IDENTIFICATION, CHARACTERIZATION, AND MANAGEMENT OF GLYPHOSATE-RESISTANT WATERHEMP (*Amaranthus rudis* Sauer.) IN MISSOURI.

A Thesis presented to the Faculty of Graduate School

University of Missouri

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

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by

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MAY 2008

The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

IDENTIFICATION, CHARACTERIZATION, AND MANAGEMENT OF GLYPHOSATE-RESISTANT WATERHEMP (*Amaranthus rudis* Sauer.) IN MISSOURI.

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ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor Dr. Kevin Bradley for all his assistance, time, knowledge, and sacrifices he has given in assisting me in accomplishing this milestone of my educational career. I feel extremely fortunate to have found an advisor not only of such knowledge, but willingness to help me further my education and expand the horizons of my future. I also could not have successfully reached this point without the help of my committee, Dr. Reid Smeda and Dr. Ray Massey.

I would also like to thank the hard working and always entertaining technicians Jim Wait and Eric Riley. I could not have accomplished what I have without your sacrifices and hard work on all of our eventful adventures in the Platte County, Missouri river bottoms. I know both of you are truly heartbroken not to returning to a place of such unpredictability.

To the graduate student office: Carl, Dan, Kristin, and Johnathan. Whether you were a member of the fun side or serious side, you all have made the long days in the office just a little shorter. Thanks for all your assistance, motivation, and for keeping me entertained and in an upbeat mode no matter to the task at hand.

Most importantly I would like to thank my family for all of their love and support. Mom and Dad, I am fortunate to have been raised by two individuals with so much love and desire. I would not be were I am today without your guidance, but most of all thanks for your never ending support. Rodney, Leon, and Alicia: whether you know it or not all three have been my ultimate role models who have inspired me in every step of my life thus far. Though I may have thought differently as a child, having the three of you as older sibling has been a real blessing, thanks for being everything I’ve ever wanted to be.

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Literature Review

Justification. There are currently 183 species of weeds in the world that have been confirmed to be resistant to herbicides (Heap 2006). As defined by the Weed Science Society of America resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. This resistance can occur naturally or may be induced through genetic engineering or mutagenesis (Heap 2006). One practice that is responsible for the rapid selection of herbicide resistant species is the continuous use of a single active ingredient or herbicides with similar modes of action (Jasieniuk et al. 1996). Since the introduction of glyphosate-resistant soybeans in 1996, the use of glyphosate on soybean acreage has increased dramatically from 2.5 million kg/yr in 1995 to 30 million kg/yr in 2002 (Young 2006). This increase in the use of glyphosate on soybean has also lead to a decrease of other active ingredients used on at least 10% of soybean acres; from 11 in 1995 to one in 2002 (Young 2006). The use of a single active ingredient on the majority of the soybean acreage in the United States has greatly increased the selection pressure that is being placed on weed species to develop resistance to glyphosate. In the state of Missouri this selection pressure may even be greater as the amount of soybean acreage in 2006 was nearly double that of corn (USDA 2006). This ratio of soybean to corn acreage indicates that a majority of Missouri acreage is planted in a continuous soybean rotation in which glyphosate is more often than not the primary herbicide used for weed control. The increased pressure for glyphosate resistance can already be seen in the number of glyphosate-resistant weeds that have been confirmed in the state of Missouri. Missouri currently has three weed species that are confirmed as glyphosate-resistant (Heap 2006). The most recent species that has been confirmed to be glyphosate-resistant is a biotype of common waterhemp (*Amaranthus rudis* Sauer.) in Platte County Missouri.

Six common waterhemp biotypes in which variable glyphosate control had been observed were screened for glyphosate resistance in greenhouse studies in 2005. The biotypes were sprayed with increasing rates of glyphosate and a GR_{50} based on fresh weight reduction was determined for each biotype. Two of the biotypes were determined to have a GR_{50} greater than the labeled rate of glyphosate at 0.86 kg/ha. The one biotype with the significantly highest GR_{50} as compared to all other biotypes was the Platte County Missouri biotype with a GR_{50} of 2.35kg ae/ha (Bradley et al. 2006). These experiments have led to the further investigation of this biotype in the field, greenhouse and laboratory. Additional experiments will be conducted to better understand glyphosate resistance at the field level, evaluate the use of alternative herbicide programs in corn and soybeans, and to evaluate the distribution of resistance in common waterhemp accession at the Platte County, Missouri site.

INTRODUCTION

Common Waterhemp (*Amaranthus rudis* Sauer.)

Common waterhemp is a member of the *Amaranthus* genus. The common name waterhemp refers to tall and common waterhemp which are considered as two separate species based on diminutive pistillate characteristics (Gleason and Cronquist, 1991; Horak et al 1994). However common waterhemp is considered to be the more common of the two species in the western portion of the Midwest (Wax 1995). Within the *Amaranthus* genus there are ten species which are considered to be problematic in the Midwestern United States: redroot pigweed (*Amaranthus retroflexus* L.), smooth pigweed (*Amaranthus hybridus* L.), Powell amaranth (*Amaranthus powellii* S. Wats.), tumble pigweed (*Amaranthus albus* L.), prostrate pigweed (*Amaranthus blitoides* S. Wats.), spiny amaranth (*Amaranthus spinosus* L.), common waterhemp (*Amaranthus rudis* Sauer.), tall waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer.), Palmer amaranth (*Amaranthus palmeri* S. Wats.) and sandhills waterhemp (*Amaranthus arenicola* I.M. Johnst.) (Gleason and Cronquist 1991; Horak et al. 1994). In a survey taken in Illinois *Amaranthus* spp. was listed as the number one weed encountered in corn and soybean fields with 75 percent of those specifically being listed as waterhemp (Hager and Sprague 2002). Common waterhemp has become a widespread problem throughout the Midwest over the last several years for a variety of reasons.

The adoption of reduced and no-tillage systems may have contributed to the increase in common waterhemp, as the small seeds of waterhemp are allowed to stay in the top layer of soil where germination is greatest (Hager et al. 1997). Buhler (1992) showed that redroot pigweed, also a member of the *Amaranthus* genus which produces small seeds, had greater densities in no-tillage systems as compared to conventional tillage systems. In a more recent study it was shown that common waterhemp emergence in a no-tillage system was twice that of a conventional tillage system (Steckel et al 2001). The increased use of conservation tillage programs is only one of several factors contributing to the increased occurrence of common waterhemp in agronomic fields.

Common waterhemp is also a prolific seed producer, with a single plant able to produce at least 250,000 seeds (Sellers et al. 2003). This seed production is 1.4 fold greater than redroot pigweed (*Amaranthus retroflexus* L.) and smooth pigweed (*Amaranthus hybridus* L.), two fold greater than Palmer amaranth (*Amaranthus palmeri* S. Wats.), and 3.4 fold greater than the seed production of tumble pigweed (*Amaranthus albus* L.) and spiny amaranth (*Amaranthus spinosus* L.) (Sellers et al. 2003). The prolific production of seed can lead to a rapid build-up of seedbanks when common waterhemp is not controlled. The seedbanks of common waterhemp have been shown to be more persistent than seedbanks of woolly cupgrass and giant foxtail, but similar to that of velvetleaf (Buhler and Hartzler 2001). Buhler and Hartzler (2001) were able to recover 12% of original common waterhemp seed from a deposited seedbank four years after burial, with the recovered seed having 95% viability. This persistence of common waterhemp seed in the soil and the prolific seed production of common waterhemp can make it difficult to deplete soil seedbanks of common waterhemp.

The emergence of common waterhemp from late April to mid-July (Steckel et al. 2001) also makes it difficult to manage common waterhemp. Hartzler et al. (1999) found that common waterhemp emerged later than either giant foxtail (*Setaria faberi* Herrm.) or woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth.), and emerged 5 to 25 days after velvetleaf (*Abutilon theophrasti* Medicus). Common waterhemp emergence also occurred over a longer period of time than these other agronomic weeds. These characteristics make it very difficult to control common waterhemp when depending on herbicides, as soil-applied herbicides often do not persist long enough to control common waterhemp and post-applied herbicides without residual activity often fail to control late-emerging common waterhemp cohorts that emerge after the post application.

Common Waterhemp Interference and Competition

The emergence and seed production characteristics of common waterhemp have made it difficult to ultimately determine the most effective time for control. Hager et al. (2002) found that allowing common waterhemp to interfere with soybeans up to 10 weeks after soybean unifoliate expansion resulted in an average of 43% soybean yield reduction over a three year period. These authors also found soybean yield losses could occur when delaying waterhemp removal until four weeks after soybean unifoliate expansion. Steckel and Sprague (2004) showed that common waterhemp that emerges before the V4-V5 stage of soybeans needed to be controlled in order to reduce soybean yield loss and to reduce common waterhemp seed production. These authors also found that common waterhemp that emerged with the crop and was allowed to have season-long competition resulted in a 44 and 37% yield reduction in 76 and 19cm row soybeans, respectively.

Similar work has been conducted with common waterhemp populations in corn. Cordes et al. (2004) found that season long interference at densities of 362 or more common waterhemp plants per m^2 reduced corn yields up to 36%. The authors also found that if high densities of common waterhemp were not controlled by the time they reached 15 cm in height, then corn yield reductions of up to 15% would occur. A 10% corn yield loss was observed when lower common waterhemp densities of 35 to 82 plants per m^2 were allowed to compete season-long.

Herbicide Resistance in Common Waterhemp

Common waterhemp, Palmer amaranth and sandhills amaranth are all dioecious, or have male and female flowers that occur on separate plants. This is in contrast to the other *Amaranthus* species that are monoecious, or contain male and female flowers on the same plant (Gleason and Cronquist, 1991; Horak et al., 1994). The dioecious biology of common waterhemp forces it to outcross with other common waterhemp plants, which creates genetic diversity and an ability to quickly adapt (Hager et al. 1997; Foes et al. 1998). This ability to adapt can be seen when observing the number of common waterhemp biotypes that have developed resistance to herbicides.

Prior to the discovery of glyphosate resistance in the common waterhemp biotype located in northwest Missouri, biotypes of common waterhemp have developed resistance to photosystem II, acetolactate synthase, and protoporphyrinogen oxidase inhibiting herbicides (Heap 2006).

Biotypes of common waterhemp resistant to photosystem II inhibiting herbicides were first reported in 1994 in Missouri, and since the initial confirmation additional biotypes have been identified in Kansas, Nebraska, Iowa, and Illinois (Heap 2006).

The most widespread occurrence of herbicide resistance in common waterhemp is resistance to acetolactate synthase (ALS) inhibiting herbicides. The first reported case occurred in Iowa in 1993, and further confirmations have occurred in Illinois, Missouri, Kansas, Ohio, Wisconsin, and Oklahoma (Heap 2006). The ability of common waterhemp to quickly adapt to repeated applications of the same herbicide is readily apparent in cases where ALS inhibitors have been applied. For example, a Kansas biotype of common waterhemp survived eight times the labeled rate of both imazethapyr and thifensulfuron after only two previous applications of these herbicides (Horak and Peterson 1995). Additional studies at the enzyme level showed that this biotype required more than 520 times the concentration of imazethapyr to inhibit ALS activity by 50 percent as compared to a susceptible biotype (Lovell et al 1996). Lovell et al. (1996) also showed that the biotype was cross-resistant to the sulfonylurea herbicides chlorimuron and thifensulfuron. In two other studies conducted in Iowa and Illinois, common waterhemp biotypes were also found to be cross-resistant to both the imidazolinone and sulfonylurea herbicides (Sprague et al. 1997; Hinz and Owen 1997).

The third herbicide group in which common waterhemp biotypes have developed resistance are the protoporophyrinogen oxidase inhibiting herbicides (PPO). A biotype in Kansas was found to be 34, 82, 8, and 4 times resistant to aciflourfen, lactofen, fomesafen, and sulfentrazone, as compared to a susceptible biotype of common waterhemp (Shoup et al. 2003). Shoup et al. (2003) also found this biotype to have cross resistance to ALS inhibiting herbicides. A biotype of common waterhemp in Missouri was found to be 9.5 and 11 times resistant to acifluorfen and lactofen as compared to a susceptible biotype; a second biotype was found to be 28 and 44 times resistant to the same herbicides as compared to a susceptible biotype (Li et al. 2004).

There are three known biotypes of common waterhemp including the previously mentioned biotype in Kansas that are resistant to multiple herbicide modes of action (Heap 2006). A biotype of common waterhemp in Illinois was found to be cross-resistant to the imidazolinone, sulfonylurea, and triazolopyrimidine sulfonanilide families of herbicides which inhibit the ALS enzyme. This same biotype was also resistant to atrazine, a photosystem II inhibiting herbicide (Foes et al. 1998). A second biotype of common waterhemp in Illinois was found to be resistant to three herbicide modes of action. This biotype was 23 times resistant to lactofen, 17,000-18,000 times resistant to imazamox and thifensulfuron, and 38 times resistant to atrazine as compared to susceptible biotypes of common waterhemp (Patzoldt et al. 2005).

The widespread occurrence of herbicide resistance in common waterhemp has limited the number of herbicides available for producers to control common waterhemp, especially in soybeans. In the Midwest, this contributed to the rapid adoption of glyphosate-resistant soybeans, especially for producers who had heavy infestations of common waterhemp.

Glyphosate

Glyphosate was first introduced onto the world market as a postemergence, non-selective herbicide by Monsanto in 1974 (Franz et al. 1997). Glyphosate was one of 51,000 compounds screened by Monsanto between 1960 and 1972, but only three of these compounds became commercially available as herbicides (Franz et al. 1997). Glyphosate is a white odorless, crystalline amino acid that has poor solubility, and is often formulated as a salt; the first commercial available form of glyphosate was formulated as a monoisopropylamine salt (Franz et al. 1997).

Glyphosate was the first herbicide with a site of action that was a single, defined enzyme target within plants (Duke 1985). Glyphosate is responsible for the inhibition of the enzyme 5-enolpyruvoylshikimate 3-phosphate synthase (EPSPS). EPSPS is a key enzyme in the shikimate pathway in which the aromatic amino acids phenylalanine, tyrosine and tryptophan are produced. These amino acids are three of the most important amino acids produced by the shikimate pathway (Franz et al. 1997) and are used for the synthesis of proteins, which are used in a variety of plant products. Though the exact events responsible for plant death due to glyphosate application are unknown, it is known that the inhibition of EPSPS and the lack of production of the aromatic amino acids play a major role in the death of susceptible plants (Franz et al 1997). The shikimate pathway is found exclusively in plants and microorganisms, and is absent in insects, birds, fish or mammals. The absence of this pathway within higher animals explains the relatively low toxicity of glyphosate to mammals, birds and fish (Franz et al. 1997).

Another favorable characteristic of glyphosate that makes it environmentally safe is its adsorption and mobility characteristics in soils. Glyphosate is readily adsorbed to a majority of soils and has limited mobility, with soil pH having little effect on glyphosate adsorption (Sprankle et al. 1975). Glyphosate is degraded primarily through microbial activity and is broken down into carbon dioxide and aminomethylphosphonic acid (AMPA). AMPA is also degraded by soil microbes and results in the release of carbon dioxide (Franz et al 1997, Rueppel et al. 1977). The rapid adsorption and degradation of glyphosate in soils decreases the possibility of contamination to ground water and water tables.

Glyphosate-Resistant Crops

Glyphosate-resistant soybean (*Glycine max* L.) was the first glyphosate-resistant crop to be released in the United States in 1996. Since the introduction of glyphosate-resistant soybeans, canola, cotton, and corn varieties have been released with the glyphosate-resistant trait (Duke 2005). Glyphosate resistance in soybean was obtained through the insertion of EPSPS enzyme that occurs naturally in the microorganism *Agrobacterium* sp. strain CP4. The CP4-EPSPS enzyme is highly tolerant to glyphosate as compared to the susceptible EPSPS (Padgette et al. 1996; Dill 2005). Canola varieties resistant to glyphosate also contain a glyphosate oxidoreductase gene which degrades glyphosate into glyoxylate and aminomethylphosphonate (Duke 2005). Certain hybrids of glyphosate-resistant corn do not use the CP4-EPSPS enzyme as a means of resistance; these varieties have a mutated EPSPS which only differs from the wild maize EPSPS at two genetic positions in which substitutions have occurred (Sidhu et al. 2000; Dill 2005). The corn hybrids in which the modified EPSPS is present are referred to as GA21 hybrids.

