CHARACTERIZATION OF HPPD-INHIBITOR RESISTANCE IN WATERHEMP
(AMARANTHUS TUBERCULATUS)

BY

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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, 2012

Urbana, Illinois

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ABSTRACT

Waterhemp, a summer annual species native to much of the Midwest, has proven to be one of the toughest weeds for Illinois producers to control. Several characteristics of waterhemp make this species ideally suited to thrive in Illinois agricultural fields, including but not limited to high seed production, extended emergence, and seed dormancy. The evolution of herbicide resistance in waterhemp has introduced an additional obstacle producers must face when trying to manage this troublesome weed. In the summer of 2009, a waterhemp population in a seed corn production field in McLean Co., IL was not adequately controlled following foliar applications of 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides. Chapter 1 of this thesis includes a review of the literature pertaining to HPPD inhibitors, namely their discovery and uses as herbicides, as well as a section on waterhemp biology. Chapter 2 discusses the initial greenhouse research used to determine if the waterhemp population from McLean Co. (designated MCR) had indeed evolved a novel form of resistance to HPPD inhibitors. Plants grown from seed collected at the suspected resistance site demonstrated reduced sensitivity to foliar applications of the HPPD inhibitors mesotrione, tembotrione, and topramezone. Furthermore, a mesotrione dose response comparing MCR with two known HPPD inhibitor-sensitive waterhemp populations revealed the level of resistance to be 10-to 35-fold. The efficacy of foliar-applied HPPD inhibitors at the McLean Co. location is addressed in Chapter 3. Similar to findings of the previous greenhouse experiments, HPPD inhibitors did not provide adequate control of this population, and mortality data indicate a high percentage of plant survival when these herbicides were applied at their recommended field use rates. Additional field and greenhouse research presented in Chapter 3 indicates that MCR also demonstrates resistance to acetolactate synthase (ALS) inhibitors and photosystem II (PSII)
inhibitors, while glyphosate, glufosinate, and protoporphyrinogen oxidase (PPO) inhibitors generally provide the greatest control of this population. Chapter 4 discusses the efficacy of various soil-applied residual herbicides to determine appropriate control options for the McLean Co. waterhemp population. Under field conditions, acetochlor, sulfentrazone, flumioxazin, metribuzin, and pyroxasulfone generally provided the highest levels of waterhemp control and greatest reduction in waterhemp density. Chapter 4 also includes a soil-applied mesotrione dose response comparing HPPD inhibitor-resistant waterhemp (MCR) and RxR progeny (derived from greenhouse crosses of HPPD-resistant waterhemp) with an HPPD inhibitor-sensitive biotype. Subsequent resistant-to-sensitive (R/S) ratios revealed a 9-to 13-fold level of resistance. Finally, Chapter 5 includes a summary of experiments, as well as implications of the research presented herein.
ACKNOWLEDGMENTS

First and foremost, I would like to thank my adviser Dr. Aaron Hager for adding a fellow Saluki to the Weed Science team here at the University of Illinois. I am also extremely grateful to my other committee members, Drs. Patrick Tranel and Dean Riechers, for the knowledge and advice they have shared with me during my Masters program.

My graduate research, both field and greenhouse, could never have been accomplished without the help of so many individuals. First are my neighbors around the corner, Doug Maxwell and Lisa Gonzini, who have always been there to lend a helping hand during any endeavor. I would also like to thank Josh Kunkel, Brad Stierwalt, and numerous student workers for the countless hours they have spent helping this graduate student conduct his experiments. Though too many to list, I am grateful for the many students, researchers, and professors I have come to know over the last two and a half years. A special thanks is reserved for my second academic family in the Animal Sciences Department. I would like to extend a great deal of gratitude to Syngenta Crop Protection for providing financial support of this research.

Finally, I would like to thank my parents for the encouragement they have given me over the years. Only time will tell where this Saluki/Illini will wind up, but I know support and free food will always be found in Pesotum, Illinois.
TABLE OF CONTENTS

CHAPTER 1 .............................................................................................................................................1
INTRODUCTION........................................................................................................................................1
1.1 History of HPPD Inhibitors .............................................................................................................1
1.2 Target Site and Mode of Action of HPPD Inhibitors .....................................................................3
1.3 Herbicidal Properties of HPPD Inhibitors .....................................................................................6
1.4 Waterhemp Biology .......................................................................................................................10
1.5 Research Objectives .....................................................................................................................13
1.6 Attributions ..................................................................................................................................14
1.7 Literature Cited ............................................................................................................................15
1.8 Figures ..........................................................................................................................................25

CHAPTER 2 ...........................................................................................................................................28
RESISTANCE TO HPPD-INHIBITING HERBICIDES IN A POPULATION OF WATERHEMP (AMARANTHUS TUBERCULATUS) FROM ILLINOIS, UNITED STATES ..................................................................................................................28
2.1 Abstract .......................................................................................................................................28
2.2 Introduction .................................................................................................................................28
2.3 Materials and Methods ...............................................................................................................30
2.4 Results and Discussion ...............................................................................................................33
2.5 Source of Materials ....................................................................................................................36
2.6 Literature Cited ............................................................................................................................37
2.7 Tables .........................................................................................................................................40
2.8 Figures .........................................................................................................................................42

CHAPTER 3 .........................................................................................................................................43
FOLIAR HERBICIDE OPTIONS TO MANAGE A WATERHEMP (AMARANTHUS TUBERCULATUS) POPULATION RESISTANT TO HPPD-INHIBITING HERBICIDES .........................................................................................43
3.1 Abstract .......................................................................................................................................43
3.2 Introduction .................................................................................................................................43
3.3 Materials and Methods ...............................................................................................................46
3.4 Results and Discussion ...............................................................................................................52
3.5 Source of Materials ....................................................................................................................59
3.6 Literature Cited ............................................................................................................................59
3.7 Tables .........................................................................................................................................63
3.8 Figures .........................................................................................................................................73

CHAPTER 4 .........................................................................................................................................74
CHARACTERIZING THE RESPONSE OF AN HPPD-RESISTANT WATERHEMP (AMARANTHUS TUBERCULATUS) POPULATION TO SOIL-RESIDUAL HERBICIDES .........................................................................................74
4.1 Abstract .......................................................................................................................................74
4.2 Introduction ................................................................................................................................75
4.3 Materials and Methods ....................................................................................................................77
4.4 Results and Discussion .......................................................................................................................81
4.5 Source of Materials ............................................................................................................................87
4.6 Literature Cited ..................................................................................................................................87
4.7 Tables ................................................................................................................................................91
4.8 Figures ...............................................................................................................................................97

CHAPTER 5 ............................................................................................................................................98
CONCLUDING REMARKS ......................................................................................................................98
5.1 Research Conclusions and Implications ...........................................................................................98
5.2 Literature Cited ................................................................................................................................102
CHAPTER 1

INTRODUCTION

1.1 History of HPPD Inhibitors

Inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) include herbicides represented by three chemical classes: the triketones, isoxazoles, and pyrazolones (Hirai et al. 2002; van Almsick 2009). The discovery of the herbicidal triketones, which include compounds such as mesotrione, sulcotrione, and tembotrione, is attributed to two key events. The first occurred when scientists at the Western Research Center of Zeneca Ag Products observed allelopathic properties of the bottlebrush plant *Calistemon* spp. (Lee et al. 1997). The chemical responsible for these toxic properties was determined to be leptospermone (Hellyer 1968) (Figure 1.1), which produced bleaching symptoms on certain grass and broadleaf weeds at a rate of 1000 g ha$^{-1}$ (Gray et al. 1980). The second event occurred during production of novel acetyl-CoA carboxylase (ACCase) inhibitors similar to sethoxydim by Zeneca Ag Products scientists in 1982 (Lee et al. 1998; Mitchell et al. 2001). Instead of the expected analog, a benzoylcyclohexanedione was created which was herbicidally inactive. However, this unexpected compound possessed antidote characteristics for soybean injury caused by thiocarbamates, and a program to develop additional analogs was implemented. One such analog, with the addition of a chlorine group, produced the same bleaching symptoms in plants treated with leptospermone, thus leading to the discovery of the herbicidal triketones (Lee et al. 1998; Michaely and Kraatz 1983; Mitchell et al. 2001). Shortly after, several other leading chemical and agricultural companies would conduct their own research with herbicidal triketones (Hawkes et al. 2007).
Initial herbicide research in the isoxazole chemical class, which includes isoxaflutole (Figure 1.2), was spearheaded by Rhone-Poulenc scientists during the 1980s and early 90s (Pallett 2000; Pallett et al. 2001). Compounds synthesized during research on hydroxymethylglutaryl coenzyme A (HMGCoA) reductase inhibitors provided the initial step towards the herbicidally active isoxazoles. These early compounds were further refined, and one product (M&B 46 206, Figure 2) produced bleaching symptoms on broadleaves and grasses following application to soil or foliage. Due to patent conflicts, however, research on this initial bleaching compound ceased. Undeterred and inspired by the weed control spectrum and symptomology, Rhone-Poulenc scientists continued to develop novel bleaching products, and eventually the first benzoyl isoxazole (RPA 200809, Figure 1.2) was produced in 1989. This compound was further refined by substituting the methyl group with a cyclopropyl group and replacing the nitrogen dioxide group with a sulfur dioxide group. This final product was the herbicide isoxaflutole, which provided control of broadleaf and grass weeds at 62 g ha\(^{-1}\) when applied to soil (Cain et al. 1993; Pallett 2000; Pallett et al. 2001).

The origin of herbicide research in the pyrazolone chemical class, which includes compounds such as pyrasulfutole and topramezone, can be traced back to rice (*Oryza sativa*) production in Japan. Pyrazolate, the first in this class, was developed during the 1970s and 80s by Sankyo researchers (Kawakubo et al. 1979; Konotsune and Kawakubo 1977; Yamaoka et al. 1988), and was soon followed by the release of pyrazoxyfen from Ishihara Sangyo Kaisha (Kimura 1984; Nishiyama et al. 1979) and Mitsubishi Petrochemical’s benzofenap (Ikeda and Goh 1991) (Figure 1.3). With the successful commercialization of these early bleaching benzoyl pyrazoles (van Almsick 2009), several other companies conducted research with pyrazolone class compounds including, but not limited to, Nissan Chemical Industries (Baba et al. 1988),
BASF (Von Deyn et al. 1996) and Dow AgroSciences (Benko et al. 1998). Aventis CropScience researchers would also contribute to the growing list of patented pyrazolones with their compound pyrasulfutole (Shmitt et al. 2001).

1.2 Target Site and Mode of Action of HPPD Inhibitors

A prominent symptom of the herbicidal triketones, isoxazoles, and pyrazolones is bleaching of plant tissue. This response naturally led researchers to speculate if the target site of these compounds was similar to other bleaching herbicides, such as the phytoene desaturase (PDS) inhibitor norflurazon (Mayer et al. 1989) which disrupts the carotenoid biosynthesis pathway. Carotenoids protect plants from photooxidation by quenching triplet chlorophyll and preventing destructive singlet oxygen formation (Siefermann Harms 1987). The loss of these protective carotenoids will result in membrane and pigment destruction, leading to characteristic bleached plant tissue. Mayonada et al. (1989) compared levels of carotenoids and phytoene, a precursor to carotenoids, in vivo in norflurazon and sulcotrione (Figure 1.1) treated soybean (Glycine max (L.) Merr.). The results showed an increase in phytotene concentration and a decrease in carotenoids after treatments of norflurazon and the novel triketone sulcotrione. Sulcotrione primarily bleached new growth whereas norflurazon bleached older growth, leading to speculation that the target site may be within the carotenoid biosynthesis pathway but not directly phytoene desaturase. This hypothesis was further supported when Sandmann et al. (1990) reported that PDS in corn (Zea mays L.) chloroplasts, Aegean wallflower (Cheiranthus cheiri) chromoplasts, and cyanobacterium (Anacystis) thylakoids was not inhibited in vitro by herbicidal triketones.
A breakthrough for determining the target site occurred during toxicology screens of these new bleaching herbicides. Lab rats treated with the triketone NTBC (Figure 1.1) produced elevated levels of tyrosine in their blood, leading researchers to focus on tyrosine pathways in mammals (Ellis et al. 1995). 4-hydroxyphenylpyruvate (HPPA) and 4-hydroxyphenyllactate (HPLA) also accumulated in the urine of these triketone-treated rats, suggesting that the second enzyme in the tyrosine degradation pathway, 4-hydroxyphenylpyruvate dioxygenase (HPPD), was the target site for mammalian systems. This proposal was confirmed with inhibition of incubated rat liver HPPD *in vitro* (Ellis et al. 1995). With mammalian toxicology screens providing guidance, the next step was to confirm HPPD as the target site in plant systems. *In vivo* work by Prisbylla et al. (1993) showed increased levels of tyrosine in sorghum (*Sorghum bicolor*), ivyleaf morningglory (*Ipomoea hederacea*), and green foxtail (*Setaria viridis*) after treatment with NTBC. The authors also performed a reversal experiment comparing sulcotrione and the PDS inhibitor fluridone with and without homogentisic acid (HGA) on duckweed (*Lemna gibba*). The product HGA is created directly from the substrate HPPA by the HPPD enzyme (Schultz et al. 1985). If HPPD is inhibited by sulcotrione, then the addition of HGA would alleviate adverse effects and promote normal plant functions. The herbicides applied alone produced typical bleaching symptoms; however, with the addition of HGA only fluridone caused injury symptoms, further suggesting HPPD as the target site in plant systems for triketones. *In vitro* HPPD analysis in corn (Prisbylla et al. 1993; Schulz et al. 1993) and barnyardgrass (*Echinochloa crus-galli*) (Secor 1994) confirmed that HPPD was the target site for these novel bleaching triketones. Eventually the HPPD enzyme would also be confirmed as the target site for the pyrazolone and isoxazole class herbicides (Matsumoto 2005; Viviani et al. 1998).
Following the identification of HPPD as the target site for these bleaching compounds, the mode of action (how the herbicide controls the plant) could be better understood. During tyrosine degradation in plant systems, plastoquinones and tocopherols are produced from HGA (Schultz et al. 1985), which is synthesized by HPPD and the HPPA substrate. Plastoquinones, among other roles, serve as electron acceptors or “cofactors” during the conversion of phytoene to phytofluene by PDS in carotenoid synthesis (Mayer et al. 1990, 1992; Norris et al. 1995; Pallett et al. 1998). When an inhibitor binds to the HPPD enzyme, HGA is depleted and subsequent products (plastoquinones, tocopherols, and carotenoids) decrease in concentration as exemplified in metabolite profiling by Pallett et al. (1998). The loss of plastoquinones and their cofactor ability derails the production of the carotenoid precursor phytofluene. Without these protective carotenoids, the plant is susceptible to photoxidation and its effects described previously. A decrease in tocopherols also plays a part in the cascade of effects leading to plant death, though not to the level of carotenoid loss. It has been established that tocopherols posses the ability to quench destructive singlet oxygen (Kaiser et al. 1990); however, research of tocopherol-deficient mutants in Arabidopsis seems to downplay the importance of tocopherols in photooxidation prevention (Havaux et al. 2005). Whole plants deficient in this singlet oxygen quencher only demonstrated classic photooxidation symptoms (bleaching, membrane destruction) under extreme conditions, i.e., 8°C and light intensity at 1100 µmol m⁻² s⁻¹. The authors propose other plant compounds, such as carotenoids, account for this deficiency under normal conditions and aid in protection. This, along with earlier research, suggest the primary mode of action for HPPD inhibitors is a loss of carotenoids followed by photooxidation. Depletion of tocopherols most likely plays a secondary or complimentary role in leading to plant death.
1.3 Herbicidal Properties of HPPD Inhibitors

Selectivity in crops (such as corn, rice, and wheat) has made 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors another potent tool used by growers to control grass and broadleaf weeds. A large portion of commercial HPPD inhibitor use occurs in corn production systems. In 2010, approximately 42% of total corn hectares in Illinois was treated with either a soil- or foliar-applied HPPD inhibitor herbicide (USDA/NASS 2011). Representative compounds from each of the three chemical classes are now commercially used in corn, including mesotrione, sulcotrione and tembotrione (triketone), isoxaflutole (isoxazole) and topramezone (pyrazolone).

