy (Mengistu et al. 2000).

Another triazine resistance mechanism has been attributed to non-target site mutations. Naturally tolerant plant species possess enhanced metabolism of atrazine via glutathione S-transferase (GST) activity and/or cytochrome P_{450} (P450) oxidative reactions (Yuan et al. 2007). Populations of velvetleaf, rigid ryegrass, blackgrass, and waterhemp have evolved triazine resistance via enhanced herbicide metabolism (Burnet et al. 1991; Gronwald et al. 1989; Patzoldt et al. 2003).

1.4 Target site and mode-of-action of synthetic auxins

Synthetic auxin herbicides are often described as synthetic versions of the phytohormone indole-3-acetic acid (IAA). These herbicides act as growth regulators within susceptible plants (Sterling and Hall 1997). The synthetic auxins' affinity for the auxin receptor and F-box protein *TIR1,* and subsequent signal transduction processes, is thought to be the primary site of herbicide action. Herbicides in this group generally regulate cell division and elongation and developmental processes including vascular tissue and floral meristem differentiation, leaf initiation, phyllotaxy, senescence, apical dominance and root formation (Grossmann 2010). IAA that occurs normally within the plant has endogenous control mechanisms that maintain its concentration at appropriate levels. However, plants have no such regulatory mechanism for synthetic auxin herbicides. Levels of these herbicides can increase to damaging concentrations, or, alternatively, at very low concentrations they can actually promote healthy cell growth (Monaco et al. 2002).

Grossmann (2010) described the deregulation of plant growth by auxin herbicides or IAA at high concentrations in three phases following uptake of an auxinic herbicide in a dicot plant. The first is the stimulation phase, which occurs within the first hours after application of the herbicide. During this phase, metabolic processes such as ethylene biosynthesis are stimulated through the induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase in the shoot tissue 1–2 hours after application. This is followed by leaf epinasty, tissue swelling, and stem curling 3–4 hours after application. Abscisic acid then accumulates after 5–8 hours.

The second phase occurs within 24 hours after application. This phase includes the inhibition of root and shoot growth, decreased internode elongation and leaf area, intensified green leaf pigmentation, stomatal closure, reduced transpiration, and overproduction of reactive oxygen species. Tissue proliferation often occurs in the stem tip, nodes, or even along the length of the entire stem. Susceptible plants will exhibit epinasty or bending and splitting of the stem, disrupting phloem/xylem transport. Roots will become thickened (or braced/fused together) and stunted. Adventitious roots (roots that grow in unusual locations) also can develop (Sterling and Hall 1997). During phase three, senescence and tissue decay lead to chlorosis, wilting, and plant death.

Auxinic herbicides have been reported to stimulate ethylene biosynthesis in many plant species. There is evidence that some of the responses induced by an auxinic herbicide, such as leaf and stem epinasty and leaf abscission, may be caused by enhanced ethylene production. Also, auxinic herbicides have been shown to promote ethylene production at higher levels in sensitive plants than in species that are resistant or tolerant to the herbicide. Although the exact role ethylene evolution plays within the mode of action is unknown, evidence suggests it is a secondary response that isn't completely responsible for plant death (Sterling and Hall 1997).

These events, all occurring in succession, cause weakened cell walls and enable growth by turgor-pressure-driven cell expansion. Synthetic auxins also promote changes in gene expression, affecting approximately 25 different auxin responsive genes (Monaco et al. 2002).

This results in an increase of DNA, RNA and other protein levels in treated tissue, with the greatest effect on RNA levels (Chen et al. 1972).

1.4.1 Synthetic auxin resistance in weed species

Currently, 32 weed species worldwide have evolved resistance to synthetic auxin herbicides (Heap 2016). The earliest cases of resistance were reported in 1957 with wild carrot (*Daucus carota)* in Canada and spreading dayflower (*Commelina diffusa)* in the United States. The most recent instance is annual sowthistle (*Sonchus oleraceus)* reported in 2015 in Australia (Heap 2016). As with other herbicide families, auxin resistance has been reported to be conferred by several different mechanisms.

The magnitude of resistance to 2,4-D is often variable: 2.5-fold for wild radish (*Raphanus raphanistrum)* (Walsh et al. 2004), 18-fold for wild mustard, 25-fold for prickly lettuce (Burke et al. 2009), and 29-fold for globe fringebrush (*Fimbristylis miliacea* (L.) Vahl) (Karim et al. 2004). Globe fringebrush demonstrated a fitness penalty and the frequency of 2,4-D resistance in rice fields declined from 86% to less than 2% of individuals after 3 years without 2,4-D (Karim et al. 2004). In contrast, MCPA and 2,4-D resistance in musk thistle did not confer an observable fitness penalty (Bonner et al. 1998). In 2009, a population of waterhemp was reported to be at least 10-fold resistant to 2,4-D (Bernards et al. 2012).

In *Arabidopsis*, research demonstrated increasing doses of 2,4-D induced up-regulation or down-regulation of different genes in the ethylene and abscisic acid pathways, indicating there might be several receptor sites depending on 2,4-D dose and plant species (Raghavan et al. 2006). This observation adds to the difficulty of finding the exact cause of resistance. Therefore,

it is not surprising to find inconsistencies in identifying probable causes of 2,4-D resistance

among various 2,4-D-resistant species.

In 1990, the first documented case of dicamba resistance occurred in wild mustard in

fields near Minto, Manitoba (Jasieniuk et al. 1995). Resistance to dicamba and related

herbicides in wild mustard is correlated with alterations in intracellular calcium levels, and may

be due to reduced herbicide binding at auxin binding sites (Deshpande and Hall 2000). In a

kochia population, resistance was reported to be a quantitative trait, resulting from a number

of relatively small changes in gene products, such as herbicide binding proteins, transporters,

and metabolic enzymes (Cranston et al. 2001). A quantitative trait could explain why resistance

to auxinic herbicides has been relatively slow to develop and has not spread rapidly (Cranston

et al. 2001). In contrast, dicamba resistance in wild mustard was shown to be conferred by a

single, dominant nuclear allele (Jasieniuk et al. 1995). Another study reported that dicamba

resistance in another kochia population is likely conferred by a single allele with a high degree

of dominance (Preston et al. 2009). As indicated by these studies, it has been difficult to resolve

the mechanism of action of auxinic herbicides due to the multiplicity of biochemical effects

within the cell, which might also play a role in the evolution of resistance (Coupland 1994).

1.5 Target site and mode-of-action of HPPD inhibitors

Herbicides that inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in the

biosynthesis of plastoquinone and α-tocopherols, encompass three chemical classes:

triketones, isoxazoles, and pyrazolones. These herbicides have been used for selective control

of weeds since the early 1990s (Grossmann and Ehrhardt 2007; Hirai et al. 2002; van Almsick 2009).

Injury symptoms caused by these herbicides include bleaching or whitening of plant tissue. Bleaching results from blocking the conversion of 4-hydroxyphenylpyruvate dioxygenase to homogentisate in the biosynthetic pathway to create plastoquinone (Mitchell et al. 2001; Norris et al. 1995). This causes an indirect inhibition of carotenoid synthesis at the phytoene desaturase site due to the depletion of plastoquinones that are needed as electron carriers between carotenoid desaturase and the photosynthetic electron transport chain. Depletion of plastoquinones reduces energy dissipation from photosynthesis (van Almsick 2009). The indirect inhibition is demonstrated by eliminating the inhibition of carotenoids by feeding supplemental homogentisate to the treated plants (Norris et al. 1995).

Carotenoids protect the chloroplast from triplet chlorophyll, singlet oxygen, lipid peroxidation, and membrane damage. Tocopherols help to protect against hydroxyl radicals and lipid peroxide radicals. The loss of these antioxidants results in the disruption of cell membranes in susceptible weeds by free radicals. The lack of carotenoids also results in the chlorophyll molecules being unprotected from UV rays and excess light. Without carotenoids, the chlorophyll is destroyed and the plant tissue becomes white (van Almsick 2009).

Research has demonstrated that a combination of an HPPD inhibitor and a PSII inhibitor can produce a synergistic effect on weed control following a foliar (Hugie et al. 2008), or soil application (Bollman et al. 2006). The synergism is believed to result from the depletion of plastoquinones and the subsequent increased binding of PSII inhibitors to the D1 protein. The

resulting triplet chlorophyll and singlet oxygen would not be quenched by the carotenoids and tocopherols that normally protect the plant since these are depleted by HPPD inhibitors (Abendroth et al. 2006).

1.5.1 HPPD inhibitor resistance in weed species

Reports of resistance to HPPD inhibitors have been relatively infrequent compared with other herbicide families, perhaps at least partly attributed to the fact they are the newest group of herbicides utilized in crop production. Waterhemp and Palmer amaranth (*Amaranthus palmeri)* are the only two species that have evolved resistance to HPPD-inhibiting herbicides. Biotypes of both species were first reported to be resistant in 2009 (Heap 2016).

The first report of a HPPD-inhibitor resistant Palmer amaranth population originated from Stafford County, Kansas. Seeds were gathered from Palmer amaranth plants that survived treatment with pyrasulfotole and bromoxynil (1:8 ratio) at 245 g ai ha^{-1} and also from plants in the same field that were not treated with a HPPD-inhibiting herbicide. Results from greenhouse and field dose response experiments indicated the populations were 7–11 times more resistant to pyrasulfotole and bromoxynil than a susceptible population (Thompson et al. 2012).

The first case of HPPD-resistant waterhemp occurred in a seed corn production field in central Illinois, USA. The population was not adequately controlled after postemergence applications of HPPD herbicides. Progeny grown from seed collected in the field survived following foliar applications of mesotrione, tembotrione, or topramazone. Addition of atrazine to these treatments increased efficacy but still failed to control the plants. Dose response

15

experiments showed that the level of resistance to mesotrione was at least 10-fold, relative to sensitive biotypes (Hausman et al. 2011).

The mechanism of resistance was not due to an alteration in HPPD sequence, HPPD expression, or reduced herbicide absorption. Metabolism of mesotrione was significantly reduced following application of the cytochrome P_{450} monooxygenase inhibitors malathion or tetcyclacis, suggesting that resistance is attributable to elevated rates of metabolism via a distinct detoxification mechanism (Ma et al. 2013). Inheritance experiments reported that metabolism-based atrazine resistance in the population is conferred by a single major gene, whereas inheritance of mesotrione resistance is much more complex (Huffman et al. 2015).

1.6 Waterhemp biology

Waterhemp is a small-seeded, summer annual broadleaf weed species within the *Amaranthaceae* family. This species is native to Illinois and was historically found on the margins of freshwater bodies, but has since spread across much of the state (Sauer 1955, 1972). Though native to Illinois, waterhemp is distributed from Texas to Maine and even extends to parts of North Dakota (Spaunhorst et al. 2014). This species is considered a problematic weed by corn and soybean growers due to its prevalence and its ability to survive various herbicides (Hager and Sprague 2002). A morphologically diverse species, waterhemp plants can reach heights of 2 meters. Leaves are lanceolate measuring 2–10 cm long and 1–3 cm wide originating from a glabrous stem (Sauer 1955). The seedlings have egg-shaped cotyledons, are hairless, and have a waxy or glossy appearance of the leaf surface.

Waterhemp is a dioecious species with the male plants producing pollen and the female

plants producing seeds (Sauer 1957). Being dioecious, waterhemp is an obligate out-crossing

species. This out-crossing allows a single female to potentially be pollinated by multiple males,

leading to an increased genetic diversity of progeny (Hager et al. 1997). Waterhemp has been

shown to periodically hybridize with other species of *Amaranthus* (Murray 1940). Female plants

are prolific seed producers capable of producing more than one million seeds (Steckel et al.

2003).

Waterhemp can produce a prodigious amount of seeds even during unfavorable growing

conditions. A single waterhemp plant that emerged 50 days after soybean planting produced

3000 seeds (Hartzler et al. 2004). Others have demonstrated waterhemp is shade tolerant, with

female plants grown under 68% shade producing up to 400,000 seeds (Steckel et al. 2003).

Waterhemp tends to produce approximately 1.5 times more seed than other pigweed species

of comparable size (Sellers et al. 2003). The seeds are very small (1–1.5 mm), and germinate at

higher rates when located close to the soil surface (Steckel et al. 2007). This characteristic

contributes to waterhemp's prevalence in no-till production systems.

Waterhemp seedlings exhibit a delayed emergence pattern that occurs later and over a

more prolonged period than other common weeds such as velvetleaf and giant foxtail (*Setaria

faberi)* (Hartzler et al. 1999). In addition to delayed emergence, dormancy allows a portion of

the seeds to remain viable in the soil for several years (Burnside et al. 1996). This ability to

remain dormant, coupled with the potential for high seed production and a delayed emergence

pattern, has allowed waterhemp to become a significant contributor to the weed seed bank in agronomic fields (Buhler et al. 2001).

Waterhemp can be competitive with agronomic crops, causing significant yield losses and reduction of income for growers. In research by Hager et al. (2002), removal of waterhemp two weeks after soybean unifoliate leaf expansion resulted in yields comparable to a weed-free control. However, allowing waterhemp to interfere with soybean for ten weeks resulted in an average yield loss of 43% over three years. Waterhemp density in this study ranged from 86–1315 plants m^{-2}. A study by Cordes et al. (2004) reported a corn yield loss of 36% occurred with waterhemp density ranging from 369–445 plants m^{-2} and full-season interference.

Waterhemp biotypes have been reported to have evolved resistance to ALS-inhibiting herbicides, PS II-inhibiting herbicides, PPO-inhibiting herbicides, HPPD-inhibiting herbicides, 2,4-D, and glyphosate (Heap 2016). Waterhemp has the ability to accumulate multiple resistances within individual plants (Patzoldt et al. 2005). Research by Bell et al. (2013) characterized a population of waterhemp from Illinois that contained individual plants resistant to herbicides from four different site-of-action groups. Producers have fewer herbicide options to control waterhemp with multiple resistances.

1.7 Research objectives

In the fall of 2012, a grower in Champaign County, Illinois reported a population of waterhemp in conventional white corn was not controlled with topramezone. The grower stated that in the past he planted conventional corn hybrids and glyphosate-resistant soybean varieties, rotating between the two each year. The grower relied on a rotation of different

herbicides including HPPD, PPO, ALS, and PSII inhibitors for weed control. Chapter 2 describes

the responses of the putative resistant waterhemp population to mesotrione, atrazine, and 2,4-

D applied post under greenhouse conditions. The greenhouse experiments were conducted

with plants grown from seed generated by crossing two resistant parents that originated from

seed gathered from the grower's field. The field population was designated CHR, and the

resistant-by-resistant (RxR) population was designated M6. The M6 population was compared

to other resistant and susceptible populations to quantify the magnitude of resistance.

Chapter 3 investigates the response of the CHR population to foliar treatments of

different herbicides used in corn, soybean, and bare-ground studies. Treatments were

compared by visual ratings, plant heights, and plant dry weight to measure the population's

response to different rates of various herbicides with different sites of action. Adding atrazine

to an HPPD-inhibiting herbicide results in a synergistic effect, thereby increasing the

effectiveness of a treatment (Woodyard et al. 2009). Several treatments in corn were repeated

with the addition of atrazine to determine if there was an increase in effectiveness.

Chapter 4 details the response of the population to soil-applied herbicides from different

site-of-action groups applied at different rates. The effectiveness of the treatments was

measured using visual ratings and stand counts compared with a non-treated control.