Since the introduction of glyphosate-resistant soybeans and the subsequent introduction of glyphosate-resistant corn, cotton, and canola, the use of glyphosate has increased dramatically. In 1997 the total percentage of acreage of herbicide tolerant soybeans and cotton in the United States was 17 and 10% respectively. These percentages have increased over the past nine years to 89 and 65% of soybean and cotton acres, respectively (USDA 2007). Though glyphosate-resistant soybean and cotton acreage has dramatically increased over the past nine years the amount of herbicide tolerant corn has increased slowly with 52% of corn acreage being herbicide tolerant in 2007 (USDA 2007). The dramatic increase in glyphosate-resistant soybean and cotton acreage can be attributed to the fact that glyphosate is not only a broad spectrum herbicide, but is also economical and convenient as compared to other herbicide options (Dill 2005). The lag in the amount of corn acreage planted to glyphosate-resistant corn may be due to the fact that many alternative corn herbicides are as effective and economical as glyphosate (Dill 2005; Duke 2005). With the increased use of glyphosate-resistant crops across the United States has come an increase in the concern of potential weed shifts and occurrence of glyphosate-resistant weeds due to the extensive use of glyphosate.

Economics of Glyphosate-Resistant Crops and Weeds

The increased use of glyphosate and glyphosate-resistant crops is due not only to the effectiveness and convenience of the herbicide, but also to the economic value of the system. Although several authors have suggested that the economic advantages of glyphosate-resistant corn are minimal when compared to conventional herbicide programs (Nolte and Young 2002; Johnson et al. 2000; Hellwig et al. 2003), studies of the economics of glyphosate resistant soybeans have revealed that growers will gain an economic advantage with the use of glyphosate-resistant soybeans. Reddy and Whiting (2000) compared the economics of a glyphosate-resistant soybean system in which glyphosate was used as the only herbicide for weed control to both conventional soybean systems and a sulfonylurea-tolerant soybean system. Data from this study showed that the net returns from the glyphosate-resistant system was $407/ha as compared to $271/ha and $317/ha in the sulfonylurea-tolerant and conventional soybean systems, respectively. Though the glyphosate-resistant system had the highest net return, Reddy and Whiting (2000) suggested that the yield potential and price of the soybean cultivar should be the ultimate factors in deciding which system is the most economically sound. In a similar study, glyphosate-resistant soybean programs were compared economically to 11 conventional soybean programs in which conventional soybean cultivars were used

(Roberts et al. 1999). Roberts et al. (1999) found that seven of the conventional soybean programs, despite the lower cost of conventional soybean seed, had a higher cost than that of the glyphosate-resistant soybean program. Similar to the suggestions of Redding and Whiting (2000), Roberts et al. (1999) suggested that the yield potential of the conventional soybean hybrid would be the deciding factor of which system would be more profitable. Roberts et al. (1999) also suggested that the non-budget factors of glyphosate resistant soybean such as ease of use, timeliness, and reduced weed control risk would also become a factor when considering the use of glyphosate-resistant soybeans.

Not only have the economics of glyphosate-resistant versus conventional soybean programs been examined, but most recently the economics behind glyphosate-resistant weeds have also been examined (Mueller et al. 2005). Mueller et al. (2005) compared the economics of proactive versus reactive management of glyphosate-resistant weeds in a glyphosate-resistant cropping system. In a case study of the possibility of a common waterhemp population becoming resistant to glyphosate, the authors determined that the cost of a proactive management strategy using a preemergence herbicide followed by glyphosate postemergence would add $4.52/ha per year in a rotation of conventional corn and glyphosate-resistant soybeans. This is in comparison to the additional cost of managing a population of common waterhemp that had become glyphosate-resistant, in which the added expense was determined to be $44.25/ha per year in the same corn and soybean rotation. Additionally the authors determined that the critical time in which a common waterhemp population would have to become resistant was 29 years. The authors referred to this critical time as the amount of time a population would have to remain susceptible to a herbicide under a reactive management system in order to be economically feasible compared to the proactive management system. This suggest that if the population became resistant to glyphosate in less than 29 years then the proactive approach is more profitable, however if it took longer than 29 years the reactive approach is more economical. In the case of the Missouri common waterhemp biotype in which the biotype developed resistance before the 29 year critical time limit, this research indicates that it would have been better economically to employ a proactive resistance management program.

Weed Shifts and Glyphosate-Resistant Weeds

Changes in management of an agriculture crop and the repeated use of a new management strategy can put increased pressure onto a weed community and cause a change in the weed species that occur in that community (Aldrich and Kremer 1997; Culpepper 2006). One such management change that has taken place throughout the past twenty years has been the adoption of conservation tillage systems. Many researchers believe that these systems have caused a shift towards more annual grassy and small seeded broadleaf weed species (Tuesca et al 2001; Hager et al. 1997; Buhler 1992). In addition to tillage, herbicide use is also a management tactic that may cause weed shifts (Owen and Zelaya 2005). The use of a single herbicide over time can cause a shift towards weed species that are more tolerant of that herbicide (Shaner 2000). With the increased use of glyphosate on glyphosate resistant crops throughout the United States, the possibility that weed shifts are occurring has been studied extensively in recent years. The ability of ivyleaf morningglory (*Ipomoea hederacea* (L.) Jacq.) to tolerate glyphosate applications, and the emergence characteristic of common sunflower (*Helianthus annuus* L), common waterhemp (*Amaranthus rudis* Sauer), shattercane (*Sorghum bicolor* (L.) Moench), and woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth.) have resulted in shifts towards higher populations of these species in glyphosate-based weed management systems (Hilgenfeld et al. 2004). A survey of weed scientists across the United States showed that morningglory, spiderwort, lambsquarters, waterhemp, and winter annual species were becoming more problematic in glyphosate-resistant soybeans due to weed shifts (Culpepper 2006). The increased use of glyphosate may not only cause weed shifts, but the increased use of a single herbicide can also place selection pressure on weeds to develop resistance.

There are currently nine weed biotypes in the United States, including the northwestern Missouri common waterhemp population, that have been confirmed to be glyphosate-resistant. Throughout the world there are a total of thirteen glyphosate-resistant weed species (Heap 2008).

The first case of glyphosate resistance occurred in a biotype of rigid ryegrass (*Lolium rigidum*) in Australia, which was 9.5 times more resistant to glyphosate than a susceptible biotype (Pratley et al 1999). Since the first confirmation of glyphosate-resistant rigid ryegrass, several other resistant biotypes have been identified throughout Australia including a biotype that was resistant to glyphosate, the ALS inhibiting herbicides sulfometuron and chlorsulfuron, and the ACCase inhibitor diclofop (Neve et al. 2004). The first confirmation of glyphosate-resistant rigid ryegrass (*Lolium rigidum*) in the United States occurred in 1998 in an almond orchard in California that had received multiple applications of glyphosate yearly for the previous 15 years (Simarmata et al 2005). Biotypes of rigid ryegrass have also been identified as glyphosate-resistant in South Africa (Heap 2006).

Further studies into the inheritance and the mechanism of resistance have been examined with many of the biotypes of rigid ryegrass in Australia and California. Experiments conducted in Australia showed that glyphosate resistance was inherited on a single nuclear gene (Lorraine-Colwill et al. 2001; Wakelin and Preston 2006a), though the California biotype showed the inheritance of resistance to be multi-genic and incompletely dominant (Simarmata et al 2005). Further investigations into the mechanisms of resistance showed that glyphosate-resistant biotypes of rigid ryegrass in Australia had EPSPS enzymes that were equally susceptible to glyphosate as the EPSPS of a known sensitive biotype (Lorraine-Cowill et al. 2003). Investigations into translocation of glyphosate applied to susceptible and resistant plants showed that glyphosate was translocated to the roots and meristematic regions in susceptible plants, while glyphosate was translocated to leaf tips in the resistant plants, suggesting that differential translocation between susceptible and resistant biotypes played a role in the mechanism of resistance (Lorraine-Cowill et al. 2003; Wakelin et al. 2004). Another biotype of rigid ryegrass in Australia was shown to be resistant to glyphosate through a mutation on the EPSPS gene and differential translocation did not play a role in the mechanism of resistance in this species (Wakelin and Preston 2006b).

Shortly following the first confirmation of glyphosate-resistant rigid ryegrass in Australia, a biotype of goosegrass (*Eleucine indica* (L) Gaertn) in Malaysia was found to be 8 to 12 times resistant to glyphosate (Lee and Ngim 2000). Further investigations of this biotype revealed that glyphosate resistance was inherited through a single nuclear incompletely dominant gene and that a mutation at the 106 position on the EPSPS gene was responsible for resistance in this biotype (Ng et al. 2004; Baerson et al. 2002). To date, this is the only confirmed glyphosate-resistant goosegrass biotype in the world (Heap 2006).

In the United States, glyphosate-resistant horseweed (*Conyza canadensis* (L.) Cronq.) is now the most widespread glyphosate-resistant weed and has now been confirmed in 14 states (Heap 2006). The first biotype of glyphosate-resistant horseweed was confirmed in 2000 in Delaware and was 8 to 13 times more tolerant of glyphosate than a susceptible horseweed biotype (VanGessel 2001). Since this first confirmation of glyphosate resistance in horseweed, glyphosate-resistant biotypes of horseweed have been identified throughout the United States (Koger et al 2004). Investigations into the inheritance of glyphosate resistance in the Delaware biotype suggested that inheritance was through a single, nuclear, incompletely dominant allele (Zelaya et al. 2004). Additional studies of horseweed biotypes from across the United States showed that reduced translocation was responsible for resistance in some of these horseweed biotypes (Koger and Reddy 2005; Feng et al. 2004).

In 2002 a biotype of common ragweed (*Ambrosia artemisiifolia* L.) was confirmed to be resistant to glyphosate in the state of Missouri. The biotype had an I_{50} value 9.6 times greater than that of a susceptible biotype. The biotype also showed to have three times less shikimic acid accumulation as compared to a susceptible biotype. (Pollard et al. 2004)

A biotype of Italian ryegrass (*Lolium multiflorum* Lam.) in Oregon was confirmed to be glyphosate-resistant in 2004 (Heap 2006). The biotype occurred in an orchard in which multiple applications of glyphosate had been sprayed annually for the previous 15 years. The biotype was five times more resistant to glyphosate than a susceptible biotype. In analyzing shikimic acid build-up, the susceptible biotype had five times more shikimic acid accumulation as compared to the resistant biotype. However when the EPSPS genes from the susceptible and resistant biotypes were compared, no amino acid differences were found (Perez-Jones et al. 2005).

The most recently confirmed biotypes of glyphosate-resistant weeds have been Palmer amaranth (*Amaranthus palmeri* S. Wats.), giant ragweed (*Ambrosia trifida* L.) and common waterhemp (*Amaranthus rudis* Sauer.) (Heap 2006). The Palmer amaranth biotype discovered in Georgia required 7 to 8 times the labeled rate of glyphosate to reduce plant growth by 50% (Vencill et al. 2006). In initial investigation of glyphosate-resistant giant ragweed in Ohio and Indiana, two applications of glyphosate provided 50 to 76% control of resistant biotypes as compared to 93% of a susceptible biotype. In these same biotypes 5 to 59% of the plants that survived applications of glyphosate had substantial regrowth (Stachler et al.). Further details of the glyphosate-resistant common waterhemp biotype will be investigated in this research.

Summary and Objectives

Since the introduction of first glyphosate-resistant crop in 1996 the adoption of glyphosate-resistant crops and the use of glyphosate have rapidly increased over the past decade. The intensive use of glyphosate and reliance on a single herbicide has increased the number of weeds in which glyphosate resistance has occurred. The possibility of a glyphosate-resistant common waterhemp population has been feared because of its tolerance and resistance to many herbicide modes of action, emergence characteristics, prolific seed production, and occurrence throughout the Midwest as a problematic weed. A common waterhemp population in Platte County Missouri was discovered to have a GR_{50} of 2.35 kg acid equivalent of glyphosate per hectare, in comparison to a susceptible biotype with a GR_{50} of 0.012 kg ae/ha. The objectives of this research are to: 1) evaluate the level of resistance of the Platte County Missouri biotype at the field level, 2) evaluate alternative herbicide programs for management of the northwestern Missouri biotype in both corn and soybean systems, and 3) evaluate the distribution of resistance in common waterhemp accession at the Platte County, Missouri site.

LITERATURE CITED

Aldrich, R.J. and R.J. Kremer. 1997. Principles in Weed Management. 2nd Ed. Ames, IA: Iowa State University Press. Pg37-38.

Anderson D.D., F.W. Roeth, and A.R. Martin. 1996. Occurrence and control of triazine-resistant common waterhemp (*Amaranthus rudis*) in field corn (*Zea mays*). Weed Technol. 10:570-575.

Baerson, S.R., D.J. Rodriguez, M. Tran, Y. Feng, N.A. Biest, and G.M. Dill. 2002. Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiol. 129:1265-1275.

Bradley, K.W., J. Li, and N.H. Monnig. 2006. Greenhouse investigations of suspected glyphosate-resistant common waterhemp populations form Missouri. Proc Weed Sci. Soc Amer. 46.

Buhler D.D. 1992. Population dynamics and control of annual weeds in corn (*Zea mays*) as influenced by tillage systems. Weed Sci. 40:241-248.

Buhler D.D. and R.G. Hartzler. 2001. Emergence and persistence of seed of velvetleaf, common waterhemp, woolly cupgrass, and giant foxtail. Weed Sci. 49:230-235.

Culpepper, A.S. 2006. Glyphosate-induced weed shifts. Weed Technol. 20:277-281.

Cordes, J.C., W.G. Johnson, P. Scharf, and R.J. Smeda. 2004. Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. Weed Technol. 18:999-1005.

Dill G.M. 2005. Glyphosate-resistant crops: history, status, and future. Pest Manag. Sci. 61:219-224.

Duke, S.O. 2005. Taking stock of herbicide-resistant crops ten years after introduction. Pest Manag. Sci. 61:211-218.

Duke, S.O. 1985. Weed Physiology, Vol. 2, Herbicide Physiology. CRC Press: Boca Raton, FL, 1985.

Feng, P.C.C., M. Tran, T. Chiu, R.D. Sammons, G.R. Heck, C.A. Jacob. 2004. Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake, translocation, and metabolism. Weed Sci. 52:498-505.

Foes, M.J., L. Liu, P.J. Tranel, L.M. Wax, E.W. Stoller. 1998. A biotype of common waterhemp (Amaranthus *rudis*) resistant to triazine and ALS herbicides. Weed Sci. 46:514-520.

Franz J.E., M.K. Mao, and J.A. Sikorski. 1997. Glyphosate A Unique Global Herbicide. American Chemical Society. Washington D.C.

Gleason, H.A. and A. Cronquist. 1991. Manual of vascular plants of Northeastern United States and adjacent Canada. 2nd ed. New York: Botanical Garden. Pp 104-108.

Hinz, J.R. and M.K. Owen. 1997. Acetolactate synthase resistance in a common waterhemp (*Amaranthus rudis*) population. Weed Technol. 11:13-18.

Hager A., L. Wax, and M. McGlamery. 1997. Biology of a troublesome species. The bulletin. University of Illinois. No. 4.

Hager A. and C. Sprague. 2002. Weeds on the horizon. The Bulletin. University of Illinois. No. 6.

Hager A.G., L.M. Wax, E.W. Stoller, and G.A. Bollero. 2002. Common waterhemp (*Amaranthus rudis)* interference in soybean. Weed Sci. 50: 607-610.

Hartzler R.G., D.D. Buhler, and D.E. Stoltenberg. 1999. Emergence characteristics of four annual weed species. Weed Sci. 47:578-584.

Heap, I. The International Survey of Herbicide Resistant Weeds. Online. Internet. April 23, 2008. Available www.weedscience.com.

Hellwig, K.B., W.G. Johnson, and R.E. Massey. 2003. Weed management and economic returns in no-tillage herbicide-resistant corn (*Zea mays*). Weed Technol. 17:239-248.

Hilgenfeld, K.L., A.R. Martin, D.A. Mortensen, and S.C. Mason. 2004. Weed management in a glyphosate resistant soybean system: weed species shifts. Weed Technol. 18:284-291.

Hinz, J.R.R. and M.D.K. Owen. 1997. Acetolactate synthase resistance in common waterhemp (*Amaranthus rudis*) population. Weed Technol. 11:13-18.