The triketones mesotrione and sulcotrione were developed by Zeneca Ag scientists (now Syngenta). Rates of mesotrione typically range from 100–225 g ha\(^{-1}\) for applications to soil and 70–150 g ha\(^{-1}\) when applied to plant foliage. Mesotrione’s weed control spectrum includes various annual broadleaf species such as velvetleaf (Abutilon theophrasti), common cocklebur (Xanthium strumarium), giant ragweed (Ambrosia trifida), common lambsquarters (Chenopodium album), Amaranthus spp., and suppression or control of the annual grass species large crabgrass (Digitaria sanguinalis) and barnyardgrass (Wichert et al. 1999). Sutton et al. (2002) demonstrated the importance of this herbicide, as well as other HPPD inhibitors, when their research revealed no cross-resistance to mesotrione in several acetolactate synthase (ALS) and photosystem II (PSII) inhibitor resistant weed biotypes. Sulcotrione, registered in Europe at rates of 300–450 g ha\(^{-1}\) foliar applied for a weed spectrum similar to mesotrione, is much weaker on Setaria spp. (Beraud et al. 1991). Tembotrione is another triketone produced by Bayer CropScience for use in corn production with typical foliar applied rates of 75–100 g ha\(^{-1}\) (Santel 2009). This product contains the safener isoxadifen-ethyl and controls several broadleaf species.
including velvetleaf, *Amaranthus* spp., common lambsquarters, and eastern black nightshade (*Solanum ptycanthum*) with a wider spectrum of grass weeds (including foxtails) as compared with other triketones (Santel 2009; Schulte and Köcher 2009).

Isoxaflutole, a member of the isoxazole chemical class and currently produced by Bayer CropScience, controls a wide spectrum of broadleaf and grass weeds following soil or foliar applications (Luscombe and Pallett 1996; Luscombe et al. 1995). Foliar applications are possible by addition of the safener cyprosulfamide in newer formulations of isoxaflutole, but must be made before corn reaches V2 (Watteyne et al., 2009). Interestingly, Pallet et al. (1998) and Viviani et al. (1998) have reported isoxaflutole is not herbicidally active, but rather the metabolic derivative diketonitrile (DKN) is the actual compound binding to the HPPD enzyme. Whether in soil (Luscombe and Pallett 1996) or within the plant (Pallett et al. 1998), the isoxazole ring of isoxaflutole is opened by metabolism creating DKN. “Pro-drug” or “pro-herbicide” are terms commonly used to describe inactive herbicides that are converted into active forms within plants. Pallet et al. (2001) has also reported absorption of isoxaflutole is much higher in shoots and roots of target weeds than the active DKN, indicating isoxaflutole as the more appropriate delivery system. Topramezone is a member of the pyrazolone chemical class developed by BASF and licensed to AMVAC Chemical Corporation for North American markets (Porter et al. 2005). Similar to tembotrione, this compound controls a wide spectrum of broadleaf and grass weeds with foliar applications of 12–18 g ha⁻¹ (Anonymous, 2006).

Selectivity in corn has been attributed to reduced uptake, rapid metabolism, and an inherently less sensitive target site, though the relative importance of each can differ from one HPPD inhibitor to the next.¹⁴C work conducted by Mitchell et al. (2001) showed foliar absorption of mesotrione was between 55% and 90% in three susceptible weed species, while
corn uptake was only 45% 24 hours after treatment (HAT). Conversely, corn uptake of
topramezone and tembotrione 24 HAT was not different than susceptible weeds, with absorption
of 80% and 86%, respectively (Grossmann and Ehrhardt 2007; Schulte and Köcher 2009). The
pro-herbicide nature of isoxaflutole and its derivative DKN also contributes to reduced uptake
by the crop. The lipophilic isoxaflutole stays close to the soil surface (0–3 cm) where weed
seeds are germinating, while converted DKN (which is more water soluble) travels farther down
towards corn roots, yet is not as easily absorbed (Pallet et al. 2001).

Rapid metabolism of HPPD inhibitors plays a large part in crop selectivity for these
commercial bleaching herbicides. \(^{14}\text{C}\) mesotrione research by Wichert et al. (1999) found 42%,
34%, and 10% of total radioactive compounds outside the treated leaf of common lambsquarters,
barnyardgrass, and redroot pigweed (*Amaranthus retroflexus*) was still parent mesotrione (active
form) 7 days after treatment (DAT). Conversely, none of the radioactive compounds found
outside the treated corn leaf 7 DAT were active mesotrione, suggesting rapid metabolism in the
crop. Pallet et al. (1998) reported 82% of the radioactivity in \(^{14}\text{C}\) isoxaflutole-treated velvetleaf
was still active DKN 6 DAT, while only 29% DKN remained in corn. The rest of the radioactive
compounds were found to be inactive benzoic acid and other polar metabolites. Similar results
have been reported with topramezone and tembotrione treated corn and weed species
(Grossmann and Ehrhardt 2007; Schulte and Köcher 2009).

Research with \(^{14}\text{C}\) mesotrione found that hydroxylated mesotrione was a major early
metabolite, leading scientists to speculate if cytochrome P450s were involved in crop
metabolism. P450s are membrane bound enzymes that can oxidize substrates in the presence of
oxygen, and these hydroxylated compounds can be further metabolized by the plant (Davies and
Caseley 1999). Hawkes et al. (2001) tested this hypothesis by applying \(^{14}\text{C}\) mesotrione to corn
pre-treated with malathion, a known P450 inhibitor. Plants treated with mesotrione or malathion alone showed no injury symptoms, whereas corn treated with malathion followed by mesotrione showed injury (bleaching). Analysis of metabolites showed a significant decrease in hydroxylated mesotrione, implicating P450s as an important contributor of crop selectivity. Research by Williams and Pataky (2010) has further supported this, with data indicating increased injury by mesotrione, tembotrione, and topramezone in sweet corn hybrids with P450 mutations. Some commercial formulations of HPPD inhibitors include safeners, such as cyprosulfamid (isoxaflutole) and isoxadifen (tembotrione). These compounds reduce injury potential by inducing herbicide metabolism in the crop without reducing toxic effects in target weeds (Davies and Caseley 1999).

An inherently less sensitive HPPD target site in grasses may also play a part in crop selectivity for certain HPPD inhibitors. In vitro analysis demonstrated mesotrione forming a more stable complex with Arabidopsis HPPD than wheat HPPD (Hawkes et al. 2001). The authors also reported that tobacco plants genetically altered to express wheat HPPD were several fold more resistant to mesotrione compared with unaltered plants. This selectivity characteristic may be unique to certain HPPD inhibitors though, as other HPPD inhibitors have a much wider spectrum of grass control.

HPPD inhibitors are commonly tank mixed with PSII-inhibiting herbicides to increase weed control and spectrum in corn production systems. PSII inhibitors, such as, atrazine compete with plastoquinones for the D1 protein binding site, disrupting electron transfer in photosystem II. The inability to transfer electrons creates triplet chlorophyll and singlet oxygen which destroy plant membranes (Hess 2000). Employing the Colby equation (Colby 1967), several reports have shown combinations of HPPD and PSII inhibitors have synergistic effects
on weed control following foliar (Abendroth et al. 2006; Hugie et al. 2008), or soil applications (Bollman et al. 2006). This synergism has even been detected in certain PSII inhibitor-resistant species (Hugie et al. 2008; Sutton et al. 2002). It is hypothesized (Abendroth et al. 2006; Armel et al. 2005) that synergism with HPPD inhibitors arises from depletion of plastoquinones, which allows increased binding of PSII inhibitors to the D1 protein. The resulting triplet chlorophyll and singlet oxygen would not be quenched by carotenoids and tocopherols since these are depleted by HPPD inhibitors.

1.4 Waterhemp Biology

Waterhemp [Amaranthus tuberculatus (Moq.) Sauer] is a small-seeded, summer annual broadleaf weed species native to Illinois and taxonomically classified within the Amaranthus family (Sauer 1955, 1957). A morphologically diverse species, erect waterhemp stems can reach heights over 2 meters (Horak and Loughin 2000; Sauer 1955) with oblong to lanceolate leaves measuring 2–10 cm long and 1–3 cm wide (Sauer 1955). Waterhemp posses the C₄ carbon fixation pathway that results in high photosynthetic rates during periods of elevated temperature and light intensity. Historically found on the margins of freshwater bodies, Sauer (1955) astutely reported that waterhemp could be invasive in disturbed areas such as fields and gardens. Though native, this plant is now considered a problematic weed by Illinois corn and soybean producers not only because of its prevalence but also because of its ability to survive various herbicides (Hager and Sprague 2002).

Waterhemp emergence events or “flushes” occur later and over a more prolonged period (late May through early August) than other commonly encountered weeds such as velvetleaf and foxtail (Hartzler et al. 1999). Waterhemp is a dioecious species (Murray 1940; Sauer 1955,
1957) with male plants producing pollen and female plants producing seeds. Female waterhemp plants are prolific seed producers capable of producing more than one million seeds under ideal conditions (Hartzler et al. 2004; Steckel et al. 2003). Equally amazing is this plant’s ability to produce seeds under unfavorable conditions. Individual waterhemp that had emerged 50 days after soybean planting still produced 3000 seeds female⁻¹ (Hartzler et al. 2004), while Steckel et al. (2003) reported that plants growing in a 68% shaded environment generated up to 400,000 seeds. Dormancy allows a portion of waterhemp seeds to remain viable in the soil for several years (Buhler and Hartzler 2001; Burnside et al. 1996). Seed dormancy, coupled with high seed output and later emergence, makes waterhemp a significant contributor to the weed seed bank in agronomic fields (Buhler et al. 2001).

Waterhemp is an obligate out-crossing species. As a result, a single female can be pollinated by multiple males, leading to increased genetic diversity of progeny (Hager et al. 1997). Waterhemp has also been shown to hybridize with other species of the *Amaranthus* family (Murray 1940), adding more variability to an already diverse species. Waterhemp presence in Illinois agriculture has increased over the last twenty years and is most likely a result of several factors (Hager et al. 1997). Along with its high genetic diversity, this species is naturally adapted to certain agricultural practices. Waterhemp seeds are very small (1–1.5mm), and tend to germinate at much higher rates when close to the soil surface (Steckel et al. 2007). Conventional tillage tends to bury seeds to greater depths, reducing germination and seedling emergence (Leon and Owen 2006; Steckel et al. 2007). However, an increase of no-till hectares in Illinois (Horowitz et al. 2010) has resulted in more waterhemp seeds remaining closer to the soil surface, thus increasing the probability for successful germination. Additionally, early emerging waterhemp, which can be controlled mechanically prior to crop planting, must be
controlled by other methods in no-till production systems (Hager et al. 1997). Decreased use of soil-applied herbicides and over reliance on foliar-applied herbicides also has contributed to increased waterhemp prevalence (Hager et al. 1997). Several foliar-applied herbicides that are used to control emerged waterhemp have little to no soil residual activity. Waterhemp that emerge (Hartzler et al. 1999) after these foliar applications might produce (Hartzler et al. 2004). Complete waterhemp control is difficult, as many herbicides do not possess the sufficient residual activity needed to control late emerging plants.

Evolution of herbicide resistance has greatly enhanced waterhemp prevalence across much of the Midwest. Prior to 2009, waterhemp had evolved resistance to herbicides representing four different site-of-action groups (Heap 2012). Reports of waterhemp resistance to ALS and PSII inhibitors first began in the early 1990s and has quickly spread to multiple states (Anderson et al. 1996; Hinz and Owen 1997; Horak and Peterson 1995; Sprague et al. 1997). The first case of resistance to protoporphyrinogen oxidase (PPO) inhibitors was reported in Kansas (Shoup et al. 2003) and eventually followed by glyphosate resistance in Missouri (Legleiter and Bradley 2008). Waterhemp has the ability to stack multiple resistances within individual plants (Foes et al. 1998; Patzoldt et al. 2005). Bell et al. (2009) identified a population of waterhemp from Illinois that contains individuals resistant to herbicides from four site-of-action groups.

Waterhemp is one more challenging weed species confronting Illinois growers. Research by Hager et al. (2002) demonstrated a 43% reduction in soybean yield when waterhemp was allowed to compete with the crop for 10 weeks. A multiple year and location trial by Bensch et al. (2003) reported similar results with an estimated yield reduction range of 27 to 63% under season long competition. Corn yields also are adversely affected. Cordes et al. (2004) reported a
36% decrease in corn yield at high waterhemp densities (369–445 m²). Waterhemp is known to greatly affect corn yields during periods of crop stress such as drought. During a three year field trial, season-long waterhemp competition reduced yields 74% during two years of moisture stress, as compared with 11% during a third year of adequate rainfall (Steckel and Sprague 2004). Producers must develop management plans, which may include chemical methods, to properly control waterhemp infestations. However, this species is known to evolve and stack resistance to herbicides represented by different site-of-action groups. The development of novel herbicide resistances will severely limit growers’ options for controlling this troublesome species.

1.5 Research Objectives

HPPD inhibitors, which include mesotrione, tembotrione, and isoxaflutole, were applied to approximately 42% of Illinois corn hectares in 2010 (USDA/NASS 2011). In the summer of 2009, a waterhemp population in a seed corn production field in McLean Co., IL was not adequately controlled following foliar applications of HPPD inhibitors. Reduced herbicidal efficacy observed in the field could potentially be due to herbicide resistance, adverse weather conditions, or applicator error. Therefore, Chapter 2 describes the responses of waterhemp grown from field collected seed and two known sensitive biotypes after foliar applications of HPPD inhibitors under controlled greenhouse conditions.

Previous research has demonstrated waterhemp growth stage at the time of herbicide application can influence herbicide efficacy. Chapter 3 investigates the response of the McLean Co. population to HPPD-inhibiting herbicides applied at various plant heights under field conditions. The accumulation of waterhemp biomass and mortality under field conditions was
measured using a rate response with HPPD inhibitors alone and in combination with atrazine. Potential resistance to multiple herbicide groups in the McLean Co. waterhemp population is also described in Chapter 3.

Certain inhibitors of HPPD, such as isoxaflutole and mesotrione, can be applied to the soil for residual control of annual weed species including *Amaranthus* spp. (Luscombe and Pallett 1996; Wichert et al. 1999). Field and greenhouse experiments described in Chapter 4 investigate the efficacy of soil-applied HPPD inhibitors on the McLean Co. waterhemp population. Various soil-applied herbicides representing several site-of-action groups were also evaluated under field conditions to determine effective control options for this population.

### 1.6 Attributions

The material presented in Chapter 2 was previously published in *Pest Management Science* 2011, volume 67, pages 258–261 and titled “Resistance to HPPD-inhibiting herbicides in a population of waterhemp (*Amaranthus tuberculatus*) from Illinois, United States” by Nicholas E. Hausman, Sukhvinder Singh, Patrick J. Tranel, Dean E. Riechers, Shiv S Kaundun, Nicholas D Polge, David A Thomas, and Aaron G Hager. Technical assistance with greenhouse experiments was provided by Doug Maxwell and Lisa Gonzini.

Chapters 3 and 4 will be submitted for publication in *Weed Technology* in collaboration with Patrick J. Tranel, Dean E. Riechers, Doug Maxwell, Lisa Gonzini and Aaron G. Hager. David Thomas and Doug Maxwell operated tillage and planting equipment for field research, while Dr. Donald Bullock provided consultation for statistical analysis of field and greenhouse data in Chapters 3 and 4. Statistical analysis and advice using R software in Chapter 4 was provided by Dr. Adam Davis.
1.7 Literature Cited

Anonymous. 2006. Impact Herbicide, technical information and use guide for field corn, sweet corn and popcorn. AMVAC, Los Angeles, Calif.


USDA/NASS. 2011. Statistics by Subject-Environmental.


1.8 Figures

Figure 1.1 HPPD inhibitor leptospermone with novel triketone class herbicides (Lee et al. 1997).

leptospermone

sulcotrione

NTBC
Figure 1.2 HPPD inhibitors developed by Rhone-Poulenc scientists including the isoxazole class herbicide isoxaflutole (Pallett et al. 2001).