1.8 Literature cited

Abendroth JA, Martin AR, Roeth FW (2006) Plant response to combinations of mesotrione and
photosystem II inhibitors. Weed Technol. 20:267–274

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

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

Bollman SL, Kells JJ, Penner D (2006) Weed response to mesotrione and atrazine applied alone
and in combination preemergence. Weed Technol. 20:903–907

Bonner K, Rahman A, James T, Nicholson K, Wardle D (1998) Relative intra-species competitive
ability of nodding thistle biotypes with varying resistance to the herbicide 2, 4-D. New
Zeal. J. Agr. Res. 41:291–297

Brown HM (1990) Mode of action, crop selectivity, and soil relations of the sulfonylurea
herbicides. Pestic Sci. 29:263–281

Brown HM, Kearney PC (1991) Plant biochemistry, environmental properties, and global impact
of the sulfonylurea herbicides. ACS Sym. Ser. 443:32–49

Buhler DD, Kohler KA, Thompson RL (2001) Weed seed bank dynamics during a five-year crop
rotation. Weed Technol. 15:170–176

Burke IC, Yenish JP, Pittman D, Gallagher RS (2009) Resistance of a prickly lettuce (*Lactuca
serriola*) biotype to 2,4-D. Weed Technol. 23:586–591

Burnet MWM, Hart Q, Holtum JAM, Powles SB (1994) Resistance to nine herbicide classes in a
population of rigid ryegrass (*Lolium rigidum*). Weed Sci. 42:369–377

Burnet MWM, Hildebrand OB, Holtum JAM, Powles SB (1991) Amitrole, triazine, substituted urea, and metribuzin resistance in a biotype of rigid ryegrass (*Lolium rigidum*). Weed Sci. 39:317–323

Burnside OC, Wilson RG, Weisberg S, Hubbard KG (1996) Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci. 44:74–86

Chen L, Switzer C, Fletcher R (1972) Nucleic acid and protein changes induced by auxin-like herbicides. Weed Sci.20:53–55

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

Coupland D (1994) Resistance to the auxin analog herbicides. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: CRC:171–214

Cranston HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE (2001) Dicamba resistance in kochia. Weed Sci.49:164–170

Dayan FE, Daga PR, Duke SO, Lee RM, Tranel PJ, Doerksen RJ (2010) Biochemical and structural consequences of a glycine deletion in the alpha-8 helix of protoporphyrinogen oxidase. Biochim. Biophys. Acta. 1804:1548–56

Dayan FE, Owens DK, Tranel PJ, Preston C, Duke SO (2014) Evolution of resistance to phytoene desaturase and protoporphyrinogen oxidase inhibitors - state of knowledge. Pest Manag. Sci.70:1358–1366

Deshpande S, Hall JC (2000) Auxinic herbicide resistance may be modulated at the auxin-

binding site in wild mustard (*Sinapis arvensis L.*): a light scattering study. Pestic.

Biochem. Phys. 66:41–48

Duggleby RG, Pang SS (2000) Acetohydroxyacid synthase. J. Biochem. Mol. Biol. 33:1–36

Duke SO (1990) Overview of herbicide mechanisms of action. Environ. Health Persp. 87:263–

271

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen LP, Jr., Matsumoto H (1991)

Protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci. 39:465–473

Durner J, Gailus V, Boger P (1991) New aspects on inhibition of plant acetolactate synthase by

chlorsulfuron and imazaquin. Plant Phys. 95:1144–1149

Frear DS, Swanson HR, Mansager ER (1983) Acifluorfen metabolism in soybean - diphenylether

bond-cleavage and the formation of homoglutathione, cysteine, and glucose conjugates.

Pestic. Biochem. Phys.20:299–310

Gerwick BC, Subramanian MV, Loneygallant VI, Chandler DP (1990) Mechanism of action of the

1,2,4-triazolo[1,5-a] pyrimidines. Pestic. Sci. 29:357–364

Gronwald JW, Andersen RN, Yee C (1989) Atrazine resistance in velvetleaf (*Abutilon

theophrasti*) due to enhanced atrazine detoxification. Pestic. Biochem. Phys. 34:149–163

Grossmann K (2010) Auxin herbicides: current status of mechanism and mode of action. Pest

Manag. Sci. 66:113–120

Grossmann K, Ehrhardt T (2007) On the mechanism of action and selectivity of the corn

herbicide topramezone: a new inhibitor of 4 hydroxyphenylpyruvate dioxygenase. Pest

Manag. Sci. 63:429–439

Grossmann K, Hutzler J, Caspar G, Kwiatkowski J, Brommer CL (2011) Saflufenacil (Kixor (TM)): biokinetic properties and mechanism of selectivity of a new protoporphyrinogen IX oxidase inhibiting herbicide. Weed Sci. 59:290–298

Grossmann K, Schiffer H (1999) Protoporphyrinogen oxidase-inhibiting activity of the new, wheat-selective isoindoldione herbicide, cinidon-ethyl. Pestic. Sci. 55:687–695

Hager AG, Sprague CL (2002) Weeds on the horizon. Univ. of Illinois. The Pest Manag. Crop Develop Bulletin. 2:6

Hager AG, Wax LM, Simmons FW, Stoller EW (1997) Waterhemp management in agronomic crops. Univ. of Illinois Bulletin 855:12

Hager AG, Wax LM, Stoller EW, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci. 50:607–610

Hartzler RG, Bruce B, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci. 52:242–245

Hartzler RG, Buhler DD, Stoltenberg DE (1999) Emergence characteristics of four annual weed species. Weed Sci. 47:578–584

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 Manag. Sci. 67:258–261

Heap, I. The International Survey of Herbicide Resistant Weeds. Online. Internet. Thursday, April 4, 2016 . Available www.weedscience.com

Hess DF (2000) Light-dependent herbicides: an overview. Weed Sci. 48:160–170

Hirai K, Uchida A, Ohno R (2002) Major synthetic routes for modern herbicide classes and

agrochemical characteristics. Pages 221–229 *in* Boger P, Wakabayashi K, Hirai K, eds.

Herbicide Classes in Development. New York, NY: Springer-Verlag

Hirschberg J, Mcintosh L (1983) Molecular basis of herbicide resistance in *Amaranthus hybridus*.

Science 222:1346–1349

Huffman J, Hausman NE, Hager AG, Riechers DE, Tranel PJ (2015) Genetics and inheritance of

nontarget-site resistances to atrazine and mesotrione in a waterhemp (*Amaranthus

tuberculatus*) population from Illinois. Weed Sci. 63:799–809

Hugie JA, Bollero GA, Tranel PJ, Riechers DE (2008) Defining the rate requirements for

synergism between mesotrione and atrazine in redroot pigweed (*Amaranthus

retroflexus*). Weed Sci. 56:265–270

Jacobs JM, Jacobs NJ, Sherman TD, Duke SO (1991) Effect of diphenyl ether herbicides on

oxidation of protoporphyrinogen to protoporphyrin in organellar and plasma membrane

enriched fractions of barley. Plant Phys. 97:197–203

Jasieniuk M, Morrison IN, Brûlé-Babel AL (1995) Inheritance of dicamba resistance in wild

mustard (*Brassica kaber*). Weed Sci. 43:192–195

Karim RS, Man AB, Sahid IB (2004) Weed problems and their management in rice fields of

Malaysia: an overview. Weed Biol. Manag. 4:177–186

Kraemer AF, Marchesan E, Avila LA, Machado SLO, Grohs M (2009) Environmental fate of

imidazolinone herbicides - a review. Planta Daninha 27:629–639

Larossa RA, Schloss JV (1984) The sulfonylurea herbicide sulfometuron methyl is an extremely

  potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium. J.*

  Biol. Chem. 259:8753–8757

Lee HJ, Duke SO (1994) Protoporphyrinogen IX-oxidizing activities involved in the mode of

  action of peroxidizing herbicides. J. Agr. Food Chem. 42:2610–2618

Lee RM, Hager AG, Tranel PJ (2008) Prevalence of a novel resistance mechanism to PPO-

  inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Sci. 56:371–375

Li J, Smeda RJ, Nelson KA, Dayan FE (2004) Physiological basis for resistance to diphenyl ether

  herbicides in common waterhemp (*Amaranthus rudis*). Weed Sci. 52:333–338

Li X, Nicholl D (2005) Development of PPO inhibitor-resistant cultures and crops. Pest Manag.

  Sci. 61:277–285

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

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 Phys. 163:363–377

Matsumoto H (2002) Inhibitors of protoporphyrinogen oxidase: a brief update. Pages 151–161

  in Böger P, Wakabayashi K, Hirai K, eds. Herbicide Classes in Development: Springer

  Berlin, Heidelberg

Mengistu LW, Mueller-Warrant GW, Liston A, Barker RE (2000) psbA mutation (valine219 to

  isoleucine) in *Poa annua* resistant to metribuzin and diuron. Pest Manag. Sci. 56:209–

  217

Mitchell G, Bartlett DW, Fraser T, Hawkes TR, Holt DC, Townson JK, Wichert RA (2001)

Mesotrione: a new selective herbicide for use in maize. Pest Manag. Sci. 57:120–128

Monaco TJ, Weller SC, Ashton FM (2002) Weed Science: Principles and Practices: John Wiley &

Sons pg. 301–308

Murray MJ (1940) Colchicine induced tetraploids in dioecious and monoecious species of the

Amaranthaceae. J. Hered. 31:477–485

Norris SR, Barrette TR, DellaPenna D (1995) Genetic dissection of carotenoid synthesis in

Arabidopsis defines plastoquinone as an essential component of phytoene desaturation.

Plant Cell 7:2139–49

Patzoldt WL, Hager AG, McCormick JS, Tranel PJ. (2006). A codon deletion confers resistance to

herbicides inhibiting protoporphyrinogen oxidase. Proc. Natl. Acad. Sci. *USA* 103:12329–

34

Patzoldt WL, Dixon BS, Tranel PJ (2003) Triazine resistance in *Amaranthus tuberculatus* (Moq)

Sauer that is not site-of-action mediated. Pest Manag. Sci. 59:1134–1142

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 (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with

multiple resistance across three herbicide sites of action. Weed Sci. 53:30–36

Preston C, Belles DS, Westra PH, Nissen SJ, Ward SM (2009) Inheritance of resistance to the

auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Sci. 57:43–47

Raghavan C, Ong E, Dalling M, Stevenson T (2006) Regulation of genes associated with auxin,

    ethylene and ABA pathways by 2,4-dichlorophenoxyacetic acid in *Arabidopsis*. Funct.

    Integr. Genomics 6:60–70

Ray TB (1986) Sulfonylurea herbicides as inhibitors of amino-acid biosynthesis in plants. Trends

    Biochem. Sci. 11:180–183

Ryan GF (1970) Resistance of common groundsel to simazine and atrazine. Weed Sci. 18:614–

    616

Sauer J (1955) Revision of the dioecious *amaranths*. Madrono 13:5–46

Sauer J (1957) Recent migration and evolution of the dioecious *Amaranths*. Evolution 11:11–31

Sauer J (1972) The dioecious A*maranths*: a new species name and major range extensions.

    Madrono 21:426–434

Scoggan AC, Santel HJ, Wollam JW, Rudolph RD (1999) BAY MKH 6561: a new herbicide for

    grass and broadleaf weed control in cereals. 1999 Brighton Conference: Weeds, Vols 1–

    3:93–98

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six

    Amaranthus species in Missouri. Weed Sci. 51:329–333

Shaner DL, Anderson PC, Stidham MA (1984) Imidazolinones - potent inhibitors of

    acetohydroxyacid synthase. Plant Phys. 76:545–546

Spaunhorst DJ, Siefert-Higgins S, Bradley KW (2014) Glyphosate-resistant giant ragweed

    (*Ambrosia trifida*) and waterhemp (*Amaranthus rudis*) management in dicamba-

    resistant soybean (*Glycine max*). Weed Technol. 28:131–141

Steckel LE, Sprague CL, Hager AG, Simmons FW, Bollero GA (2003) Effects of shading on

    common waterhemp (*Amaranthus rudis*) growth and development. Weed Sci. 51:898–

    903

Steckel LE, Sprague CL, Stoller EW, Wax LM, Simmons FW (2007) Tillage, cropping system, and

    soil depth effects on common waterhemp (*Amaranthus rudis*) seed-bank persistence.

    Weed Sci. 55:235–239

Sterling T, Hall J (1997) Mechanism of action of natural auxins and the auxinic herbicides. Rev.

    Toxicol. 1:111–142

Stidham MA (1991) Herbicides that inhibit acetohydroxyacid synthase. Weed Sci. 39:428–434

Thompson, C. R., D. Peterson, and N. G. Lally. (2012). Characterization of HPPD resistant Palmer

    amaranth. http://wssaabstracts.com/public/9/abstract-413.html. Accessed Nov. 2nd,

    2015

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we

    learned? Weed Sci. 50:700–712

van Almsick A (2009) New HPPD-inhibitors-a proven mode of action as a new hope to solve

    current weed problems. Outlook. Pest Manag. 20:27–30

Walsh MJ, Powles SB, Beard BR, Parkin BT, Porter SA (2004) Multiple-herbicide resistance

    across four modes of action in wild radish (*Raphanus raphanistrum*). Weed Sci. 52:8–13

Woodyard AJ, Hugie JA, Riechers DE (2009) Interactions of mesotrione and atrazine in two

    weed species with different mechanisms for atrazine resistance. Weed Sci. 57:369–378

Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. Pest

    Manag. Sci. 70:1340–1350

Yuan JS, Tranel PJ, Stewart Jr CN (2007) Non-target-site herbicide resistance: a family business.

Trends Plant Sci. 12:6–13

CHAPTER 2

RESISTANCE TO 2,4-D AND, HPPD-, PPO-, ALS-, AND PSII-INHIBITING HERBICIDES IN A POPULATION OF WATERHEMP (*AMARANTHUS TUBERCULATUS*) FROM ILLINOIS

2.1 Abstract

In 2012, a grower reported a population of waterhemp was not controlled with topramezone. Plants grown from field-collected seed were subjected to an initial screening process to elucidate if the population expressed resistance to various herbicides. Preliminary results from this screening indicated the population demonstrated reduced sensitivity to HPPD inhibitors, ALS inhibitors, PSII inhibitors, PPO inhibitors, and 2,4-D. Greenhouse and laboratory experiments were performed to quantify the response of plants from this population to herbicides from these five site-of-action groups. Confirmation of ALS and PPO inhibitor resistance via target-site mutation was accomplished through utilization of molecular assays. Dose-response experiments were utilized to confirm HPPD, PSII, and 2,4-D resistance. The dose-response experiments conducted on progeny from the field population indicated that the level of resistance is 16-, 30-, and 253-fold to mesotrione, 2,4-D, and atrazine respectively, when compared to a sensitive population. These experiments support the hypothesis that this waterhemp population demonstrates resistance to herbicides from five site-of-action groups.

2.2 Introduction

Waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] is a small seeded, dioecious,

summer annual broadleaf species native to much of the Midwest (Sauer 1955). This

competitive weed has been shown to reduce soybean yield in excess of 40% when uncontrolled

for ten weeks (Hager et al. 2002), and corn yield by 74% when uncontrolled for the growing

season (Steckel and Sprague 2004a). Female waterhemp plants are capable of producing in

excess of one million seeds (Hartzler et al. 2004) that can remain dormant in the soil for

extended periods of time (Burnside et al. 1996; Hartzler et al. 1999). These characteristics,

coupled with an extended duration of emergence, allow waterhemp to emerge late in the

growing season after pre-emergence herbicides have dissipated or after post-emergence

herbicides have been applied (Steckel and Sprague 2004b).

Waterhemp biotypes have evolved resistance to herbicides from six site-of-action

groups, including inhibitors of acetolactate synthase (ALS) (Guo et al. 2015), photosystem II (PS

II) (Patzoldt et al. 2003), protoporphyrinogen oxidase (PPO) (Shoup et al. 2003),

hydroxyphenylpyruvate dioxygenase (HPPD) (Hausman et al. 2011), EPSPS (Zelaya and Owen

2005), and synthetic auxins (Bernards et al. 2012). A survey by Patzoldt et al. (2002) used

randomly collected waterhemp samples in Illinois to assess their response to herbicides from

several site-of-action groups. Results indicated that approximately 90% of the sampled

populations contained resistance to ALS inhibitors, and 25% of the populations were resistant

to multiple herbicide site-of-action groups. Bell et al. (2013) characterized a waterhemp

population demonstrating resistance to glyphosate, atrazine, lactofen, and imazamox.

To control a herbicide-resistant weed population, growers generally apply another herbicide with an alternative site-of-action. This method, however, has the potential of selecting for multiple herbicide resistances. Populations of waterhemp demonstrating resistance to multiple herbicides groups have reduced herbicide control options (Bell et al. 2009; Patzoldt et al. 2005). The increase in herbicide-resistant waterhemp populations has contributed to an increased presence of this species in Illinois agronomic fields over the last decade (Hager et al. 1997).

To reduce the likelihood of evolving multi-resistant populations, several strategies have been proposed. Hager et al. (1997) suggest the utilization of integrated management systems, including the use of dissimilar soil-residual herbicides along with post-applied herbicides, is essential for control of waterhemp. A study by Evans et al. (2015) concluded that mixing multiple, effective herbicide sites of action in tank mixtures greatly reduced the selection for glyphosate-resistant waterhemp.

The research presented herein describes the characterization of a novel combination of herbicide resistances in an Illinois waterhemp population. This population was identified in 2012 in a Champaign County, Illinois field dedicated to conventional corn and glyphosate-resistant soybean production. Herbicide application records indicate various herbicide site-of-action groups (HPPD, PPO, ALS, and PSII inhibitors) had been used for weed control. The data presented herein provides evidence that this population, designated CHR, is resistant to HPPD inhibitors, ALS inhibitors, PSII inhibitors, PPO inhibitors, and 2,4-D.