Horak, M.J. and D.E. Peterson. 1995. Biotypes of palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol. 9:192-195.

Horak, M.J., D.E. Peterson, D.J. Chessman, and L.M. Wax. 1994. Pigweed Identification: A pictorial guide to the common pigweeds of the great plains. Manhattan, KS: Kansas State University. Pp12.

Jasieniuk, M. A.L. Brule-Babel, and I.N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 44:176-193.

Johnson, W.G., P.R. Bradley, S.E. Hart, M.L. Buesinger, and R.E. Massey. 2000. Efficacy and economics of weed management in glyphosate-resistant corn (*Zea mays*). Weed Technol. 14:57-65.

Koger, C.H., D.H. Poston, R.M. Hayes, and R.F. Montgomery. 2004. Glyphosate-resistant horseweed (Conyza *canadensis*) in Mississippi. Weed Technol. 18:820-825.

Koger, C.H. and K.N. Reddy. 2005. Role of adsorption and translocation in the mechanism of glyphosate resistance in horseweed (Conyza *canadensis*). Weed Sci. 53:84-89.

Lee, L.J., and J. Ngim. 2000. A first report of glyphosate-resistant goosegrass (*Eleucine indica* (L) Gaertn) in Malaysia. Pest Manag. 56:336-339.

Li J., R.J. Smeda, K.A. Nelson, and F.E. Dayan. 2004. Physiological basis for resistance to diphenyl ether herbicides in common waterhemp (Amaranthus *rudis*). Weed Sci. 52:333-338.

Lorraine-Colwill, D.F., S.B. Powles, T.R. Hawkes, and C. Preston. 2001. Inheritance of evolved glyphosate resistance in *Lolium rigidum* (Gaud.). Theor. Appl. Genet. 102:545-550.

Lorraine-Colwill, D.F., S.B. Powles, T.R. Hawkes, P.H. Hollinshead, S.A.J. Warner, and C. Preston. 2003. Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. Pesticide Biochem. and Phys. 74:62-67.

Lovell S.T., L.M. Wax, M.J. Horak, and D.E. Peterson. 1996. Imidazolinone and sulfonylurea resistance in a biotype of common waterhemp (*Amaranthus rudis*). Weed Sci. 44:789-794.

Mueller T.C., P.D. Mitchell, B.G. Young, and A.S. Culpepper. 2005. Proactive versus reactive management of glyphosate-resistant or –tolerant weeds. Weed Technol. 19:924-933.

Neve, P., J. Sadler, and S.B. Powles. 2004. Multiple herbicide resistance in a glyphosate-resistant rigid ryegrass (*Lolium rigidum*) population. Weed Sci. 52:920-928.

Ng, C.H., W. Ratnam, S. Surif, and B.S. Ismail. 2004. Inheritance of glyphosate resistance in goosegrass (*Eleucine indica*). Weed Sci. 52:564-570.

Nolte, S.A. and B.G. Young. 2002. Efficacy and economic return on investment for conventional and herbicide-resistant corn (*Zea mays*). Weed Technol. 16:371-378.

Owen, M.D.K. and I.A. Zelaya. 2005. Herbicide-resistant crops and weed resistance to herbicides. Pest Manag. Sci. 61:301-311.

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichholtz, X. Delannay, R.L. Fuchs, G.M. Kishore, and R.T Fraley. 1996. New weed control opportunities: development of soybeans with a roundup ready gene. *In* Herbicide resistant crops: agriculture, environmental, economic, regulatory and technical aspects, ed by S.O. Duke. CRC Press. Boca Raton, FL.

Patzoldt, W.L., P.J. Tranel, and A.G. Hager. 2005. A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci. 53:30-36.

Perez-Jones, A., K.W. Park, J. Colquhoun, C. Mallory-Smith, and D. Shaner. 2005. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. Weed Sci. 53:775-779.

Pollard, J.M., B.A. Sellers, and R.J. Smeda. 2004. Differential response of common ragweed to glyphosate. Proc. N. Cent. Weed Sci. 59:27.

Pratley, J., N. Urwin, R. Stanton, P. Baines, J. Broster, K. Cullis, D. Schafer, J. Bohn, and R. Krueger. 1999. Resistance to glyphosate in *Lolium rigidum* I. Bioevaluation. Weed Sci. 47: 405-411.

Reddy, K.N. and K. Whiting. 2000. Weed control and economic comparisons of glyphosate-resistant, sulfonylurea-resistant, and conventional soybean (*Glycine max*) systems. Weed Technol. 14:204-211.

Roberts, R.K., R. Pendergrass, and R.M. Hayes. 1999. Economic analysis of alternative herbicide regimes on roundup ready soybeans. Journal of Production Agriculture 12:449-454.

Rueppel M.L., B.B. Brightwell, J. Schaefer, and J.T. Marvel. 1977. Metabolism and Degradation of glyphosate in soil and water. Journal of Agriculture and Food Sci.. 25: 517-528.

Sellers, B.A., R.J. Smeda, W.G. Johnson, J.A. Kendig, and M.R. Ellersieck. 2003. Comparative growth of six *Amaranthus* species in Missouri. Weed Sci. 51:329-333.

Shaner, D.L. 2000. The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. Pest Manag. Sci. 56:320-326.

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

Sidhu R.S., B.G. Hammond, R.L. Fuchs, J.N. Mutz, L.R. Holden, B. George, and T. Olson. 2000. Glyphosate-tolerant corn: The composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays* L.). J. of Agricul. and Food Chem. 48:2305-2312.

Simarmata, M., S. Bughrara, and D. Penner. 2005. Inheritance of glyphosate resistance in rigid ryegrass (Lolium *rigidum*) from California. Weed Sci. 53:615-619.

Sprague C.L., E.W. Stoller, L.M. Wax, and M.J. Horak. 1997. Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. Weed Sci. 45:192-197.

Sprankle, P, W.F. Meggitt, and D. Penner. 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 23:229-234.

Stachler, J.M., M.M. Loux, W.G. Johnson, and A.M. Westhoven. 2006. Management of giant ragweed populations that are difficult to control with glyphosate. Proc. N. Cent. Weed Sci. 61:226.

Steckel, L.E., C.L. Sprague, F.W. Simmons, G. Bollero, A. Hager, E.W. Stoller, and L.M. Wax. 2001. Tillage and cropping effects on common waterhemp (*Amaranthus rudis*) emergence and seed bank distribution over four years. Weed Sci. Soc. of Amer. Abstract. 41:321.

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

Tuesca, D., E. Puricelli, and J.C. Papa. 2001. A long-term study of weed flora shifts in different tillage systems. Weed Research 41: 369-382.

USDA. United States Department of Agriculture. Agriculture Statistics Board. 2006. June Acreage Report.

USDA. United States Department of Agriculture. Economic Research service. Adoption of Genetically Engineered Crops in the U.S.: Extent of Adoption. April 23, 2008. Web Internet. http://www.ers.usda.gov/data/biotechcrops/adoption.htm.

VanGessel, M.J. 2001. Glyphosate-resistant horseweed in Delaware. Weed Sci. 49:703-705.

Vencill, W.K., J.B. Haider, T.L. Grey, and A.S. Culpepper. 2006. Physiological aspects of glyphosate-resistant Palmer amaranth (Amaranthus *palmeri*). Proc. N. Cent. Weed Sci. 61:96.

Wakelin, A.M., D.F. Lorraine-colwill, and C. Preston. 2004. Glyphosate resistance in four different populations of *Lolium rigidum* is associated with reduced translocation of glyphosate to meristematic zones. Weed Research 44:453-459.

Wakelin, A.M., and C. Preston. 2006a. Inheritance of glyphosate resistance in several populations of rigid ryegrass (*Lolium rigidum*) from Australia. Weed Sci. 54:212-219.

Wakelin, A.M., and C. Preston. 2006b. A target-site mutation is present in a glyphosate-resistant *Lolium rigidum* population. Weed Research 46:432-440.

Wax L.M., 1995. Pigweeds of the Midwest-distribution, importance, and management. Proceedings Iowa Integrated Crop Management conference. 7:239-242.

Young, B.G. 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol. 20:301-307.

Zelaya, I.A., M.D.K. Owen, M.J. VanGessel. 2004. Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L) Cronq. Theor. Appl. Genet. 110:58-70.

CHAPTER II

Glyphosate and Multiple Herbicide Resistance in Waterhemp (*Amaranthus rudis)* Populations from Missouri[1]

Abstract: Field and greenhouse experiments were conducted to determine the level of glyphosate resistance in waterhemp (*Amaranthus rudis* Sauer) populations from Platte County (MO1) and Holt County, Missouri (MO2), and also to determine the level and distribution of resistance to glyphosate, acetolactate synthase (ALS)-inhibiting herbicides, and protoporophyrinogen oxidase (PPO)-inhibiting herbicides across the MO1 site. Results from greenhouse experiments revealed that the MO1 and MO2 waterhemp populations were 19- and 9-times more resistant to glyphosate, respectively, than a susceptible waterhemp population. In 2006 and 2007 field experiments, greater than 54% of waterhemp at the MO1 site survived applications of 1.7 kg glyphosate ae ha $^{-1}$ six weeks after treatment (WAT), which represents twice the labeled use rate (2X) for the control of waterhemp less than 15-cm in height. Tank-mix combinations of ALS- and PPO-inhibiting herbicides with glyphosate also failed to provide complete control of the waterhemp population at the MO1 site. Collection and screening of seed from individual female waterhemp accessions revealed multiple resistance to glyphosate, ALS-, and PPO-inhibiting herbicides across the MO1 site. All 14 waterhemp accessions collected across the MO1 site exhibited greater than 65% survival to 2X rates of glyphosate and thifensulfuron, and these accessions were spread across a 5-km^2 (503 ha) area. Four waterhemp accessions collected across a 0.9-km^2 (87 ha) area also exhibited

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[1] Received for publication ___ and in revised form ___

26 to 38% survival to 2X rates of lactofen. The results from these experiments provide evidence and confirmation of the first glyphosate-resistant waterhemp population in the United States and reveal that multiple resistance to glyphosate, ALS-, and PPO-inhibiting herbicides can occur in waterhemp.

Nomenclature: Glyphosate; lactofen; thifensulfuron; common waterhemp, *Amaranthus rudis* Sauer; soybean, *Glycine max* L.

Key Words: Acetolactate synthase, multiple herbicide resistance, glyphosate resistance, protoporophyrinogen oxidase.

INTRODUCTION

In 2007 there were eight species of weeds that are resistant to glyphosate in the United States and 13 species worldwide (Heap 2007). All of these weeds have been discovered since the introduction of glyphosate-resistant crops. Another effect of the introduction of glyphosate-resistant soybeans has been the use of glyphosate on the soybean acreage in the United States, which has dramatically increased from 2.5 million kg ai per year in 1995 to 30 million kg ai per year in 2002 (Young 2006). The increase in the use of glyphosate on soybean has also led to a decrease in the use of active ingredients applied on at least 10% of the soybean acreage; from 11 in 1995 to one in 2002 (USDA 2008; Young 2006). One practice that leads to the selection of herbicide-resistant weed biotypes is the continuous use of a single active ingredient or herbicides with a similar mode of action over time (Jasieniuk et al. 1996). For example, glyphosate-resistant rigid ryegrass (*Lolium rigidum* Gaudin) and Italian ryegrass (*Lolium multiflorum* Lam.) were both identified in orchards where glyphosate had been used continuously for at least a 14-year period (Perez-Jones et al. 2005; Simarmata et al. 2005). Glyphosate-resistant horseweed [*Conyza canadensis* (L.) *Cronq.*] and common ragweed (*Ambrosia artemisiifolia* L.) were identified after continuous applications of glyphosate were made on glyphosate-resistant soybeans over three and six years, respectively (Pollard et al. 2004; VanGessel 2001). The continuous use of glyphosate as the primary postemergence herbicide utilized in glyphosate-resistant cotton (*Gossypium hirsutum* L.) for a four year time period also led to the selection of glyphosate-resistant palmer amaranth (*Amaranthus palmeri* S. Wats.) in Georgia, although this field had also been treated with pendimethalin and paraquat during this same time period (Culpepper et al. 2006).

Waterhemp has been listed as the most encountered and troublesome weed in soybeans in Missouri as well as the most encountered broadleaf weed in corn and soybeans in Illinois (Webster 2005; Hager and Sprague 2002). Waterhemp is a prolific seed producer, able to produce about 1.5 times more seed than most other species in the *Amaranthus* genus (Sellers et al. 2003). Average waterhemp plants generally produce about 250,000 seed per plant, although some plants can produce as many as 1,000,000 seed when growing under optimal conditions in noncompetitive environments (Sellers et al. 2003). Waterhemp seeds have a discontinuous emergence pattern and are able to germinate later in the season than most other summer annual weed species (Hartzler et al. 1999; Steckel et al. 2007). Additionally, waterhemp seed can persist in the soil for as many as four years and maintain high viability during this period (Buhler and Hartzler 2001; Steckel et al. 2007).

Waterhemp is also considered a troublesome weed because of its dioecious nature and ability to outcross with other waterhemp plants and other *Amaranthus* species like smooth pigweed and palmer amaranth (Franssen et al. 2001; Trucco et al. 2005; Wetzel et al. 1999). This creates genetic diversity and an ability to quickly adapt to consecutive applications of the same herbicide or herbicides with the same mechanism of action (Foes et al. 1998; Hager et al. 1997; Nordby et al. 2007). Currently, waterhemp biotypes have been identified with resistance to photosystem II, acetolactate synthase (ALS), and protoporophyrinogen oxidase- (PPO) inhibiting herbicides (Heap 2007). Waterhemp biotypes resistant to ALS-inhibiting herbicides are perhaps the most widespread occurring on at least 810,000 hectares within eight states across the Midwest, while waterhemp biotypes resistant to PPO-inhibiting herbicides have also been discovered in Kansas, Illinois, and Missouri (Heap 2007). Additionally, waterhemp biotypes with multiple resistance to photosystem II and ALS-inhibiting herbicides and to photosystem II, ALS-, and PPO-inhibiting herbicides have been identified in Illinois (Foes et al. 1998; Patzoldt et al. 2005), while waterhemp biotypes with multiple resistance to PPO- and ALS-inhibiting herbicides have been identified in Kansas (Shoup et al. 2003).

In 2004 two soybean producers in Platte and Holt counties in northwest Missouri reported a failure to control waterhemp following consecutive applications of glyphosate. Both sites had a history of continuous glyphosate-resistant soybean production for a period of at least six years, with at least one and usually two applications of glyphosate each year. The objectives of this research were to characterize the level of glyphosate resistance in the waterhemp populations from each location, to determine the level and distribution of glyphosate resistance at the field level at the Platte County (MO1) site, and to characterize the level and distribution of ALS- and PPO-inhibitor resistance in the waterhemp population at the MO1 site.

MATERIALS AND METHODS

General Procedures for all Greenhouse Experiments. In all greenhouse trials, 0.25 g of seed from the respective waterhemp population was broadcast into 25- by 50-cm plastic greenhouse flats containing a 3:1 mixture of commercial potting medium[1] to sand. This same mixture was used to cover the seedbed at a thickness of approximately 6 mm. After emergence, waterhemp seedlings were thinned to twenty plants per flat. All plants were maintained in a greenhouse at 25 to 30 C, watered and fertilized as needed, and provided with artificial lighting from metal halide lamps (600 µmol photon m^{-2} s^{-1}) simulating a 16-h photoperiod day. All waterhemp were sprayed with herbicides when average plant height reached 15 cm. Herbicide treatments were applied with a compressed air laboratory spray chamber equipped with a even flat-fan spray nozzle[2] delivering 220 L/ha at 234 kPa. Visual control ratings were taken at 1, 2, and 3 weeks after treatment (WAT) and were based on a scale of 0 to 100, with 0 equal to the appearance and vigor of waterhemp in untreated flats and 100 equal to complete waterhemp death. At 3 WAT, counts of all surviving waterhemp plants in each pot were recorded, and aboveground biomass of all waterhemp plants in each flat was harvested and weighed. Fresh weights were converted to a percentage of the untreated control using the untreated plants from each respective waterhemp population.