M&B 46 206  
RPA 200 809  
Isoxaflutole
Figure 1.3 Early commercial HPPD inhibitors of the pyrazolone class (Hawkes et al. 2007).

pyrazolate
prazoxyfen
benzofenap
CHAPTER 2
RESISTANCE TO HPPD-INHIBITING HERBICIDES IN A POPULATION OF WATERHEMP (AMARANTHUS TUBERCULATUS) FROM ILLINOIS, UNITED STATES

2.1 Abstract

A population of waterhemp in a seed maize production field in central Illinois, USA was not adequately controlled after postemergence applications of herbicides that inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD). Progeny from the field population survived following treatment with mesotrione, tembotrione or topramezone, applied to the foliage either alone or in combination with atrazine in greenhouse experiments. Dose-response experiments indicated that the level of resistance to the HPPD inhibitor mesotrione is at least 10-fold, relative to sensitive biotypes. These studies confirm that waterhemp has evolved resistance to HPPD-inhibiting herbicides.

2.2 Introduction

Herbicides are used extensively worldwide to control weeds in diverse cropping systems. Even with widespread utilization of herbicides, complete control or eradication of weeds is seldom achieved. Weeds persist in agroecosystems that are heavily dependent on herbicides by several mechanisms, including the evolution of herbicide-resistant biotypes.

Waterhemp [Amaranthus tuberculatus (Moq.) Sauer] is a summer annual weed species (Sauer 1957) that can reduce yield of maize (Zea mays) (Steckel and Sprague 2004), soybean (Glycine max) (Hager et al. 2002b), and grain sorghum (Sorghum bicolor) (Feltner et al. 1969).
Waterhemp infestations in Illinois agronomic crops have become common during the last two decades (Hager et al. 1997), attributable to changes in cropping practices, differential susceptibility to herbicides and evolution of herbicide-resistant biotypes. Agronomic crops frequently are treated one or more times with herbicides to control waterhemp, in part because its germination and emergence extends longer into the summer growing season than is common for other summer annual weed species (Hartzler et al. 1999). The high reproduction potential of waterhemp (Steckel et al. 2003) provides a wealth of genetic variants on which herbicide selection can act. Furthermore, being a dioecious species, and thus an obligate outcrosser, waterhemp is ideally suited for evolving herbicide resistance by sharing resistance genes among populations and biotypes (Steckel 2007). Resistance to herbicides that inhibit acetolactate synthase (ALS) (Horak and Peterson 1995), photosystem II (PSII)(Anderson et al. 1996), protoporphyrinogen oxidase (PPO)(Shoup et al. 2003), and glyphosate (Legleiter and Bradley 2008) has been documented, as has the phenomenon of waterhemp biotypes resistant to multiple herbicide families (Bell et al. 2009; Legleiter and Bradley 2008; Patzoldt et al. 2005; Shoup et al. 2003).

Herbicides that inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD; EC 1.13.11.27) constitute one of the newest commercially available herbicide classes for use in maize and other cereal crops. HPPD catalyzes the conversion of 4-hydroxymethylpyruvate to homogentisate in the biosynthesis of plastoquinone and tocopherols (Grossmann and Ehrhardt 2007). Inhibition of HPPD by herbicides leads to photooxidative destruction of chlorophyll and destruction of photosynthetic membranes in emerging shoot tissue, resulting in a characteristic bleaching of new leaf tissues. Several HPPD inhibitors are commercially available for use in cereal crops. Favorable characteristics of these herbicides, such as broad-spectrum weed control and excellent
crop tolerance, have contributed to their widespread integration into maize production systems (Beaudegnies et al. 2009).

The research presented within describes a novel herbicide resistance in an Illinois waterhemp biotype. This biotype was discovered during August 2009 in a McLean County, IL, USA field dedicated to seed maize production for the previous six years. Each season during that same period, the field was treated with a combination of S-metolachlor and simazine applied prior to crop and weed emergence followed by one or more foliar applications of HPPD-inhibiting herbicides (Table 2.1). The data presented herein provide evidence that this waterhemp biotype is resistant to HPPD-inhibiting herbicides.

2.3 Materials and Methods

2.3.1 Waterhemp populations

During August 2009, inflorescences from female waterhemp plants that were not controlled following foliar applications of tembotrione and mesotrione were collected and dried at room temperature. Seeds were manually harvested and stratified in 0.1% agarose solution at 4°C for 30 days. Seeds from individual female plants were considered to be unique accessions. Seed and plants derived from the putative HPPD-resistant population were designated MCR. The responses of three MCR accessions (12, 15 and 16) were compared with two known HPPD-inhibitor-sensitive waterhemp populations (Patzoldt et al. 2005), designated ACR and WCS.

2.3.2 Greenhouse plant culture

All plants used in these experiments were germinated from seeds sown in 12×12 cm flats containing a commercial potting medium¹. Emerged seedlings (2 cm) were transplanted into 950
cm³ pots (one seedling per pot) containing a 3:1:1:1 mixture of potting mix:soil:peat:sand that included a slow-release fertilizer². Greenhouse conditions were maintained at 28/22 °C day/night with a 16:8 h 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 Response to foliar-applied HPPD inhibitors alone or combined with atrazine

Uniformly sized waterhemp plants (10–12 cm tall) were treated with one of three commercially available formulations of HPPD-inhibiting herbicides, atrazine alone or a tank-mix combination of HPPD inhibitor and atrazine. The HPPD inhibitors and their respective application rates included topramezone at 18 g ha⁻¹, tembotrione at 92 g ha⁻¹ and mesotrione at 105 g ha⁻¹. The commercially available formulation of tembotrione also contained the safener isoxadifen-ethyl. Atrazine, alone or in combination with an HPPD inhibitor, was applied at 560 g ha⁻¹. Applications were made using a compressed air research sprayer³ fitted with a TeeJet 80015 EVS nozzle⁴ calibrated to deliver 185 L ha⁻¹ at 275 kPa. All treatments included methylated seed oil (MSO, 1% v/v) and liquid ammonium sulfate (AMS, 2.5% v/v). Following application, plants were placed on greenhouse benches in a randomized complete block design. Each treatment was replicated 4 times, and the experiment was conducted twice. Visual assessment of plant response was done 7, 14 and 21 days after treatment (DAT) using a scale ranging from 0 (no plant injury) to 100 (plant mortality). Data were compared using the PROC GLM procedure⁵. All data generated from two runs of the experiment were pooled, as the homogeneity of variance test (P = 0.05) was not significant. Treatment means were compared using Fisher’s protected LSD. Additionally, the combined mean effect of the MCR accessions
(12, 15 and 16) was compared with the sensitive populations (ACR and WCS) using contrast statements in PROC GLM.

2.3.4 Quantifying resistance to HPPD inhibitors

Waterhemp plants derived from the MCR, ACR and WCS populations were grown under the greenhouse conditions described in Section 2.3.2. Mesotrione was applied to 10–12 cm tall plants at increasing rates equally spaced along a base 3.16 logarithmic scale. The rate range for ACR and WCS populations was 0.1–1050 g mesotrione ha$^{-1}$, and 1–10,500 g mesotrione ha$^{-1}$ for MCR. Treatments were applied as described in Section 2.3.3, except that all treatments included crop oil concentrate (COC, 1% v/v) and AMS (2.5% v/v). Following application, plants were placed on greenhouse benches in a randomized complete block design. Each treatment was replicated 6 times, and the experiment was conducted twice. At 21 DAT, all above-ground plant tissue was harvested and dried at 65$^{\circ}$C for 7 days, and dry weights were recorded. The dry weight of all plants within each treatment were averaged and converted to a percentage of the untreated 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

\[
y = c + \frac{d - c}{1 + \exp[b\left(\log(x) - \log(GR_{50})\right)]]}
\]

[1]

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 $GR_{50}$ is 50% reduction in dry weight. Visual
assessment of plant response was also recorded immediately prior to plant harvest, as described previously.

### 2.4 Results and Discussion

#### 2.4.1 Response to foliar-applied HPPD inhibitors alone or combined with atrazine

HPPD inhibitors caused characteristic injury (stunting and meristem bleaching) on plants from the control populations (WCS and ACR) and all three MCR accessions. In general, MCR accessions exhibited less injury than WCS or ACR. Injury to WCS and ACR 7 DAT ranged from 56–85%, but was 54% or less for MCR accessions (Table 2.2). Reduced sensitivity of MCR to HPPD inhibitors became more pronounced over time. Injury to WCS and ACR generally increased over time, whereas most MCR plants began to recover approximately 10 DAT. By 14 DAT new leaf tissue was evident on many MCR plants. Injury to WCS and ACR 21 DAT by HPPD inhibitors applied alone ranged from 88–100%, whereas injury to MCR was only 13–58%.

MCR demonstrated resistance to atrazine. This is similar to ACR, which previously has been reported to possess non-target-site-mediated resistance to atrazine (Patzoldt et al. 2003; Patzoldt et al. 2005). Atrazine injured ACR and MCR less than 10%, but injured WCS, known to be sensitive to atrazine (Patzoldt et al. 2005), more than 80% (Table 2.2). Triazine herbicides were applied to the field where MCR was discovered one or more times each of the previous seven growing seasons (Table 2.1). The mechanism of triazine resistance in MCR has not been determined.

Across all populations 7 DAT, atrazine combined with each HPPD inhibitor significantly increased injury over that of each HPPD inhibitor alone (Table 2.2). By 21 DAT, atrazine
combined with any HPPD inhibitor injured WCS and ACR at least 98%, but injured MCR only 43–89%. Combinations of HPPD and PS II inhibitors exhibit synergistic activity (Abendroth et al. 2006; Hugie et al. 2008; Sutton et al. 2002; Woodyard et al. 2009a), even on triazine-resistant biotypes (Hugie et al. 2008; Woodyard et al. 2009b). A detailed dose-response analysis is required, however, to determine if the increase in combined activity observed in MCR is additive or synergistic (Hugie et al. 2008). Regardless, these results are consistent with field observations (data not reported) that MCR is not adequately controlled with foliar-applied HPPD inhibitors alone or combined with atrazine.

Overall, the MCR accessions responded similarly to each other, and most pair-wise comparisons were not significantly different (p=0.05) (Table 2.2). Although each accession was relatively uniform in response to HPPD inhibitors and atrazine, variability in injury was observed within each accession. HPPD inhibitors caused mortality of a few plants (<10%) in each MCR accession, suggesting that the MCR population is segregating for the resistance. Since no major differences were observed among accessions, MCR accession 15 was selected to quantify resistance to HPPD inhibitors.

### 2.4.2 Quantifying resistance to HPPD inhibitors

Treatment of WCS, ACR, and MCR plants with a range of mesotrione doses resulted in typical herbicide response curves, with decreasing dry weights observed with increasing doses (Figure 2.1). GR$_{50}$ values ($\pm$ 1 standard error), determined with curve-fitting software, were calculated to be 48.5 ($\pm$ 6.8), 4.9 ($\pm$ 0.9), and 1.4 ($\pm$ 0.1) g mesotrione ha$^{-1}$ for MCR, ACR, and WCS, respectively. Based on these GR$_{50}$ values, the relative level of resistance to mesotrione in MCR was 10- or 35-fold, depending on which sensitive population was used for comparison.
Several cycles of inbreeding to increase seed supply may have reduced plant vigor and enhanced herbicide sensitivity in WCS, whereas ACR is more robust, demonstrating resistance to triazines, ALS inhibitors and PPO inhibitors (Patzoldt et al. 2005). Thus, the range in responses exhibited by WCS and ACR likely reflect the range in responses that might be observed among field populations. Despite the different responses between WCS and ACR at low mesotrione doses, dry weights of these populations were reduced greater than 90% with 105 g mesotrione ha\(^{-1}\) (a typical postemergence use rate in Illinois). In contrast, this same mesotrione dose reduced dry weight of MCR by approximately 60% (Figure 2.1). Furthermore, the MCR population likely is segregating for the HPPD resistance trait, in which case the level of resistance for the population underestimates the level of resistance for the resistant biotype.

Visual assessment of plant injury 21 DAT (data not shown) was consistent with dry weight data in revealing different dose responses among the populations. The percentage of plants rated at 100% injury (mortality) for ACR and WCS was 60 and 100%, respectively, at 105 g meotrione ha\(^{-1}\), and 100% for both populations at 315 g mesotrione ha\(^{-1}\). In contrast, mortality of MCR was 0 and 33% at 105 and 315 g mesotrione ha\(^{-1}\), respectively.

### 2.4.3 Implications and future research

Since few herbicides with novel modes of action are being commercialized (Cole et al. 2000) and resistance to currently available herbicide classes is increasing (Heap 2012), it is important to effectively and efficiently utilize herbicides currently available. It might be advantageous to utilize beneficial herbicide interactions, such as synergistic interactions between HPPD and PS II inhibitors (Hugie et al. 2008), for management of certain herbicide-resistant weeds. MCR is both HPPD- and atrazine-resistant yet displays initial injury from foliar-applied
HPPD inhibitors before recovering. It will therefore be of interest to determine if a beneficial interaction between HPPD inhibitors and either atrazine or bromoxynil exists in MCR, similar to that seen in metabolism-based, atrazine-resistant *Abutilon theophrasti* (Woodyard et al. 2009b).

Waterhemp is the first weed to evolve resistance to HPPD inhibitors, which represents the fifth herbicide mode of action to which waterhemp has evolved resistance (Heap 2012). Future research will investigate the genetics, inheritance, and mechanisms of resistance to HPPD inhibitors and atrazine in MCR. Evolution of resistance to HPPD inhibitors is a recent phenomenon (Heap 2012) and thus mechanistic research has not been reported for HPPD resistance in weeds. Waterhemp’s dioecious biology likely will facilitate the stacking of resistance to HPPD inhibitors with resistance to other herbicide families, leading to new multiple-resistant biotypes (Tranel et al. 2010). As this occurs, effective herbicide options for management of waterhemp will become even more limited.

### 2.5 Source of Materials

1. LC1. Sun Gro Horticulture, 15831 N.E. 8th Street, Bellevue, WA 98008.


4. TeeJet 80015EVS. TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187.

2.6 Literature Cited


2.7 Tables

Table 2.1  Herbicide use history at the Illinois field location where a putative HPPD-resistant waterhemp biotype was discovered.

<table>
<thead>
<tr>
<th>Year</th>
<th>Herbicides applied&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>mesotrione + atrazine</td>
</tr>
<tr>
<td>2004</td>
<td>mesotrione + atrazine</td>
</tr>
<tr>
<td>2005</td>
<td>mesotrione + atrazine</td>
</tr>
<tr>
<td>2006</td>
<td>topramezone + atrazine</td>
</tr>
<tr>
<td>2007</td>
<td>topramezone + atrazine</td>
</tr>
<tr>
<td>2008</td>
<td>tembotrione followed by mesotrione</td>
</tr>
<tr>
<td>2009</td>
<td>tembotrione followed by mesotrione</td>
</tr>
</tbody>
</table>

<sup>a</sup> Herbicides listed in Table 2.1 were applied with spray additives per label recommendations after crop and weed emergence. S-metolachlor + simazine was applied each year before crop and weed emergence.
Table 2.2 Mean (n=8) injury for HPPD-inhibitor-sensitive populations WCS and ACR and three accessions (12, 15, and 16) of the HPPD-inhibitor-resistant MCR population at 7, 14, and 21 days after treatment with an HPPD inhibitor and/or atrazine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% injury 7 DAT</th>
<th>% injury 14 DAT</th>
<th>% injury 21 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>WCS</td>
<td>ACR</td>
<td>12</td>
</tr>
<tr>
<td>mesotrione</td>
<td>85</td>
<td>63</td>
<td>40</td>
</tr>
<tr>
<td>mesotrione + atrazine</td>
<td>93</td>
<td>83</td>
<td>71</td>
</tr>
<tr>
<td>tembotrione</td>
<td>84</td>
<td>56</td>
<td>40</td>
</tr>
<tr>
<td>tembotrione + atrazine</td>
<td>95</td>
<td>87</td>
<td>67</td>
</tr>
<tr>
<td>topramezone</td>
<td>84</td>
<td>69</td>
<td>41</td>
</tr>
<tr>
<td>topramezone + atrazine</td>
<td>95</td>
<td>81</td>
<td>62</td>
</tr>
<tr>
<td>atrazine</td>
<td>83</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>LSD</td>
<td>6</td>
<td>9</td>
<td>14</td>
</tr>
</tbody>
</table>

*a For comparison of treatment means among accessions/populations, alpha = 0.05.