2.3 Materials and Methods

2.3.1 Waterhemp populations

Inflorescences from 25 female waterhemp plants that were not controlled following foliar applications of lactofen were collected from the field in August 2013 and dried at room temperature. Seeds from the collected plants were first surface sterilized by a 10-min treatment with 1:1 commercial bleach[1]: water solution, then washed twice with sterilized deionized water, suspended in 0.15% (w/w) agarose, and stored for at least 30 days at 4° C to improve seed germination according to the method from Bell et al. (2013). Plants grown from collected seed were subjected to herbicide screening consisting of ALS, PSII, and HPPD inhibitors and the survivors were crossed by Janel Huffman in the greenhouse to create several seed sources. The seed source designated 'M6' was chosen for the greenhouse experiments due to the amount of seed available and the seed lots germination rate. The response of M6 was compared to several other populations in three separate dose-response experiments.

The response of M6 to foliar-applied mesotrione was compared to a resistant population crossed by Nick Hausman and designated NH40. This population was previously confirmed resistant to HPPD- and PSII-inhibiting herbicides (Hausman et al. 2011). Two other populations susceptible to HPPD inhibitors (WUS and BCR) also were included for comparison. WUS is not resistant to herbicides from any site-of-action group, while BCR was previously characterized by Bell et al. (2013) as resistant to glyphosate, ALS, PPO, and PSII inhibitors. M6, NH40, and WUS were the populations evaluated in an atrazine dose-response experiment, while the response of M6 to foliar-applied 2,4-D was compared to WUS and a Nebraska

33

waterhemp population (designated NE) previously confirmed resistant to 2,4-D (Bernards et al. 2012).

2.3.2 Greenhouse plant culture

All plants used in these three experiments were germinated from seeds sown in 12x12 cm flats containing a commercial potting medium[2]. Emerged seedlings averaging 2 cm tall were transplanted into plug inserts (one per insert) that were 7.5 cm deep. One week later, the plugs containing the seedlings were transplanted into 950 cm^3 pots containing a 3:1:1:1 mixture of potting mix:soil:peat:sand that included a slow release fertilizer[3]. Greenhouse conditions were maintained at 28/22° C during the day/night with a 16-hour photoperiod. Natural sunlight was supplemented with mercury halide lamps to provide 800 µmol $m^{-2} s^{-1}$ photon flux at the plant canopy.

2.3.3 Confirmation of resistance to ALS inhibitors

DNA was extracted from three M6 plants and compared to 2 positive (ALS resistant) and 2 negative (ALS sensitive) control populations. PCR-based molecular markers were used to detect any polymorphisms of each population in the region encoding amino acid position 574 or 653 of ALS following the methods described by Patzoldt and Tranel (2007). Confirmation of resistance was achieved by separating the products of the reaction in a 1.2% agarose gel containing 5 µg ml^{-1} ethidium bromide and then comparing the M6 bands within the gel to the positive and negative controls.

2.3.4 Confirmation of resistance to PPO inhibitors

DNA was extracted from the three M6 plants and compared to 2 positive (PPO resistant) and 2 negative (PPO sensitive) control populations using a molecular assay described by Lee et al. (2008) to test for the presence of the ΔG210 codon deletion which is the predominate PPO resistance mechanism in waterhemp (Lee et al. 2008). The assay was completed by utilizing an allele-specific polymerase chain reaction analysis. Confirmation of resistance was achieved by separating the products of the reaction in a 1.2% agarose gel containing 5 μg ml^{-1} ethidium bromide and comparing the bands of the M6 samples to the positive and negative controls.

2.3.5 PSII inhibitor resistance

Resistance to atrazine can manifest through a target-site mutation or as enhanced herbicide metabolism (Mengistu et al. 2000; Patzoldt et al. 2003). Preliminary field research indicated that soil-applied metribuzin effectively controlled CHR, suggesting a non-target-site mechanism of triazine resistance. To test this hypothesis, DNA was extracted from three M6 plants and the entire gene encoding the atrazine target protein (psbA) was sequenced.

2.3.6 Herbicide application

All herbicide treatments were applied using a moving-nozzle, compressed air research spray chamber[4] with an adjustable platform and equipped with an 80015EVS even flat-spray nozzle[5]. The nozzle was positioned approximately 45 cm above the plant canopy and the sprayer was calibrated to deliver 185 L ha^{-1} at 275 kPa. Treatments were applied to all replications in order from lowest to highest dose.

2.3.7 Herbicide dose response experiments

Uniformly-sized plants (10 cm tall) from the respective populations (M6, NH40, BCR, WUS, and NE) were treated with herbicide at increasing rates equally spaced along a base 3.16 (mesotrione), 2 (2,4-D), or 2.5 (atrazine) logarithmic scale, resulting in 9, 10, and 8 herbicide doses for mesotrione, 2,4-D, and atrazine, respectively, and one non-treated control for each population (Seefeldt et al. 1995). The mesotrione rates applied to the HPPD-sensitive populations (BCR and WUS) ranged from 0.1–1,050 g ha^{-1}, while the rate applied to M6 and NH40 ranged from 1–10,500 g ha^{-1}. Crop oil concentrate[6] (COC, 1% v/v) and ammonium sulfate (AMS[7],2.5% v/v) were included with all mesotrione-containing treatments. The 2,4-D (dimethylamine salt[8]) rates applied to the sensitive population (WUS) ranged from 4.37–2,240 g ae ha^{-1}, and from 140–17,926 g ae ha^{-1} for M6 and NE. Nonionic surfactant[9] (NIS, 0.25% v/v) and AMS (2.5% v/v) were included with all 2,4-D-containing treatments. The atrazine rate applied to the sensitive population (WUS) ranged from 11–7,002 g ai ha^{-1}, while the rates applied to M6 and NE ranged from 72–43,759 g ai ha^{-1}. All treatments included COC (1% v/v) and AMS (2.5% v/v).

Immediately after herbicide application, treated plants were placed on greenhouse benches in a randomized complete block design. Each dose was replicated 8 times, and each experiment was conducted twice. Visual assessment of plant response was conducted 7, 14 and 21 days after treatment (DAT) using a scale ranging from 0 (no plant injury) to 100 (plant mortality). At 21 DAT, all above-ground plant tissue was harvested, dried at 65° C for 7 days, and dry weights recorded. The dry weights of all plants within each dose were then averaged

and converted to a percentage of the non-treated control. All dry weight data generated from two runs of the experiment were pooled, as Levene's test for homogeneity of variance was not significant. Combined data were analyzed using a non-linear regression model with the dose-response curve package in R software (Knezevic et al. 2007). The dose-response model was constructed using the equation $y = c + (\frac{d-c}{1+\exp\{b[\log(x)-\log(GR50)]\}})$. The four-parameter non-linear logistic model is described as follows: *b* is the slope of the curve, *c* is the lower limit, *d* is the upper limit and GR50 is 50% reduction in dry weight. This model provided the effective dose (ED) which resulted in a 10, 50, or 90% reduction in dry weight in relation to non-treated plants.

2.4 Results and Discussion

2.4.1 Confirmation of resistance to ALS- and PPO-inhibiting herbicides

Marker analysis was performed to confirm that the CHR population is resistant to ALS inhibitors via an amino acid substitution at position 574 of ALS. A comparison of the bands between the positive (ALS resistant) controls and M6 indicated identical band locations, thereby confirming plants from CHR possess a target site mutation known to confer resistance to ALS-inhibiting herbicides. Confirmation of resistance to PPO inhibitors also was accomplished via molecular marker analysis. A comparison of the bands between the positive (PPO resistant) controls and M6 indicated identical band locations, thereby confirming plants from the CHR population possess the ΔG210 codon deletion known to confer resistance to PPO-inhibiting herbicides.

2.4.2 Quantifying HPPD-inhibitor resistance

HPPD inhibitors caused characteristic injury (stunting and bleaching of meristematic tissue) on plants from all populations. However, when compared with sensitive populations WUS and BCR, M6 and NH40 exhibited far less injury at each evaluation timing (data not presented). The reduced sensitivity of M6 and NH40 to HPPD inhibitors became more apparent as time progressed from the application date. Injury to WUS and BCR generally increased over time (data not presented), whereas M6 and NH40 began to recover approximately 10 DAT. By 14 DAT new, non-injured leaf tissue had developed on the majority of M6 and NH40 plants.

Treatment of WUS, BCR, M6, and NH40 with a range of mesotrione doses produced response curves illustrating decreasing dry weights with increasing doses (Figure 2.1). $ED_{10,50,90}$ values were calculated to determine the estimated effective doses of mesotrione to reduce plant dry weight 10, 50, and 90% (Table 2.1). ED_{50} values, with accompanying standard errors, were calculated to be 32 (±4.4), 40.4 (±4.9), 6.6 (±1.2), and 2.08 (±0.352) g mesotrione ha^{-1} for M6, NH40, BCR, and WUS, respectively. The relative level of resistance to mesotrione in M6 was 4.8- or 16-fold, depending on the sensitive population used for comparison.

A confirmed HPPD inhibitor resistant waterhemp population from Iowa was reported to exhibit an 8-fold decrease in sensitivity to mesotrione when compared to a sensitive population. The Iowa population required a rate of 21 g mesotrione ha^{-1} to provide 50% control (determined visually), whereas M6 required 32 g mesotrione ha^{-1} to provide a 50% reduction in dry weight 21 DAT (McMullan and Green 2011). Another HPPD resistant population from Illinois

(NH40) had an ED$_{50}$ value of 48.5 g mesotrione ha^{-1} in research conducted by Hausman et al. (2011), a value similar to that reported in this research. These data indicate M6 is exhibiting a similar level of resistance as that of other confirmed HPPD resistant waterhemp populations.

Unlike BCR, WUS is a population that has not been previously treated with herbicides (Bell et al. 2013). Although BCR was less sensitive to mesotrione than WUS, both populations were completely controlled (no green tissue) with 105 g mesotrione ha^{-1} (a typical post-emergence use rate in Illinois). In contrast, the same dose of mesotrione reduced dry weight of M6 by 69% and NH40 by 71% when averaged across all replications in both runs of the experiment. Visual assessments of the plants provided further evidence that both the M6 and NH40 populations are exhibiting resistance to mesotrione (data not shown).

2.4.3 Quantifying 2,4-D resistance

2,4-D caused characteristic injury (epinasty, leaf strapping, stunting) on plants from all populations. The dose required to cause injury to plants in the sensitive population was much less than that required to cause injury to M6 and NE. M6 and NE showed significantly less epinasty when exposed to doses typical of a field rate compared with the sensitive population (WUS). At higher doses (2,240–17,926 g ae ha^{-1}) injury symptoms not commonly observed following exposure to synthetic auxin herbicides, such as leaf chlorosis and necrosis, were readily observed on plants from the M6 and NE populations. Plant mortality was not achieved at most rates applied to M6 and NE and plants showed re-growth by 21 DAT. Failure to achieve complete control resulted in high standard errors of calculated ED values.

Treatment of the WUS, M6, and NE plants with a range of 2,4-D rates produced response curves showing decreasing dry weights with increasing doses (Figure 2.2). ED_{50} values ranged from 95, 518, and 10 g ae 2,4-D ha^{-1} for M6, NE, and WUS, respectively (Table 2.2). Based on these values, M6 is 9.5-fold resistant to 2,4-D compared to a sensitive population while NE is 5-fold more resistant than M6. Resistance ratios increase when determining the effective dose to reduce dry weight by 90%. ED_{90} values were 9,869, 44,233, and 325 g ae 2,4-D ha^{-1} for M6, NE, and WUS, respectively (Table 2.2). Based on the ED_{90} values, M6 is 30-fold more resistant to 2,4-D compared to a sensitive population, while NE is 4-fold more resistant than M6.

The 2,4-D resistant waterhemp population (NE) described by Bernards et al. (2012) is 10-fold resistant to 2,4-D when compared to a sensitive population. This was based on both ED_{50} and visual estimates of plant injury. Other weed species resistant to 2,4-D have demonstrated a wide range of resistance ratios, including 2.5-fold for wild radish (*Raphanus raphanistrum)* (Walsh et al. 2004), 18-fold for wild mustard (*Brassica kaber*), 25-fold for prickly lettuce (*Lactuca serriola*) (Burke et al. 2009), and 29-fold for globe fringebrush (*Fimbristylis miliacea* (L.) Vahl) (Karim et al. 2004).

A rate of 1,120 g ae 2,4-D ha^{-1} (a typical field rate in Illinois) reduced dry weight of M6 and NE by 60% and 39%, respectively. The same rate reduced the dry weight of WUS by 95%. These data illustrate a distinct difference in response to 2,4-D between the sensitive population (WUS) and M6.

2.4.4 Quantifying atrazine resistance

In this research, a high level of resistance to atrazine was demonstrated by both M6 and NH40. WUS displayed injury symptoms commonly observed following exposure to PSII-inhibiting herbicides, including leaf chlorosis followed by necrosis (Hess 2000). Both M6 and NH40 demonstrated little to no injury from all but the highest atrazine rates. A distinct separation of the dose response curves between the atrazine-resistant and susceptible waterhemp populations is shown in Figure 2.3. A majority of M6 and NH40 plants survived the highest dose of atrazine (43,759 g atrazine ha^{-1}).

ED$_{50}$ values (Table 2.3) were calculated to be 16,437, 20,428, and 65 g atrazine ha^{-1} for M6, NH40, and WUS, respectively. Based on these ED$_{50}$ values, M6 was 252-fold resistant to atrazine relative to the sensitive WUS population, and NH40 was 1.2-fold more resistant than M6. Reports of other atrazine-resistant waterhemp populations indicate levels of resistance ranging from 10-fold (non-target-site based resistance) (McMullan and Green 2011), 38-fold (non-target-site based resistance) (Patzoldt et al. 2005), to >185-fold (target-site based resistance) (Foes et al. 1998).

The magnitude of atrazine resistance in M6 and NH40, coupled with the inability to achieve plant mortality at the highest application rate, resulted in high estimated effective doses and large standard errors. An accurate estimated effective dose of atrazine could not be calculated with the data collected in this experiment. The high level of atrazine resistance in M6 was further investigated to determine if a change in the atrazine binding site could explain the observed level of resistance. The entire gene encoding the atrazine target protein (psbA) was

sequenced to determine if the CHR population possessed target-site or non-target-site based resistance to atrazine. Sequencing revealed the CHR population had no mutation that would confer target site resistance to PSII inhibitors (data not shown), suggesting atrazine resistance in M6 could be caused by enhanced atrazine metabolism (Ma et al. 2013).

2.4.5 Implications and future research

The research demonstrates that M6 displays resistance to herbicides from five site-of-action groups, including inhibitors of ALS, PPO, PSII, HPPD, and synthetic auxin herbicides. A population with this magnitude of multiple resistance can pose significant challenges for its effective management. This population highlights the necessity for herbicide discovery and the implementation of weed management programs not solely dependent upon herbicides.

The CHR population may be the first of many to display this level of multiple resistance to the herbicides that have been previously effective. As predicted by Tranel et al. (2010), waterhemp's dioecious biology has facilitated the stacking of resistance to HPPD inhibitors with resistance to other herbicide families. The number of populations like this could increase with additional selection pressure (Allen et al. 2011). Future research will investigate the genetics, inheritance, and metabolism displayed by this population. With more information about the mechanisms of resistance, a better understanding of how to control the population may be acquired.

2.5 Source of Materials

[1]Clorox, The Clorox Company, 1221 Broadway, Oakland, CA.

[2]LC1 Sun Gro Horticulture, 15831 N.E. 8th Street, Bellevue, WA 98008.

[3]Scotts Osmocote Classic 13–13–13, The Scotts Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[4]Generation III Research Sprayer. DeVries Manufacturing, 28081 870th Ave., Hollandale, MN 56045.

[5]TeeJet 80015EVS. TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187.

[6] Herbimax, Loveland Products, Inc., 3005 Rocky Mountain Ave, Loveland, CO 80538.

[7] N-PAK AMS, Winfield Solutions, LLC, P.O. Box 64589, St. Paul, MN 55164-0589.

[8] Weedar 64, Nufarm Inc., Burr Ridge, IL 60527.

[9] Activator 90, Loveland Products, INC., P.O. Box 1286, Greeley, CO 80632-1286.