All greenhouse experiments were arranged in a completely randomized design and each experiment was conducted twice. All treatments in the glyphosate dose-response experiments were replicated four times while treatments in the MO1 multiple resistance experiments were replicated three times. All data were subjected to ANOVA using the

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[1] Pro-Mix, Hummert International, 4500 Earth City Expressway, Earth City, MO 63045.
[2] TeeJet 8001EVS, Spraying System Co., P.O. Box 7900, Wheaton, Illinois 60189

PROC GLM procedure in SAS[3] and tested for appropriate interactions. Data were pooled when interactions between experimental runs did not occur. In the dose-response experiments, a three-parameter logistic equation; $Y=d/(1+\exp b(\log(x) - \log(e)))$ was used to calculate the herbicide dose resulting in 50% reduction in waterhemp shoot fresh weight (GR_{50}) using the R statistical software program with the drc extension package (Knezevic et al. 2007; R Development Core Team 2006). In this equation, zero is assumed as the lower limit, d represents the upper limit, e represents the GR_{50}, and b represents the slope.

Glyphosate Dose-Response. Just prior to soybean harvest in 2004, approximately 20 female waterhemp seedheads were randomly selected and clipped from plants that appeared to have survived in-crop application(s) of glyphosate within a 75-hectare soybean field in Platte County, Missouri. Mature seed was gleaned from these seedheads and combined into a collective sample representative of the waterhemp population from this field, which was designated MO1. Approximately 60% of this collective sample of seed was tested in the glyphosate dose-response experiments. Similarly, approximately 10 waterhemp seedheads were randomly selected and clipped from plants within a 20-hectare soybean field in Holt County, Missouri that also appeared to survive in-crop applications of glyphosate. Mature seed was gleaned from these seedheads and combined into a collective sample designated as MO2. Approximately 90% of this collective sample of seed was tested in the glyphosate-dose response experiments. During the same year, approximately 10 waterhemp seedheads were randomly selected and clipped from plants within a 30-hectare soybean field in Barton County, Missouri, and seed from this population was designated as the susceptible (S) waterhemp

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[3] SAS Institute, Inc., SAS Campus Drive, Cary, NC 27513.

population used for comparison with the other waterhemp populations in these experiments. This location had a traditional rotation of conventional corn followed by glyphosate-resistant soybean with glyphosate used for weed control in soybean only and herbicides with alternative modes-of-action used for weed control in corn. In addition to the S population, seed from waterhemp populations in Monticello, Missouri (M) and Sutter, Illinois (ST) were also used for comparison to the MO1 and MO2 waterhemp populations. The M and ST populations have been characterized as having a variable response to glyphosate in previous research (Smeda and Schuster, 2002). Waterhemp seed from all five populations was planted and grown in greenhouse flats as described above and treated with the isopropylamine salt of glyphosate[4] at 0, 0.42, 0.84, 1.7, 3.4, and 6.7 kg ae ha^{-1} once plants reached 15 cm in height. A non-ionic surfactant[5] was also added to all treatments at 0.25% v/v along with 2.9 kg ha^{-1} ammonium sulfate.

MO1 Multiple Resistance Characterization. In the early fall of 2006, 14 individual waterhemp seedheads were harvested from across a 5-km area at the site in Platte County, Missouri where glyphosate resistance was initially suspected. A handheld global positioning system was used to mark the location of each individual waterhemp seedhead that was harvested. Mature seed was gleaned from each seedhead and this seed was then designated with a code (W01-W14) according to the location harvested. Seed from these 14 accessions were planted and grown as described previously, along with two susceptible accessions (S1 and S2). Seed from the S1 waterhemp accession was collected in 2004 from the Bradford Research and Extension Center in Boone County, Missouri, and was known to be sensitive to glyphosate and PPO-inhibiting herbicides, but exhibited

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[4] Roundup Original, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.
[5] Induce, Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017.

a high level of resistance to ALS-inhibiting herbicides. For this reason, seed collected from a redroot pigweed (*Amaranthus retroflexus* L.) population in Illinois was utilized as a second susceptible accession (S2), as this population was reported to be sensitive to ALS-inhibiting herbicides (P. Tranel, personal communication), but was known to be resistant to protoporophyrinogen oxidase-inhibiting herbicides. All accessions were treated with the potassium salt of glyphosate[6] at 1.7 kg ae ha^{-1}, lactofen at 0.44 kg ha^{-1}, and thifensulfuron at 0.009 kg ha^{-1} once plants reached 15 cm in height. These rates represent twice the recommended use rates (2X) of these herbicides for the control of waterhemp plants at this stage of growth. A non-ionic surfactant and crop oil concentrate was added at 0.25% v/v to thifensulfuron and lactofen, respectively, and ammonium sulfate was added to all treatments at 2.9 kg ha^{-1}. A nontreated control of each population was also included for comparison.

Field Experiments. Field experiments were conducted during the summers of 2006 and 2007 at separate locations in adjacent fields at the site in Platte County, Missouri where waterhemp seed was initially harvested and glyphosate resistance was suspected. The soil type at both locations in both years was a Waldron silty clay loam (Fine, smectic, calcareous, mesic Aeric Fluvaquents). In 2006, the soil contained 3.0% organic matter and had a pH of 7.3. In 2007, the soil had a pH of 7.4 and contained 2.2% organic matter. Individual plots were 3 by 12 m in size. At all locations, Dekalb ‘93B09’ glyphosate-resistant soybeans were planted into a conventionally-tilled seedbed in rows spaced 76-cm apart at a seeding rate of 420,000 seeds ha^{-1} on May 16, 2006 and May 21, 2007. Fertilizer applications were made according to soil test recommendations provided by the University of Missouri Soil and Plant Testing Laboratory. Treatments were

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[6] Roundup Weathermax, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

arranged in a randomized complete block design and were replicated four times. All treatments were applied at a constant speed of 5 km h^{-1} with a hand-held CO_2-pressurized research backpack sprayer containing 8002 flat-fan nozzle tips that delivered 140 L ha^{-1}. In both years, soybeans were harvested from the two center rows in each plot with a small plot combine and yields were adjusted to 13% moisture content.

In order to better understand the extent of glyphosate resistance in this waterhemp population at the field level, the potassium salt of glyphosate was applied at 0.86, 1.7, 3.4, and 6.7 kg ae ha^{-1} when waterhemp plants reached approximately 15 cm in height. These rates correspond to one, two, four, and eight times the recommended glyphosate use rate for control of waterhemp at this stage of growth. Combinations of the potassium salt of glyphosate at 0.86 kg ae ha^{-1} plus flumiclorac at 0.06 kg ai ha^{-1}, lactofen at 0.14 kg ai ha^{-1}, fomesafen at 0.19 kg ai ha^{-1}, acifluorfen at 0.42 kg ai ha^{-1}, carfentrazone at 0.004 kg ai ha^{-1}, cloransulam at 0.02 kg ai ha^{-1}, and 2, 4-DB at 0.035 kg ai ha^{-1} were also applied at this same time. Ammonium sulfate was added to all treatments at 2.9 kg ha^{-1}, and a nontreated control was also included for comparison. In 2006, all treatments were applied on June 16 while in 2007 treatments were applied on June 20. Just prior to application, 20 common waterhemp plants that ranged from 10 to 15 cm in height within the middle two rows of each plot were flagged for determination of survival over time by lightly tying fluorescent orange ribbon around the base of each plant. Counts of surviving waterhemp plants in each plot were taken at two, four, and six WAT and divided by the original number of flagged plants in order to determine percent survival in response to each treatment. Dead waterhemp plants were defined as any plant with 100% necrotic tissue. In addition to percent waterhemp survival, visual weed control and soybean injury ratings were taken at regular intervals throughout the growing season. Visual ratings were based on a scale of 0 to 100, with 0 equal to the waterhemp vigor and ground cover observed in the nontreated control plots or no soybean injury and 100 equal to complete waterhemp control or complete soybean death.

RESULTS AND DISCUSSION

Glyphosate Dose-Response. Results from these experiments indicate that the MO1 and MO2 waterhemp populations were more tolerant to increasing rates of glyphosate when compared to the ST, M, and S waterhemp populations (Figure 2.1). Estimates of the glyphosate dose required to reduce shoot fresh weight biomass by 50% (GR_{50}) were 2.3 kg ae ha^{-1} for the MO1 population and 1.1 kg ae ha^{-1} for the MO2 population (Table 2.1). In both cases, the glyphosate rates required to reduce fresh weight by 50% are well above the labeled use rate of 0.77 kg ae ha^{-1} recommended for control of waterhemp ranging from 10 to 15 cm in height (Anonymous 2007). In these experiments, the M and ST populations that had previously been reported to have a variable response to glyphosate were comparable or more sensitive to glyphosate than the S biotype, which had a GR_{50} of 0.12 kg ae ha^{-1} (Table 2.1). Comparisons of the GR_{50} of each biotype with that of the S population revealed that the MO1 and MO2 populations exhibited a 19- and 9-fold level of resistance to glyphosate, respectively, when compared with the S waterhemp population. The 9-fold level of resistance observed in the MO2 waterhemp population is similar to the levels of resistance reported in glyphosate-resistant horseweed biotypes from Delaware (VanGessel 2001) and Mississippi (Koger et al. 2004), and also similar to that reported in glyphosate-resistant common ragweed from Missouri (Pollard et al.

2004). However, the 19-fold level of resistance exhibited by the MO1 waterhemp population is considerably higher than that reported in the majority of glyphosate-resistant weed biotypes thus far and also higher than that reported in palmer amaranth from Georgia (Culpepper et al. 2006). Palmer amaranth is the only other species in the *Amaranthus* genus with resistance to glyphosate reported thus far. This relatively high level of resistance in the MO1 waterhemp population suggests that an insensitive EPSP-synthase enzyme may play some role in the overall mechanism leading to glyphosate resistance in the MO1 waterhemp biotype, as an insensitive target site enzyme generally confers a higher level of resistance in a weed or crop species than other non-target site mechanisms (Powles and Preston 2006; Pline-Srnic 2006). Other authors have also suggested that several mechanisms responsible for glyphosate resistance could co-occur in a resistant weed species (Powles and Preston 2006; Westwood and Weller 1997; Zelaya and Owen 2005).

The herbicide use history of the fields in which the MO1 and MO2 waterhemp populations were discovered was likely the single most important factor that led to the selection for glyphosate resistance in these waterhemp populations. In both locations, the waterhemp populations had been exposed to at least one and usually two glyphosate applications annually for the previous six or seven years without utilization of an alternate site of action herbicide. As discussed previously, this herbicide-use pattern is very similar to that reported for the majority of glyphosate-resistant weed biotypes reported thus far.

MO1 Multiple Resistance Characterization. All 14 accessions from across the MO1 site exhibited greater than 65% survival to 2X rates of glyphosate three weeks after treatment, which was higher survival than either the S1 or S2 accessions (Figure 2.2). Similarly, all 14 accessions collected across the MO1 location exhibited greater than 70% survival to thifensulfuron. In addition to these accessions, both the S1 and S2 waterhemp accessions displayed some level of resistance to thifensulfuron, although the S2 accession utilized in this research was believed to be susceptible to ALS-inhibiting herbicides. The resistance of the S2 accession to thifensulfuron is not surprising, as the majority of the waterhemp populations in the Midwest are now resistant to ALS-inhibiting herbicides (Heap 2007). Unlike the response to glyphosate and thifensulfuron, resistance to lactofen was not apparent in all of the waterhemp accessions across the MO1 site. Survival of the W03, W09, W10, and W11 waterhemp accessions ranged from 26 to 38% in response to 2X application of lactofen (Figure 2.2). Although these levels of survival were not as substantial as those from glyphosate and thifensulfuron, these responses indicated some degree of resistance to lactofen as compared to the S1 waterhemp accession. All other accessions from the MO1 site exhibited less than 20% survival in response to 2X rates of lactofen.

Results from these experiments indicate that multiple resistance across two sites of herbicidal action is present in all of the waterhemp accessions collected from the MO1 site, and multiple resistance across three sites of herbicide action is likely to occur in at least four waterhemp accessions collected from this site (Figure 2.2). Based on the location of the waterhemp accessions harvested across the MO1 site, these results also indicate that multiple resistance to glyphosate and thifensulfuron occurred across a 5-km^2 (503 ha) area while waterhemp with multiple resistance to glyphosate, thifensulfuron, and lactofen was more sporadic and confined to a 0.9-km^2 (87 ha) area.

As discussed previously, the extent of the distribution of ALS-inhibiting herbicide resistance across the MO1 site was not unexpected as the majority of common waterhemp populations across the Midwest United States now have some level of resistance to ALS inhibiting herbicides (Heap 2007). Although the mechanism responsible for the spread of glyphosate-resistance in waterhemp across the MO1 site is unknown, based on previous examples it seems likely that the spread of glyphosate resistance across the MO1 site was pollen-mediated. For example, other authors have reported pollen-mediated transfer of glyphosate resistance in horseweed, rigid ryegrass, and goosegrass (Ng et al. 2004; Simarmata et al. 2005; Wakelin and Preston 2006; Zelaya et al. 2004). Franssen et al. (2001) have also shown that ALS-inhibiting herbicide resistance in waterhemp is pollen-mediated.

Field Experiments. There was a significant treatment by year interaction for the waterhemp survival, soybean injury and yield data, therefore results are presented separately by year (Tables 2.2 and 2.3). In 2006, survival of flagged common waterhemp plants 2 WAT in response to 0.86 to 6.7 kg glyphosate ae ha^{-1} ranged from 74 to 100 %, respectively (Table 2.2). At 6 WAT, the range of waterhemp survival in response to these same glyphosate rates declined to 53 to 98%. In 2007, waterhemp survival in response to increasing rates of glyphosate was much lower than that observed in 2006 (Table 2.2). However, 6 WAT 20% of the waterhemp still survived an application of glyphosate at 6.7 kg ae ha^{-1}, which represents eight times the recommended use rate for the control of this species. In both years, waterhemp survival in response to 3.4 and 6.7 kg glyphosate ae ha^{-1} was similar and less than that achieved with either 0.86 or 1.7 kg ae ha^{-1}. Differences in the survival of waterhemp between years are likely a result of changes in the location of the experiments from 2006 to 2007. In 2007, the trial was located on the same farm but approximately 0.75 km from the 2006 research site. Additionally, this higher degree of waterhemp survival may be partially explained by the 6-cm rainfall deficit that occurred in the two weeks before and two weeks after application in 2006 compared to 2007. Other researchers have found that plants growing under water-stressed conditions, such as the waterhemp at the time of the herbicide applications in 2006, are likely to require higher doses of glyphosate in order to achieve complete control compared to plants that are not growing under water-stressed conditions (Ruiter and Meinen, 1998).

In 2006, no glyphosate tank-mix combination increased waterhemp control compared to applications of 0.86 kg glyphosate ae ha^{-1} alone 2 and 6 WAT (Table 2.2). Waterhemp survival ranged from 94 to 98% with all of the glyphosate tank-mix combinations evaluated which was higher than the level of waterhemp survival observed with applications of glyphosate at 1.7, 3.4, and 6.7 kg ae ha^{-1} alone. In 2006, the high degree of waterhemp survival following applications of flumiclorac, carfentrazone, fomesafen, lactofen, and aciflourfen suggested the likelihood of resistance to protoporophyrinogen oxidase (PPO)-inhibiting herbicides in the MO1 waterhemp population (Figure 2.2). Waterhemp survival in response to PPO-inhibiting herbicide tank-mix combinations was much lower in 2007 than 2006, with only 15, 23, and 20% of flagged waterhemp plants surviving tank-mix combinations of glyphosate with fomesafen, lactofen, and acifluorfen, respectively. As discussed previously, this is likely due to the change in the proximity of the research sites and the differential rainfall experienced between years. As observed in the multiple resistance characterization study, resistance to PPO-inhibiting herbicides in the waterhemp population was sporadic across the MO1 site. In both years, combinations of cloransulam, 2, 4-DB, and carfentrazone with 0.86 kg glyphosate ae ha^{-1} resulted in similar levels of waterhemp survival 6 WAT as the standard rate of glyphosate alone. As determined in the multiple resistance characterization experiments, resistance to the ALS-inhibiting herbicide thifensulfuron was widespread throughout the MO1 site; therefore the lack of response to cloransulam, an ALS-inhibiting herbicide, was expected. The tank mixture of glyphosate and 2, 4-DB also failed to increase waterhemp control as compared to the 0.86 kg glyphosate rate alone. Although this is a common practice among some soybean producers, this rate of 2,4-DB is significantly lower than the labeled rate required to control waterhemp at least 10-cm in height (Anonymous 2000).