*b For comparison of accession/population means among treatments, alpha = 0.05.
2.8 Figures

**Figure 2.1** Mesotrione dose response curves for HPPD-inhibitor-sensitive populations WCS and ACR and the HPPD-inhibitor-resistant MCR population. Shoot dry weights were obtained 21 DAT. Treatment means (n=12) ±1 standard error are plotted and connected with straight lines.
CHAPTER 3

FOLIAR HERBICIDE OPTIONS TO MANAGE A WATERHEMP (AMARANTHUS TUBERculosus) POPULATION RESISTANT TO HPPD-INHIBITING HERBICIDES

3.1 Abstract

Waterhemp is an annual broadleaf weed that recently has developed resistance to HPPD-inhibiting herbicides. Experiments were conducted in 2010 and 2011 at a McLean County, Illinois seed corn production field where HPPD inhibitors failed to adequately control waterhemp in 2009. Objectives of these experiments were to characterize the response of this field population to HPPD inhibitors and determine the population’s sensitivity to herbicides from other site-of-action groups. Waterhemp treated at 10 to 15 cm with 105 g mesotrione ha\(^{-1}\), 92 g tembotrione ha\(^{-1}\), or 18 g topramezone ha\(^{-1}\) had significantly greater biomass 14 days after treatment (DAT) than waterhemp harvested the day of herbicide application, indicating plant recovery and growth. Mortality data collected in 2011 revealed 83 to 100% waterhemp survival when any HPPD inhibitor was applied at recommended use rates. Mesotrione applied at 105 g ha\(^{-1}\) alone or combined with atrazine provided significantly greater waterhemp control when applied VEPOST (2 to 5 cm weed height) compared with EPOST (5 to 10 cm) or POST (10 to 15 cm) application timings. Glyphosate, glufosinate, fomesafen, lactofen, and acifluorfen provided significantly greater waterhemp injury and stand reduction 7 and 14 DAT than mesotrione, dicamba, and 2,4-D. Atrazine, chlorimuron, and imazethapyr provided significantly less waterhemp control. Results of a greenhouse experiment using waterhemp grown from field-collected seed were similar to field data, and confirm the McLean Co. population is resistant to HPPD, PSII and ALS inhibitors.
3.2 Introduction

Waterhemp [Amaranthus tuberculatus (Moq.) Sauer] is a small-seeded, dioecious, summer annual broadleaf weed species (Sauer 1955, 1957) common in Illinois corn (Zea mays L.) and soybean (Glycine max (L.) Merr) production systems (Hager et al. 1997). Previous research has demonstrated this weed species can reduce soybean yields more than 40% (Hager et al. 2002b) and up to 74% in corn (Steckel and Sprague 2004). Once established, this species is difficult to eradicate as individual female plants can produce in excess of one million seeds (Hartzler et al. 2004; Steckel et al. 2003) that can remain dormant in the soil seed bank for years (Buhler and Hartzler 2001; Burnside et al. 1996). Waterhemp emergence occurs over a more prolonged period than weeds such as velvetleaf (Abutilon theophrasti) and foxtail (Setaria spp.) (Hartzler et al. 1999). The extended emergence of waterhemp often necessitates an integrated management system, including utilization of soil-residual and foliar-applied herbicides, to adequately manage waterhemp.

The evolution of waterhemp populations resistant to various foliar-applied herbicides has effectively reduced the number of herbicide options that remain viable to control waterhemp. Prior to 2009, waterhemp had evolved resistance to four herbicide site-of-action groups (Heap 2012): acetolactate synthase (ALS) inhibitors, photosystem II (PSII) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors. Waterhemp with resistance to multiple herbicide groups further limits control options (Foes et al. 1998; Patzoldt et al. 2005). Bell et al. (2009) identified a population of waterhemp in which individual plants demonstrated resistance to herbicides from four site-of-action groups.
Resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides is a relatively recent phenomenon (Heap 2012). Waterhemp populations from McLean Co., IL (Hausman et al. 2011) and Henry Co., IA (McMullan and Green 2011) have been reported to demonstrate resistance to HPPD-inhibiting herbicides. Two biotypes of Palmer amaranth from Kansas also have demonstrated reduced sensitivity to various HPPD-inhibiting herbicides (Lally et al. 2010). In greenhouse research, waterhemp grown from seed collected at the McLean Co., IL location displayed resistance to foliar-applied mesotrione, tembotrione, and topramezone at rates of 105, 92, and 18 g ha\(^{-1}\), respectively, and resistance to the PSII inhibitor atrazine (Hausman et al. 2011). Additional greenhouse research revealed the Illinois population demonstrated a 10- to 35-fold level of resistance to the HPPD inhibitor mesotrione (Hausman et al. 2011). McMullan and Green (2011) reported similar greenhouse results, with the Iowa population demonstrating an 8-fold level of resistance to mesotrione as well as resistance to atrazine and thifensulfuron.

The objectives of this research were to further characterize the HPPD inhibitor-resistant population from Illinois. One objective was to characterize biomass accumulation under field conditions using a rate response experiment with HPPD inhibitors applied alone or in combination with atrazine. Herbicide applications under greenhouse conditions (Hausman et al. 2011) were made to waterhemp of a consistent size, which is atypical under field growing conditions. Therefore, a second objective was to characterize the response of this population to HPPD-inhibiting herbicides applied at various waterhemp growth stages under field conditions. A final objective was to determine the response of this population to various foliar-applied herbicides representing seven herbicide site-of-action groups under both field and greenhouse conditions.
3.3 Materials and Methods

3.3.1 General methods for field experiments

Field experiments were conducted in 2010 and 2011 at the location (McLean Co., IL) from which the HPPD-resistant waterhemp population was initially identified. The soil was a Sable silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls), with a pH of 6.4 and 3.2% organic matter. Preplant tillage was performed each spring to prepare the seedbed for planting and to control emerged weeds. Experiments were conducted either in corn (GH 8914RRLL 3000 GT) or soybean (S30-F5), planted in 76 cm rows. Corn and soybean were planted on May 6 in 2010, while in 2011 corn and soybean were planted on May 5 and May 19, respectively. 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 AIXR110025 nozzles spaced 51 cm apart on a 3-m boom calibrated to deliver 187 L ha$^{-1}$ at 276 kPa.

Statistical analysis for all field experiments was performed using PROC Mixed in SAS 9.2 with herbicide treatment considered a fixed effect. Random effects included year and block within year and all interactions containing either of these effects. Initial analysis revealed no significant year by treatment interactions ($\alpha = .05$); thus, data were pooled and results presented as a combination of 2010 and 2011.

3.3.2 HPPD inhibitors applied alone or combined with atrazine in corn

Annual grass weed species were controlled in this experiment with S-metolachlor plus simazine applied prior to planting at 1780 and 1120 ai g ha$^{-1}$, respectively. Waterhemp (10–15
cm tall) were treated with one of three HPPD-inhibiting herbicides, atrazine, or a combination of HPPD inhibitor and atrazine. The HPPD-inhibiting herbicides mesotrione, tembotrione, and topramezone were applied at 1, 2 and 4x the recommended field use rate. All herbicide application rates are presented in Table 3.1. All treatments containing HPPD inhibitors included crop oil concentrate (COC) at 1% (v/v) and 28% urea ammonium nitrate (UAN) at 2.5% (v/v); COC was included with the atrazine only treatment.

Visual estimates of percent waterhemp control were recorded 7 and 14 days after treatment (DAT) using a scale of 0 (no control) to 100 (complete control). These estimates were based on waterhemp injury, biomass, and stand reduction when compared with the nontreated control. In addition to visual estimates, four uniformly-sized waterhemp plants per plot (twelve per treatment including untreated) were selected to quantify aboveground biomass accumulation after herbicide treatment. Immediately prior to herbicide application, selected waterhemp plants (10 to 15 cm) were marked by placing a small plastic stake near each plant. All other waterhemp plants within a 15-cm diameter of each marked plant were carefully removed to ensure full spray interception by the marked plants. Twelve additional plants (10 to 15 cm) from the untreated plots were harvested prior to application to determine pre-treatment biomass. All marked waterhemp plants were harvested 14 DAT, dried at 65°C for 7 days, and dry weights recorded.

Dry weights of treated waterhemp and plants collected prior to treatment application were averaged within plots (four per plot) and converted to a percentage of the nontreated plants harvested 14 DAT. Data displayed a normal distribution of error (p-value .2121). Levene’s test for homogeneity of variance was significant at α = .05 (p-value = .0001) suggesting variances between treatments might be different. Several different data transformations failed to increase the p-value of an equal variance more than 0.01. Estimates of variance for each treatment fell
within a range of 2.23 to 290.15, providing further evidence that a common variance was very unlikely. This is not unexpected as herbicide treatments that provide high levels of weed control generally produce consistent results across all replications, resulting in small variances. Less efficacious herbicide treatments are more likely to produce less consistent results across replications, resulting in larger variances (Littell et al. 2006). To account for unequal variances in this experiment, the SAS statement `REPEATED / GROUP = treatment` was used to estimate variances for each treatment when comparing treatment means. Additionally, fit statistics revealed the unequal variance structure fit the data better (AIC 740.6) than the common variance structure (AIC 751.2). All herbicide treatment means were compared with the pretreatment mean to assess any potential biomass accumulation 14 DAT using the Dunnett’s SAS procedure with $\alpha = .05$. In 2011, mortality data were recorded at harvest for the twelve marked waterhemp plants per treatment to determine uniformity of the field population response to HPPD inhibitors.

### 3.3.3 Mesotrione application timings in corn

Mesotrione and atrazine were applied alone or in combination to emerged waterhemp at three different waterhemp growth stages. The application timings and corresponding waterhemp heights were: very early post (VEPOST) applied when waterhemp was 2 to 5 cm tall, early post (EPOST) applied when waterhemp was 5 to 10 cm tall, and post (POST) applied when waterhemp was 10 to 15 cm tall. Mesotrione was applied at 105 g ai ha$^{-1}$ while atrazine was applied at 560 g ai ha$^{-1}$. All treatments included COC at 1% (v/v) and ammonium sulfate (AMS) at 2.5% (v/v).

Visual estimates of percent waterhemp control were recorded 7 and 14 DAT using the criteria described previously. Evaluations 21 DAT were taken only during the 2011 growing
season. Single degree of freedom contrast statements \((\alpha = .05)\) were used to determine differences in waterhemp control by application timing 14 DAT with mesotrione or mesotrione plus atrazine. Data were not subject to transformation as data displayed a normal distribution of error with a probability value \((p\text{-value})\) of .4072, and Levene’s test for homogeneity of variance was not significant at \(\alpha = .05\) \(p\text{-value} = .6073\).

3.3.4 Response of the Illinois HPPD-resistant waterhemp population to herbicides from other site-of-action groups under field conditions

The response of the HPPD-resistant waterhemp population to eleven herbicides representing seven herbicide site-of-action families was determined under field conditions. These herbicides along with their respective sites of action, rates, and adjuvants, are presented in Table 3.2. Field experiments were conducted in soybean at the McLean Co., IL location described previously. Herbicides were applied when the majority of emerged waterhemp reached 10 to 15 cm tall. Visual estimates of percent waterhemp control were recorded 7 and 14 DAT. To meet the assumption of normality, estimates of control were subject to an arcsine square root transformation. Levene’s test for homogeneity of variance on transformed data was not significant at \(\alpha = .05\) for both 7 and 14 DAT \(p\text{-values} = .1088\) and \(.2842\) respectively. Mean estimates of percent waterhemp control at 7 and 14 DAT were separated using the SAS macro \%pdmix800\ (Saxton 1998) with \(\alpha = .05\). Treatments with the same letter designation represent herbicides that were not significantly different in control of this waterhemp population.
3.3.5 Response of the Illinois HPPD-resistant waterhemp population to herbicides from other site-of-action groups under greenhouse conditions

3.3.5.1 Waterhemp populations

Inflorescences of several female waterhemp not controlled with foliar applied HPPD-inhibiting herbicides were collected from the McLean Co. location in August 2009. Seed collected from individual females were treated as unique accessions. Twelve accessions were chosen to characterize the resistance and sensitivity of this population (designated MCR) to different herbicide site-of-action groups. One ml of seeds from each accession was combined in a glass vile and shaken thoroughly to ensure adequate mixing. Seeds from this mixture were stratified in a 0.1% agarose solution at 4°C for 30 days. The response of the MCR population to various foliar-applied herbicides was compared with the responses of two other waterhemp populations (designated ACR and WCS). The ACR population demonstrates resistance to ALS-, PSII-, and PPO-inhibiting herbicides (Patzoldt et al. 2005), while WCS displays no resistance to any herbicide site-of-action group (Patzoldt et al. 2002).

3.3.5.2 Plant culture

Waterhemp plants from all three populations were germinated from seeds sown in 12-cm by 12-cm flats containing a commercial potting medium. Emerged seedlings (2 cm) were transplanted into 950 cm³ pots (one seedling per pot) containing a 3:1:1:1 mixture of potting mix:soil:peat:sand that included a slow-release fertilizer. Greenhouse conditions were maintained at 28/22 C 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.
3.3.5.3 Response to foliar-applied herbicides

Uniformly-sized waterhemp plants (10 to 12 cm tall) from MCR, ACR, and WCS were treated with one of eight herbicides. Herbicides, their respective sites of action, application rates, and spray additives are presented in Table 3.3. All herbicides were applied with a compressed air research sprayer fitted with a TeeJet 80015 EVS nozzle. The nozzle was situated 46 cm above the plant canopy, and the sprayer was calibrated to deliver 185 L ha$^{-1}$ at 275 kPa. After herbicide application, plants were moved back to the greenhouse in a completely randomized design (CRD). Treatments applied to WCS and ACR were replicated 4 times, while treatments applied to MCR were replicated 48 times, and the experiment was conducted twice. Visual assessment of plant response was recorded 7, 14 and 21 DAT using a scale ranging from 0 (no plant injury) to 100 (plant mortality). At 21 DAT, all above ground tissue was harvested and dried for 7 days at 65$^\circ$C. Plants were then weighed and dry weights recorded.

3.3.5.4 Statistical analysis

Statistical analysis was performed using PROC Mixed in SAS 9.2 with waterhemp population, herbicide treatment, and subsequent interaction considered fixed effects. Random effects included experiment run and interactions containing run with a fixed effect. Dry weight data were subject to square root transformation to increase normality of residual distribution, while Levene’s test for homogeneity of variance was highly significant at $\alpha = .05$ (p-value = .0001) suggesting variances among populations and their respective treatments may be different. Additional transformations failed to increase the probability of a common variance, while estimates of variances for waterhemp population by treatment fell within a range of .005 to 2.55, further suggesting an unequal variance structure. Fit statistics showed the unequal variance
structure fit the data better (AIC 584.9) than the common variance structure (AIC 947.8). Thus, statistical analysis in SAS was done using the \texttt{REPEATED / GROUP = population*treatment} statement.

Dry weight means for MCR were separated using the SAS macro \texttt{MCB} (Hsu 1996), which compares the treatment resulting in the highest plant biomass to all other treatments. Single degree of freedom contrasts statements ($\alpha = .05$) were used to compare responses of MCR with ACR and WCS by treatment. Additionally, dry weights of the three biotypes by treatment were converted to a percent of the untreated and displayed as a bar graph ($\pm 1$ SEM) for visual representation of the entire experiment.

3.4 Results and Discussion

3.4.1 HPPD inhibitors applied alone or combined with atrazine in corn

Regardless of application rate, mesotrione, tembotrione, or topramezone provided less than 40% control of the waterhemp population (Table 3.4). These results from a field-based experiment are similar to those reported by Hausman et al. (2011) who evaluated the response of this same waterhemp population to these herbicides under greenhouse growing conditions. The addition of atrazine to each HPPD inhibitor generally increased waterhemp control compared with each HPPD inhibitor alone. With the exception of the 4x rate of mesotrione combined with atrazine, control of waterhemp was 58% or less 14 DAT across all treatments.

By 14 DAT, biomass of waterhemp treated with the 1x rates of mesotrione, tembotrione, or topramezone were significantly greater than the biomass of pre-treatment waterhemp (Table 3.4) indicating substantial plant recovery and growth. Biomass of waterhemp plants treated with the 2x rate of tembotrione or atrazine also were significantly greater than pre-treatment biomass.
Although average biomass was either significantly less or not different from pre treatment biomass for all other treatments, waterhemp control estimates recorded 7 and 14 DAT indicate generally poor control of this population. Mortality data were collected in 2011 as an additional metric to describe the response of this waterhemp population to foliar-applied HPPD-inhibiting herbicides.