2.6 Literature Cited

Allen J, Hinz J, Essner R, Fischer J, Van Wert S (2011) Introducing a new soybean event with glyphosate and HPPD tolerance. Proc. North Central Weed Sci. Soc. 66:144

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

Bell MS, Tranel PJ, Hager AG (2009) Introducing quad-stack waterhemp: populations containing individuals resistant to four herbicide modes of action. Proc. North Central Weed Sci. Soc. 64:40

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

Burke IC, Yenish JP, Pittman D, Gallagher RS (2009) Resistance of a prickly lettuce (*Lactuca serriola*) biotype to 2,4-D. Weed Technol. 23:586–591

Burnside OC, Wilson RG, Weisberg S, Hubbard KG (1996) Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci. 44:74–86

Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, Davis AS (2015) Managing the evolution of herbicide resistance. Pest Manag. Sci. doi: 10.1002/ps.4009

Foes M J, Tranel PJ, Wax LM, Stoller EW (1998). A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci. 514–520

Guo JQ, 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 AG, Wax LM, Simmons FW, Stoller EW (1997) Waterhemp management in agronomic

   crops. Univ. of Illinois Bulletin 855:12

Hager AG, Wax LM, Stoller EW, Bollero GA (2002) Common waterhemp (*Amaranthus rudis*)

   interference in soybean. Weed Sci. 50:607–610

Hartzler RG, Battles B, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*)

   emergence date on growth and fecundity in soybean. Weed Sci. 52:242–245

Hartzler RG, Buhler DD, Stoltenberg DE (1999) Emergence characteristics of four annual weed

   species. Weed Sci. 47:578–584

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 Manag. Sci. 67:258–261

Hess DF (2000) Light-dependent herbicides: an overview. Weed Sci. 48:160–170

Karim RS, Man AB, Sahid IB (2004) Weed problems and their management in rice fields of

   Malaysia: an overview. Weed Biol. Manag. 4:177–186

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies:

   the concept and data analysis. Weed Technol. 21:840–848

Lee RM, Hager AG, Tranel PJ (2008) Prevalence of a novel resistance mechanism to PPO-

   inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Sci. 56:371–375

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 Phys. 163:363–377

McMullan PM, Green JM (2011) Identification of a tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in Iowa. Weed Technol. 25:514–518

Mengistu LW, Mueller-Warrant GW, Liston A, Barker RE (2000) psbA mutation (valine219 to isoleucine) in *Poa annua* resistant to metribuzin and diuron. Pest Manag. Sci. 56:209–217

Patzoldt WL, Dixon BS, Tranel PJ (2003) Triazine resistance in *Amaranthus tuberculatus* (Moq) Sauer that is not site-of-action mediated. Pest Manag. Sci. 59:1134–1142

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 Prot. 21:707–712

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

Sauer J (1955) Revision of the dioecious *amaranths*. Madrono 13:5–46

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. Weed technol. 218–227

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci. 51:145–150

Steckel LE, Sprague CL (2004a) Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci. 52:359–364

Steckel LE, Sprague CL (2004b) Late-season common waterhemp (*Amaranthus rudis*) interference in narrow- and wide-row soybean. Weed Technol. 18:947–952

Tranel PJ, Riggins CW, Bell MS, Hager AG (2010) Herbicide resistances in *Amaranthus tuberculatus*: A call for new options. Journal of Agri. and Food Chem. 59:5808–5812

Walsh MJ, Powles SB, Beard BR, Parkin BT, Porter SA (2004) Multiple-herbicide resistance across four modes of action in wild radish (*Raphanus raphanistrum*). Weed Sci. 52:8–13

Zelaya IA, Owen MDK (2005) Differential response of *Amaranthus tuberculatus* (Moq ex DC JD Sauer) to glyphosate. Pest Manag. Sci. 61:936–950

2.7 Figures

Figure 2.1 Mesotrione dose response curves for population M6 compared with HPPD-inhibitor-sensitive populations WUS and BCR and the HPPD-inhibitor-resistant NH40 populations. Above ground dry weights were obtained 21 DAT. The vertical line through response curves signifies a typical field use rate.
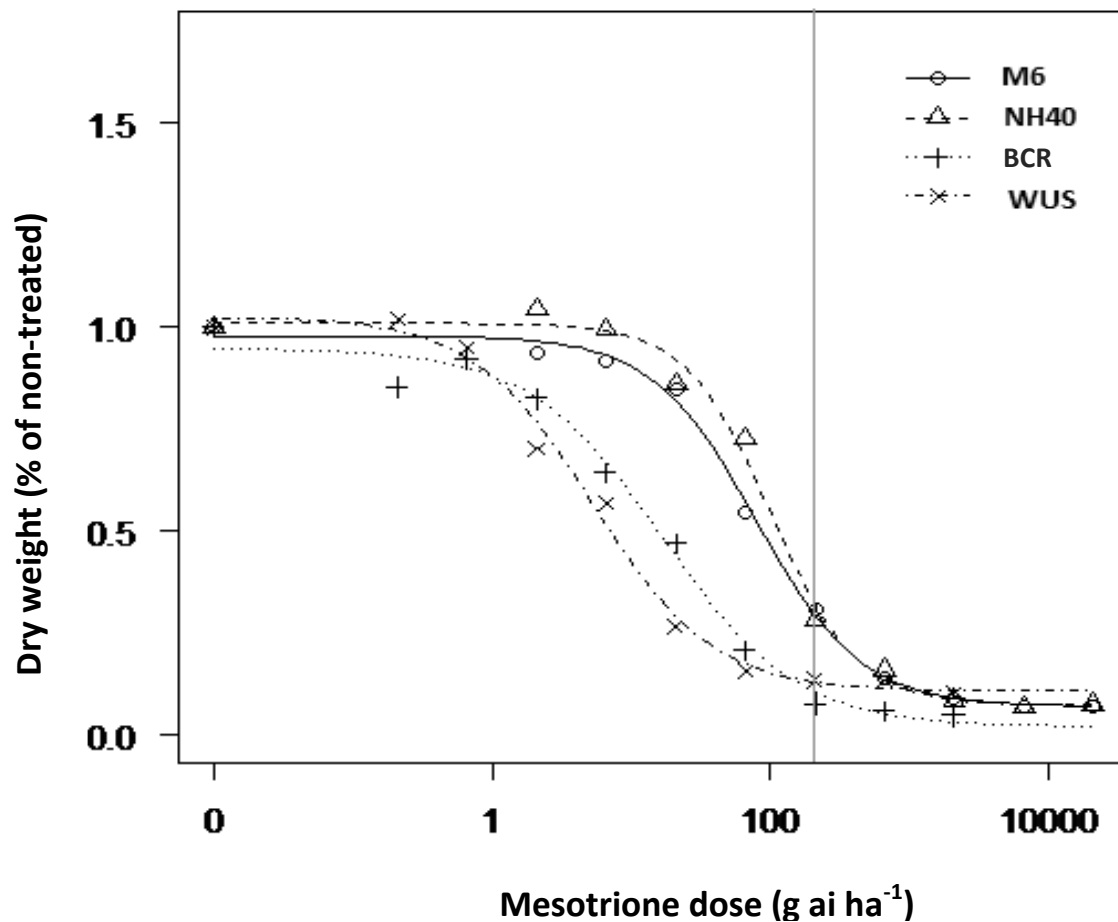
Figure 2.2 2,4-D dose response curve for population M6 compared with 2,4-D resistant population NE, and the sensitive population WUS. Above ground dry weights were obtained 21 DAT. The vertical line through response curves signifies a typical field use rate.
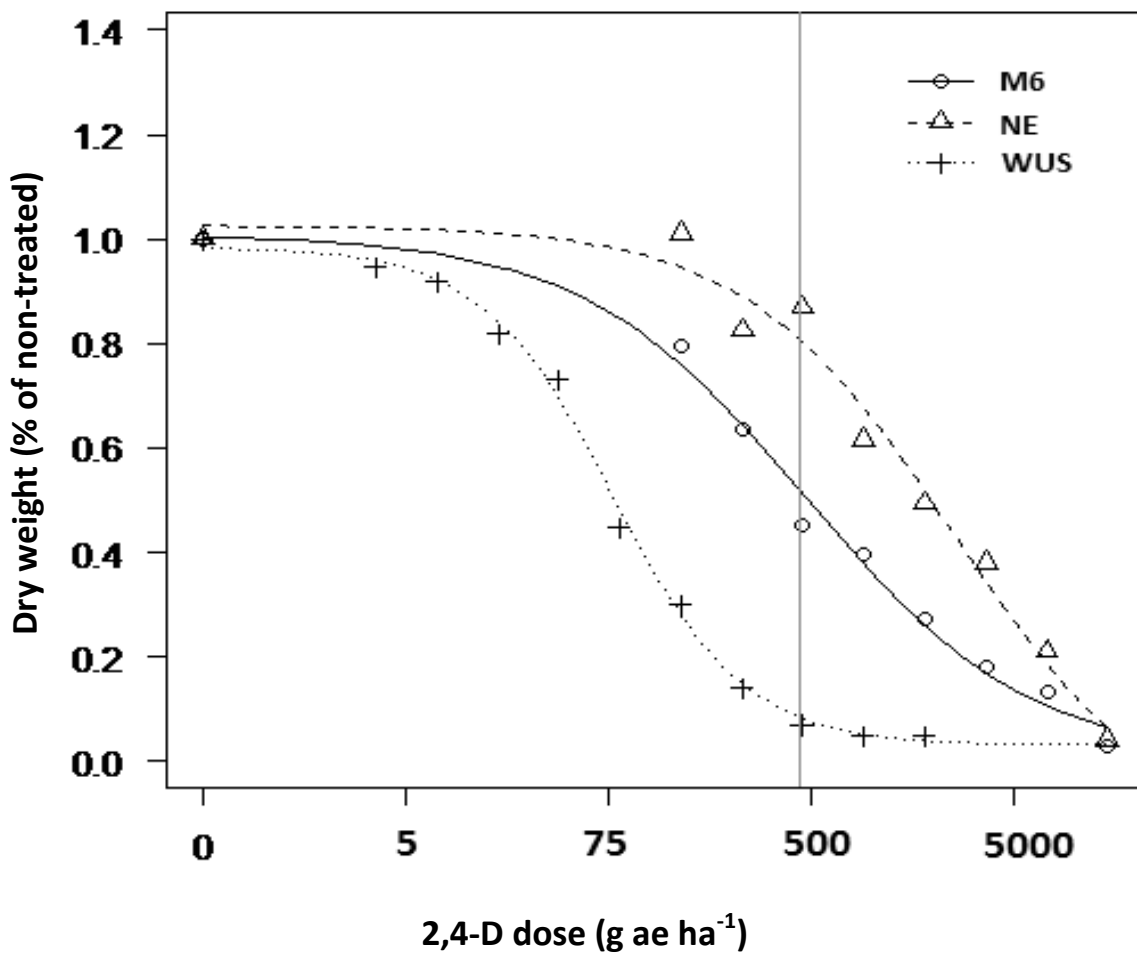
Figure 2.3 Atrazine dose response curve for population M6 compared with atrazine resistant population NH4, and the sensitive population WUS. Above ground dry weights were obtained 21 DAT. The vertical line through response curves signifies a typical field use rate.
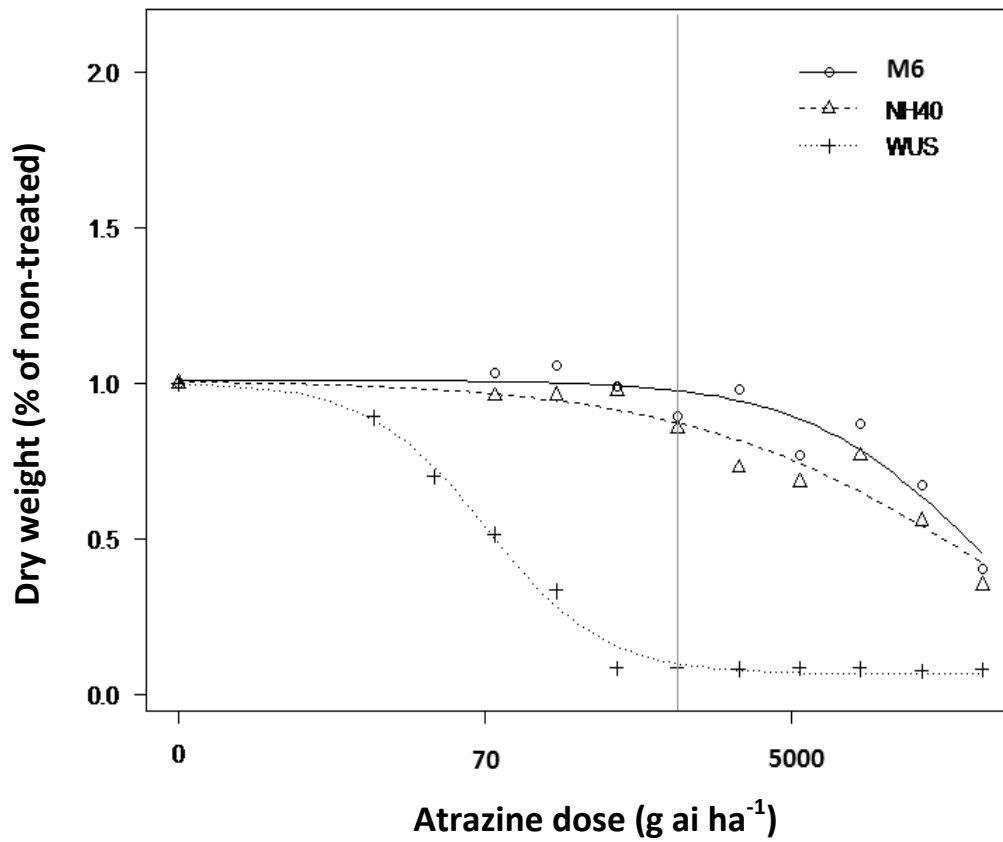
2.8 Tables

Table 2.1 Estimated effective dose in g mesotrione ha^{-1}

Pop. ED	Estimated effective dose	Std. Error
BCR:10	.58	.248
BCR:50	6.6	1.2
BCR:90	75	32.4
M6:10	4.7	1.5
M6:50	32	4.6
M6:90	213	70.8
NH40:10	8.6	2.6
NH40:50	40	4.9
NH40:90	191	54
WUS:10	.23	.08
WUS:50	2.1	.35
WUS:90	18.5	6

Table 2.2 Estimated effective dose in g 2,4-D ha^{-1}

Pop. ED	Estimated effective dose	Std. Error
NE:10	37	11.6
NE:50	518	210
NE:90	7131	6049
M6:10	5.6	2.4
M6:50	95	26
M6:90	1589	1166
WUS:10	2	.56
WUS:50	10.5	1.2
WUS:90	54.2	15.05

Table 2.3 Estimated effective dose in g atrazine ha^{-1}. High dose estimates occurred as a result of a lack of plant mortality.

Pop. ED	Estimated effective dose	Std. Error
NH40:10	132	1168.2
NH40:50	20428	17896
NH40:90	3151800	492720
M6:10	917	244.1
M6:50	16437	41941
M6:90	294450	9685600
WUS:10	9.3	4.92
WUS:50	65	16.89
WUS:90	464	30.662

CHAPTER 3

FOLIAR HERBICIDE OPTIONS TO MANAGE A WATERHEMP (*AMARANTHUS TUBERCULATUS)* POPULATION RESISTANT TO HERBICIDES FROM FIVE SITE-OF-ACTION GROUPS

3.1 Abstract

Field experiments were conducted in 2014 and 2015 to characterize the response of a waterhemp population resistant to herbicides from five site-of-action groups to herbicides from various site-of-action groups, and determine the influence of application timing on the efficacy of herbicides applied at field use rates. Herbicides commonly used in corn and soybean were applied at 1x and 2x the recommended field use rate when waterhemp plants were 8–10 cm tall. ALS-inhibiting herbicides did not control this waterhemp population, based on visual assessment of herbicide efficacy 21 days after treatment, while control with PPO-inhibiting herbicides was not greater than 70% regardless of application rate. HPPD inhibitors applied at a 1x rate did not control waterhemp more than 66%, although there were differences in the level of control among herbicides in this group. Atrazine applied in combination with HPPD-inhibiting herbicides increased control over that obtained with HPPD inhibitors alone. Activity with atrazine did not exceed 3%. A 2x application rate of 2,4-D provided less than 40% control, however a 1x application rate of dicamba provided 80% control. At least 90% control was achieved only with glyphosate at either application rate and the 2x application rate of glufosinate. Herbicide application timing results indicated that applications made to small waterhemp plants at the early post (EP) timing provided more control compared with

applications made to larger plants at the post (P) and late post (LP) application timings.

Additional waterhemp emergence following the EP application of herbicides that lack

appreciable soil residual activity did reduce control ratings at later evaluation timings, which

could necessitate the need for a second application of a foliar-applied herbicide.

3.2 Introduction

Waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] is a small seeded, dioecious,

summer annual broadleaf species indigenous to much of the Midwest (Sauer 1955). Individual

female plants are capable of producing in excess of one million seeds, making this species

especially difficult to eradicate (Hartzler et al. 2004). Previous research by Hager et al. (2002)

and Cordes et al. (2004) described significant yield reductions in soybean and corn by

waterhemp interference. The utilization of soil-residual herbicide followed by one or two

applications of foliar-applied herbicides and/or inter-row mechanical cultivation is often

necessary to achieve adequate control.