Soybean injury did not exceed 5% in response to any treatment 2 WAT and by 4 WAT no injury was recorded in either year (data not shown). In both years the highest level of soybean injury 1WAT was in response to glyphosate plus carfentrazone (Table 2.3). Tank mixtures of glyphosate with all of the other herbicides evaluated resulted in less than 7% injury in 2006 and less than 15% injury in 2007. Few differences in soybean yields were observed between treatments in 2006 or 2007, although all herbicide treatments resulted in significantly higher yields than the untreated control (Table 2.3). It is likely that these herbicide treatments provided some reductions in early-season interference and competition, but little season-long control of the glyphosate-resistant waterhemp population. Other researchers have found that near season-long waterhemp competition can reduce soybean yield up to 43 % (Hager et al. 2002). In both years, the combination of glyphosate plus 2, 4-DB resulted in some of the lowest yields of any of the herbicide treatments. This may be a reflection of the poor waterhemp control provided by this treatment but more likely is a result of soybean injury resulting from applications of this herbicide. Culpepper et al. (2001) also reported a 6% decrease in soybean yield across several locations as a result of 2, 4-DB applications at 0.04 kg ha^{-1}.

LITERATURE CITED

Anonymous. 2000. Butyrac 200 herbicide product label. Ankeny IA: Albaugh, Inc. 3 p.

Anonymous. 2007. Roundup Weathermax herbicide product label. Monsanto Company Publication No. 63003F4-40. St. Louis, MO: Monsanto Company. 20 p.

Buhler, D. D., and R. G. Hartzler. 2001. Emergence and persistence of seed of velvetleaf, common waterhemp, woolly cupgrass, and giant foxtail. Weed Sci. 49:230-235.

Culpepper, A. S., A. E. Gimenez, A. C. York, R. B. Batts, and J. W. Wilcut. 2001. Morningglory (*Ipomoea* spp.) and large crabgrass (*Digitaria sanguinalis*) control with glyphosate and 2, 4-DB mixtures in glyphosate-resistant soybean (*Glycine max*). Weed Technol. 15:56-61.

Culpepper, A. S., T. L. Grey, W. K. Vencill, J. M. Kichler, T. M. Webster, S. M. Brown, A. C. York, J. W. Davis, and W. W. Hanna. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci. 54:620-626.

Foes, M. J., L. Liu, P. J. Tranel, L. M. Wax, and E. W. Stoller. 1998. A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci. 46:514-520.

Franssen, A. S., D. Z. Skinner, K. Al-Khatib, M. J. Horak, and P. A. Kulakow. 2001. Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. Weed Sci. 49:598-606.

Hager, A. and C. Sprague. 2002. Weeds on the horizon. *In* The Bulletin, Issue no. 6. Champaign, IL: University of Illinois.

Hager, A., L. Wax, and M. McGlamery. 1997. Waterhemp-Biology of a troublesome species. *In* The Bulletin, Issue no. 4. Champaign, IL: University of Illinois.

Hager, A. G., L. M. Wax, E. W. Stoller, and G. A. Bollero. 2002. Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci. 50:607-610.

Hartzler, R. G., D. D. Buhler, and D. E. Stoltenberg. 1999. Emergence characteristics of four annual weed species. Weed Sci. 47:578-584.

Heap, I. 2007. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/in.asp. Accessed December 6, 2007.

Jasieniuk, M., A. L. Brule-Babel, and I. N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 44:176-193.

Knezevic, S. Z., J. C. Streibig, and C. Ritz. 2007. Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol. 21:840–848.

Koger, C. H., D. H. Poston, R. M. Hayes, and R. F. Montgomery. 2004. Glyphosate-resistant horseweed (*Conyza canadensis*) in Mississippi. Weed Technol. 18:820-825.

Ng, C. H., W. Ratnam, S. Surif, and B. S. Ismail. 2004. Inheritance of glyphosate resistance in goosegrass (*Eleucine indica*). Weed Sci. 52:564-570.

Nordby, D., R. G. Hartzler, and K. W. Bradley. 2007. Biology and management of waterhemp. Glyphosate, Weeds, and Crop Series Publication GWC-13. 12 p.

Patzoldt, W. L, P. J. Tranel, and A. G. Hager. 2005. A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci. 53:30-36.

Perez-Jones, A., K. W. Park, J. Colquhoun, C. Mallory-Smith, and D. Shaner. 2005. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. Weed Sci. 53:775-779.

Pline-Srnic, W. 2006. Physiological mechanisms of glyphosate resistance. Weed Technol. 20:290-300.

Pollard, J. M., B. A. Sellers, and R. J. Smeda. 2004. Differential response of common ragweed to glyphosate. Proc. N. Cent. Weed Sci. Soc. 59:27.

Powles, S. B. and C. Preston. 2006. Evolved glyphosate resistance in plants: biochemical and genetic basis of resistance. Weed Technol. 20:282-289.

R Development Core Team. 2006. R: A Language and Environment for Statistical Computing. 2006. Vienna, Austria: R Foundation for Statistical Computing. URL www.r-project.org.

Ruiter, H. D., and E. Meinen. 1998. Influence of water stress and surfactant on the efficacy, absorption, and translocation of glyphosate. Weed Sci. 46:289-296.

Sellers, B. A., R. J. Smeda, W. G. Johnson, J. A. Kendig, and M. R. Ellersieck. 2003. Comparative growth of six *Amaranthus* species in Missouri. Weed Sci. 51:329-333.

Shoup, D. E., K. Al-Khatib, and D. E. Peterson. 2003. Common waterhemp (*Amaranthus rudis*) resistance to protoporophyrinogen oxidase-inhibiting herbicides. Weed Sci. 51:145-150.

Simarmata, M., S. Bughrara, and D. Penner. 2005. Inheritance of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) from California. Weed Sci. 53:615-619.

Smeda, R. J. and C. L. Schuster. 2002. Waterhemp resistance to glyphosate: Fact or fiction? Proc. N Cent. Weed Sci. Soc. 57:209.

Steckel, L. E., C. L. Sprague, E. W. Stoller, L. M. Wax, and F. W. Simmons. 2007. Tillage, cropping system, and soil depth effects on common waterhemp (*Amaranthus rudis*) seed-bank persistence. Weed Sci. 55:235-239.

Trucco, F., M. R. Jeschke, A. L. Rayburn, and P. J. Tranel. 2005. Promiscuity in weedy amaranths: high frequency of female tall waterhemp (*Amaranthus tuberculatus*) x smooth pigweed (*A. hybridus*) hybridization under field conditions. Weed Sci. 53:46-54.

[USDA] United States Department of Agriculture. 2008. National Agriculture Statistics Service. Agriculture Chemical Use Database: Web page: http//www.pestmanagement.info/nass/app_useage.cfm. Accessed: March 6, 2008.

VanGessel, M.J. 2001. Glyphosate-resistant horseweed in Delaware. Weed Sci. 49:703-705.

Wakelin, A. M., and C. Preston. 2006. Inheritance of glyphosate resistance in several populations of rigid ryegrass (*Lolium rigidum*) from Australia. Weed Sci. 54:212-219.

Webster, T. M. 2005. Weed survey-southern states. Proc. S. Weed Sci. 58:291-306.

Wetzel, D. K., M. J. Horak, D. Z. Skinner, and P. A. Kulakow. 1999. Transferal of herbicide resistance traits from *Amaranthus palmeri* to *Amaranthus tuberculatus*. Weed Sci. 47:538-543.

Westwood, J. H. and S. C. Weller. 1997. Cellular mechanisms influence differential glyphosate sensitivity in field bindweed (*Convolvulus arvensis*) biotypes. Weed Sci. 42:2-11.

Young, B.G. 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol. 20:301-307.

Zelaya, I. A. and M. D. K. Owen. 2005. Differential responses of *Amaranthus tuberculatus* (Moq ex DC) JD Sauer to glyphosate. Pest Manag. Sci. 61:936-950.

Zelaya, I.A., M.D.K. Owen, and M.J. VanGessel. 2004. Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L) Cronq. Theor App Genet. 110:58-70.

Table 2.1 Estimates of the glyphosate dose resulting in 50% reduction in shoot biomass (GR_{50}) and resistance ratios for the Missouri 1 (MO1), Missouri 2 (MO2), Monticello (M), and Sutter (ST) waterhemp populations when compared to a susceptible (S) control.

	Estimate[a]	
Population	GR_{50}	R/S Ratio[b]
	---- kg ae ha^{-1} ----	
MO1	2.3 ± 0.24	19.2
MO2	1.1 ± 0.02	9.2
M	0.007 ± 0.5	0.06
ST	0.12 ± 0.09	1.0
S	0.12 ± 0.10	1.0

[a] Values represent mean ± SE.
[b] R/S Ratio=GR_{50} of respective waterhemp population divided by GR_{50}(S).

Table 2.2. Influence of glyphosate and glyphosate tank-mix combinations on common waterhemp (AMATA) survival at 2 and 6 weeks after treatment (WAT) in Platte County, Missouri during 2006 and 2007.

Treatments[b]	Rate	AMATA Survival[a]			
		2006		2007	
		2WAT	6WAT	2WAT	6WAT
	-- kg ae ha^{-1} --	% [c]			
Glyphosate	0.86	100 a	98 a	90 ab	78 a
Glyphosate	1.7	94 b	89 a	76 c	55 bc
Glyphosate	6.7	74 d	53 b	36 d	20 e
Glyphosate + flumiclorac	0.86 + 0.06	100 a	98 a	61 c	43 cd
Glyphosate + carfentrazone	0.86 + 0.004	100 a	96 a	64 c	63 ab
Glyphosate + fomesafen	0.86 + 0.19	99 a	95 a	23 d	15 e
Glyphosate + lactofen	0.86 + 0.14	100 a	94 a	33 d	23 e
Glyphosate + acifluorfen	0.86 + 0.42	100 a	95 a	35 d	20 e
Glyphosate + cloransulam	0.86 + 0.02	99 a	95 a	85 ab	73 ab
Glyphosate + 2,4-DB	0.86 + 0.04	100 a	98 a	99 a	70 ab

[a] AMATA survival expressed as a percentage of the total plants flagged prior to treatment (n=80).
[b] All treatments applied with 2.9 kg ha^{-1} ammonium sulfate.
[c] Means followed by the same letter within a column are not significantly different, $P<0.05$.

Table 2.3. Influence of glyphosate and glyphosate tank-mix combinations on soybean injury 1 week after treatment (WAT) and yield in Platte County, Missouri during 2006 and 2007.

		Soybean			
		Injury 1WAT		Yield	
Treatments[a]	Rate	2006	2007	2006	2007
	-- kg ae ha^{-1}--	----------------------%[b]---------------------		--------------------kg ha^{-1} [b]-------------	
Glyphosate	0.86	0 d	0 e	2314 ab	3139 a-d
Glyphosate	1.7	0 d	0 e	2318 ab	3136 a-d
Glyphosate	3.4	0 d	5 d	2581 a	3210 abc
Glyphosate	6.7	1 d	10 c	2346 ab	3001 bcd
Glyphosate + flumiclorac	0.86 + 0.06	4 bc	10 c	2284 ab	3247 abc
Glyphosate + carfentrazone	0.86 + 0.004	9 a	17 a	2437 ab	2932 cd
Glyphosate + fomesafen	0.86 + 0.19	2 cd	12 bc	2593 a	3294 ab
Glyphosate + lactofen	0.86 + 0.14	5 b	14 b	2336 ab	3367 a
Glyphosate + acifluorfen	0.86 + 0.42	6 b	13 bc	2524 ab	3279 ab
Glyphosate + cloransulam	0.86 + 0.02	1 d	1 e	2288 ab	2992 bcd
Glyphosate + 2,4-DB	0.86 + 0.04	0 d	10 c	2036 b	2842 d
Untreated		0 d	0 e	939 c	1119 e

[a] All treatments applied with 2.9 kg ha^{-1} ammonium sulfate.

[b] Means followed by the same letter within a column are not significantly different, $P<0.05$

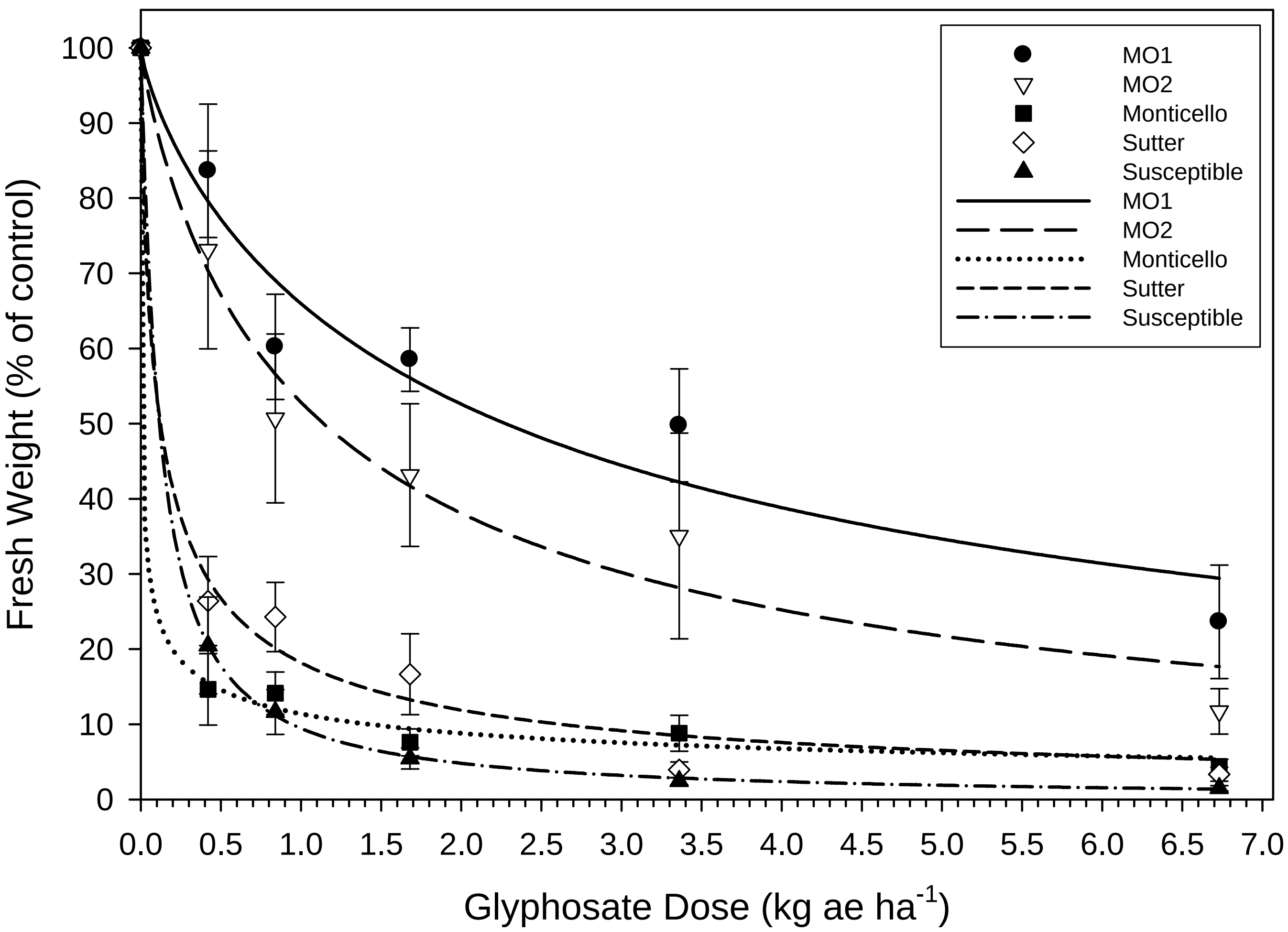

Figure 2.1. Shoot biomass response of the Missouri 1 (MO1), Missouri 2 (MO2), Sutter (ST), Monticello (M), and susceptible (S) waterhemp populations to increasing rates of glyphosate. Symbols and lines represent actual and predicted responses, respectively. Vertical bars represent ± the standard error of the mean.