Mortality of the twelve waterhemp plants marked prior to herbicide application and treated with 1x rates of mesotrione, tembotrione, or topramezone ranged from 0 to 17% (Table 3.5). Plants were considered survivors if they displayed actively growing, non-bleached tissue around the apical meristem. As herbicide rates increased, plant survival varied among HPPD inhibitors. Mortality following application of 4x mesotrione and topramezone was 25 and 67%, respectively. The addition of atrazine to the 1x rates of HPPD inhibitors increased waterhemp mortality to 25 to 58%. Mortality was greatest among the 2x and 4x rates of each HPPD inhibitor plus atrazine, ranging from 75 to 83% mortality. Waterhemp densities at the McLean Co. location over two years averaged 450 plants m\(^{-2}\), thus at the highest rate of mortality approximately 75 waterhemp plants m\(^{-2}\) would survive a 4x rate of HPPD inhibitor plus atrazine. Anecdotal observations suggested many of the surviving plants that had resumed growth and by the end of the growing season would likely have produced seed.

These results are in general agreement with other published reports. McMullan and Green (2011) reported waterhemp control was less than 70% at the Henry Co., IA location 30 DAT following applications of mesotrione, tembotrione, or topramezone at twice the recommended field use rate. Mortality of waterhemp generated from seed collected from the McLean Co., IL location and treated with 315 g ai ha\(^{-1}\) mesotrione was only 33% (Hausman et al. 2011). Lally et al. (2010) reported two putative HPPD-resistant Palmer amaranth biotypes
required 11 and 5 times more pyrasulfotole plus bromoxynil than a sensitive control to achieve 50% mortality.

### 3.4.2 Mesotrione application timings in corn

By 7 DAT, regardless of application timing, mesotrione caused characteristic HPPD-inhibitor symptomatology including bleaching of treated foliage and stunted growth (Table 3.6). Combining atrazine with mesotrione generally increased waterhemp control compared with mesotrione alone, but control with atrazine alone did not exceed 8% regardless of application timing, similar to greenhouse results reported in Hausman et al. (2011). Waterhemp control generally decreased by 14 DAT for all treatments and timings except mesotrione plus atrazine applied VEPOST. The decrease in control was attributed to recovery of treated plants, manifest by new, non-injured leaf tissue emerging from apical meristems and axillary buds.

Contrast statements (Table 3.6) comparing application timings 14 DAT demonstrated control of waterhemp was significantly greater when mesotrione was applied VEPOST compared with either EPOST or POST (45 and 56% greater, respectively). There was no significant difference in waterhemp control between EPOST and POST application timings. Results were similar when mesotrione was combined with atrazine. Waterhemp control following the VEPOST application was significantly greater when compared with EPOST and POST timings (44 and 54% greater, respectively). EPOST or POST application timings were not significantly different in regards to waterhemp control with mesotrione plus atrazine.

Greater weed control when mesotrione was applied to smaller weeds has been reported previously. Similar to waterhemp response to the VEPOST application timing, Johnson et al. (2002) reported increased control of common cocklebur (*Xanthium strumarium*) and yellow
nutsedge (Cyperus esculentus) 14 DAT following early postemergence mesotrione applications as compared with control following later applications. Woodyard et al. (2009) reported decreased common lambsquarters (Chenopodium album) control with mesotrione plus atrazine between years, partially due to increased weed height one year. While waterhemp control with mesotrione alone or combined with atrazine was greatest following the VEPOST application, a substantial number of waterhemp plants were able to recover and resume growth, which is attributed to resistance to HPPD inhibitors and atrazine. Additional management practices, such as inter-row cultivation or utilization of a different site-of-action herbicide, would be needed to prevent further weed interference and seed production. More waterhemp plants survived following the EPOST and POST herbicide application timings, reflected in the estimates of control. The results of the present experiments demonstrate foliar-applied HPPD inhibitors are not viable options for control of this Illinois waterhemp population. Additional research was undertaken to determine what other foliar-applied herbicides remain viable options for control of this population.

3.4.3 Response of the Illinois HPPD-resistant waterhemp population to herbicides from other site-of-action groups under field conditions

By 7 DAT, control of this waterhemp population was greatest with acifluorfen, fomesafen, lactofen, glyphosate and glufosinate, ranging from 81 to 93% (Table 3.7). Poor control with mesotrione (30%) and atrazine (8%) was consistent with previously reported results. 2,4-D and dicamba provided less than 35% control, while control provided by the ALS inhibitors chlorimuron and imazethapyr was 12% or less. The PPO inhibitors and glyphosate provided the greatest waterhemp control (75 to 89%) by 14 DAT. Control with glufosinate (68%) was
significantly less than glyphosate and all PPO inhibitors except acifluorfen. Waterhemp control ranged from 16 to 26% with mesotrione, 2,4-D, and dicamba, while atrazine, chlorimuron, and imazethapyr provided less than 10% control.

The response of this population to atrazine and ALS inhibitors was not unexpected. A survey of 59 Illinois waterhemp populations in 1998–1999 revealed 22% of populations contained individuals displaying resistance to both PSII and ALS inhibitors (Patzoldt et al. 2002). Obligate outcrossing coupled with production of large quantities of mobile seeds (Tranel et al. 2010) has undoubtedly aided the spread of these resistance traits throughout Illinois over the last decade. Additionally, this particular field was treated with soil- and foliar-applied triazine herbicides for at least seven continuous years (Hausman et al. 2011), increasing the selection for triazine-resistant waterhemp. Reduced waterhemp control with glufosinate 14 DAT was attributed primarily to regrowth of treated plants. Hoss et al. (2003) reported similar findings, with glyphosate providing greater waterhemp control than glufosinate 14 DAT due to recovery of glufosinate-treated plants. Recovery of waterhemp treated with PPO-inhibiting herbicides was less than recovery of glufosinate-treated waterhemp. Hager et al. (2003) observed a similar response when a small percentage of PPO inhibitor-treated waterhemp plants recovered from lower leaf axils 14 DAT with the same PPO-inhibiting herbicides evaluated in the present research. The presence of recovering plants at the McLean Co. location is most likely due to the high population density (450 plants m⁻²) and not additional herbicide resistance.

PPO inhibitors and glufosinate cause rapid lipid peroxidation and cell membrane destruction, but do not translocate throughout the plant (Hess 2000; Matsumoto 2002). Thus, thorough spray coverage is essential for these products to control weeds, and the high waterhemp density may have prevented thorough spray coverage on a small percentage of plants. Waterhemp treated
with the synthetic auxins dicamba and 2,4-D displayed epinasty and leaf malformation; however, little stand reduction or mortality of treated plants had occurred by 14 DAT. In order to eliminate the potentially confounding effects of varying climatic conditions and non-uniform spray coverage, additional research to characterize the response of the MCR population to various herbicide site-of-action groups was conducted under controlled greenhouse conditions.

### 3.4.4 Response of the Illinois HPPD-resistant waterhemp population to herbicides from other site-of-action groups under greenhouse conditions

Mean visual estimates of control for WCS, ACR, and MCR by treatment are presented in Table 3.8. MCR response to mesotrione in the greenhouse was similar to field results. Injury was greatest by 7 DAT (64%) and thereafter declined over subsequent evaluations (26% by 21 DAT). By 14 DAT plants were demonstrating signs of recovery from initial injury, manifested by emergence of new, noninjured leaf tissue near the apical meristem and resumption of growth. Atrazine, chlorimuron and imazethapyr provided the least control of MCR, ranging from 1 to 7% 21 DAT, while glyphosate, lactofen, dicamba, and glufosinate provided the greatest control (70 to 93%). MCR dry weight data revealed the highest amount of accrued plant biomass occurred in the plants treated with atrazine, imazethapyr, or chlorimuron (Table 3.9). MCR accumulated significantly less biomass in all other treatments, with the probability of similar means at .0001 (Table 3.9).

MCR biomass following glyphosate application was significantly higher than the combined average of WCS and ACR (Table 3.9); however, MCR dry weight in this treatment only accounted for approximately 10% of the untreated (Figure 1). The overall magnitude of this difference was small in comparison to the response difference of MCR and ACR with WCS to
atrazine or ALS inhibitors. MCR had the same mean biomass as WCS and ACR following
treatment with glufosinate or dicamba (Table 3.9). Visual estimates of dicamba injury and injury
symptomatology (epinasty and leaf malformation) were generally similar among all three
biotypes (Table 3.8). Estimates for dicamba control of MCR were much greater in the
greenhouse than the field, perhaps since more emphasis was placed on evaluating individual
plants rather than total biomass reduction in the field.

The response of MCR to mesotrione (105 g ai ha\(^{-1}\)) was similar to that observed in field
experiments. All MCR plants treated with mesotrione (96 plants in total across two runs of the
greenhouse experiment) survived to the 21 DAT harvest, though the amount of injury varied
from plant to plant. Conversely, all replicates (8 plants in total across two runs of the greenhouse
experiment) of WCS and ACR were completely controlled with mesotrione by 21 DAT (Table
3.8). Additionally, the accumulated biomass of MCR following treatment with mesotrione was
greater (p-value .001) than the average of WCS and ACR. Biomass accumulation of MCR
plants treated with lactofen was compared with ACR and WCS separately, since ACR is known
to be resistant to PPO-inhibiting herbicides (Patzoldt et al. 2005). MCR biomass was
significantly less (p-value .005) than ACR, while MCR biomass was significantly similar to the
PPO inhibitor-sensitive WCS (p-value .1422).

Collectively, results from these greenhouse and field experiments confirm the MCR
waterhemp population demonstrates multiple herbicide resistance to HPPD, PSII, and ALS
inhibitors. Furthermore, biomass and mortality data suggest a high percentage of plants survive
when labeled use rates of HPPD inhibitors are applied to 10 to 15 cm tall waterhemp.
Mesotrione alone or in combination with atrazine applied VEPOST (2 to 5 cm waterhemp)
increased control of MCR, although numerous plants survived. In general, PPO inhibitors,
glufosinate, and glyphosate provided the greatest control of MCR in both field and greenhouse experiments. HPPD inhibitor resistance is present in multiple states (Heap 2012) and could increase with additional selection intensity (Allen et al. 2011).

3.5 Source of Materials

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


3LC1. Sun Gro Horticulture, 15831 N.E. 8th Street, Bellevue, WA 98008.


3.6 Literature Cited


3.7 Tables

Table 3.1 Application rates of HPPD inhibitors applied alone or in combination with atrazine at McLean Co., IL (2010–2011).

<table>
<thead>
<tr>
<th>Herbicide(^{a})</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g ai ha(^{-1})</td>
</tr>
<tr>
<td>mesotrione</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>420</td>
</tr>
<tr>
<td>tembotrione</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>368</td>
</tr>
<tr>
<td>topramezone</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>72</td>
</tr>
<tr>
<td>mesotrione + atrazine</td>
<td>105 + 560</td>
</tr>
<tr>
<td></td>
<td>210 + 560</td>
</tr>
<tr>
<td></td>
<td>420 + 560</td>
</tr>
<tr>
<td>tembotrione + atrazine</td>
<td>92 + 560</td>
</tr>
<tr>
<td></td>
<td>184 + 560</td>
</tr>
<tr>
<td></td>
<td>368 + 560</td>
</tr>
<tr>
<td>topramezone + atrazine</td>
<td>18 + 560</td>
</tr>
<tr>
<td></td>
<td>36 + 560</td>
</tr>
<tr>
<td></td>
<td>72 + 560</td>
</tr>
<tr>
<td>atrazine</td>
<td>560</td>
</tr>
</tbody>
</table>

\(^{a}\)Herbicide treatments containing HPPD inhibitors included crop oil concentrate (COC 1% v/v) and 28% urea ammonium nitrate (UAN, 2.5% v/v); COC was included with the atrazine only treatment.
Table 3.2 Application rates of foliar-applied herbicides from seven site-of-action groups at McLean Co., IL (2010–2011).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site of Action&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rate&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Additives&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorimuron</td>
<td>ALS</td>
<td>11.2</td>
<td>NIS + UAN</td>
</tr>
<tr>
<td>imazethapyr</td>
<td>ALS</td>
<td>70</td>
<td>COC + UAN</td>
</tr>
<tr>
<td>glyphosate</td>
<td>EPSPS</td>
<td>840&lt;sup&gt;c&lt;/sup&gt;</td>
<td>AMS</td>
</tr>
<tr>
<td>glufosinate</td>
<td>GS</td>
<td>450</td>
<td>AMS</td>
</tr>
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<td>mesotrione</td>
<td>HPPD</td>
<td>105</td>
<td>COC + UAN</td>
</tr>
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<td>acifluorfen</td>
<td>PPO</td>
<td>420</td>
<td>COC + UAN</td>
</tr>
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<td>fomesafen</td>
<td>PPO</td>
<td>395</td>
<td>COC + UAN</td>
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<td>lactofen</td>
<td>PPO</td>
<td>140</td>
<td>COC + UAN</td>
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<tr>
<td>atrazine</td>
<td>PSII</td>
<td>1680</td>
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<td>2,4-D</td>
<td>synthetic auxin</td>
<td>270</td>
<td>-</td>
</tr>
<tr>
<td>dicamba</td>
<td>synthetic auxin</td>
<td>280&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NIS</td>
</tr>
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<sup>a</sup> 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.

<sup>b</sup> Abbreviations for additives: NIS, nonionic surfactant at 0.25% (v/v); UAN, 28% urea ammonium nitrate at 2.5% (v/v); COC, crop oil concentrate at 1% (v/v); AMS, ammonium sulfate at 2.5% (v/v).

<sup>c</sup> Acid equivalent (g ae ha<sup>-1</sup>).
Table 3.3 Application rates of foliar-applied herbicides from seven site-of-action groups in the greenhouse.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site of Action(^a)</th>
<th>Rate</th>
<th>Additives(^b)</th>
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<tbody>
<tr>
<td>chlorimuron</td>
<td>ALS</td>
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<td>NIS + UAN</td>
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<td>imazethapyr</td>
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<td>70</td>
<td>COC + UAN</td>
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<td>EPSPS</td>
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<td>AMS</td>
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<td>glufosinate</td>
<td>GS</td>
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<td>mesotrione</td>
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<tr>
<td>dicamba</td>
<td>synthetic auxin</td>
<td>280(^c)</td>
<td>NIS</td>
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\(^a\) 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.

\(^b\) Abbreviations for additives: NIS, nonionic surfactant at 0.25% (v/v); UAN, 28% urea ammonium nitrate at 2.5% (v/v); COC, crop oil concentrate at 1% (v/v); AMS, ammonium sulfate at 2.5% (v/v).

\(^c\) Acid equivalent (g ae ha\(^{-1}\)).
Table 3.4 Visual estimates of control and mean biomass of McLean Co. waterhemp.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Rate</th>
<th>7 DAT</th>
<th>14 DAT</th>
<th>% biomass of untreated</th>
<th>estimated difference$^b$</th>
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<tr>
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<td>17</td>
<td>.0065$^*$</td>
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<td>92 + 560</td>
<td>35</td>
<td>31</td>
<td>19</td>
<td>-2</td>
<td>.5281</td>
</tr>
<tr>
<td></td>
<td>184 + 560</td>
<td>48</td>
<td>50</td>
<td>12</td>
<td>-9</td>
<td>.0014</td>
</tr>
<tr>
<td></td>
<td>368 + 560</td>
<td>48</td>
<td>58</td>
<td>11</td>
<td>-10</td>
<td>.0001</td>
</tr>
<tr>
<td>topramezone + atrazine</td>
<td>18 + 560</td>
<td>35</td>
<td>33</td>
<td>16</td>
<td>-5</td>
<td>.2763</td>
</tr>
<tr>
<td></td>
<td>36 + 560</td>
<td>38</td>
<td>35</td>
<td>14</td>
<td>-7</td>
<td>.0049</td>
</tr>
<tr>
<td></td>
<td>72 + 560</td>
<td>40</td>
<td>46</td>
<td>12</td>
<td>-9</td>
<td>.0018</td>
</tr>
<tr>
<td>atrazine</td>
<td>560</td>
<td>4</td>
<td>3</td>
<td>82</td>
<td>61</td>
<td>.0001$^*$</td>
</tr>
</tbody>
</table>

$^a$ Significant at $\alpha = .05$, of interest were treatments with positive differences indicating growth.
Table 3.4 (cont.)

\(^a\) Plants harvested the day of spraying to assess biomass accumulation after herbicide application.