Waterhemp has evolved resistance to acetolactate synthase-(ALS) inhibiting herbicides

(Guo et al. 2015), photosystem II- (PSII) inhibiting herbicides (Patzoldt et al. 2003),

protoporphyrinogen oxidase- (PPO) inhibiting herbicides (Shoup et al. 2003),

hydroxyphenylpyruvate dioxygenase- (HPPD) inhibiting herbicides (Hausman et al. 2011), 2,4-D

(Bernards et al. 2012), and enolpyruvylshikimate-3-phosphate synthase- (EPSPS) inhibitors

(Zelaya and Owen 2005). The evolution of herbicide-resistant waterhemp populations has

effectively reduced the number of viable herbicide options growers can utilize for management

in corn and soybean. This management challenge is especially problematic when populations

demonstrate resistance to herbicides from multiple site-of-action groups, such as the four-way resistant population described by Bell et al. (2013).

Evans et al. (2015) described a waterhemp population resistant to herbicides from five site-of-action groups found in Champaign Co., Illinois. Molecular marker assays indicated plants from this population possessed changes in the coding regions of genes producing herbicide target site proteins that are known confer resistance to ALS- and PPO-inhibiting herbicides. Greenhouse dose-response experiments indicated the level of resistance demonstrated by this population (CHR) to mesotrione, 2,4-D, and atrazine is 16-, 30-, and 253-fold, respectively, when compared with one or more sensitive populations. Additional field experiments were conducted on the grower's field where CHR was first identified to further characterize the response of the CHR population to various foliar-applied herbicides.

One objective of this research was to characterize the response of CHR to 1x and 2x application rates of herbicides from different site-of-action groups under field conditions. Research conducted by Hausman et al. (2011) and Ma et al. (2013) indicated that HPPD-inhibiting herbicides provided greater control of an HPPD-resistant waterhemp population when applied to small (less than 8 cm) waterhemp plants compared to when applications were made to larger plants. Therefore, a second objective was to characterize CHR's response to foliar-applied herbicides applied at three weed growth stages.

3.3 Materials and Methods

3.3.1 General methodology for field experiments

Field experiments were conducted in 2014 and 2015 at the location in Champaign Co.,
Illinois where CHR was initially identified. Soil samples were collected in the fall of 2014 and
sent to a commercial laboratory for chemical analysis. The soil is a Flanagan silt loam (fine,
smectitic, mesic Aquic Argiudolls) with a pH of 5.5, cation exchange capacity of 19.5 meq/100g$^-$
^{1}, and an organic matter content of 4.8%. Preplant tillage was performed each spring to prepare
the seedbed for planting and to control any existing vegetation. Experiments (except the
herbicide application timing experiment) were conducted in either corn (DKC62-77RIB) or
soybean (Asgrow 3231 RR2), planted in 76-cm rows. Planting dates in 2014 were May 7th (corn)
and May 26th (soybean), while in 2015 dates were May 14th (corn) and May 22nd (soybean).
Experiments were performed in a randomized complete block design with three replications of
each treatment. Individual replications were plots measuring 3 by 7.6 meters that included four
crop rows. Herbicides were applied using a pressurized CO_2 backpack sprayer equipped with
Teejet[1] AIXR110025 nozzles, spaced 51 cm apart on a 3-meter boom calibrated to deliver 187 L
ha^{-1} at 276 kPa. Environmental conditions were recorded during each application. Herbicides
and associated spray additives routinely applied in Illinois corn and soybean crops were
selected for evaluation.

Statistical analysis for all field experiments was performed using PROC GLM in SAS 9.2[2],
with herbicide treatment considered a fixed effect, while year and block nested within year
were considered random effects. Initial analysis revealed no significant year by treatment

interactions (α=.05) thus, data from both years were pooled. Treatment means for all metrics were separated by Proc GLM in SAS. The differences between the dry weights of treated plants harvested 21 DAT and dry weights of pre-treatment plants also were calculated. The difference values were utilized to determine if treated plants recovered and began to re-grow following treatment with the herbicide.

3.3.2 Postemergence herbicide options in corn

Twenty-three treatments (including a non-treated control) were evaluated in the corn experiment. Treatments (Table 3.1) included 1x and 2x recommended field application rates of three HPPD inhibitors, two plant growth regulators (PGR), one glutamine synthetase (GS) inhibitor, one EPSP synthase inhibitor, one PSII inhibitor, and combinations of each HPPD herbicide with 560 g ai ha^{-1} of atrazine. The combinations of HPPD-inhibiting herbicides and atrazine were included to determine if control of a population resistant to both herbicide groups could be increased over that obtained with each herbicide applied alone. A synergistic interaction between HPPD-inhibiting herbicides and certain PS II inhibitors has been documented by many researchers, but this synergistic interaction may not always overcome resistance (Hausman et al. 2011; Ma et al. 2013; Woodyard et al. 2009).

Herbicide treatments were applied when waterhemp plants were 8–10 cm tall. Corn growth stage at the time of application was V4 in 2014 and V5 in 2015. Prior to herbicide application, five uniformly-sized waterhemp plants per plot (15 per treatment) were marked by placing a wooden garden stake near each plant. These plants were subsequently harvested 21

days after treatment (DAT) to evaluate the treatment's effect on biomass accumulation. Fifteen additional plants were harvested from non-treated plots to determine pre-treatment biomass.

Herbicide efficacy was visually determined and recorded 7, 14, and 21 DAT using a scale of 0 (no control) to 99 (complete control). These ratings took into account waterhemp injury, biomass and stand reduction, and any recovery of treated plants when compared with a non-treated control. In addition to visual estimates of herbicide efficacy, the height of each marked plant was recorded 14 and 21 DAT (data not presented). All marked waterhemp plants were harvested 21 DAT, dried at 65° C for seven days, and dry weights recorded. Dry weights for all 15 plants from each treatment were averaged across all three replications.

3.3.3 Postemergence herbicide options in soybean

Seventeen treatments (including a non-treated control) were evaluated in the soybean experiment. Treatments (Table 3.2) included 1x and 2x recommended field application rates of four PPO inhibitors, one EPSPS inhibitor, one GS inhibitor, and two ALS inhibitors. Herbicide treatments were applied when waterhemp plants were 8–10 cm tall. Soybean growth stage at the time of application was V3 both years. Prior to herbicide application, five uniformly-sized waterhemp plants per plot (15 per treatment) were marked by placing a wooden garden stake near each plant. These plants were subsequently harvested 21 DAT to evaluate the treatment's effects on biomass accumulation. Fifteen additional plants were harvested from non-treated plots to determine pre-treatment biomass. Visual estimates of herbicide efficacy, heights and dry weights of marked waterhemp plants were recorded as described previously.

3.3.4 Effect of herbicide application timing

A selection of ten herbicides was applied at three application timings based on waterhemp growth stage each year. A single application rate, representing a typical recommended rate, for each herbicide was selected. Each combination of herbicide and timing was considered a separate treatment (Table 3.3). Herbicide applications were made when waterhemp plants were 5–8 cm (early post(EP)), 10–14 cm (post(P)), or 15–23 cm (late post (LP)) tall. Prior to herbicide application, five uniformly-sized waterhemp plants per plot (15 per treatment) were marked by placing a wooden garden stake near each plant. These plants were subsequently harvested 21 DAT to evaluate the treatment's effects on biomass accumulation. Fifteen additional plants were harvested from non-treated plots to determine pre-treatment biomass. The herbicides selected for this experiment represent those routinely used in Illinois corn and soybean crops, therefore, no crop was planted. Visual estimates of herbicide efficacy, heights and dry weights of marked waterhemp plants were recorded as described previously.

3.4 Results and Discussion

3.4.1 Postemergence herbicide options in corn

Mean control ratings and dry weights of marked plants harvested 21 DAT are presented in Table 3.4. The differences between the dry weights of treated plants harvested 21 DAT and dry weights of pre-treatment plants also are presented. PROC GLM t test (LSD) groupings, presented in Table 3.5, were generated for both visual estimates of waterhemp control and dry weights of treated plants harvested 21 DAT

Control of waterhemp with HPPD-inhibiting herbicides was variable among the three

HPPD inhibitors evaluated. Visual estimates of control 21 DAT indicated the 1x application

rates of mesotrione and tembotrione provided a similar level of control, although control did

not exceed 66%. Topramezone failed to control waterhemp more than 49% regardless of

application rate. The 2x application rate of each HPPD inhibitor increased control compared

with the 1x application rate, but the higher application rates did not reduce plant dry weights

more than the 1x application rates. Regardless of application rate, treated waterhemp plants

developed injury symptoms characteristic of herbicides in this group, but began to recover and

re-grow by 14 DAT. However, estimated difference values of pre-treatment plants and treated

plants harvested 21 DAT reveal that plants treated with HPPD inhibitors generally

demonstrated small amounts of re-growth. Only plants treated with the 1x application rate of

topramezone produced significant re-growth 21 DAT. Competition with the crop might have

slowed the regrowth of treated waterhemp plants compared with the rate of recovery

observed in greenhouse experiments (data not presented). Adding 560 g ai ha^{-1} atrazine to each

HPPD inhibitor increased control of CHR at each application rate, however this did not result in

differences in plant dry weight. The response of CHR to HPPD inhibitors under field conditions

is similar to another HPPD-resistant waterhemp population described by McMullan and Green

(2011), who reported less than 60% control with labeled use rates of three HPPD herbicides.

Control of CHR with HPPD inhibitors, however, generally was greater than the control of a

McLean county HPPD-resistant waterhemp (MCR) population described by Hausman et al.

(2011), for which visual estimates of control with the same HPPD inhibitors applied at similar

rates were 24% or less.

The response of CHR to two plant growth regulators differed significantly. Dicamba controlled CHR 80–94% depending on application rate, while control with 2,4-D did not exceed 36% regardless of application rate. Treated plants demonstrated some injury symptoms, including minor leaf cupping and epinasty, but rapidly recovered and resumed growth. There were no differences in plant dry weight between the dicamba application rates, but the 2x application rate of 2,4-D reduced dry weight more than the 1x application rate. Estimated difference values indicate significant levels of plant re-growth and dry weight accumulation 21 DAT with either application rate of 2,4-D, but no differences with either application rate of dicamba.

Atrazine provided very low control at either application rate and produced little to no injury symptoms on treated plants. Estimated difference values indicate significant levels of plant re-growth and dry weight accumulation 21 DAT with either application rate of atrazine. Glyphosate provided at least 90% control regardless of application rate, while a similar level of control with glufosinate required a 2x application rate. However, plant dry weight was similar among all application rates of glyphosate and glufosinate. Estimated difference values indicate no significant plant regrowth and dry weight accumulation occurred after application of glyphosate or glufosinate.

3.4.2 Post-applied herbicide options in soybean

Mean control ratings and dry weights of marked plants harvested 21 DAT are presented in Table 3.6. The estimated differences between the dry weights of treated plants harvested 21 DAT and dry weights of pre-treatment plants also are presented. PROC GLM t test (LSD)

62

groupings, presented in Table 3.7, were generated for both visual estimates of waterhemp control and dry weights of treated plants harvested 21 DAT.

ALS-inhibiting herbicides chlorimuron and imazethapyr provided no control of CHR 21 DAT regardless of application rate. Plants treated with either rate of these ALS herbicides had significant weight increases by 21 DAT compared to pre-treatment plants, and had weights comparable to non-treated plants harvested 21 DAT. This magnitude of ALS resistance is consistent with reports of other ALS-resistant waterhemp populations that demonstrate target-site resistance to ALS-inhibiting herbicides (Patzoldt and Tranel 2007).

Plants treated with PPO inhibitors exhibited rapid tissue chlorosis, which became necrotic within a few days after application. However, treated plants began to recover from this initial injury approximately 7 DAT, although degree of injury and speed of recovery varied among the PPO inhibitors. The PPO inhibitors typically applied after crop and weed emergence (acifluorfen, fomesafen, lactofen) controlled CHR 58% or less, regardless of application rate. Saflufenacil, a PPO inhibitor typically applied prior to soybean emergence, controlled CHR 60–70%. Increasing application rates only increased control of CHR with fomesafen. There were no differences in dry rate among the PPO inhibitors nor did dry weights vary by application rate. Positive estimated difference values for all PPO inhibitor treatments indicate that treated plants had recovered to some extent by 21 DAT, but only plants treated with acifluorfen applied at the 1x rate had significant dry weight gain when compared to pre-treatment plants.

Similar to results from the corn herbicide experiment, glyphosate and glufosinate provided the greatest control of CHR. Regardless of application rate, control of CHR with

glyphosate was at least 94% while control with glufosinate ranged from 81–91% 21 DAT. Dry weights were not different between glyphosate and glufosinate regardless of application rate. Negative estimated difference values for both treatments indicate that no plant growth occurred after treatment with glyphosate or glufosinate.

3.4.3 Effect of herbicide application timing

Mean control ratings and dry weights of marked plants harvested 21 DAT, are presented in Table 3.8. The estimated differences between the dry weights of treated plants harvested 21 DAT and dry weights of pre-treatment plants taken at each of the three application timings also are presented.

PROC GLM t test (LSD) groupings, presented in Table 3.9, were generated for both visual estimates of waterhemp control and dry weights of treated plants harvested 21 DAT. Single degree of freedom contrasts, presented in Table 3.10, represent dry weights of plants harvested 21 DAT for separate application timings within each treatment.

ALS-inhibiting herbicides did not control CHR regardless of application timing, although chlorimuron applied EP reduced dry weight more than when applied P or LP. Positive estimated difference values indicated plant re-growth following all timings except for chlorimuron EP (Pr>F = 0.1259). These results are consistent with the results from the soybean herbicide experiment previously described and further support the existence of ALS resistance within the population.

PPO-inhibiting herbicides provided varying levels of control when applied at different plant heights. The greatest control was achieved when fomesafen or lactofen was applied EP compared with either P or LP. Additionally, control was greater when these herbicides were applied P compared with LP. These results are similar to those of Hager et al. (2003), who reported greater control of waterhemp when PPO-inhibiting herbicides were applied to smaller plants compared to when applications were made to larger plants. Estimated difference values for all PPO-inhibiting herbicide application timings indicate plant recovery by 21 DAT. Plant dry weight gain when compared to pre-treatment plant dry weight was significant only in LP applications of lactofen. Contrast statements for fomesafen application timings indicated no difference in control between EP and P, but significant increases in control between P vs LP and EP vs LP (Table 3.10).

Application timing influenced control of CHR with HPPD-inhibiting herbicides. There was no difference in control with mesotrione applied EP and P, but both of these application timings resulted in greater control of CHR than when applied LP. Tembotrione applied EP controlled CHR more than when applied LP. Differences in control of CHR according to application timing did not equate to differences in dry weights by application timing. Dry weights of CHR following application of mesotrione or tembotrione were similar regardless of application timing. Although not significantly different, positive estimated difference values were calculated for mesotrione applied EP and P and for tembotrione applied EP and P. This indicates small weight gains when compared to pre-treatment plant dry weights. Contrasts among mesotrione application timings show significant differences between P vs LP and EP vs LP (Table 3.10).

Application of plant growth regulators at various stages resulted in different levels of control dependent upon the herbicide. Control of CHR with 2,4-D at all timings was 34% or less, but application EP or P increased control compared with the LP application. Control with dicamba ranged from 67–80%, and also varied according to application timing. Dicamba applied EP timing had the lowest control rating, but an average plant dry weight equal to that of the P timing application. Additional waterhemp emergence between the EP and P application timings contributed to the lower control ratings at the 21 DAT evaluation timing. Estimated difference values indicate very small weight gains occurred after dicamba was applied. Estimated difference values were greater for 2,4-D application timings with the LP application timing resulting in a significant increase in dry weight. Contrasts of 2,4-D timings indicate no significant difference in 21 DAT dry weight occurred. A contrast between 2,4-D and dicamba revealed estimated differences in dry weight differed significantly, and that dicamba provided an estimated difference value of 45% more control compared with 2,4-D (Table 3.10).

Control of CHR with glufosinate and glyphosate was greatest when these herbicides were applied P. Glufosinate and glyphosate applied EP controlled existing plants, as illustrated by the dry weight values 21 DAT. Additional waterhemp emergence between the EP and P application timings contributed to the lower whole plot control ratings at the 21 DAT evaluation timing. Control of larger plants at the LP timing was greater with glyphosate than glufosinate. P and LP applications of both herbicides resulted in negative estimated difference values when compared to pre-treatment plant dry weights.