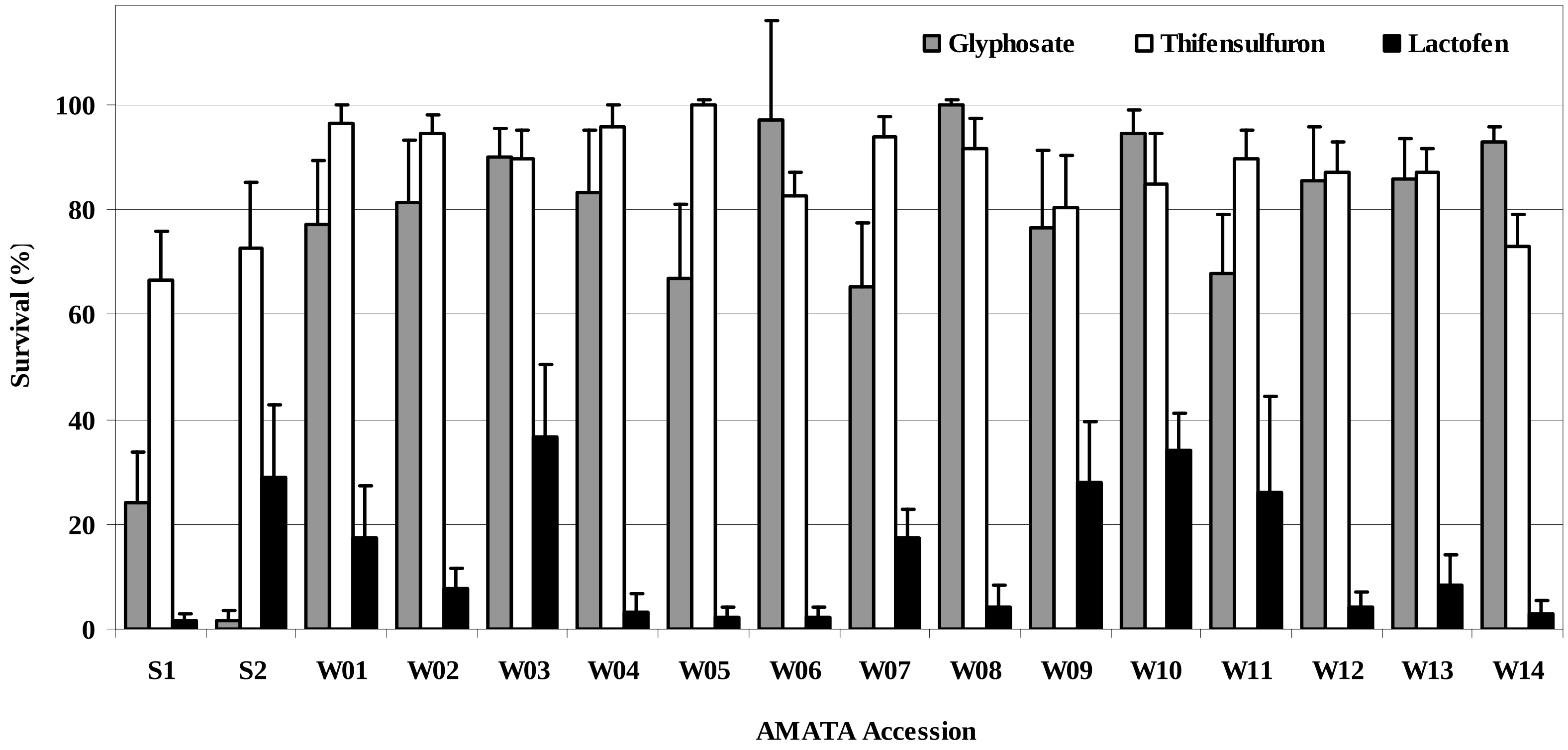

Figure 2.2. Survival of 14 waterhemp accessions from Platte County, Missouri (W01-W14) and two susceptible waterhemp accessions (S1 and S2) to 2X rates of glyphosate, thifensulfuron, and lactofen. Vertical bars represent ± the standard error of the mean, n=80.

CHAPTER III

Evaluation of Herbicide Programs for the Management of Glyphosate-resistant Waterhemp (*Amaranthus rudis*) in Corn[1]

Abstract: Field experiments were conducted in corn to evaluate the influence of herbicide treatments on glyphosate resistant waterhemp control in Platte County, Missouri during 2006 and 2007. Preemergence (PRE), preemergence followed by postemergence (PRE fb POST), and postemergence-only (POST-only) herbicide programs were evaluated for use in conventional, glyphosate-resistant, or glufosinate-resistant corn hybrids. All programs containing a preemergence herbicide, conventional postemergence herbicide, or glufosinate resulted in greater than 98% control of glyphosate-resistant waterhemp and reduced seed production by at least 99%. Waterhemp densities were also reduced by programs that contained a PRE herbicide, conventional POST herbicide, or glufosinate. The highest waterhemp density and poorest control occurred with a sequential glyphosate program. Corn yields did not differ between herbicide treatments in 2006 however several conventional PRE and PRE fb POST programs increased yields compared to the sequential glyphosate program in 2007. Results from this research indicate that several herbicide options are available for the control of glyphosate-resistant common waterhemp in corn. However, the lack of crop rotation at this field site for a period of at least 15 consecutive years and lack of residual herbicide applications typically utilized in corn likely contributed to the increased sensitivity of this waterhemp population to PRE-only corn herbicide programs.

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[1] Received for publication ___ and in revised form ___

Nomenclature: Glufosinate, glyphosate; common waterhemp, *Amaranthus rudis* Sauer; corn, *Zea mays* L.

Key Words: Glyphosate, glyphosate resistance, herbicide programs, waterhemp.

INTRODUCTION

Waterhemp is considered one of the ten most common and troublesome weeds in corn and soybeans throughout Missouri and much of the Midwest (Webster 2005). A survey conducted in Illinois listed *Amaranthus* species as the most common broadleaf weed in corn and soybeans with the vast majority of respondents listing waterhemp specifically (Hager and Sprague 2002). Producers and agricultural retailers also listed waterhemp as the most common weed encountered in corn and soybean production in a survey conducted in Missouri (Bradley et al. 2007).

When allowed to compete with corn for an entire growing season, waterhemp reduced yields by 74% in a year of limited precipitation (Steckel and Sprague 2004). Although most growers would not allow waterhemp to compete season-long, several researchers have found that early season waterhemp competition is most detrimental to corn yields. Cordes et al. (2004) found that corn yields were reduced by 1107 kg/ha when waterhemp was allowed to reach 15 cm in height at densities of 369 to 445 plant/m^2. Steckel and Sprague (2004) found that waterhemp that emerged prior to corn and was allowed to compete up to the V6 growth stage reduced yields up to 50%. The authors also found that waterhemp emerging after the V6 corn growth stage did not significantly affect corn yields. These results suggest that early-season removal of waterhemp is essential to avoiding yield loss, and that later emerging waterhemp does not significantly affect corn yields.

Waterhemp emergence occurs later in the growing season and over a longer period of time than most other agronomic weeds like velvetleaf (*Abutilon theophrasti* Medik.), giant foxtail (*Setaria faberi* Herrm.), and woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth.) (Hartzler, et al. 1999). This discontinuous emergence pattern of waterhemp allows this weed to escape many preemergence herbicide applications and often allows waterhemp to flourish after postemergence applications of non-residual herbicides like glyphosate have been made. Eliminating waterhemp escapes is essential to avoiding future infestations as waterhemp is a very prolific seed producer. Waterhemp that is allowed to compete with soybeans for the length of a growing season is capable of producing 309,000 to 2.3 million seeds per plant (Hartzler and Battles 2004). Waterhemp seed also persist in the soil seedbank for an elongated period of time with 95% of the seed remaining viable after four years of burial (Buhler and Hartzler 2001). All of these factors have contributed to the increased prevalence and difficulty in controlling waterhemp in Midwestern corn and soybean production systems.

Waterhemp is also dioecious in nature and has an ability to outcross with other waterhemp plants and other *Amaranthus* species like smooth pigweed and palmer amaranth (Franssen et al. 2001; Trucco et al. 2005; Wetzel et al. 1999). This creates genetic diversity and an ability to quickly adapt to consecutive applications of the same herbicide or herbicides with the same mechanism of action (Foes et al. 1998; Hager et al. 1997; Nordby et al. 2007). Currently, waterhemp biotypes have been identified with resistance to photosystem II, acetolactate synthase (ALS), and protoporophyrinogen oxidase- (PPO) inhibiting herbicides. A biotype with multiple resistance to PPO and ALS-inhibitors has also been identified in Kansas and a biotype resistant to all three modes of action has been identified in Illinois (Heap 2008; Foes et al. 1998; Patzoldt et al. 2005; Shoup et al. 2003).

Glyphosate resistance has been identified in nine weed species in the United States including waterhemp biotypes that were first identified in Platte County, Missouri in 2005 (Bradley et al. 2006; Heap 2008). After this initial confirmation of glyphosate-resistant waterhemp in Missouri, waterhemp biotypes with resistance to glyphosate have now been identified in Illinois, Kansas and Minnesota (Heap 2008). All of these weed species have developed resistance to glyphosate after continuous exposure to glyphosate over space and time. Glyphosate-resistant horseweed [*Conyza canadensis* (L.) *Cronq.*] and common ragweed (*Ambrosia artemisiifolia* L.) were identified after continuous applications of glyphosate were made on glyphosate-resistant soybeans over three and six years, respectively (Pollard et al. 2004; VanGessel 2001). Similarly, in the Platte County, Missouri location where glyphosate-resistant waterhemp was first identified, at least one application of glyphosate had been applied to glyphosate-resistant soybeans each year for a period of seven years (Bradley et al. 2006; Legleiter and Bradley 2008).

The identification of glyphosate-resistant waterhemp populations in Missouri, Illinois, Kansas, and Minnesota represents a significant threat to Midwestern corn and soybean production systems due to the prevalence of waterhemp as a problem weed and heavy reliance upon glyphosate for weed management in these systems. The objectives of this research were to identify herbicide programs for use in conventional, glyphosate-resistant, or glufosinate-resistant corn production systems for the effective control of glyphosate-resistant waterhemp.

MATERIALS AND METHODS

General Materials and Methods. Field trials were conducted during 2006 and 2007 in Platte County, Missouri where glyphosate-resistant waterhemp was previously identified (Bradley et al. 2006; Legleiter and Bradley 2008). The soil at this site was a Waldron silty clay loam (Fine, smectitic, calcareous, mesic Aeric Fluvaquents) with a pH of 7.3 and 7.4, and organic matter content of 3.0% and 2.2% in 2006 and 2007, respectively. On April 19 in 2006 and on May 21 in 2007 Pioneer '34H35' glyphosate-resistant and glufosinate-resistant corn was planted in 76 cm rows at 70,889 seeds/ha. In 2007, corn planting was severely delayed due to excessive spring rainfall and wet soil conditions experienced at this location (Table 3.1). Fertilizer applications were made according to soil test recommendations provided by the University of Missouri Soil and Plant Testing Laboratory. The experiment was conducted in a randomized complete block design with four replications and individual plots were 3 by 12 meters in length. All herbicide treatments were applied using a hand-held CO_2-pressurized research backpack sprayer containing flat fan nozzle tips[3] that delivered 140 L/ha at a speed of 5 km/h.

Herbicide Treatments. Herbicide treatments evaluated in both years included preemergence only (PRE), preemergence followed by postemergence (PRE fb POST), and postemergence only (POST) treatments. All herbicide programs and rates evaluated are listed in Table 3.2. Application dates and waterhemp and soybean height and density at the time of each application are listed in Table 3.3.

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[3] TeeJet XR8002, Spraying System Co., P.O. Box 7900, Wheaton, Illinois 60189

Treatment Evaluation and Data Collection. Visual waterhemp control, waterhemp density over time, and waterhemp seed production were evaluated in response to each herbicide treatment. Visual evaluations were taken at two week intervals up to eight weeks after each treatment. Visual ratings were based on a scale of 0 to 100%, with 0 equal to the waterhemp vigor and ground cover observed in the untreated control plots or no corn injury, and 100 equal to complete waterhemp control or complete corn death. A 1-m^2 area was permanently established in each plot prior to the first postemergence herbicide applications for measurement of residual waterhemp control over time. Waterhemp densities in each 1-m^2 area were recorded at one and three weeks after the final postemergence herbicide applications. Additionally, prior to corn harvest all female waterhemp seedheads within the two center corn rows in each plot (9.3 m^2 area) were harvested, placed into paper bags, and allowed to dry naturally under greenhouse conditions. Mature seed was then carefully hand gleaned from dried plants and all additional debris removed using a Clipper FR seed cleaner[4] to minimize seed loss. Twenty random samples of pure waterhemp seed were counted each year to provide an average number of waterhemp seed per gram. Waterhemp seed samples from each plot were weighed and seed production in response to each treatment was extrapolated using these previously determined averages. Percent waterhemp seed reduction in response to each treatment was calculated from the number of seed produced in the untreated checks each year. In both years, corn was harvested from the two center rows in each plot with a small plot combine and yields were adjusted to 15.5% moisture content.

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[4] Clipper FR, Blount/Ferrell-Ross, 785 S. Decker Dr., Bluffton, IN 46714

Data analysis. Visual waterhemp control ratings, waterhemp seed production, and corn yield data were subjected to ANOVA using the PROC GLM procedure in SAS[5] and tested for appropriate interactions. Waterhemp density counts recorded throughout the season were combined across herbicide programs rather than listed separately as illustrated in Table 3.2. Waterhemp density data were subject to ANOVA using PROC MIXED in SAS to determine differences between herbicide programs following application. Considerations of orthogonal differences were made due to differing number of samples in the herbicide programs. Data were pooled when interactions between experimental years did not occur.

RESULTS AND DISCUSSION

Waterhemp Control. There was not a significant interaction between years for the visual waterhemp control data, therefore results from both years were combined. Five weeks after the final POST applications, all treatments except the sequential glyphosate treatment provided nearly complete control of glyphosate-resistant waterhemp (Table 3.4). The glyphosate-only program resulted in only 46% control of glyphosate-resistant waterhemp, which is likely a reflection of a heterogeneous population of waterhemp that contained both glyphosate-resistant and susceptible biotypes. There were no differences in waterhemp control between any of the PRE-only or PRE fb POST herbicide treatments evaluated in these trials, which was unexpected and inconsistent with previous research results. Other authors have found that preemergence applications of *S*-metolachlor plus atrazine and acetochlor plus atrazine will only suppress waterhemp germination for a period of 27 to 38 days after application (Schuster and Smeda 2007). These authors also

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[5] SAS Institute, Inc., SAS Campus Drive, Cary, NC 27513

reported that a PRE-only application of *S*-metolachlor plus atrazine plus isoxaflutole resulted in 60% control of waterhemp in comparison to a variety of PRE fb POST programs that resulted in almost complete control of waterhemp.

The especially high level of waterhemp control we are reporting in this research from PRE-only herbicide treatments like atrazine may be due to the history of herbicide use at this location. For example, this field site had been in soybean production for at least 15 consecutive years with glyphosate-resistant soybeans planted consecutively for seven years and glyphosate utilized as the only herbicide during that time period. Therefore, the lack of crop rotation and application of residual herbicides typically utilized in corn likely contributed to the increased sensitivity of this waterhemp population to PRE-only corn herbicide programs.

Waterhemp Seed Reduction. Waterhemp plants produced an average of 686,400 seed per 9.3 m^2 area in 2006 and 101,700 seed per 9.3 m^2 area in 2007. The reduction in waterhemp seed production observed in 2007 was likely a result of the late planting date and different environmental conditions experienced in 2007 compared to 2006 (Table 3.1). Excessive rainfall and cool conditions delayed corn planting until late May in 2007, leading to the removal of previously-emerged waterhemp with tillage prior to planting. Although additional flushes of waterhemp emerged during the course of the growing season, this resulted in lower densities of waterhemp in 2007 compared to 2006. Steckel and Sprague (2004) also found that waterhemp plants that emerged at the V6 corn growth stage produced 90 to 1,200 seeds per plant while waterhemp that emerged with corn produced from 3,000 to 16,000 seeds per plant.