\(^b\) Estimated difference in dry weight (as a % of the untreated) between 12 herbicide treated plants and 12 pre treatment plants.
Table 3.5 Mortality of twelve marked waterhemp per treatment used in assessment of biomass accumulation, expressed as a percent. Mortality data were collected only in 2011.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Rate</th>
<th>Mortality (14 DAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g ai ha(^{-1})</td>
<td>%</td>
</tr>
<tr>
<td>mesotrione</td>
<td>105</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>420</td>
<td>25</td>
</tr>
<tr>
<td>tembotrione</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>368</td>
<td>50</td>
</tr>
<tr>
<td>topramezone</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>67</td>
</tr>
<tr>
<td>mesotrione + atrazine</td>
<td>105 + 560</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>210 + 560</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>420 + 560</td>
<td>83</td>
</tr>
<tr>
<td>tembotrione + atrazine</td>
<td>92 + 560</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>184 + 560</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>368 + 560</td>
<td>75</td>
</tr>
<tr>
<td>topramezone + atrazine</td>
<td>18 + 560</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>36 + 560</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>72 + 560</td>
<td>83</td>
</tr>
<tr>
<td>atrazine</td>
<td>560</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3.6 Visual estimates of McLean Co. waterhemp control, and contrast statements, after foliar applications of mesotrione and atrazine alone or in combination. Applications timings based on waterhemp height: 2 to 5 cm (VEPOST), 5 to 10 cm (EPOST), 10 to 15 cm (POST).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Application Timing</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEPOST</td>
<td>EPOST</td>
<td>POST</td>
<td>DAT</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>mesotrione</td>
<td>69</td>
<td>66</td>
<td>47</td>
<td>27</td>
<td>21</td>
<td>13</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>atrazine</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>mesotrione + atrazine</td>
<td>78</td>
<td>80</td>
<td>63</td>
<td>53</td>
<td>36</td>
<td>30</td>
<td>30</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>Estimated difference(^b)</th>
<th>Standard error</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>mesotrione</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEPOST vs. EPOST</td>
<td>45</td>
<td>6.56</td>
<td>.0005*</td>
</tr>
<tr>
<td>VEPOST vs. POST</td>
<td>56</td>
<td>6.56</td>
<td>.0002*</td>
</tr>
<tr>
<td>EPOST vs. POST</td>
<td>11</td>
<td>6.56</td>
<td>.1515</td>
</tr>
<tr>
<td>mesotrione + atrazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEPOST vs. EPOST</td>
<td>44</td>
<td>6.56</td>
<td>.0006*</td>
</tr>
<tr>
<td>VEPOST vs. POST</td>
<td>54</td>
<td>6.56</td>
<td>.0002*</td>
</tr>
<tr>
<td>EPOST vs. POST</td>
<td>10</td>
<td>6.56</td>
<td>.1800</td>
</tr>
</tbody>
</table>

\(^a\) Significant at \(\alpha = .05\).

\(^b\) 21 DAT ratings only taken in 2011.

\(^a\) Estimated difference of mean control between application timings within mesotrione alone or mesotrione plus atrazine treatments.
Table 3.7 Visual estimates of McLean Co. waterhemp control with herbicides representing seven site-of-action groups. Ratings with the same letter within a column are not significantly different at \( \alpha = .05 \) (separated by the SAS macro \%pdmix800).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site of Action(^a)</th>
<th>7 DAT</th>
<th>14 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorimuron</td>
<td>ALS</td>
<td>8 c</td>
<td>6 e</td>
</tr>
<tr>
<td>imazethapyr</td>
<td>ALS</td>
<td>12 c</td>
<td>6 de</td>
</tr>
<tr>
<td>glyphosate</td>
<td>EPSPS</td>
<td>88 a</td>
<td>89 a</td>
</tr>
<tr>
<td>glufosinate</td>
<td>GS</td>
<td>81 a</td>
<td>68 b</td>
</tr>
<tr>
<td>mesotrione</td>
<td>HPPD</td>
<td>30 b</td>
<td>19 cd</td>
</tr>
<tr>
<td>acifluorfen</td>
<td>PPO</td>
<td>86 a</td>
<td>75 ab</td>
</tr>
<tr>
<td>fomesafen</td>
<td>PPO</td>
<td>93 a</td>
<td>89 a</td>
</tr>
<tr>
<td>lactofen</td>
<td>PPO</td>
<td>90 a</td>
<td>87 a</td>
</tr>
<tr>
<td>atrazine</td>
<td>PSII</td>
<td>8 c</td>
<td>8 de</td>
</tr>
<tr>
<td>2,4-D</td>
<td>synthetic auxin</td>
<td>23 bc</td>
<td>16 cde</td>
</tr>
<tr>
<td>dicamba</td>
<td>synthetic auxin</td>
<td>33 b</td>
<td>26 c</td>
</tr>
</tbody>
</table>

\(^a\) 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.
Table 3.8  Mean visual estimates of MCR, ACR and WCS waterhemp populations 7, 14, and 21 days after herbicide treatment under greenhouse conditions.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site of Action(^a)</th>
<th>7 DAT</th>
<th>14 DAT</th>
<th>21 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WCS</td>
<td>ACR</td>
<td>MCR</td>
</tr>
<tr>
<td>chlorimuron</td>
<td>ALS</td>
<td>88</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>imazethapyr</td>
<td>ALS</td>
<td>89</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>glyphosate</td>
<td>EPSPS</td>
<td>96</td>
<td>96</td>
<td>76</td>
</tr>
<tr>
<td>glufosinate</td>
<td>GS</td>
<td>95</td>
<td>94</td>
<td>92</td>
</tr>
<tr>
<td>mesotrione</td>
<td>HPPD</td>
<td>91</td>
<td>88</td>
<td>64</td>
</tr>
<tr>
<td>lactofen</td>
<td>PPO</td>
<td>96</td>
<td>48</td>
<td>88</td>
</tr>
<tr>
<td>atrazine</td>
<td>PSII</td>
<td>91</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>dicamba</td>
<td>synthetic auxin</td>
<td>82</td>
<td>86</td>
<td>81</td>
</tr>
</tbody>
</table>

\(^a\) 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.
Tables 3.9  Mean separation of MCR dry weights after herbicide application, as well as single degree of freedom contrast statements comparing MCR with ACR and WCS under greenhouse conditions. Plants harvested 21 DAT.

<table>
<thead>
<tr>
<th>MCR treatment comparison&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Estimate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Std. error&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>atrazine</td>
<td>2.62</td>
<td>.043</td>
<td>-</td>
</tr>
<tr>
<td>imazethapyr</td>
<td>2.49</td>
<td>.043</td>
<td>.2140</td>
</tr>
<tr>
<td>chlorimuron</td>
<td>2.45</td>
<td>.043</td>
<td>.1153</td>
</tr>
<tr>
<td>mesotrione</td>
<td>1.26</td>
<td>.043</td>
<td>.0001</td>
</tr>
<tr>
<td>dicamba</td>
<td>1.20</td>
<td>.043</td>
<td>.0001</td>
</tr>
<tr>
<td>glyphosate</td>
<td>0.95</td>
<td>.043</td>
<td>.0001</td>
</tr>
<tr>
<td>lactofen</td>
<td>0.89</td>
<td>.043</td>
<td>.0001</td>
</tr>
<tr>
<td>glufosinate</td>
<td>0.70</td>
<td>.043</td>
<td>.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contrasts&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Num DF</th>
<th>Den DF</th>
<th>Estimated difference&lt;sup&gt;d&lt;/sup&gt;</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCR vs. WCS, ACR glyphosate</td>
<td>1</td>
<td>5.24</td>
<td>.3268</td>
<td>11.91</td>
<td>.0169*</td>
</tr>
<tr>
<td>MCR vs. WCS, ACR glufosinate</td>
<td>1</td>
<td>4.75</td>
<td>.0789</td>
<td>0.73</td>
<td>.4335</td>
</tr>
<tr>
<td>MCR vs. WCS, ACR mesotrione</td>
<td>1</td>
<td>6.7</td>
<td>.5542</td>
<td>30.32</td>
<td>.0010*</td>
</tr>
<tr>
<td>MCR vs. ACR lactofen</td>
<td>1</td>
<td>11.8</td>
<td>-.6508</td>
<td>11.79</td>
<td>.0050*</td>
</tr>
<tr>
<td>MCR vs. WCS lactofen</td>
<td>1</td>
<td>5.92</td>
<td>.1908</td>
<td>2.86</td>
<td>.1422</td>
</tr>
<tr>
<td>MCR vs. WCS, ACR dicamba</td>
<td>1</td>
<td>7.7</td>
<td>.2374</td>
<td>5.38</td>
<td>.0502</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant at α = .05.

<sup>a</sup> MCR %MCB mean separation test that compares the herbicide treatment resulting in the highest dry weight with all other herbicide treatments.

<sup>b</sup> Estimated mean dry weight per treatment for MCR. Data subject to square root transformation.

<sup>c</sup> Single degree of freedom contrast statements. MCR dry weight is compared with the average of WCS and ACR except in lactofen comparison.

<sup>d</sup> Differences of mean dry weight between MCR and sensitive biotypes ACR and WCS.
3.8 Figures

Figure 3.1 Response of MCR, ACR and WCS following foliar applications of herbicides representing seven site-of-action groups under greenhouse conditions. Data are dry weights collected 21 DAT, and presented as a percent of the untreated (± 1 SEM).

CHAPTER 4
CHARACTERIZING THE RESPONSE OF AN HPPD-RESISTANT WATERHEMP
(AMARANTHUS TUBERCULATUS) POPULATION TO SOIL-RESIDUAL
HERBICIDES

4.1 Abstract

Field experiments were conducted in 2010 and 2011 at a Mclean County, IL seed corn production field where resistance to foliar-applied 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors was confirmed in waterhemp. Corn herbicides were applied at 1 and 2x the recommended field use rate, while soybean herbicides were applied only at 1x the recommended rate. Waterhemp control and density were determined 30 and 60 days after treatment (DAT) of soil-applied herbicides. At 30 DAT in corn, 1x rates of mesotrione, safened and unsafened isoxaflutole formulations, atrazine, and S-metolachlor provided less than 70% control of waterhemp, while control with acetochlor was greater than 80%. Mesotrione and unsafened isoxaflutole at 2x rates increased control compared with their respective 1x rates in 2010 and 2011, while the 2x rate of safened isoxaflutole increased control in 2010. At 30 DAT in corn, 1 and 2x rates of acetochlor, and 2x rates of mesotrione and unsafened isoxaflutole, provided the greatest reduction of waterhemp density across years. At 60 DAT, waterhemp control and density generally declined for all treatments in 2010, while densities in several treatments were generally similar to their 30 DAT results in 2011. At 30 DAT in soybean, sulfentrazone, flumioxazin, metribuzin and pyroxasulfone provided the highest levels of waterhemp control (84 to 92%), as well as the greatest reduction in waterhemp density in both years. Similar to results in corn, waterhemp control and density generally declined between evaluation timings for treatments in
2010, but the decrease in density was not present in 2011. A dose response experiment with soil-applied mesotrione was performed under controlled greenhouse conditions using three waterhemp populations; MCR15 (derived from seed collected from the McLean Co. site), NH41 (RxR progeny derived from a MCR15 by MCR16 cross), and a sensitive control. Count data collected 21 DAT demonstrated higher seedling survival of MCR15 and NH41 at mesotrione rates of 105 g ha\(^{-1}\) or less compared with the sensitive control. Resistant-to-sensitive (R/S) ratios for NH41 and MCR15 were 12.7 and 8.8, respectively.

4.2 Introduction

Waterhemp \(\textit{Amaranthus tuberculatus}\) (Moq.) Sauer is a small-seeded, dioecious, summer annual broadleaf species native to much of the Midwest (Sauer 1955, 1957). This weed species can significantly reduce the seed yield of corn \(\textit{Zea mays}\) L.) and soybean \(\textit{Glycine max}\) (L.) Merr) through competition for limited resources (Bensch et al. 2003; Cordes et al. 2004; Hager et al. 2002b; Steckel and Sprague 2004). A prolific seed producer, individual female waterhemp plants can produce in excess of one million seeds (Hartzler et al. 2004; Steckel et al. 2003) that can remain dormant for years (Buhler and Hartzler 2001; Burnside et al. 1996). Hartzler et al. (1999) reported waterhemp emergence generally occurs over a more prolonged period relative to other annual weeds. These characteristics can increase the likelihood of waterhemp seeds augmenting the weed seed bank of agronomic fields (Buhler et al. 2001).

The evolution of waterhemp populations resistant to various herbicides has contributed to the increased presence of this weed species in Illinois agronomic fields over the last decade (Hager et al. 1997). Prior to 2009, waterhemp had evolved resistance to four herbicide site-of-action groups (Heap 2012): acetyl-CoA synthase (ALS) inhibitors, photosystem II (PSII)
inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) inhibitors. Populations of waterhemp demonstrating resistance to multiple herbicide groups further reduces chemical control options (Bell et al. 2009; Foes et al. 1998; Patzoldt et al. 2005). Integrated management systems, which often include the utilization of soil-residual herbicides, are essential for control of waterhemp (Hager et al. 1997).

Sweat et al. (1998) reported control of a Kansas waterhemp biotype with preemergence (PRE) herbicides was greater than 85% by 28 days after treatment (DAT); however, ALS inhibitors provided only 70% or less control of an Iowa biotype. Shoup and Al-Khatib (2004) reported all soil-applied herbicides controlled a Kansas waterhemp population resistant to foliar-applied PPO inhibitors greater than 75% prior to any foliar herbicide application. Certain inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD), such as isoxaflutole and mesotrione, can be applied to the soil for residual control of annual weeds including Amaranthus spp. (Luscombe and Pallett 1996; Wichert et al. 1999). Vyn et al. (2006) reported waterhemp control was greater than 95% 70 days after corn emergence (DAE) with PRE-applied mesotrione alone or isoxaflutole plus atrazine.

Weed resistance to foliar-applied HPPD inhibitors is a relatively recent phenomenon (Heap 2012). Waterhemp populations from McLean Co., IL (Hausman et al. 2011) and Henry Co., IA (McMullan and Green 2011), as well as two biotypes of Palmer amaranth from Kansas (Lally et al. 2010), have demonstrated reduced sensitivity to various foliar-applied HPPD inhibitors. However, the response of these populations to soil-residual herbicides applied before weed emergence has not been well documented. Therefore, this research was initiated to characterize the response of the Illinois HPPD-resistant waterhemp population to several soil-applied herbicides under field conditions. Furthermore, this research also compares and
quantifies the responses of HPPD inhibitor-resistant waterhemp, RxR progeny derived from greenhouse crosses of HPPD-resistant waterhemp, and a known HPPD inhibitor sensitive biotype to a range of soil-applied mesotrione rates under greenhouse conditions.

### 4.3 Materials and Methods

#### 4.3.1 Response of the Illinois HPPD-resistant waterhemp population to soil-applied herbicides under field conditions

**4.3.1.1 General field methods**

Field experiments were conducted in 2010 and 2011 at the McLean Co., IL field location where the HPPD-resistant waterhemp population was initially identified. Herbicides applied at this location in previous seasons are presented in Table 4.1. The soil was a Sable silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls), with a pH of 6.4 and 3.2% organic matter. Preplant tillage was performed each spring to prepare the seedbed for planting and to control emerged weeds. Experiments were conducted either in corn (GH 8914RRLL 3000 GT) or soybean (S30-F5), planted in 76 cm rows. Corn and soybean were planted on May 6 in 2010, while in 2011 corn and soybean were planted on May 5 and May 19, respectively. Precipitation data during the duration of the experiments (May, June, and July) are presented in Table 4.2. 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 frequently used in Illinois cropping systems and that represent several site-of-action families were selected for evaluation. These active ingredients, their respective rates and site-of-action group, are presented in Tables 4.3 (corn experiment) and 4.4 (soybean experiment).
Herbicides in the corn experiment were applied at 1 and 2x their recommended field use rate based on soil type and organic matter content, except S-metolachlor which was applied at the 1x rate only. All herbicides in the soybean experiment were applied only at their label-recommended rate. All soil-applied herbicides were applied the day of crop planting. Herbicides were applied using a pressurized CO₂ backpack sprayer calibrated to deliver 187 L ha⁻¹ at 276 kPa with a 3-m spray boom fitted with AI110025VS nozzles spaced 51 cm apart.