Collectively, these results indicate CHR is poorly controlled following foliar applications

of herbicides that inhibit ALS, PPO, HPPD, PSII, and the synthetic auxin 2,4-D. Adding 560 g ai

ha^{-1} of atrazine to HPPD-inhibiting herbicides increased control 16% on average, but control did

not exceed 78% with any combination of HPPD inhibitor and atrazine. Dicamba and glyphosate

controlled CHR 80% and 90–94%, respectively, when applied at recommended field rates, while

control with glufosinate at a similar recommended rate ranged from 75–81%. Across the

herbicides evaluated, applications made to small waterhemp plants at the EP timing provided

more control compared with applications made to larger plants at the P and LP application

timings. Additional waterhemp emergence following the EP application of herbicides that lack

appreciable soil residual activity did reduce control ratings at later evaluation timings, which

could necessitate the need for a second application of a foliar-applied herbicide.

3.5 Source of Materials

[1] TeeJet 80015EVS. TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187.

[2] Statistical Analysis Software (SAS) 9.2. SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513.

3.6 Literature cited

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

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

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

Evans CM, Tranel PJ, Riechers DE, Davis AS, Maxwell DJ, Gonzini LC, Hager AG (2015)

Characterization of a novel five-way resistant population of waterhemp (*Amaranthus*

tuberculatus). North Central Weed Sci. Soc. Proc. 70:127

Guo JQ, 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 AG, Wax LM, Bollero GA, Simmons FW (2002) Common waterhemp (*Amaranthus rudis*

Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.).

Crop Prot. 21:277–283

Hager AG, Wax LM, Bollero GA, Stoller EW (2003) Influence of diphenylether herbicide

application rate and timing on common waterhemp (*Amaranthus rudis*) control in

soybean (*Glycine max*). Weed Technol. 17:14–20

Hartzler RG, Battles B, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*)

emergence date on growth and fecundity in soybean. Weed Sci. 52:242–245

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 Manag. Sci. 67:258–261

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 Phys. 163:363–377

McMullan PM, Green JM (2011) Identification of a tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in Iowa. Weed Technol. 25:514–518

Patzoldt WL, Dixon BS, Tranel PJ (2003) Triazine resistance in *Amaranthus tuberculatus* (Moq) Sauer that is not site-of-action mediated. Pest Manag. Sci. 59:1134–1142

Patzoldt WL, Tranel PJ (2007) Multiple ALS mutations confer herbicide resistance in waterhemp (*Amaranthus tuberculatus*). Weed Sci. 55:421–428

Sauer J (1955) Revision of the dioecious *amaranths*. Madrono 13:5–46

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci. 51:145–150

Woodyard AJ, Hugie JA, Riechers DE (2009) Interactions of mesotrione and atrazine in two weed species with different mechanisms for atrazine resistance. Weed Sci. 57:36–-378

Zelaya IA, Owen MDK (2005) Differential response of *Amaranthus tuberculatus* (Moq ex DC JD Sauer) to glyphosate. Pest Manag. Sci. 61:936–950

3.7 Tables

Table 3.1 Herbicides, their respective sites of action, and application rates that were applied in the corn herbicide experiment at Champaign Co. Il (2014–2015).

Herbicide[a]	Site of Action[b]	Rate
		g ai ha^{-1}
2,4-D	Auxin receptor	560[c]
		1120[c]
Atrazine	PSII	1681
		3361
Dicamba	Auxin receptor	560[c]
		1120[c]
Glufosinate	GS	448
		896
Glyphosate	EPSPS	840[c]
		1681[c]
Mesotrione	HPPD	105
		211
Mesotrione+ATZ	HPPD, PSII	105+560
		211+560
Tembotrione	HPPD	92
		184
Tembotrione+ATZ	HPPD, PSII	92+560
		184+560
Topramezone	HPPD	18
		36
Topramezone+ATZ	HPPD, PSII	18+560
		36+560
Untreated	—	0

[a]Herbicide treatments containing HPPD inhibitors and atrazine included crop oil concentrate (COC 1% v/v) and 28% urea ammonium nitrate (UAN, 2.5% v/v); treatments with synthetic auxins included a nonionic surfactant at 0.25% (v/v).
[b]Abbreviations for site of action: ALS, acetolactate synthase; EPSPS, enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; HPPD, hydroxyphenylpyruvate dioxygenase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.
[c]Acid equivalent (g ae ha^{-1})

Table 3.2 Herbicides, their respective sites of action, and application rates that were applied in the soybean herbicide experiment at Champaign Co. Il (2014–2015).

Herbicide	Site of Action[a]	Rate	Spray additive
		g ai ha^{-1}	
Acifluorfen	PPO	280	COC+AMS
		560	COC+AMS
Chlorimuron	ALS	13	COC+AMS
		26	COC+AMS
Fomesafen	PPO	347	COC+AMS
		683	COC+AMS
Glufosinate	GS	594	AMS
		1188	AMS
Glyphosate	EPSPS	840[b]	AMS
		1681[b]	AMS
Imazethapyr	ALS	71	COC+AMS
		141	COC+AMS
Lactofen	PPO	218	COC+AMS
		437	COC+AMS
Saflufenacil	PPO	25	MSO
		49	MSO
Untreated	-	0	-

[a]Abbreviations for site of action: ALS, acetolactate synthase; EPSPS, enolpyruvylshikimate-3-phosphate synthase; GS, glutamine synthetase; PPO, protoporphyrinogen oxidase;
[b]Acid equivalent (g ae ha^{-1})

Table 3.3 Herbicide application rates and timings on bare-ground plots at Champaign Co. Il (2014–2015).

Herbicide	Timing	Rate
		g ai ha^{-1}
2,4-D	EP	560[b]
	P	
	LP	
Chlorimuron	EP	13
	P	
	LP	
Dicamba	EP	560[b]
	P	
	LP	
Fomesafen	EP	347
	P	
	LP	
Glufosinate	EP	594
	P	
	LP	
Glyphosate	EP	840[b]
	P	
	LP	
Imazethapyr	EP	71
	P	
	LP	
Lactofen	EP	218
	P	
	LP	
Mesotrione	EP	105
	P	
	LP	
Tembotrione	EP	92
	P	
	LP	
Untreated	—	0

[a] Waterhemp heights measured 5–8 cm EP (Early Post), 10–14 cm P (Post), and 15–23 cm LP (Late Post)

[b] Acid equivalent (g ae ha^{-1})

[c] Spray additives were the same for each herbicide as indicated in the previous two experiments

Table 3.4 Waterhemp control, dry weight, and estimated difference between the dry weights of treated plants harvested 21 DAT and dry weights of pre-treatment plants in the corn herbicide experiment, averaged over 2014 and 2015.

Herbicide	Rate	21 DAT		Estimated difference[d]	
	g ai ha^{-1}	Control (%)	Biomass (g)		Pr>F
2,4-D	560[b]	22	2.85	2.562	<0.0001*
	1120[b]	36	1.86	1.572	<0.0001*
Atrazine	1680	0	2.66	2.372	<0.0001*
	3360	3	2.15	1.862	<0.0001*
Dicamba	560[b]	80	0.94	0.652	0.0564
	1120[b]	94	0.55	0.262	0.407
Glufosinate	448	75	0.33	0.042	0.8935
	896	90	0.12	-0.168	0.5933
Glyphosate	840[b]	90	0.14	-0.148	0.6266
	1680[b]	97	0.16	-0.128	0.6837
Mesotrione	105	66	0.27	-0.018	0.9415
	210	76	0.23	-0.058	0.8534
Mesotrione+ATZ	105+560	78	0.14	-0.148	0.6266
	210+560	88	0.16	-0.128	0.6721
Tembotrione	92	57	0.42	0.132	0.6745
	184	69	0.23	-0.058	0.8534
Tembotrione+ATZ	92+560	76	0.2	-0.088	0.7792
	184+560	90	0.15	-0.138	0.6606
Topramezone	18	30	0.89	0.602	0.0464*
	36	49	0.47	0.182	0.5631
Topramezone+ATZ	18+560	48	0.6	0.312	0.3249
	36+560	64	0.2	-0.088	0.7792
Untreated	-	-	5.07	4.782	<.0001
Untreated at app.[c]	-	-	0.288	-	-
LSD[a]		9.6	0.65		

* Significant at α = .05, of interest were treatments with positive differences indicating growth.

[a] Separated by PROC GLM in SAS, α = .05

[b] Acid equivalent (g ae ha^{-1})

[c] Plants harvested the day of spraying to assess biomass accumulation after herbicide application.

[d] Estimated difference in dry weight between herbicide-treated plants and pre-treatment plants.

Table 3.5 Foliar herbicide options for maize LSD groupings. Ratings with the same letter within a column are not significantly different at α = .05 (separated by PROC GLM in SAS)

Herbicide	PROC GLM t Test (LSD) for Y		
	g ai ha^{-1}	Biomass^b	Efficacy^c
2,4-D	560^b	B	I
	1120^b	D	HI
Atrazine	1681	BC	J
	3361	CD	J
Dicamba	560^b	E	BC
	1120^b	EFG	A
Glufosinate	448	EFG	BCD
	896	G	A
Glyphosate	840^b	G	A
	1681^b	G	A
Mesotrione	105	FG	DEF
	211	FG	BC
Mesotrione+ATZ	105+560	G	B
	210+560	G	A
Tembotrione	92	EFG	FG
	184	FG	CDE
Tembotrione+ATZ	92+560	G	BC
	184+560	G	A
Topramezone	18	EF	HI
	36	EFG	G
Topramezone+ATZ	18+560	EFG	G
	36+560	G	EF
untreated	0	A	J

^{a}Acid equivalent (g ae ha^{-1})

^{b}Biomass is grouped heaviest=A to lightest =G (LSD=0.65 g)

^{c}Efficacy is grouped highest % control=A to least=J (LSD= 9 .6 %)

Table 3.6 Waterhemp control, dry weight, and estimated difference between the dry weights of treated plants harvested 21 DAT and dry weights of pre-treatment plants in the soybean herbicide experiment, averaged over 2014 and 2015.

Herbicide	Rate	21 DAT		Estimated Difference[d]	
	g ai ha^{-1}	Control %	Biomass (g)		Pr>F
Acifluorfen	280	35	6.18	5.752	0.0404*
	560	40	4.09	3.665	0.2176
Chlorimuron	13	0	11.11	10.688	0.0016*
	26	0	11.11	10.679	0.0017*
Fomesafen	347	49	3.17	2.741	0.3513
	683	58	2.93	2.507	0.3931
Glufosinate	594	81	0.38	-0.043	0.9862
	1188	91	0.11	-0.321	0.9108
Glyphosate	840[b]	94	0.11	-0.316	0.9122
	1681[b]	97	0.07	-0.362	0.8999
Imazethapyr	71	0	12.85	12.423	0.0004*
	141	0	10.17	9.742	0.0034*
Lactofen	218	49	3.28	2.851	0.3328
	437	55	2.63	2.203	0.4521
Saflufenacil	25	60	4.47	4.039	0.1761
	49	70	1.32	0.891	0.7593
Untreated	-	-	12.43	12.002	0.0006
Untreated at app.[c]	-	-	.43	-	-
LSD[a]		11	6.00		

* Significant at α = .05, of interest were treatments with positive differences indicating growth.

[a] Separated by PROC GLM in SAS, α = .05

[b] Acid equivalent (g ae ha^{-1})

[c] Plants harvested the day of spraying to assess biomass accumulation after herbicide application.

[d] Estimated difference in dry weight between herbicide treated plants and pre-treatment plants.

Table 3.7 Foliar herbicide options for soybean LSD groupings. Ratings with the same letter within a column are not significantly different at α = .05 (separated by PROC GLM in SAS)

Herbicide	Proc GLM t Test (LSD) for Y		
	g ai ha^{-1}	Weight[a]	Efficacy[b]
Acifluorfen	280	BCD	FG
	560	DE	G
Chlorimuron	13	AB	H
	26	AB	H
Fomesafen	347	DE	EF
	683	DE	E
Glufosinate	594	DE	BC
	1188	E	AB
Glyphosate	840[c]	E	A
	1681[c]	E	A
Imazethapyr	71	A	H
	141	ABC	H
Lactofen	218	DE	EF
	437	DE	E
Saflufenacil	25	CDE	DE
	49	DE	CD
Untreated	0	A	H

[a]Biomass is grouped heaviest=A to lightest =G (LSD=6 g)

[b]Efficacy is grouped highest % control=A to least=J (LSD= 11 %)

[c] Acid Equivalent (g ae ha^{-1})

Table 3.8 Post herbicide application timing field data. Visual estimates of control presented as a percentage of the untreated plot. Rating and biomass are presented as averages across 2014 and 2015. Average plant dry weight at treatment across both years was 0.142g (EP), 0.654g (P), and 1.238g (LP).

Herbicide	Timing	Rate	21 DAT		Estimated Difference[c]	
		g ai ha^{-1}	Control (%)	Biomass (g)		Pr>F
2,4-D	EP	560[a]	34	2.67	2.53	0.2223
	P		34	3.25	2.60	0.2108
	LP		21	5.08	3.84	0.0472*
Chlorimuron	EP	13	0	3.33	3.19	0.1259
	P		0	8.09	7.44	0.0009*
	LP		0	7.81	6.57	0.0028*
Dicamba	EP	560[a]	67	1.12	0.98	0.6345
	P		80	1.11	0.46	0.8251
	LP		77	1.59	0.35	0.8623
Fomesafen	EP	347	66	1.13	0.99	0.6316
	P		53	2.54	1.89	0.3605
	LP		26	3.32	2.08	0.3120
Glufosinate	EP	594	78	0.27	0.13	0.9492
	P		89	0.06	-0.59	0.7729
	LP		71	0.35	-0.89	0.6646
Glyphosate	EP	840[a]	52	0.83	0.69	0.7379
	P		94	0.37	-0.28	0.8896
	LP		93	0.62	-0.62	0.7625
Imazethapyr	EP	71	0	5.26	5.12	0.0167*
	P		0	7.34	6.69	0.0024*
	LP		0	8.52	7.28	0.0011*
Lactofen	EP	218	79	0.42	0.28	0.8912
	P		55	3.66	3.01	0.1490
	LP		40	5.97	4.73	0.0261*
Mesotrione	EP	105	79	0.37	0.23	0.9094
	P		71	0.52	-0.13	0.9478
	LP		43	1.61	0.37	0.8568
Tembotrione	EP	92	65	0.86	0.72	0.7265
	P		57	1.47	0.82	0.6890
	LP		47	1.2	-0.04	0.9856
Untreated		0	0	11.24	10.59	<0.0001
LSD[b]			11	4.2		

* Significant at α = .05, of interest were treatments with positive differences indicating growth.

[a]Acid equivalent (g ae ha^{-1})

[b] Separated by PROC GLM in SAS, α = .05

[c]Estimated difference in dry weight between 30 herbicide treated plants and 30 pre-treatment plants.

Table 3.9 Post herbicide application timing LSD groupings. Ratings with the same letter within a column are not significantly different at α = .05 (separated by PROC GLM in SAS).

Herbicide	Timing	Rate	Proc GLM t Test (LSD) for Y	
		g ai ha^{-1}	Biomass[a]	Efficacy[b]
2,4-D	EP	560	EFG	KL
	P		DEFG	KL
	LP		BCDEF	M
Chlorimuron	EP	13	DEFG	N
	P		AB	N
	LP		ABC	N
Dicamba	EP	560	FG	CDE
	P		G	BC
	LP		FG	CD
Fomesafen	EP	347	FG	DEF
	P		EFG	GHI
	LP		DEFG	LM
Glufosinate	EP	594	G	BC
	P		G	AB
	LP		G	CDE
Glyphosate	EP	840	G	HIJ
	P		G	A
	LP		G	A
Imazethapyr	EP	71	BCDEF	N
	P		ABCD	N
	LP		AB	N
Lactofen	EP	218	G	BC
	P		CDEFG	FGHI
	LP		BCDE	JK
Mesotrione	EP	105	G	BC
	P		G	CDE
	LP		FG	IJK
Tembotrione	EP	92	G	EFG
	P		FG	FGH
	LP		FG	HIJ
Untreated	P	0	A	N

[a]Biomass is grouped heaviest=A to lightest=G (LSD=4.2g)

[b]Efficacy is grouped highest %control=A to least=J (LSD= 11 %)

[c] Acid equivalent (g ae ha^{-1})

Table 3.10 Post herbicide timing treatment contrasts.