Waterhemp seed reduction was closely correlated with visual ratings of waterhemp control 5 WAT. Waterhemp seed production was reduced by at least 99% for all treatments except for the sequential glyphosate treatment, which reduced seed production by 81% (Table 3.4). Although the sequential glyphosate treatment resulted in less seed reduction than all other treatments, 81% seed reduction was higher than expected for this treatment. In field observations indicated that glyphosate-resistant waterhemp was not controlled with the sequential glyphosate treatment, but waterhemp plant growth was slowed significantly by the glyphosate applications compared to the untreated check. The temporary stunting of waterhemp and competitive ability of corn likely contributed to the high level of waterhemp seed reduction in response to the sequential glyphosate treatment. Uscanga-Mortera et al. (2007) also found that corn can reduce waterhemp seed production by as much as 90%.

Waterhemp Density in Response to Herbicide Programs. There was a significant interaction between waterhemp density in 2006 and 2007, therefore results are presented separately by year. In both years PRE-only, PRE fb conventional postemergence herbicides, PRE fb glyphosate, and PRE fb glufosinate programs were similar and provided significantly lower waterhemp density than the untreated control at all time intervals after treatment (Tables 3.5 and 3.6). Herbicide programs consisting of POST glyphosate + atrazine, POST glufosinate fb glufosinate, POST glufosinate + atrazine fb glufosinate and POST mesotrione + atrazine all resulted in significant reductions in waterhemp density from 0 to 1 WAT and were significantly lower than the untreated control at all time intervals after application. POST glyphosate fb glyphosate programs failed to reduce waterhemp density compared to the untreated control at any timing in either year. When grouped into programs, these results indicate that glyphosate-resistant waterhemp can be effectively controlled with a variety of conventional PRE and POST herbicide programs, with glufosinate-based herbicide programs in glufosinate-resistant corn, and with PRE herbicide programs in glyphosate-resistant corn.

Corn Yield. Corn yield in response to herbicide treatments ranged from 9,002 to 12,654 kg/ha in 2006 and from 8,568 to 10,585 in 2007 (Table 3.4). Although waterhemp control was relatively low with the sequential glyphosate treatment, corn yields were similar to all other herbicide treatments in both years (Table 3.4). In-field observations during both years indicated that glyphosate applications temporarily stunted waterhemp growth and allowed the corn crop to gain a competitive advantage in response to the sequential glyphosate treatment. In 2007, several conventional PRE and PRE fb POST programs increased yields compared to the sequential glyphosate program but few treatments increased yield above that of the untreated control. This is likely due to the late emergence, slow growth, and relatively low density of waterhemp in 2007 compared to 2006 (Table 3.3). In 2007, waterhemp density was approximately 4-fold lower than in 2006 in plots with no preemergence herbicide treatment and these plants did not reach 6 cm in height until June 27. By this time, corn plants had gained a 70-cm height advantage. Cordes et al. (2004) observed a similar response in that low waterhemp densities allowed to compete season long resulted in less than 10% corn yield reduction. Steckel and Sprague (2004) also found that late season competition of waterhemp that emerged after the V6 growth stage had minimal effects on corn yield.

The results from this research indicate that several herbicidal options are available for the control of glyphosate-resistant common waterhemp in corn. All PRE herbicides provided adequate control and seed reduction of glyphosate-resistant waterhemp when applied alone or followed by a POST application of a conventional herbicide, glyphosate, or glufosinate. Programs containing glufosinate following a PRE herbicide application or applied sequentially with or without atrazine provided excellent control of glyphosate-resistant waterhemp and offers an alternative non-selective herbicide option for use in glufosinate-resistant corn. Glyphosate-only programs failed to control glyphosate-resistant waterhemp. However, due to the extraordinarily high sensitivity of this population to PRE corn herbicides, glyphosate-resistant corn and POST glyphosate applications were still an effective method for the control of glyphosate-resistant waterhemp when applied after a PRE herbicide or in combination with a conventional corn herbicide.

LITERATURE CITED

Bradley, K.W., T. Legleiter, L. Hunter, C. Nichols, C. Foresman. 2007. The status of glyphosate-resistant waterhemp in Missouri. Proc N. Cent. Weed Sci. Soc. 62:192.

Bradley, K.W., J. Li, and N.H. Monnig. 2006. Greenhouse investigations of suspected glyphosate-resistant common waterhemp populations from Missouri. Proc Weed Sci. Soc Amer. 46.

Buhler, D. D. and R. G. Hartzler. 2001. Emergence and persistence of seed of velvetleaf, common waterhemp, woolly cupgrass, and giant foxtail. Weed Sci. 49:230-235.

Cordes, J. C., W. G. Johnson, P. Scharf and R. J. Smeda. 2004. Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. Weed Technol. 18:999-1005.

Foes, M. J., L. Liu, P. J. Tranel, L. M. Wax, and E. W. Stoller. 1998. A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci. 46:514-520.

Franssen, A. S., D. Z. Skinner, K. Al-Khatib, M. J. Horak, and P. A. Kulakow. 2001. Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. Weed Sci. 49:598-606.

Hager, A. and C. Sprague. 2002. Weeds on the horizon. *In* The Bulletin, Issue no. 6. Champaign, IL: University of Illinois.

Hager, A., L. Wax, and M. McGlamery. 1997. Waterhemp-Biology of a troublesome species. *In* The Bulletin, Issue no. 4. Champaign, IL: University of Illinois.

Hartzler, R.G., B.A. Battles. 2004. Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci. 52:242-245.

Hartzler, R. G., D. D. Buhler and D. E. Stoltenberg. 1999. Emergence characteristics of four annual weed species. Weed Sci. 47:578-584.

Heap, I. 2007. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/in.asp. Accessed March 26, 2008.

Legleiter T.R. and K.W. Bradley. *In press*. Glyphosate and multiple herbicide resistance in waterhemp (*Amaranthus rudis)* populations from Missouri. Weed Sci.

Nordby, D., R. G. Hartzler, and K. W. Bradley. 2007. Biology and management of waterhemp. Glyphosate, Weeds, and Crop Series Publication GWC-13. 12 p.

Patzoldt, W. L, P. J. Tranel, and A. G. Hager. 2005. A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci. 53:30-36.

Pollard, J. M., B. A. Sellers, and R. J. Smeda. 2004. Differential response of common ragweed to glyphosate. Proc. N. Cent. Weed Sci. Soc. 59:27.

Shoup, D. E., K. Al-Khatib, and D. E. Peterson. 2003. Common waterhemp (*Amaranthus rudis*) resistance to protoporophyrinogen oxidase-inhibiting herbicides. Weed Sci. 51:145-150.

Schuster, C.L. and R.J. Smeda. 2007. Management of *Amaranthus rudis* S. in glyphosate-resistant corn (*Zea mays* L) and soybean (*Glycine max* L. Merr.). Crop Prot. 26:1436-1443.

Steckel, L. E. and C. L. Sprague. 2004. Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci. 52:359-364.

Trucco, F., M. R. Jeschke, A. L. Rayburn, and P. J. Tranel. 2005. Promiscuity in weedy amaranths: high frequency of female tall waterhemp (*Amaranthus tuberculatus*) x smooth pigweed (*A. hybridus*) hybridization under field conditions. Weed Sci. 53:46-54.

Uscanga-Mortera, E., S.A. Clay, F. Forcella, and J. Gunsolus. 2007. Common waterhemp growth and fecundity as influenced by emergence date and competing crop. Agron. J. 99:1265-1270

VanGessel, M.J. 2001. Glyphosate-resistant horseweed in Delaware. Weed Sci. 49:703-705.

Webster, T. M. 2005. Weed survey-southern states. Proc. S. Weed Sci. 58:291-306. Wetzel, D. K., M. J. Horak, D. Z. Skinner, and P. A. Kulakow. 1999. Transferal of herbicide resistance traits from *Amaranthus palmeri* to *Amaranthus tuberculatus*. Weed Sci. 47:538-543.

Table3.1. Average monthly temperature and total monthly precipitation from April through October in 2006 and 2007.

	Temperature		Precipitation	
Month	2006	2007	2006	2007
	--------------------C---------------------		--------------------mm-------------------	
April	17	11	105	78
May	20	20	42	151
June	25	23	32	106
July	28	25	81	25
August	27	28	195	43
September	19	22	56	63
October	14	15	84	164
Total	--	--	596	630

Table 3.2. Preemergence and postemergence herbicide treatments and herbicide programs evaluated in all experiments.

Preemergence Treatment		Postemergence Treatment		
Herbicide	Rate[a]	Herbicide[bc]	Rate[a]	Herbicide Program[d]
	-kg ai/ha-		--------kg ai/ha--------	
acetochlor + atrazine	2.79 + 1.4	mesotrione	0.11	PRE fb conventional post
		diflufenzopyr + dicamba	0.06 + 0.15	PRE fb conventional post
		glufosinate	0.47	PRE fb glufosinate
		glyphosate	0.86	PRE fb glyphosate
		none	----	PRE only
S-metolachlor + mesotrione + atrazine	1.46 + 0.19 + 1.46	mesotrione	0.11	PRE fb conventional post
		diflufenzopyr + dicamba	0.06 + 0.15	PRE fb conventional post
		glufosinate	0.47	PRE fb glufosinate
		glyphosate	0.86	PRE fb glyphosate
		none	----	PRE only
atrazine	2.24	mesotrione	0.11	PRE fb conventional post
		diflufenzopyr + dicamba	0.06 + 0.15	PRE fb conventional post
		glufosinate	0.47	PRE fb glufosinate
		glyphosate	0.86	PRE fb glyphosate
		none	----	PRE only
flufenacet + isoxaflutole	0.69 + 0.08	mesotrione	0.11	PRE fb conventional post
		diflufenzopyr + dicamba	0.06 + 0.15	PRE fb conventional post
		glufosinate	0.47	PRE fb glufosinate
		glyphosate	0.86	PRE fb glyphosate
		none	----	PRE only

none	-----	glufosinate fb glufosinate	0.47 fb 0.41	POST glufosinate fb glufosinate
		glufosinate + atrazine fb glufosinate	0.47 + 2.24 fb 0.41	POST glufosinate + atrazine fb glufosinate
		glyphosate fb glyphosate	0.86 fb 0.86	POST glyphosate fb glyphosate
		glyphosate + atrazine fb glyphosate	0.86 +2.24 fb 0.86	POST glyphosate + atrazine fb glyphosate
		mesotrione + atrazine	0.07 + 1.1	POST mesotrione + atrazine
		none	----	Untreated

[a] Glyphosate rates presented in kg acid equivalent per hectare; all others presented in kg active ingredient per hectare

[b] fb=followed by

[c] Ammonium sulfate added to all glyphosate and glufosinate treatments at 2.8 kg/ha and 3.3 kg/ha, respectively. Mesotrione applied with 1% v/v crop oil concentrate and 2.5%v/v urea and ammonium nitrate.

[d] PRE= preemergence herbicide application; POST= postemergence herbicide application

Table 3.3. Application dates and average height and density of glyphosate-resistant waterhemp (AMATA) and corn at the time of each application in the 2006 and 2007 field experiments.

Application	Application Date		Average Height				Average Density			
			AMATA[a]		Corn		AMATA[a]		Corn	
Timing	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
			cm				m^2			
Preemergence	April 19	May 22	-	-	-	-	-	-	-	-
E-Postemergence[b]	June 1	June 27	8	6	43	76	254	58	36	36
Postemergence[c]	June 13	July 5	10	8	76	198	5	2	36	36
L-Postemergence[d]	June 20	July 9	13	5	102	203	71	13	36	36

[a] AMATA=waterhemp

[b] Early postemergence applications made to plots that did not receive a preemergence treatment.

[c] Postemergence application made to plots that received a preemergence treatment.

[d] Late postemergence application made plots that received early postemergence treatment.

Table 3.4. Influence of preemergence and postemergence herbicides and combinations on glyphosate-resistant waterhemp control, seed reduction, and corn yield in 2006 and 2007.

		Waterhemp		Corn Yield	
Preemergence Herbicide	Postemergence Herbicide	Visual Control[a]	Seed Reduction[b]	2006	2007
		----------------------%	--------------------	-----------kg/ha	---------
acetochlor + atrazine	mesotrione	100	100	10793	10006
	diflufenzopyr + dicamba	100	100	11829	8948
	glufosinate	98	100	10863	8861
	glyphosate	98	100	10305	9350
	none	99	100	9602	9618
S-metolachlor + mesotrione + atrazine	mesotrione	100	100	11309	10283
	diflufenzopyr + dicamba	100	100	11623	10067
	glufosinate	100	100	11847	8741
	glyphosate	100	100	11838	9274
	none	98	100	10800	8880
atrazine	mesotrione	100	100	9656	9052
	diflufenzopyr + dicamba	100	100	11058	9791
	glufosinate	99	100	10223	9438
	glyphosate	99	100	10941	9046
	none	98	100	12654	9366
flufenacet + isoxaflutole	mesotrione	100	100	10853	8568
	diflufenzopyr + dicamba	99	100	10504	10585
	glufosinate	100	100	10227	9182
	glyphosate	100	100	9624	9137
	none	90	99	10954	9748
none	glufosinate fb glufosinate	98	100	10124	9620
	glufosinate + atrazine fb glufosinate	99	100	9002	8719

glyphosate fb glyphosate	46	81	9923	8740
glyphosate + atrazine fb glyphosate	100	100	9667	7909
mesotrione + atrazine	100	100	10194	9360
none	0	0	4259	9131
LSD (0.05)	5	7	2820	1380

[a] Visual waterhemp control five weeks after the final postemergence herbicide application.

[b] Percent reduction in waterhemp seed as compared to the untreated control.

Table 3.5. Influence of corn herbicide programs on glyphosate-resistant waterhemp density at 0, 1, and 3 weeks after the postemergence herbicide applications in 2006.

	Waterhemp Density[a]					
	Weeks After Final Postemergence Treatment					
Herbicide Program[b]	0		1		3	
	---Waterhemp Plants/m^2---					
PRE only	0	aA	1	aA	1	aA
PRE fb conventional post	0	aA	0	aA	0	aA
PRE fb glyphosate	0	aA	0	aA	0	aA
PRE fb glufosinate	0	aA	0	aA	0	aA
POST glyphosate fb glyphosate	429	cB	408	bB	453	bB
POST glyphosate + atrazine fb glyphosate	189	abB	0	aA	0	aA
POST glufosinate fb glufosinate	346	bcC	71	aB	47	aAB
POST glufosinate + atrazine fb glufosinate	186	abB	0	aA	0	aA
POST mesotrione + atrazine	353	bcB	0	aA	0	aA
Untreated	405	bcB	410	bB	409	bB

[a] Means with different lower-case letters represent differences in waterhemp density between herbicide programs within an evaluation interval (columns), P<0.05. Means with different upper-case letters represent differences in waterhemp density within a herbicide program over time (rows), P<0.05.

[b] PRE=preemergence herbicide application; fb=followed by; POST=postemergence application.

Table 3.6. Influence of corn herbicide programs on glyphosate-resistant waterhemp density at 0, 1, and 3 weeks after the postemergence herbicide applications in 2007.

	Waterhemp density[a]					
	Weeks After Final Postemergence Treatment					
Herbicide Program[b]	0		1		3	
	---------------------------------------Waterhemp Plants/m^2---------------------------------------					
PRE only	0	aA	1	aA	1	aA
PRE fb conventional post	0	aA	0	aA	0	aA
PRE fb glyphosate	0	aA	0	aA	0	aA
PRE fb glufosinate	0	aA	0	aA	0	aA
POST glyphosate fb glyphosate	42	dC	17	bB	12	bAB
POST glyphosate + atrazine fb glyphosate	26	bcB	4	aA	1	aA
POST glufosinate fb glufosinate	34	cdB	3	aA	0	aA
POST glufosinate + atrazine fb glufosinate	22	bB	0	aA	0	aA
POST mesotrione + atrazine	20	bB	1	aA	0	aA
Untreated	18	bBC	22	bC	11	abB

[a] Means with different lower-case letters represent differences in waterhemp density between herbicide programs within an evaluation interval (columns), $P<0.05$. Means with different upper-case letters represent differences in waterhemp density within a herbicide program over time (rows), $P<0.05$.

[b] PRE=preemergence herbicide application; fb=followed by; POST=postemergence application.