Visual estimates of waterhemp control were recorded 30 and 60 DAT using a scale of 0 (no control) to 100 (complete control). These estimates were based on waterhemp injury, biomass, and stand reduction when compared with the nontreated control. Waterhemp density within a 0.25 m² quadrat was recorded 30 and 60 DAT. Density counts were conducted between the two middle crop rows and recorded from the same location for both measurements.

4.3.1.2 Statistical analysis

Statistical analysis was performed using PROC Mixed in SAS 9.2² with herbicide treatment considered a fixed effect. Random effects included year, block within year, and all interactions containing either of these effects. Mean estimates of waterhemp control and density were separated using the SAS macro %pdmix800 (Saxton 1998) with α = .05. To meet the assumptions of normality and equal variance, estimates of control were subject to an arcsine square root transformation, and density counts subject to a square root transformation. Initial analysis revealed significant year by treatment interactions in both corn and soybean experiments (α = .05); thus, estimates of control and density are presented by year.
4.3.2 Soil-applied mesotrione dose response under greenhouse conditions

4.3.2.1 Waterhemp populations

Inflorescences of several female waterhemp not controlled with foliar applied HPPD-inhibiting herbicides were collected from the McLean Co. location in August 2009. Seed collected from individual females were treated as unique accessions and stratified in a 0.1% agarose solution at 4°C for 30 days. One of these accessions, MCR15, was selected to characterize the field population response to soil-applied mesotrione. The response of MCR15 was compared with the response of RxR progeny (designated NH41 and described in subsequent paragraph) and a population confirmed sensitive to HPPD inhibitors (designated S).

4.3.2.2 Development of RxR progeny (designated NH41)

A resistant by resistant cross (RxR) was conducted to enhance uniformity of the McLean Co. waterhemp population in its response to HPPD inhibitors. Paternal and maternal waterhemp used to produce the RxR progeny (designated NH41) were derived from the unique accessions MCR15 and MCR16 collected from the McLean Co. field location. Previous research had reported waterhemp grown from these two accessions were resistant to foliar-applied mesotrione, tembotrione, or topramezone (Hausman et al. 2011). Plants grown in the greenhouse from MCR15 and MCR16 were treated with 158 g mesotrione ha⁻¹ plus crop oil concentrate (COC) at 1% (v/v) and liquid ammonium sulfate (AMS) at 2.5% (v/v) when they were 10 cm tall. Individual waterhemp demonstrating the highest levels of resistance were selected, and a male MCR15 was crossed with a female MCR16. Crossing was performed within an enclosed growth chamber to exclude all foreign pollen. At seed maturation, seeds were removed from the female inflorescence, cleaned and stratified as describe previously.
4.3.2.3 Plant culture

Plastic pots (720 cm$^3$) were filled to the top with growth medium (1:1:1 mixture of soil, peat and sand, with a pH of 6.8 and 3.5% organic matter) then tamped to produce a smooth and level planting surface. The perforated pots were allowed to soak in water for 12 hours to ensure uniform moisture distribution. After soaking, 25 seeds from one of three waterhemp populations were sown on the surface in a 5 by 5 grid with a 1-cm spacing between the pot sides and the seeds of outside rows. After sowing, an additional 50 ml of the same growth medium was passed through a 3.35 mm testing sieve, spread evenly across the top, and tamped down to produce a flat surface. Pots were then watered over the top with a 1.9 liter per minute (LPM) mister nozzle until the medium surface was moist to the touch. Greenhouse conditions were maintained at 28/22 C 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 medium surface.

4.3.2.4 Mesotrione application and statistical analysis

Mesotrione was applied to the pots the same day of planting at increasing rates equally spaced along a base 3.16 logarithmic scale. The S population was treated with a rate range of 3.2 to 1050 g mesotrione ha$^{-1}$, while the rate range for MCR15 and NH41 was 10.5 to 3310 g mesotrione ha$^{-1}$. Mesotrione was applied using a compressed air research sprayer$^3$ fitted with a TeeJet 80015 EVS nozzle$^1$ positioned 46 cm above the medium surface. The sprayer was calibrated to deliver 185 L ha$^{-1}$ at 275 kPa. After all herbicide treatments were applied, the spray chamber was fitted with an 8005 E nozzle and recalibrated to deliver 7 ml of water per pot to move the applied herbicide into the growth medium. Pots were then moved back into the greenhouse and arranged in a randomized complete block design with each treatment replicated.
four times, and the experiment conducted twice. Pots were surface watered with a 1.9 LPM mister nozzle twice a day until the medium surface was moist to the touch.

At 21 DAT, seedling survivors per pot were counted. Count data were converted as a percentage of the untreated control. Initial analysis performed with PROC Mixed in SAS 9.2 revealed no significant difference between experiment runs and no significant run by treatment interaction ($\alpha = .05$); thus, data were pooled. Combined data were analyzed using non-linear regression with the dose-response curve package in R software (Knezevic et al. 2007). The dose-response model was constructed using the equation

$$y = c + \frac{d - c}{1 + \exp \{b[\log(x) - \log(LD_{50})]\}}$$  \hspace{1cm} [1]

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 $LD_{50}$ is 50% reduction in seedling survival.

4.4 Results and Discussion

4.4.1 Response of the Illinois HPPD-resistant waterhemp population to soil-applied herbicides under field conditions

4.4.1.1 Corn experiment

At 30 DAT, waterhemp control in 2010 and 2011 was less than 70% (Table 4.5) for all herbicides applied at 1x except acetochlor which provided 87 and 83% control, respectively.

Across both years, the 1x rates of mesotrione and both isoxaflutole formulations provided similar control (53 to 68%). In 2010, atrazine (1 and 2x rates) and S-metolachlor provided the lowest levels of waterhemp control. While waterhemp control with atrazine increased in 2011, the level
of control did not exceed 80% regardless of application rate. In 2010, waterhemp control increased with the 2x rate of each HPPD inhibitor formulation (73 to 90%) compared with the corresponding 1x rates, but a similar increase did not occur with 2x rates of acetochlor or atrazine. In 2011, only the 2x rates of mesotrione and the unsafened isoxaflutole formulation increased control compared with their respective 1x rates.

Waterhemp density in the untreated plots averaged 1067 and 260 plants m\(^{-2}\) 30 DAT in 2010 and 2011, respectively (Table 4.5). With the exception of S-metolachlor and 1x rate of atrazine, all herbicide treatments reduced waterhemp density compared with the untreated in 2010. The greatest reduction in density occurred with 1 or 2x rates of acetochlor, and the 2x rates of each HPPD inhibitor, though numerous waterhemp plants remained (48 to 191 plants m\(^{-2}\)). In 2011, 1x rates of atrazine, both isoxaflutole formulations and S-metolachlor did not decrease waterhemp density compared with the untreated. The greatest reduction in waterhemp density across both years occurred with 1 or 2x rates of acetochlor and the 2x rates of mesotrione and the unsafened isoxaflutole formulation.

At 60 DAT, waterhemp control was generally lower than at 30 DAT across both years (Table 4.5), although the decrease in waterhemp control between evaluation timings was more substantial in 2011 than in 2010. Decreased control was often attributable to the presence of several large, healthy waterhemp plants that had survived the herbicide rather than substantial emergence of additional waterhemp plants. Several surviving plants were larger than individual waterhemp plants in the untreated due to decreased competition; a similar response also was observed by Shoup and Al-Khatib (2004). Acetochlor, regardless of application rate, and the 2x application rates of both isoxaflutole formulations provided 82% or greater waterhemp control in 2010. Control with either rate of mesotrione did not exceed 50%, while control with atrazine or
S-metolachlor was less than 10%. In 2011, only the 2x rate of acetochlor provided control greater than 80% 60 DAT.

Waterhemp density generally decreased by 60 DAT compared with density at 30 DAT, although the reduction in density was more pronounced in 2010 than in 2011 (Table 4.5). Waterhemp density in the untreated plots decreased 66 and 44% in 2010 and 2011, respectively. In 2010, the greatest reduction in waterhemp density 60 DAT occurred with 1 or 2x rates of acetochlor and the 2x rates of each HPPD inhibitor. In 2011, the greatest reduction in waterhemp density occurred with both rates of acetochlor and the 2x rate of mesotrione.

These results indicate that the soil-applied HPPD inhibitors included in this research provided partial control of a known HPPD inhibitor-resistant waterhemp population (Hausman et al. 2011), although control at recommended field use rates (1x) did not exceed 68% across years. These results are in contrast to previous research evaluating the efficacy of soil-applied HPPD-inhibiting herbicides on sensitive waterhemp populations. Shoup and Al-Khatib (2004) reported 100% waterhemp control with isoxaflutole applied PRE prior to application of foliar-applied herbicides. Mesotrione applied PRE controlled waterhemp greater than 95% up to 70 DAE in a multiple location study by Vyn et al. (2006). The authors also reported waterhemp density 70 DAE in mesotrione-treated plots ranged from 0 to 6 plants m⁻², depending on location. In the present research, waterhemp control with S-metolachlor 30 DAT was less than 20%, in contrast with Steckel et al. (2002) who reported 95% control with S-metolachlor at a similar evaluation timing. At 60 DAT, waterhemp densities in atrazine-treated plots were similar to the untreated, consistent with the response of a PSII inhibitor-resistant waterhemp population reported by Vyn et al. (2006). Hausman et al. (2011) reported waterhemp grown from seed collected at the McLean Co. location displayed resistance to foliar-applied atrazine. Repeated use of S-
metolachlor and triazine herbicides (Table 4.1) most likely contributed to the reduced waterhemp sensitivity observed in these treatments. Acetochlor effectively controlled this waterhemp population, a finding that has been repeatedly documented in the scientific literature (Shoup and Al-Khatib 2004; Steckel et al. 2002; Sweat et al. 1998).

4.4.1.2 Soybean experiment

Across both years, sulfentrazone, flumioxazin, metribuzin, and pyroxasulfone provided the greatest control of waterhemp (84 to 92%) 30 DAT (Table 4.6). In 2010, cloransulam and saflufenacil provided the least control (5 and 20%, respectively), while in 2011 cloaransulam, pendimethalin, and linuron provided the lowest control (30 to 38%). Waterhemp density in the untreated 30 DAT was 395 and 96 plants m⁻² in 2010 and 2011, respectively (Table 4.6). Saflufenacil, linuron, and S-metolachlor failed to reduce waterhemp density compared with the untreated in both years. Additionally, cloransulam and imazethapyr did not reduce waterhemp density in 2010, while pendimethalin and dimethenamid failed to decrease waterhemp density in 2011. Treatments that provided the greatest reduction in waterhemp density 30 DAT in both years included sulfentrazone, flumioxazin, metribuzin, and pyroxasulfone, which was similar to control rating results.

Waterhemp control generally declined between evaluations for the majority of treatments both years (Table 4.6). At 60 DAT, sulfentrazone, flumioxazin, and metribuzin controlled waterhemp 90% or greater in 2010, while no treatment controlled waterhemp greater than 82% in 2011. Treatments that demonstrated the greatest reduction in waterhemp density by 60 DAT in 2010 and 2011 included sulfentrazone, flumioxazin, alachlor, metribuzin, and pyroxasulfone. Waterhemp density was generally reduced between 30 and 60 DAT evaluations in 2010.
However, this trend was not observed between evaluations in 2011 as waterhemp densities at 60 DAT were similar to their respective 30 DAT densities. A similar response was observed in the corn experiment. Decreased intra-specific competition among waterhemp plants may have contributed to this, as waterhemp densities in untreated plots were much smaller in 2011 compared with 2010. Even though extended waterhemp emergence late in the season (Hartzler et al. 1999) could have influenced density 60 DAT, the majority of counted waterhemp plants were larger plants (greater than 15 cm), suggesting they were most likely present at the 30 DAT evaluation.

Effective waterhemp control was observed with the PPO inhibitors sulfentrazone and flumioxazin, consistent with previous research (Hager et al. 2002a; Shoup and Al-Khatib 2004; Sweat et al. 1998). With the exception of metribuzin, results were similar to Hager et al. (2002) who reported control of waterhemp eight weeks after PRE application was less than 50% with metribuzin, metolachlor, dimethenamid, pendimethalin, and linuron. By 28 DAT, Sweat et al. (1998) reported decreased control of an Iowa waterhemp population with soil-applied ALS inhibitors, a response similar to that observed with the McLean Co. waterhemp population. Results of field experiments conducted in 2010 and 2011 with various foliar-applied herbicides including chlorimuron and imazethapyr (data not shown) suggest the McLean Co. population demonstrates reduced sensitivity to ALS inhibitors. Several factors can influence the efficacy of soil-applied herbicides, such as precipitation and timing of weed emergence. Thus, waterhemp response to a soil-applied HPPD inhibitor under controlled greenhouse conditions was conducted and described in the next section.
4.4.2 Soil-applied mesotrione dose response under greenhouse conditions

Emergence of the three waterhemp biotypes was uniform and the majority of waterhemp plants (approximately 90%) began to emerge 4 to 5 days after planting. Injury, when observed on emerged seedlings, became noticeable within 1 day after emergence and included bleached cotyledons, stunted growth, and necrotic tissue, which became more pronounced as herbicide rate increased. The severity of injury at lower mesotrione rates was more prevalent in the S biotype than either MCR15 or NH41. By 21 DAT, complete mortality of the S population was achieved at 31.5 g mesotrione ha$^{-1}$, while some pots of MCR15 and NH41 treated with 315 g mesotrione ha$^{-1}$ contained actively growing waterhemp with at least one true leaf (Figure 4.1). LD$_{50}$ values (± 1 standard error) were calculated to be 63.3 (± 8.2), 43.8 (± 4.9), and 5.0 (± 0.5) g mesotrione ha$^{-1}$ for NH41, MCR15, and S respectively. Thus, resistant-to-sensitive (R/S) ratios for NH41 and MCR15 were 12.7 and 8.8, respectively. Hausman et al. (2011) reported a similar R/S ratio between MCR15 and a HPPD inhibitor sensitive waterhemp population when mesotrione was applied to the foliage, although these values were derived using plant dry weight measurements as opposed to seedling survival reported herein. The smaller R/S ratio of MCR15 as compared with NH41 is not surprising as MCR15 was field-collected seed, while NH41 was derived from an additional round of selection for resistance to HPPD inhibitors.

Results of field and greenhouse experiments indicate the McLean Co. waterhemp population demonstrates reduced sensitivity to soil-residual HPPD inhibitors applied at recommend use rates. In field research, herbicides with alternative sites of action, such as acetochlor, sulfentrazone, flumioxazin, metribuzin, and pyroxasulfone, generally provided the highest levels of waterhemp control and greatest reduction of waterhemp density. However, it should be noted that complete control of waterhemp with soil-applied herbicides was not
achieved at either 30 or 60 DAT, thus necessitating the need for an integrated management approach for the McLean Co. population. This could include utilizing soil-residual herbicides followed by cultivation and/or application of postemergence herbicides (Hager et al. 1997). Several studies have reported effective waterhemp control using multiple herbicide application strategies (Schuster and Smeda 2007; Shoup and Al-Khatib 2004; Steckel et al. 2002). Herbicide resistance in waterhemp (Heap 2012) severely limits foliar-applied herbicide options producers can use to manage waterhemp, undoubtedly placing more importance on soil-applied residual herbicides.

4.5 Source of Materials

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


4.6 Literature Cited


population of waterhemp (Amaranthus tuberculatus) from Illinois, United States. Pest
Manage. Sci. 67:258-261.


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

Lally, N. G., C. R. Thompson, and D. Peterson. 2010. Palmer amaranth differential response to

7:29-32.

tuberculatus) biotype resistant to HPPD-inhibiting herbicides, atrazine, and

bio-type with multiple resistance across three herbicide sites of action. Weed Sci. 53:30-
36.


Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in Proc


### 4.7 Tables

**Table 4.1** Herbicide use history at the McLean Co., IL field location.

<table>
<thead>
<tr>
<th>Year</th>
<th>Soil-applied</th>
<th>Foliar-applied&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>S-metolachlor + simazine</td>
<td>mesotrione + atrazine</td>
</tr>
<tr>
<td>2004</td>
<td>S-metolachlor + simazine</td>
<td>mesotrione + atrazine</td>
</tr>
<tr>
<td>2005</td>
<td>S-metolachlor + simazine</td>
<td>mesotrione + atrazine</td>
</tr>
<tr>
<td>2006</td>
<td>S-metolachlor + simazine</td>
<td>topramezone + atrazine</td>
</tr>
<tr>
<td>2007</td>
<td>S-metolachlor + simazine</td>
<td>topramezone + atrazine</td>
</tr>
<tr>
<td>2008</td>
<td>S-metolachlor + simazine</td>
<td>tembotrione followed by mesotrione</td>
</tr>
<tr>
<td>2009</td>
<td>S-metolachlor + simazine</td>
<td>tembotrione followed by mesotrione</td>
</tr>
</tbody>
</table>

<sup>a</sup>Herbicides were applied with spray additives per label recommendations after crop and weed emergence.
Table 4.2 Precipitation recorded at the McLean Co., IL field location during the field experiments of 2010 and 2011.

<table>
<thead>
<tr>
<th>Month</th>
<th>Precipitation</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cm</td>
<td>cm</td>
</tr>
<tr>
<td>May</td>
<td></td>
<td>11.8</td>
<td>6.6</td>
</tr>
<tr>
<td>June</td>
<td></td>
<td>5.9</td>
<td>13.9</td>
</tr>
<tr>
<td>July</td>
<td></td>
<td>4.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22.5</td>
<td>21.1</td>
</tr>
</tbody>
</table>
Table 4.3 Application rates of soil-applied herbicides at McLean Co., IL (2010–2011).

Herbicides were applied preemergence (PRE) in corn.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site of Action</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoxaflutole + safener</td>
<td>HPPD</td>
<td>105</td>
</tr>
<tr>
<td>isoxaflutole + safener</td>
<td>HPPD</td>
<td>210</td>
</tr>
<tr>
<td>isoxaflutole</td>
<td>HPPD</td>
<td>105</td>
</tr>
<tr>
<td>isoxaflutole</td>
<td>HPPD</td>
<td>210</td>
</tr>
<tr>
<td>mesotrione</td>
<td>HPPD</td>
<td>210</td>
</tr>
<tr>
<td>mesotrione</td>
<td>HPPD</td>
<td>420</td>
</tr>
<tr>
<td>atrazine</td>
<td>PSII</td>
<td>1680</td>
</tr>
<tr>
<td>atrazine</td>
<td>PSII</td>
<td>3360</td>
</tr>
<tr>
<td>acetochlor</td>
<td>VLCFAE</td>
<td>1680</td>
</tr>
<tr>
<td>acetochlor</td>
<td>VLCFAE</td>
<td>3360</td>
</tr>
<tr>
<td>S-metolachlor</td>
<td>VLCFAE</td>
<td>1600</td>
</tr>
</tbody>
</table>

*Abbreviations for site of action: HPPD, hydroxyphenylpyruvate dioxygenase; PSII, photosystem II; VLCFAE, very long chain fatty acid elongases.

*b cyprosulfamide.
Table 4.4 Application rates of soil-applied herbicides at McLean Co., IL (2010–2011).

Herbicides were applied preemergence (PRE) in soybean.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Site of Action⁠a</th>
<th>Rate ( \text{g ai ha}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>cloransulam</td>
<td>ALS</td>
<td>18</td>
</tr>
<tr>
<td>imazethapyr</td>
<td>ALS</td>
<td>70</td>
</tr>
<tr>
<td>clomazone</td>
<td>DOXPS</td>
<td>840</td>
</tr>
<tr>
<td>pendimethalin</td>
<td>tubulin</td>
<td>1335</td>
</tr>
<tr>
<td>flumioxazin</td>
<td>PPO</td>
<td>70</td>
</tr>
<tr>
<td>saflufenacil</td>
<td>PPO</td>
<td>25</td>
</tr>
<tr>
<td>sulfentrazone</td>
<td>PPO</td>
<td>280</td>
</tr>
<tr>
<td>linuron</td>
<td>PSII</td>
<td>840</td>
</tr>
<tr>
<td>metribuzin</td>
<td>PSII</td>
<td>420</td>
</tr>
<tr>
<td>alachlor</td>
<td>VLCFAE</td>
<td>2240</td>
</tr>
<tr>
<td>dimethenamid</td>
<td>VLCFAE</td>
<td>940</td>
</tr>
<tr>
<td>pyroxasulfone</td>
<td>VLCFAE</td>
<td>210</td>
</tr>
<tr>
<td>S-metolachlor</td>
<td>VLCFAE</td>
<td>1425</td>
</tr>
</tbody>
</table>

⁠a Abbreviations for site of action: ALS, acetolactate synthase; DOXPS, deoxy-D-xyulose 5-phosphate synthase; PPO, protoporphyrinogen oxidase; PSII, photosystem II; VLCFAE, very long chain fatty acid elongases.
Table 4.5 Mean estimates of control and density of McLean Co., IL waterhemp 30 and 60 days after treatment (DAT) of soil-applied herbicides in corn. Means with the same letter within a column are not significantly different at α = .05 (separated by the SAS macro %pdmix800).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Rate</th>
<th>Control 30 DAT</th>
<th>Control 60 DAT</th>
<th>Density 30 DAT</th>
<th>Density 60 DAT</th>
<th>Control 30 DAT</th>
<th>Control 60 DAT</th>
<th>Density 30 DAT</th>
<th>Density 60 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoxaflutole + safener&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105</td>
<td>68 cd</td>
<td>60 bc</td>
<td>263 def</td>
<td>69 def</td>
<td>62 c</td>
<td>27 de</td>
<td>263 a</td>
<td>145 a</td>
</tr>
<tr>
<td>isoxaflutole + safener&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210</td>
<td>87 ab</td>
<td>85 a</td>
<td>137 fg</td>
<td>32 efg</td>
<td>73 bc</td>
<td>38 cd</td>
<td>105 bc</td>
<td>89 abc</td>
</tr>
<tr>
<td>isoxaflutole</td>
<td>105</td>
<td>65 cd</td>
<td>57 c</td>
<td>443 cde</td>
<td>103 cde</td>
<td>62 c</td>
<td>25 de</td>
<td>217 ab</td>
<td>120 ab</td>
</tr>
<tr>
<td>isoxaflutole</td>
<td>210</td>
<td>90 a</td>
<td>87 a</td>
<td>48 g</td>
<td>9 fg</td>
<td>83 ab</td>
<td>48 bc</td>
<td>55 cde</td>
<td>55 cd</td>
</tr>
<tr>
<td>mesotrione</td>
<td>210</td>
<td>53 d</td>
<td>50 c</td>
<td>417 cde</td>
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<td>58 c</td>
<td>38 cd</td>
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<td>mesotrione</td>
<td>420</td>
<td>73 bc</td>
<td>48 c</td>
<td>191 efg</td>
<td>51 efg</td>
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<td>62 b</td>
<td>33 de</td>
<td>25 de</td>
</tr>
<tr>
<td>atrazine</td>
<td>1680</td>
<td>8 e</td>
<td>7 d</td>
<td>859 ab</td>
<td>292 ab</td>
<td>58 c</td>
<td>17 e</td>
<td>191 ab</td>
<td>141 a</td>
</tr>
<tr>
<td>atrazine</td>
<td>3360</td>
<td>13 e</td>
<td>8 d</td>
<td>520 bcd</td>
<td>248 abc</td>
<td>78 bc</td>
<td>22 de</td>
<td>115 bc</td>
<td>129 a</td>
</tr>
<tr>
<td>acetochlor</td>
<td>1680</td>
<td>87 ab</td>
<td>82 ab</td>
<td>125 fg</td>
<td>49 defg</td>
<td>83 ab</td>
<td>62 b</td>
<td>19 de</td>
<td>16 e</td>
</tr>
<tr>
<td>acetochlor</td>
<td>3360</td>
<td>93 a</td>
<td>88 a</td>
<td>93 fg</td>
<td>4 g</td>
<td>94 a</td>
<td>85 a</td>
<td>5 e</td>
<td>5 e</td>
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<tr>
<td>S-metolachlor</td>
<td>1600</td>
<td>17 e</td>
<td>7 d</td>
<td>596 abc</td>
<td>215 abc</td>
<td>18 d</td>
<td>17 e</td>
<td>200 ab</td>
<td>120 a</td>
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<td>-</td>
<td>1067 a</td>
<td>363 a</td>
<td>-</td>
<td>-</td>
<td>260 a</td>
<td>145 a</td>
</tr>
</tbody>
</table>

<sup>a</sup> cyprosulfamide.
Table 4.6 Mean estimates of control and density of McLean Co., IL waterhemp 30 and 60 days after treatment (DAT) of soil-applied herbicides in soybean. Means with the same letter within a column are not significantly different at $\alpha = .05$ (separated by the SAS macro %pdmix800).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Rate</th>
<th>Control 30 DAT</th>
<th>Control 60 DAT</th>
<th>Density 30 DAT</th>
<th>Density 60 DAT</th>
<th>2010 30 DAT</th>
<th>2010 60 DAT</th>
<th>2011 30 DAT</th>
<th>2011 60 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Herbicide</strong></td>
<td><strong>Rate</strong></td>
<td><strong>30 DAT</strong></td>
<td><strong>60 DAT</strong></td>
<td><strong>30 DAT</strong></td>
<td><strong>60 DAT</strong></td>
<td><strong>30 DAT</strong></td>
<td><strong>60 DAT</strong></td>
<td><strong>30 DAT</strong></td>
<td><strong>60 DAT</strong></td>
</tr>
<tr>
<td>cloransulam</td>
<td>18 g ai ha$^{-1}$</td>
<td>5 f</td>
<td>6 d</td>
<td>407 a</td>
<td>268 a</td>
<td>30 f</td>
<td>13 e</td>
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96
4.8 Figures

Figure 4.1 Waterhemp seedling survival 21 days after treatment (DAT) of soil-applied mesotrione. (MCR15 - HPPD inhibitor-resistant waterhemp, NH41 - RxR progeny derived from greenhouse crosses of HPPD inhibitor-resistant waterhemp, S - a known HPPD inhibitor-sensitive population).
5. 1 Research Conclusions and Implications

Weed resistance to herbicides continues to be an ever present challenge to producers who are trying to meet the needs of an increasing world population for food, fuel, and fiber. When a weed species evolves resistance to herbicides, management options available to a grower to maximize crop yield are drastically reduced. Waterhemp [Amaranthus tuberculatus (Moq.) Sauer] has proven to be one of the toughest weeds for Illinois producers to control (Hager et al. 1997). If uncontrolled, dense populations of waterhemp can reduce soybean yields over 40% (Hager et al. 2002) and up to 74% in corn (Steckel and Sprague 2004). The competitive nature of waterhemp is aided by its ability to evolve new forms of herbicide resistance, as well as its ability to “stack” multiple resistances to different groups of herbicides within an individual plant (Bell et al. 2009; Foes et al. 1998; Patzoldt et al. 2005).

Prior to 2009, waterhemp had evolved resistance to four herbicide site-of-action groups (Heap 2012): acetolactate synthase (ALS) inhibitors, photosystem II (PSII) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, and 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) inhibitors. In the summer of 2009, a waterhemp population in a seed corn production field in McLean Co., IL was not adequately controlled following foliar applications of 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors. Experiments described throughout this thesis were conducted at the McLean Co. seed corn production site, as well as under controlled greenhouse conditions. The overall purpose of this research was to determine if a
novel form of resistance to HPPD inhibitors had indeed evolved in waterhemp, and if so, what control options were available for effective management of this particular population.

The greenhouse experiments described in Chapter 2 represent the initial research into HPPD inhibitor resistance. Waterhemp plants grown from seed collected at the McLean Co. field location (designated MCR) were treated with foliar-applied HPPD inhibitors applied or in combination with atrazine. The response of MCR was compared with two known HPPD inhibitor sensitive biotypes. Furthermore, a foliar-applied mesotrione dose response was conducted to quantify the level of resistance in the MCR population. Results of these experiments suggest the MCR field population has indeed evolved resistance to HPPD inhibitors. By 21 days after treatment (DAT), HPPD inhibitors controlled the sensitive biotypes 88% or greater. Conversely, the range of control for MCR was 13-58%. The addition of atrazine with each HPPD inhibitor increased injury to MCR, though never controlled the population greater than 90%. Results of the dose response study indicate the MCR population demonstrates a 10-to 35-fold level of resistance to mesotrione.

The implications of these greenhouse studies are crucial as this brings to six the number of herbicide site-of-action groups to which waterhemp has evolved resistance. What is equally troubling is that resistance to HPPD inhibitors has not been limited to Illinois. A waterhemp population from Henry Co. Iowa (McMullan and Green 2011), as well as two biotypes of Palmer amaranth from Kansas (Lally et al. 2010), have demonstrated this form of resistance. It is highly probable that this resistance trait could spread to surrounding areas due to waterhemp characteristics such as forced outcrossing and substantial production of small mobile, seeds. Selection for resistant biotypes could increase as HPPD inhibitors comprise a significant portion of herbicide use in Illinois corn production (USDA/NASS 2011).
With confirmation of HPPD inhibitor resistance, foliar-applied herbicide experiments presented in Chapter 3 were conducted at the McLean Co. seed corn production site. Results of one study indicated substantial biomass accumulation, as well as low individual mortality, of this population after treatments of HPPD inhibitors applied at normal field use rates. Results of a second study indicated the growth stage at which the McLean Co. population is treated influences the herbicidal efficacy of an HPPD inhibitor. Mesotrione applied alone or in combination with atrazine provided significantly higher waterhemp control when applied VEPOST (2–5 cm weed height) as opposed to EPOST (5–10 cm) and POST (10–15 cm) application timings. However, a substantial number of waterhemp plants were able to recover and resume growth at all treatment application timings, an ability attributed to HPPD inhibitor resistance.

The importance of these studies was to confirm that foliar-applied HPPD inhibitors are not viable options for control of this waterhemp population. Therefore, Chapter 3 included additional research to determine what other foliar-applied herbicide options provided effective control of this population. Results of both field and greenhouse experiments indicate that PPO inhibitors, glufosinate, and glyphosate provide the greatest control of the McLean Co. population. It should be noted that adequate spray coverage is essential for PPO inhibitors and glufosinate as they do not translocate throughout the plant (Hess 2000; Matsumoto 2002). Multiple herbicide resistance was confirmed in this population as plants demonstrated reduced sensitivity to PSII and ALS inhibitors under both field and greenhouse conditions.

Integrated management systems are essential for controlling waterhemp, especially populations like the one in McLean Co. that demonstrates resistance to multiple herbicide groups. Application of a soil-applied residual herbicide can be a crucial component of this
management approach. In Chapter 4, the efficacy of various soil-applied herbicides, including HPPD inhibitors, was evaluated to determine effective control options for this population.

Under field conditions, herbicides with alternative target sites, such as acetochlor, sulfentrazone, flumioxazin, metribuzin, and pyroxasulfone, generally provided the greatest waterhemp control and greatest reduction in waterhemp density. McLean Co. waterhemp demonstrated reduced sensitivity to soil-residual HPPD inhibitors applied at recommend use rates. Therefore, LD$_{50}$ values and subsequent resistant-to-sensitive (R/S) ratios were determined for this population using a soil-applied mesotrione dose response under greenhouse conditions. HPPD inhibitor-resistant waterhemp and RxR progeny (derived from greenhouse crosses of HPPD-resistant waterhemp) demonstrated an 8.8 and 12.7 fold level of resistance, respectively, when compared with a sensitive biotype.

Results presented in Chapter 4 enforce the need for an integrated management approach for the McLean Co. population as plots treated with the most effective soil-applied herbicides still contained several waterhemp plants m$^{-2}$. The reduction in waterhemp density provided by these treatments, however, could increase the efficacy of future foliar herbicide applications as high plant densities may prevent thorough spray coverage. This is crucial for foliar-applied herbicides that do not translocate, such as glufosinate and PPO inhibitors.

By no means do the results presented within this thesis signal the end of research for this population. Experiments are currently under way to understand the mechanism(s) and inheritance of HPPD inhibitor resistance, and research will continue at the McLean Co. field location to further evaluate potential control options. If and when HPPD inhibitor resistance spreads, it is hoped this thesis will serve as a useful reference for future weed science research.
Proper management, which includes an understanding of several disciplines, will be needed to combat waterhemp, a weed species that is ideally suited to thrive in Illinois agriculture.

5.2 Literature Cited


USDA/NASS. 2011. Statistics by Subject-Environmental.