Contrasts	Estimated Difference[a]	Pr > F
Mesotrione		
Early Post vs Post	8	0.4976
Post vs Late Post	28	0.0227*
Early Post vs Late Post	36	0.0043*
2,4-D		
Early Post vs Post	0	1
Post vs Late Post	13	0.2558
Early Post vs Late Post	13	0.2558
Dicamba		
Early Post vs Post	-13	0.5247
Post vs Late Post	3	0.766
Early Post vs Late Post	-10	0.7338
Fomesafen		
Early Post vs Post	13	0.2558
Post vs Late Post	27	0.0275*
Early Post vs Late Post	40	0.0016*
2,4-D vs Dicamba[b]	-45	<0.0001*

*Significant at α = .05

[a] Estimated difference of mean control between application timings

[b] Grouped across all timings

CHAPTER 4

SOIL-APPLIED HERBICIDES TO MANAGE A MULTIPLE RESISTANT WATERHEMP (*AMARANTHUS TUBERCULATUS)* POPULATION

4.1 Abstract

Field experiments were conducted in 2014 and 2015 to characterize the response of a putative 5-way resistant population of waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] to soil-applied herbicides. Soil-residual herbicides, representing various site-of-action groups, were applied at rates of .5x, 1x, and 2x the recommended field rate. Efficacy ratings and waterhemp density were taken 14, 28, 42, and 56 days after treatment (DAT). Only 4 of the 17 soil-applied herbicides provided more than 80% control by 28 DAT in both years at a 1x rate. Acetochlor and metribuzin provided the highest control ratings, ranging from 88–95% control 28 DAT, respectively.

4.2 Introduction

Waterhemp is a small seeded, dioecious, summer annual broadleaf species common in much of the Midwest (Sauer 1955). Females of this species are capable of producing prodigious amounts of seed (Hartzler et al. 2004). Waterhemp also tends to emerge over a more prolonged period during the growing season when compared to other summer annual weed species (Hartzler et al. 1999). Steckel et al. (2007) reported emergence of common waterhemp began in late April, peaked in early June, and slowed by July. These patterns of extended emergence present significant management difficulties in production systems relying on

herbicides for weed control. Utilization of soil-residual and foliar-applied herbicides is recommended to achieve adequate control (Hager et al. 1997).

Research has shown that populations of waterhemp resistant to PPO inhibitors demonstrate varying responses to soil-applied PPO-inhibiting herbicides (Wuerffel et al. 2015). Soil-applied PPO-inhibiting herbicides are applied at higher rates to achieve residual control. This higher rate can overwhelm the resistance mechanism to a limited degree. Shoup and Al-Khatib (2004) reported >85% control was achieved with pre-applied PPO-inhibiting herbicides on a population displaying resistance to foliar-applications of PPO inhibitors ~21 days after treatment.

Field and greenhouse experiments conducted on an HPPD-resistant population from McLean Co., Illinois (MCR) demonstrated reduced sensitivity to soil-applied HPPD inhibitors at recommended field rates (Hausman et al. 2013). Results from soil-applied herbicide experiments conducted on MCR indicate that acetochlor, sulfentrazone, flumioxazin, metribuzin, and pyroxasulfone provided the greatest reduction of waterhemp density. However, the researchers also noted complete control was not achieved by any of the soil-applied herbicides 30 DAT, thus necessitating the use of foliar-applied herbicides or cultivation (Hausman et al. 2013).

The population designated CHR was previously confirmed resistant to foliar-applied HPPD, PPO, ALS, PSII, and synthetic auxin herbicides via greenhouse, laboratory, and field experiments (Evans et al. 2015). The following research was initiated to elucidate the population's response to soil-residual herbicides from multiple site-of-action groups. Soil-

applied herbicides were applied at .5x, 1x, and 2x the recommended field rates and evaluated via plant density and visual evaluations of herbicide efficacy. Furthermore, this research characterizes the response of the population to pre- and post-applied combinations of herbicides.

4.3 Materials and Methods

4.3.1 Field experiments

Field experiments were conducted in 2014 and 2015 at the location in Champaign Co., Illinois from which the resistant population was initially identified. The soil was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) with a soil pH of 5.5, cation exchange capacity 19.5 meq/100g^{-1}, and 4.8% organic matter. Pre-plant tillage was performed each spring to prepare the seedbed for planting and to control any existing vegetation.

Experiments were conducted in either corn (DKC62-77RIB) or soybean (Asgrow 3231 RR2), planted in rows 76 cm apart. Planting dates in 2014 were May 7th (corn) and May 26th (soybean), and May 14th (corn) and May 22nd (soybeans) in 2015. Experiments were designed as randomized complete blocks with three replications of each treatment. Individual replications were plots measuring 3 by 7.6 meters that included four crop rows. Herbicides were applied using a pressurized CO_2 backpack sprayer equipped with Teejet[1] AI110025 nozzles for pre- and AIXR110025 for post-applications, spaced 51 cm apart on a 3-meter boom calibrated to deliver 187 L ha^{-1} at 276 kPa.

Statistical analysis for all field experiments was performed using PROC GLM in SAS 9.2[2], with herbicide treatment considered a fixed effect and year a random effect. Likely due to increased rainfall totals in 2015, waterhemp density was higher and ratings were lower on average when compared to 2014. Although there was a difference in years, the order of effectiveness in which each herbicide performed did not significantly change so data were pooled over years. Environmental measurements and conditions were recorded during each application. Rainfall accumulation is presented in Table 4.1.

4.3.2 Soil-applied herbicide options in corn and soybean

Twenty five herbicide treatments (including a non-treated control) were evaluated in corn. Treatments (Table 4.2) included .5x, 1x, and 2x rates of commercially available corn herbicides. Application occurred immediately after planting. To evaluate herbicide effectiveness, a single stake was placed at the middle of each plot as a consistent reference point for waterhemp counts. Emerged waterhemp plants per 1/3 m^2 were determined 14, 28, and 42 days after pre (DAP). Visual ratings, presented as percent control when compared with a non-treated plot, were taken at 14, 28, 42, and 56 DAP (Table 4.3).

Twenty seven herbicide treatments (including a non-treated control) were evaluated in soybean. Treatments presented in Table 4.4 included .5x, 1x, and 2x rates of commercially available herbicides commonly used in Illinois soybean production. Applications were made immediately after soybean planting. Data were collected as previously described and are presented in (Table 4.5).

4.3.3 Sequential applications of PPO, ALS, HPPD, and PSII inhibitors

Research has shown that control of resistant waterhemp populations can vary by application timing and plant growth stage (Hausman et al. 2013; Shoup and Al-Khatib 2004; Wuerffel et al. 2015). Combinations of ALS-, PPO-, HPPD-, and PSII-inhibiting herbicides were applied to plots in order to evaluate the response of CHR to sequential-applied herbicides to which the population has previously demonstrated resistance (Table 4.6). Pre-emergent herbicides were applied to bare ground with no crop planted. Stakes were placed in the middle of each plot and data collected as previously described. Post treatments were applied 29 and 27 days after pre-application, in 2014 and 2015 respectively, when waterhemp plants were 10 cm tall. Herbicide efficacy was assessed visually 21 days after pre, 0 days after post, 14 days after post, and 21 days after post. Waterhemp density per 1/3 m^2 was determined 0, and 14 days after post and are presented in Table 4.7.

4.4 Results and Discussion

4.4.1 Soil-applied herbicide options in corn

Data collected from this experiment for two growing seasons were compared using PROC GLM. PROC GLM t test (LSD) groupings, presented in Table 4.8, were made using data consisting of visual ratings and waterhemp density. As previously mentioned, there was a difference in years when comparing the pre-applied herbicide experiments. This difference may be due to a rain event occurring 2 days after application in 2015. This event resulted in 5 cm of rain in a short period of time. In the days following the rain event, the soil dried and cracks had

formed across the ground. Visual observations revealed that the higher emergence counts were due to plants emerging through the cracks in the soil, thus avoiding contact with the herbicide.

Atrazine at a 1x rate provided no more than 19% control 28 DAT resulting in plant counts averaging 130 per 1/3 m^2 (Table 4.3). This was expected due to the high level of resistance displayed in the greenhouse dose-response experiments. Field use rates of dicamba, saflufenacil, S-metolachlor, and acetochlor (encapsulated) provided less than 70% control 28 DAT. Control decreased to less than 40% for all these treatments by 42 DAT.

Control by applications of .5, 1x, and 2x rates of HPPD-inhibiting herbicides ranged from 54–82% 28 DAT and decreased to 16–51% by 42 DAT. This level of control is in contrast to previous research evaluating the efficacy of soil-applied HPPD inhibitors on a sensitive population of waterhemp. Vyn et al. (2006) reported 100% control of waterhemp 28 days after crop emergence (DAE) with HPPD-inhibiting herbicides.

Acetochlor provided the highest observable control. At 28 DAT, the 1x and 2x rates provided 88% and 95% control, respectively. The 1x rate of acetochlor resulted in waterhemp density reductions of 94% 28 DAT and 92% 42 DAT when compared to the non-treated control. Control with this herbicide decreased to 70%–85% by 42 DAT. Results of this research are consistent with previous research on an HPPD-resistant population. Hausman et al. (2013) reported 87–83% control with 1x rates of acetochlor, 53–68% control with 1x rates of HPPD-inhibiting herbicides 30 DAT.

4.4.2 Soil-applied herbicide options in soybean

Data collected from this experiment for two growing seasons were compared using PROC GLM. PROC GLM t test (LSD) groupings, presented in Table 4.9, were made using data consisting of visual ratings and waterhemp density.

Pendimethalin, chlorimuron, and S-metolachlor failed to provide control greater than 46% at a 1x rate 28 DAT, decreasing to below 20% by 42 DAT. The 1x rates of three soil-applied PPO herbicides provided control ranging from 60–70% at 28 DAT, decreasing to below 46% 42 DAT (Table 4.5). The VLCFA inhibitors pyroxasulfone and dimethenamid-P provided control ranging from 62–75% at 28 DAT, decreasing to 30–40% by 42 DAT. Metribuzin provided high levels of control at the 1x and 2x rates, ranging from 95–98% control 28 DAT and 88–97% 42 DAT. The 1x rate of metribuzin resulted in an 85% reduction in plant density when compared to a non-treated control both 28 and 42 DAT.

The control displayed by the 1x rates of PPO soil-applied herbicides flumioxazin, saflufenacil, and sulfentrazone did not surpass 70% 28 DAT. This contrasts with results reported by previous research. Harder et al. (2012) and Shoup et al. (2003) reported soil-residual PPO-inhibiting herbicides remained efficacious on PPO-resistant waterhemp with greater than 90% residual control 14 DAT depending on the active ingredient. Comparing the results of their research to the results from the CHR experiments, it is apparent a reduction in the duration of control is occurring with the PPO-inhibiting herbicides.

4.4.3 Sequential herbicide applications of PPO, ALS, HPPD, and PSII inhibitors

The groupings presented in Table 4.10 represent the data collected at 0 days and 21 days after post-application. The treatment combinations provide an opportunity to analyze the performance of the herbicides when pre-applied alone and when in combination with a post-applied herbicide.

Visual ratings taken at post application did not surpass 20% for the ALS- and PSII-inhibiting herbicides when applied to the soil. Pre-applied treatments of flumioxazin reduced plant emergence counts ranging from 26–33 plants per 1/3 meter^2 and control ratings at post application ranged from 52–61%. Mesotrione applied as a soil-residual treatment provided the highest control in comparison to the other treatments. Ratings taken at post application ranged from 83–85% control which is greater than that of the other three site-of-action herbicide groups.

Results from the counts and ratings from 21 DAT demonstrate the efficacy of both the pre- and post-applied herbicides. A post treatment of imazethapyr or atrazine did not increase control for any of the treatments. Visual ratings of treatments with post-applied ALS- and PSII-inhibiting herbicides ranged from 0–43% 21 DAT. Foliar applications of fomesafen resulted in slight decreases in waterhemp density when compared to the densities of the pre-applied herbicides alone. Control following post treatments of fomesafen ranged from 32–70%. Significant increases in control resulted from the addition of a foliar application of mesotrione, increasing control ratings to 63–83%. Mesotrione followed by mesotrione provided the highest control at 83%.

4.5 Concluding remarks

4.5.1 Research conclusions and implications

Crop production systems currently rely on herbicides for weed management. When a species evolves resistance to herbicides, options available to producers for weed management become diminished. This reduction of options has pressed growers to utilize alternative management options that result in increased cost, time allocation, and potential yield loss. In the past, resistance to herbicides has been remedied by a simple switch to herbicides from a different site-of-action group to provide control. Iteration of this method, however, can become ineffective with the accumulation of multiple resistance mechanisms within a population.

Waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] has proven to be one of the toughest weeds for Illinois producers to control (Hager et al. 1997). This species exhibits prolific seed production, prolonged emergence patterns, and a highly competitive nature (Steckel and Sprague 2004a; Steckel and Sprague 2004b). Waterhemp has also demonstrated an ability to not only evolve resistance to different herbicide groups, but also "stack" resistance to multiple groups of herbicide within an individual plant (Bell et al. 2013; Patzoldt et al. 2005). To date, waterhemp has evolved resistance to herbicides from six site-of-action groups (Heap 2016): acetolactate synthase (ALS) inhibitors, photosystem II (PSII) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) inhibitors, 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, and plant growth regulators (PGR).

In 2012, a grower reported the failure to control a waterhemp population in conventional white corn with topramezone, an HPPD inhibitor. Preliminary investigation and screening suggested that the population might be resistant to herbicides from five site-of-action families. Experiments portrayed in this thesis were conducted in the grower's field, as well as under controlled greenhouse conditions. The overall purpose of this research was to determine if the population had in fact developed resistance to herbicides from five sites-of-action, and if so, what control options are available to the grower for the effective management of the population.

To quantify the magnitude of resistance demonstrated by the population designated Champaign County Resistant (CHR), foliar dose-response experiments were conducted with mesotrione, 2,4-D, and atrazine on progeny collected from the field population. These experiments yielded results indicating that the magnitude of resistance is 16-, 30-, and 253-fold to mesotrione, 2,4-D, and atrazine when compared to a sensitive population. The CHR population demonstrated levels of resistance consistent with that of a confirmed HPPD, and atrazine resistant population. CHR's magnitude of resistance to 2,4-D appears to be substantial, but not to the same level as that of the Nebraska 2,4-D resistant waterhemp population. This may be a result of the Nebraska population's exposure to a higher level of selection pressure in the past when compared to the CHR population.

To confirm resistance to ALS and PPO-inhibiting herbicides via altered target-site in the CHR population, plants were subjected to marker analysis using methods described by Lee et al. (2008) and Patzoldt and Tranel (2007). Samples taken from the CHR population tested positive

90

for altered amino acid position 574 conferring ALS resistance and the presence of a codon deletion (ΔG210), which confers PPO resistance. The CHR population was also subjected to DNA sequence analysis to examine if atrazine resistance was mediated by an altered target-site or non-target-site based resistance. Analysis revealed no change to the gene encoding the target-site.

Field experiments further support results observed under greenhouse conditions. Foliar-applied studies revealed that PPO inhibitors, ALS inhibitors, 2,4-D and atrazine provided very little control. Plants treated with HPPD-inhibiting herbicides exhibited typical symptomatology, but re-growth of tissue would usually occur 14–21 days after treatment and a majority of plants would survive. Addition of atrazine to an HPPD treatment resulted in improved efficacy when compared to the HPPD treatment alone. Glyphosate, glufosinate, and dicamba provided the highest comparative level of control. Glufosinate's effectiveness decreased with high population density and increasing plant size. Glyphosate performed extremely well, being the only herbicide applied (in crop) at a 1x recommended field rate to provide >90% control.

Utilization of soil-residual herbicides as a part of integrated management systems are essential for the control of waterhemp (Hager et al. 1997). A variety of herbicides were evaluated under field conditions over a two-year period. Results from these experiments reveal very limited management options to effectively control this population of waterhemp. In both corn and soybean, many soil-residual herbicides provided poor control. Interestingly, PPO-inhibiting residual herbicides provided poor control. These results are contrary to previous research reported by Harder et al. (2012) in which soil-applied PPO-inhibiting herbicides

provided high levels of control of a PPO resistant population. Applications of PPO inhibitors, ALS inhibitors, atrazine, and some very long chain fatty acid (VLCFA) inhibitors resulted in poor control. HPPD soil-residual herbicides were partially effective in lowering emergence counts, but still ineffective in providing control. This matches the results observed in another HPPD-resistant waterhemp population reported by Hausman et al. (2013). High levels of control were provided by metribuzin and un-encapsulated acetochlor only. The results from this experiment may be highly influenced by several factors inherent to the research site. High waterhemp population densities, rainfall events, and high levels of organic matter may have decreased efficacy of some treatments over the two-year period.

Research is by no means completed on this population and further experimentation is necessary. One objective would be to understand the mechanism of 2,4-D resistance (possibly cytochrome P450 mediated metabolism) and if there is a potential correlation between HPPD and 2,4-D resistance. This researcher speculates the possibility that some of the same P450 enzymes that metabolize HPPD inhibitors may be able to somewhat increase metabolism of 2,4-D. A potential test could be if the addition of malathion or another P450 inhibitor increases efficacy of 2,4-D. Also, future experimentation with soil-residual herbicides in a controlled greenhouse setting would be extremely insightful. From the results of the field experiments, it is apparent that there are very few soil-residual herbicides that provide adequate control. To test if this was due to the overall robust nature of the population or if the reduction in efficacy was due to something more site specific like the high organic matter content, soil-residual greenhouse experiments would need to be conducted.

With the confirmation of this 5-way resistant population of waterhemp, growers must be aware that the resources we use for chemical weed control may not be an effective option if misused in the future. Once producers have a population that is displaying multiple resistances, will the limited options for control influence a rapid buildup of resistance to the few remaining tools the producer has available for use? From what we have learned so far the answer is seemingly "yes". I hope that this research can be utilized as a guide for current control of the CHR population and used as a tool to aid in the effort of helping producers realize the importance of being pro-active in their management system decisions to avoid losing all chemical control options in the future.

4.6 Source of Materials

[1] TeeJet 80015EVS. TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187.

[2] Statistical Analysis Software (SAS) 9.2. SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513.

4.7 Literature cited

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

Evans CM, Tranel PJ, Riechers DE, Davis AS, Maxwell DJ, Gonzini LC, Hager AG (2015)

Characterization of a novel five-way resistant population of waterhemp (*Amaranthus*

tuberculatus). North Central Weed Sci. Soc. Proc. 70:127

Hager AG, Wax LM, Bollero GA, Simmons FW (2002) Common waterhemp (*Amaranthus rudis*

Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.).

Crop Prot. 21:277–283

Hager AG, Wax LM, Simmons FW, Stoller EW (1997) Waterhemp management in agronomic

crops. Univ. of Illinois Bulletin 855:12

Harder DB, Nelson KA, Smeda RJ (2012) Management options and factors affecting control of a

common waterhemp (*Amaranthus rudis*) biotype resistant to protoporphyrinogen

oxidase-inhibiting herbicides. Int. J. Agron. 2012

Hartzler RG, Battles B, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*)

emergence date on growth and fecundity in soybean. Weed Sci. 52:242–245

Hartzler RG, Buhler DD, Stoltenberg DE (1999) Emergence characteristics of four annual weed

species. Weed Sci. 47:578–584

Hausman NE, Tranel PJ, Riechers DE, Maxwell DJ, Gonzini LC, Hager AG (2013) Responses of an

HPPD inhibitor-resistant waterhemp (*Amaranthus tuberculatus*) population to soil-

residual herbicides. Weed Technol. 27:704–711

Heap, I. The international survey of herbicide resistant weeds. Online. Internet. Thursday, April 14, 2015. Available www.weedscience.com

Lee RM, Hager AG, Tranel PJ (2008) Prevalence of a novel resistance mechanism to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Sci. 56:371–375

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 (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci. 53:30–36

Sauer J (1955) Revision of the dioecious *amaranths*. Madrono 13:5–46

Shoup DE, Al-Khatib K (2004) Control of protoporphyrinogen oxidase inhibitor-resistant common waterhemp (*Amaranthus rudis*) in corn and soybean. Weed Technol. 18:332–340

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci. 51:145–150

Steckel LE, Sprague CL, Stoller EW, Wax LM, Simmons FW (2007) Tillage, cropping system, and soil depth effects on common waterhemp (*Amaranthus rudis*) seed-bank persistence. Weed Sci. 55:235–239

Steckel LE, Sprague CL (2004a) Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci. 52:359–364

Steckel LE, Sprague CL (2004b) Late-season common waterhemp (*Amaranthus rudis*) interference in narrow- and wide-row soybean. Weed Technol. 18:947–952

Vyn JD, Swanton CJ, Weaver SE, Sikkema PH (2006) Control of *Amaranthus tuberculatus* var.

rudis (common waterhemp) with pre and post-emergence herbicides in *Zea mays* L.

(maize). Crop Prot. 25:1051–1056

Wuerffel RJ, Young JM, Matthews JL, Young BG (2015) Characterization of PPO-inhibitor-

resistant waterhemp (*Amaranthus tuberculatus*) response to soil-applied PPO-inhibiting

herbicides. Weed Sci. 63(2):511–521

4.8 Tables

Table 4.1 Precipitation for 2014 and 2015.

Month	Precipitation	
	2014	**2015**
	——————————cm——————————	
May	6.4	13.8
June	17	22.8
July	12	7.2
Total	35.4	43.8

Table 4.2 Soil-applied treatments in corn at Champaign Co. Il (2014–2015).

Herbicide	Site of Action	Rate
		g ai ha^{-1}
Acetochlor (Encap)[a]	VLCFA	627
		1255
		2510
Acetochlor	VLCFA	1098
		2196
		4392
Atrazine	PSII	1120
		2240
		4480
Dicamba	PGR	280
		560
		1120
Isoxaflutole	HPPD	53
		105
		211
Mesotrione	HPPD	105
		211
		421
Saflufenacil	PPO	37
		75
		150
S-metolachlor	VLCFA	795
		1591
		3182

[a] Encapsulated formulation

Table 4.3 Soil-applied options for maize field data. Visual estimates of control presented as a percentage of the untreated plot. Counts and ratings are presented as averages across 2014 and 2015.

Herbicide	Rate	Counts[a]				Visual Ratings	
	g ai ha^{-1}	28 DAT	28 DAT % reduction from UT	42 DAT	42 DAT % reduction from UT	28 DAT	42 DAT
Acetochlor(Encap)[a]	627	130	17%	135	14%	3	0
	1255	128	18%	137	13%	22	5
	2510	73	53%	75	52%	43	16
Acetochlor	1098	29	81%	29	82%	79	54
	2196	10	94%	12	92%	88	70
	4392	4	97%	7	96%	95	85
Atrazine	1120	170	0%	175	0%	13	0
	2240	130	17%	108	31%	19	0
	4480	105	33%	113	28%	26	0
Dicamba	280	147	6%	153	3%	10	0
	560	91	42%	103	34%	32	3
	1120	78	50%	76	52%	53	22
Isoxaflutole	53	53	66%	54	66%	54	16
	105	27	83%	31	80%	80	40
	211	24	85%	30	81%	88	61
Mesotrione	105	30	81%	30	81%	71	25
	211	26	83%	37	76%	77	38
	421	12	92%	41	74%	82	51
Saflufenacil	37	79	49%	102	35%	18	0
	75	87	44%	81	48%	45	15
	150	14	91%	33	79%	84	47
S-metolachlor	795	95	39%	140	11%	23	5
	1591	55	65%	99	37%	39	5
	3182	63	60%	73	54%	60	25
Untreated		156	0%	157	0%	0	0

[a]Plants per 1/3 meter[2]

Table 4.4 Soil-applied treatments in soybean at Champaign Co. Il (2014–2015).

Herbicide	Site of Action	Rate
		g ai ha^{-1}
Chlorimuron	ALS	13
		26
		52
Dimethenamid-P	LCFA	420
		840
		1681
Flumioxazin	PPO	36
		72
		143
Metribuzin	PSII	280
		560
		1120
Pendimethalin	Mitotic Disrupter	532
		1064
		2129
Pyroxasulfone	LCFA	59
		119
		238
Saflufenacil	PPO	37
		75
		150
S-metolachlor	LCFA	795
		1591
		3182
Sulfentrazone	PPO	175
		350
		699

Table 4.5 Soil-applied options for soybean field data. Visual estimates of control presented as a percentage of the untreated plot. Counts and ratings are presented as averages across 2014 and 2015.

Herbicide	Rate	Counts[a]				Visual Ratings	
	g ai ha^{-1}	28 DAT	28 DAT % reduction from UT	42 DAT	42 DAT % reduction from UT	28 DAT	42 DAT
Chlorimuron	**13**	76	21%	77	15%	16	0
	26	125	0%	120	0%	13	0
	52	123	0%	118	0%	15	0
Dimethenamid-P	**420**	74	23%	71	22%	47	19
	840	48	50%	49	46%	62	29
	1681	44	54%	43	53%	76	58
Flumioxazin	**36**	87	9%	87	4%	35	18
	72	58	40%	60	34%	61	24
	143	50	48%	51	44%	72	44
Metribuzin	**280**	41	57%	40	56%	79	67
	560	14	85%	14	85%	95	88
	1120	2	98%	2	98%	98	97
Pendimethalin	**532**	86	10%	85	7%	12	2
	1064	130	0%	113	0%	15	11
	2129	70	27%	68	25%	54	27
Pyroxasulfone	**59**	79	18%	79	13%	48	17
	119	41	57%	40	56%	75	43
	238	21	78%	20	78%	84	75
Saflufenacil	**37**	71	26%	71	22%	46	22
	75	55	43%	63	31%	67	46
	150	54	44%	55	40%	69	48
S-metolachlor	**795**	91	5%	89	2%	20	5
	1591	80	17%	81	11%	46	16
	3182	24	75%	24	74%	62	39
Sulfentrazone	**175**	71	26%	71	22%	47	13
	350	64	33%	63	31%	69	43
	699	8	92%	8	91%	86	66
Untreated		96	0%	91	0%	0	0

[a]Plants per 1/3 meter[2]

Table 4.6 Sequential program treatments in bareground at Champaign Co. Il (2014–2015).

Herbicide		Site of Action		Rate	
				g ai ha^{-1}	
PRE	POST	PRE	POST	PRE	POST
Atrazine	Fomesafen	PSII	PPO	2241	347
Atrazine	Mesotrione	PSII	HPPD	2241	105
Atrazine	Imazethapyr	PSII	ALS	2241	71
Atrazine	Atrazine	PSII	PSII	2241	1681
Chlorimuron	Fomesafen	ALS	PPO	53	347
Chlorimuron	Mesotrione	ALS	HPPD	53	105
Chlorimuron	Atrazine	ALS	PSII	53	1681
Chlorimuron	Imazethapyr	ALS	ALS	53	71
Flumioxazin	Mesotrione	PPO	HPPD	72	105
Flumioxazin	Imazethapyr	PPO	ALS	72	71
Flumioxazin	Fomesafen	PPO	PPO	72	347
Flumioxazin	Atrazine	PPO	PSII	72	1681
Mesotrione	Imazethapyr	HPPD	ALS	211	71
Mesotrione	Fomesafen	HPPD	PPO	211	347
Mesotrione	Mesotrione	HPPD	HPPD	211	105
Mesotrione	Atrazine	HPPD	PSII	211	1681

Table 4.7 Sequential program field data. Visual estimates of control presented as a percentage of the untreated plot. Counts and ratings are presented as averages across 2014 and 2015.

Herbicide		% Control		Plant Density[a]	
PRE	POST	0 DAP	21 DAP	0 DAP	21 DAP
Atrazine	Atrazine	8	0	95	92
Atrazine	Imazethapyr	11	0	83	86
Atrazine	Fomesafen	11	33	60	42
Atrazine	Mesotrione	23	71	70	30
Chlorimuron	Atrazine	0	0	104	91
Chlorimuron	Imazethapyr	0	0	120	110
Chlorimuron	Fomesafen	2	32	78	46
Chlorimuron	Mesotrione	0	63	97	23
Flumioxazin	Imazethapyr	53	8	32	39
Flumioxazin	Atrazine	61	19	26	30
Flumioxazin	Fomesafen	52	42	33	24
Flumioxazin	Mesotrione	56	78	26	12
Mesotrione	Atrazine	83	36	18	17
Mesotrione	Imazethapyr	85	43	13	15
Mesotrione	Fomesafen	85	70	14	9
Mesotrione	Mesotrione	84	83	14	4
Untreated	Untreated	0	0	124	106

[a]Plants per $1/3$ meter^2

Table 4.8 Soil-applied herbicide options for corn LSD groupings. Ratings with the same letter within a column are not significantly different at α = .05 (separated by PROC GLM in SAS)

Herbicide	Rate	Proc GLM Count groupings		Proc GLM Rating groupings	
	g ai ha^{-1}	28 DAT	42 DAT	28 DAT	42 DAT
Acetochlor (Encap)	627	ABCD	ABCDE	MN	H
	1255	ABCD	ABDC	JKLM	GH
	2510	DEFGHI	EFGHI	GHI	FGH
Acetochlor	1098	GHIJ	IJK	ABC	BCD
	2196	IJ	JK	AB	AB
	4392	J	K	A	A
Atrazine	1120	A	A	KLMN	H
	2240	ABCD	BCDEFG	KLM	H
	4480	BCDE	ABCDEF	IJKL	H
Dicamba	280	ABC	AB	LMN	H
	560	CDEFG	BCDEFG	IJK	GH
	1120	DEFGH	DEFGHI	FGH	EFG
Isoxaflutole	53	EFGHIJ	FGHIJK	EFGH	FGH
	105	GHIJ	IJK	ABC	DE
	211	HIJ	IJK	AB	BC
Mesotrione	105	GHIJ	IJK	BCDE	EF
	211	HIJ	IJK	ABCD	DE
	421	IJ	HIJK	ABC	BCD
Saflufenacil	37	DEFGH	BCDEFGH	KLMN	H
	75	CDEFGH	CDEFGHI	GHI	FGH
	150	IJ	IJK	ABC	CD
S-metolachlor	795	BCDEF	ABC	JKL	GH
	1591	EFGHIJ	BCDEFGH	HIJ	GH
	3182	EFGHIJ	FGHIJ	DEFG	EF
Untreated		AB	AB	N	H
		LSD=64	LSD=62	LSD=18	LSD=20

Table 4.9 Soil-applied herbicide options for soybean LSD groupings. Ratings with the same letter within a column are not significantly different at α = .05 (separated by PROC GLM in SAS)

Herbicide	Rate	Proc GLM Count groupings		Proc GLM Rating groupings	
	g ai ha^{-1}	28 DAT	42 DAT	28 DAT	42 DAT
Chlorimuron	13	ABCDEF	ABCDE	JK	N
	26	A	A	JK	N
	52	AB	A	JK	N
Dimethenamid-P	420	ABCDEFG	ABCDEF	GH	JKLMN
	840	CDEFGH	BCDEFG	DEFG	GHIJK
	1681	CDEFGH	CDEFG	BCD	CDEF
Flumioxazin	36	ABCDE	ABCD	HI	JKLMN
	72	BCDEFGH	ABCDEFG	DEFG	HIJKLM
	143	CDEFGH	BCDEFG	CDE	EFGH
Metribuzin	280	CDEFGH	CDEFG	BCD	BCD
	560	FGH	FG	AB	AB
	1120	H	G	A	A
Pendimethalin	532	ABCDE	ABCD	JK	MN
	1064	A	AB	IJK	KLMN
	2129	ABCDEFG	ABCDEFG	EFGH	GHIJKLM
Pyroxasulfone	59	ABCDEF	ABCDE	FGH	KLMN
	119	CDEFGH	CDEFG	CD	FGHI
	238	EFGH	DEFG	ABC	ABC
Saflufenacil	37	ABCDEFG	ABCDEF	GH	IJKLMN
	75	CDEFGH	ABCDEFG	CDEF	DEFG
	150	CDEFGH	ABCDEFG	CDE	DEFG
S-metolachlor	795	ABCD	ABC	IJ	LMN
	1591	ABCDEF	ABCD	GH	KLMN
	3182	DEFGH	CDEFG	DEFG	FGHIJ
Sulfentrazone	175	ABCDEFG	ABCDEF	GH	KLMN
	350	ABCDEFGH	ABCDEFG	CDE	FGHI
	699	GH	FG	ABC	BCDE
Untreated		ABC	ABC	K	N
		LSD=66	LSD=67	LSD=19.5	LSD=22

Table 4.10 Sequential program LSD groupings. Ratings with the same letter within a column are not significantly different at α = .05 (separated by PROC GLM in SAS)

Herbicide		% Control Groupings		Plant Density Groupings	
PRE	POST	0 DAP	21 DAP	0 DAP	21 DAP
Atrazine	Atrazine	CD	F	ABCD	A
Atrazine	Imazethapyr	CD	F	ABCD	A
Atrazine	Fomesafen	CD	CD	DEF	BC
Atrazine	Mesotrione	C	AB	CDE	BCDE
Chlorimuron	Atrazine	D	F	ABC	A
Chlorimuron	Imazethapyr	D	F	AB	A
Chlorimuron	Fomesafen	D	CD	BCD	B
Chlorimuron	Mesotrione	D	B	ABCD	BCDE
Flumioxazin	Imazethapyr	B	EF	EFG	BCD
Flumioxazin	Atrazine	B	DE	FG	BCDE
Flumioxazin	Fomesafen	B	C	EFG	BCDE
Flumioxazin	Mesotrione	B	A	FG	CDE
Mesotrione	Atrazine	A	C	FG	BCDE
Mesotrione	Imazethapyr	A	C	G	BCDE
Mesotrione	Fomesafen	A	AB	G	DE
Mesotrione	Mesotrione	A	A	G	E
Untreated	Untreated	D	F	A	A
		LSD=16.7	LSD=14.8	LSD=42.6	LSD=33.1