CHAPTER IV

Glyphosate-Resistant Waterhemp (*Amaranthus rudis*) Control and Economic Returns with Herbicide Programs in Soybean[1]

Abstract: Field experiments were conducted in Platte County, Missouri during 2006 and 2007 to evaluate preemergence (PRE), postemergence (POST), and preemergence followed by postemergence (PRE fb POST) herbicide programs for the control of glyphosate-resistant waterhemp in soybean. All PRE fb POST treatments resulted in at least 66 and 70% control of glyphosate-resistant waterhemp in 2006 and 2007, respectively. Control of glyphosate-resistant waterhemp was less than 23% with lactofen and acifluorfen in 2006, but at least 64% in 2007. This is likely a result of differences in trial locations and a population of protoporphyrinogen oxidase- (PPO) resistant waterhemp at the Platte County site in 2006 compared to 2007. In both years, glyphosate resulted in less than 21% control of glyphosate-resistant waterhemp and provided the least control of all herbicide programs. Programs containing PRE herbicides resulted in waterhemp densities of less than 5 plants/m^2 while the POST glyphosate treatment resulted in 38 to 70 plants/m^2. Waterhemp seed production was reduced by at least 78% in all PRE fb POST programs, from 55 to 71% in POST programs containing lactofen and acifluorfen, and by only 21% in the POST glyphosate treatment. Soybean yields corresponded to the level of waterhemp control achieved in both years, with the lowest yields resulting from programs that provided poor waterhemp control. PRE applications of *S*-metolachlor plus metribuzin provided one of the highest net incomes in both years,

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[1] Received for publication __ and in revised form __.

and resulted in \$271/ha to \$340/ha greater net income than the glyphosate-only treatment. Collectively, the results from these experiments illustrate the effectiveness of PRE herbicide applications for the control of glyphosate-resistant waterhemp in glyphosate-resistant soybeans, and the inconsistency of PPO-inhibiting herbicides or PPO-inhibiting herbicide combinations for the control of waterhemp populations with multiple resistance to glyphosate and PPO-inhibiting herbicides.

Nomenclature: Acifluorfen; flumioxazin; glyphosate; lactofen; metribuzin; *S*-metolachlor; sulfentrazone; common waterhemp, *Amaranthus rudis* Sauer; soybean *Glycine max* L.

Key Words: Acetolactate synthase, net income, glyphosate resistance, herbicide programs, protoporphyrinogen oxidase.

INTRODUCTION

Herbicide-resistant waterhemp biotypes occur across nine states throughout the Midwest (Heap 2008). In 1993, Horak and Peterson (1995) found that a waterhemp biotype in Douglas County, Kansas survived eight times the normal use rate of the acetolactate synthesis (ALS)-inhibiting herbicides thifensulfuron and imazethapyr. Further research revealed that this biotype was 130-fold resistant to imazethapyr, 330-fold resistant to chlorimuron and 490-fold resistant to thifensulfuron (Lovell et al. 1996). In 2008, eight states had reported occurrences of ALS-resistant waterhemp on at least 810,000 hectares (Heap 2008). Triazine-resistant waterhemp has also been reported in Illinois, Kansas, Nebraska, and Missouri (Heap 2008). The occurrence of a waterhemp biotype resistant to both ALS-inhibitors and triazines was confirmed in 1996 in Bond

County, IL. This biotype exhibited greater than 1,000-fold resistance to imazethapyr and 185-fold resistance to atrazine (Foes et al. 1998). A second case of multiple resistance occurred in 2000 in a Kansas waterhemp biotype that exhibited resistance to protoporphyrinogen oxidase (PPO)-inhibiting and ALS-inhibiting herbicides (Shoup et al. 2003). Shortly after the identification of multiple resistance in Kansas, an Illinois waterhemp biotype was found to be resistant to atrazine, ALS-inhibiting, and PPO-inhibiting herbicides (Patzoldt et al. 2005).

The first confirmation of glyphosate-resistant waterhemp occurred in Platte County, Missouri in 2005. This waterhemp biotype was found to be 19-times more resistant to glyphosate than a susceptible biotype and was also confirmed to be resistant to ALS and PPO-inhibiting herbicides (Bradley et al. 2006; Legleiter and Bradley 2008). Since this initial confirmation, glyphosate-resistant waterhemp biotypes have also been confirmed in Illinois, Kansas, and Minnesota (Heap 2008). Waterhemp is the one of nine weed species to develop resistance to glyphosate in the United States (Heap 2008). In Delaware, glyphosate-resistant horseweed was the first resistant species identified in Delaware in 2000 and is now the most widespread glyphosate-resistant weed in the United States (VanGessel 2001; Heap 2008). The other glyphosate-resistant weeds that have been identified in the United States include: Italian ryegrass (*Lolium multiflorum* Lam.), rigid ryegrass (*Lolium rigidum* Gaudin), palmer amaranth (*Amaranthus palmeri* S. Wats.), hairy fleabane [*Conyza bonariensis* (L.) Cronq.], johnsongrass [*Sorghum halepense* (L.) Pers.], and common (*Ambrosia artemisiifolia* L.) and giant ragweed (*Ambrosia trifida* L.) (Heap 2008).

The occurrence of glyphosate-resistant waterhemp is a significant concern for Midwestern corn and soybean producers due to the prevalence of this species throughout the region. Surveys have listed waterhemp as one of the most common and troublesome weeds of corn and soybean production in Illinois and Missouri (Bradley et al. 2007; Hager and Sprague 2002). Factors that have contributed to the troublesome nature of waterhemp include its prolific seed production of up to 2.3 million seeds per plant, discontinuous emergence pattern from late April through mid-July, and dioecious nature that forces out-crossing and leads to genetic diversity (Foes et al. 1998; Hager et al. 1997; Hartzler, et al. 1999; Hartzler and Battles 2004; Steckel et al. 2001).

Glyphosate resistance in waterhemp is also a concern due to the heavy reliance on glyphosate for weed control in glyphosate-resistant soybean systems. Although a number of preemergence and postemergence PPO-inhibiting herbicides have been shown to provide adequate control of waterhemp in soybean, the effectiveness, simplicity, and affordability of glyphosate has resulted in this herbicide being applied to the majority of glyphosate-resistant soybeans in the United States (Johnson et al. 2000; Reddy and Whiting 2000; Sweat et al. 1998). For example, in 2006 glyphosate was applied to 92% of the soybean acreage in the United States while ten years previously glyphosate was only applied to 25% of the acreage (USDA 2008).

Previous research conducted at a field site in Platte County, Missouri has revealed that waterhemp accessions resistant to ALS-inhibitors and glyphosate occur across a 503-ha area and within this area accessions also resistant to PPO-inhibitors occur across an 87-ha area (Legleiter and Bradley 2008). The objectives of this research were to identify herbicide programs that provide effective control of glyphosate-resistant waterhemp, maximize soybean seed yields, and provide highest net incomes in glyphosate-resistant soybean.

MATERIALS AND METHODS

Field trials were conducted during 2006 and 2007at a field site in Platte county, Missouri where waterhemp was previously confirmed to be resistant to glyphosate (Bradley et al. 2006; Legleiter and Bradley 2008). Trials were conducted in adjacent fields at this site approximately 0.75 km apart. Soil types at both locations were a Waldron silty clay loam (Fine, smectitic, calcareous, mesic Aeric Fluvaquents). In 2006, the soil contained 3.0% organic matter and had a pH of 7.3. In 2007, the soil had a pH of 7.4 and contained 2.2% organic matter. Dekalb '38-52' glyphosate-resistant soybeans were planted into a conventionally tilled seedbed at 420,000 seed/ha in 76 cm rows on May 16, 2006 and May 21, 2007. Fertilizer applications were made according to soil test recommendations provided by the University of Missouri Soil and Plant Testing Laboratory. Experiments were conducted in a randomized complete block design with four replications, with individual plots that measured 3 by 12 meters in length. Herbicide treatments were applied using a hand-held CO_2-pressurized research backpack sprayer containing 8002 flat fan nozzle tips[1] that delivered 140 L/ha at a speed of 5 km/h. In both years, soybeans were harvested from the two center rows in each plot with a small plot combine and yields were adjusted to 13% moisture content.

The programs evaluated each year consisted of preemergence only (PRE), postemergence only (POST), and preemergence followed by postemergence (PRE fb POST) herbicide treatments. Herbicide treatments and rates evaluated in each herbicide

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[1] TeeJet XR8002, Spraying System Co., P.O. Box 7900, Wheaton, Illinois 60189

program are listed in Table 4.1. Average monthly temperatures and total monthly precipitation amounts are recorded in Table 4.2. Application dates and waterhemp and soybean height and density at the time of each application are listed in Table 4.3.

Visual ratings of waterhemp control and soybean injury were taken at two week intervals up to eight weeks after each application. Visual ratings were based on a scale of 0 to 100, with 0 equal to the waterhemp vigor and ground cover observed in the untreated control plots, and 100 equal to complete waterhemp control. Prior to the first postemergence treatment, a 1-m^2 area was established between the center two soybean rows in each plot. Waterhemp densities were recorded in these areas at the first postemergence application and at 2-wk intervals up to eight weeks after treatment. Prior to soybean harvest, female seedheads from within the center two rows of soybeans in each plot (9.3 m^2 area) were clipped and placed in paper bags and allowed to dry naturally. Mature seed were hand-gleaned from the dried plants, and any additional debris was removed from the pure seed sample using a Clipper FR seed cleaner[2]. Cleaned seed from all treatments were weighed and seed production in response to each treatment was determined from the average number of waterhemp seed in a 0.10 g sample. Each year, twenty random samples of waterhemp seed were counted to provide the average number of waterhemp seed per 0.10 gram. Seed production in each plot was compared to that of the untreated check to provide a percent reduction of waterhemp seed in response to each herbicide program.

Economic Analysis. The net income in response to each herbicide program was calculated by subtracting the estimated treatment costs from gross income. Gross income

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[2] Clipper FR, Blount/Ferrell-Ross, 785 S. Decker Dr., Bluffton, IN 46714

was calculated in $/ha and was determined by multiplying the soybean yield from each treatment by the average soybean price of $10.16/bu. This is a projected soybean price for the next 5 years forecasted by the Food and Agricultural Policy Institute (FAPRI 2008a). The cost of each treatment was calculated from a recent wholesale price sheet of herbicides and adjuvants provided by a major agricultural retailer in the Midwest. A custom application fee of $12.36/ha was also included for each herbicide application made within a program. Additional soybean production costs of $826/ha were also subtracted from the gross income of each treatment. This the estimated soybean production cost including operating and ownership cost as estimated by the Food and Agriculture Policy Institute (FAPRI 2008b).

Data Analysis. Visual waterhemp control ratings, percent seed reduction, and soybean yield data were subjected to ANOVA using PROC GLM in SAS[3] and tested for appropriate interactions. Waterhemp density data from the 1-m^2 areas were combined across treatments into eight soybean herbicide programs as described in Table 4.1. Waterhemp density data were subjected to a mixed model using PROC MIXED in SAS[3] to determine differences between herbicide programs over time with considerations of orthogonal differences due to differing number of samples in the herbicide programs. There was a significant interaction between years for the waterhemp control, waterhemp density, and soybean yield data; therefore results are presented separately by year. Interactions between experimental years did not occur for the waterhemp seed reduction data, therefore results were pooled across years.

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[3] SAS Institute, Inc., SAS Campus Drive, Cary, NC 27513

RESULTS AND DISCUSSION

Waterhemp Control. Visual ratings of waterhemp control 3 months after planting in both years revealed that PRE and PRE fb POST programs containing sulfentrazone and *S*-metolachlor plus metribuzin provided greater than 80% control of glyphosate-resistant waterhemp (Tables 4.4 and 4.5). Other authors have also reported excellent control of waterhemp with applications of sulfentrazone at 0.17 to 0.35 kg/ha (Dirks et al. 2000; Hager et al. 2002a; Krausz et al. 1998; Sweat et al. 1998). Sweat et al. (1998) reported excellent waterhemp control 4 WAT with PRE applications of alachlor, but our results indicate that by 8 WAT, waterhemp control will range from only 45 to 72%.

POST-only herbicide programs resulted in greatly different levels of glyphosate-resistant waterhemp control between years (Tables 4.4 and 4.5). Glyphosate-resistant waterhemp control was less than 23% with lactofen and acifluorfen in 2006, but provided at least 64% control of glyphosate-resistant waterhemp in 2007. The inconsistency of these PPO-inhibiting herbicides on glyphosate-resistant waterhemp is likely a result of a heterogeneous population of PPO-resistant and susceptible waterhemp at the Platte County site. Previous research conducted at this site revealed that PPO-resistant waterhemp accessions occur sporadically across an 87 ha area of this site (Legleiter and Bradley 2008). The trial location in 2006 was well within this defined area, while in 2007 the trial location was located on the outer edge of this area. This likely accounts for the improved control of waterhemp in 2007 compared to 2006.

In both years the POST glyphosate-only treatment resulted in less than 21% control of glyphosate-resistant waterhemp and provided the least control of all herbicide programs (Tables 4.4 and 4.5). The addition of a PRE herbicide treatment prior to POST glyphosate applications greatly improved waterhemp control in both years.

Waterhemp Density. During both years PRE only, PRE fb glyphosate, PRE fb glyphosate + PPO, and PRE fb PPO programs had lower densities at all intervals after application compared to POST glyphosate programs and the untreated control (Tables 4.6 and 4.7). As with the visual control ratings, differences in waterhemp density occurred with the POST PPO and POST glyphosate + PPO programs between 2006 and 2007. During the 2006 season both programs failed to reduce waterhemp densities and were similar to the untreated control at all time intervals after application (Table 4.6). In 2007, POST PPO and POST glyphosate + PPO programs resulted in lower waterhemp densities than the untreated control and were similar to programs containing PRE herbicide applications (Table 4.7). During both years, the POST glyphosate treatments failed to reduce waterhemp densities and were similar the untreated control at all time intervals after application (Tables 4.6 and 4.7). These results illustrate the effectiveness of PRE herbicide applications for the control of glyphosate-resistant waterhemp in glyphosate-resistant soybeans, and the inconsistency of PPO-inhibiting herbicides or PPO-inhibiting herbicide combinations for the control of waterhemp populations with multiple resistance to glyphosate and PPO-inhibiting herbicides.

Waterhemp Seed Production. Waterhemp seed production was reduced by at least 78% for all PRE fb POST programs (Table 4.8). PRE treatments containing sulfentrazone and *S*-metolachlor plus metribuzin resulted in greater seed reduction than those containing flumioxazin or alachlor, which correlated with visual waterhemp control ratings. Other authors have also reported that waterhemp plants that emerge after an established crop produce less seed than those that emerge with the crop (Hartzler and Battles 2004; Steckel and Sprague 2004). For this reason, PRE herbicide applications likely contributed to the decrease in waterhemp seed production in the PRE and PRE fb POST programs.

Despite variable waterhemp control and densities between years, percent waterhemp seed reduction in response to POST herbicide programs was similar between years (Table 4.8). The POST programs containing lactofen and acifluorfen resulted in seed reductions between 55 to 71%, and were lower than the majority of programs that received PRE herbicide applications. The POST-only glyphosate treatment resulted in a 21% reduction in waterhemp seed, which was the lowest reduction of all the herbicide programs evaluated in this research (Table 4.8). Waterhemp at densities of 29 plants/m^2 produced approximately 82,900 seeds/m^2 in response to the POST-only glyphosate program (data not shown). Similarly, Bensch et al. (2003) reported that waterhemp densities of 8 plants/m row resulted in approximately 51,800 waterhemp seeds/m^2.

Soybean Yield. Soybean yields were higher with PRE fb POST programs than all POST programs in 2006, but similar to POST programs containing lactofen and acifluorfen in 2007 (Tables 4.9 and 4.10). This is likely a result of the increased efficacy of PPO-inhibiting herbicides on glyphosate-resistant waterhemp in 2007 compared to 2006 (Tables 4.4 and 4.5). Increased soybean yield with programs containing PRE herbicide applications can also be attributed to the delay in waterhemp emergence, as waterhemp emerging after the V4-V5 soybean stage will only result in minor soybean yield reductions (Steckel and Sprague 2004). Soybean yields from the PRE *S*-metolachlor plus metribuzin program were higher than the PRE alachlor and PRE flumioxazin programs in

2006 and 2007, respectively. This treatment was also one of the highest-yielding treatments of all treatments evaluated in both years of this study (Tables 4.9 and 4.10).

In 2006, some of the lowest soybean yields occurred in response to the POST glyphosate and PPO-inhibiting herbicide treatments, since the location in 2006 contained waterhemp with multiple resistance to glyphosate and PPO-inhibiting herbicides (Table 4.9). In 2007 however, POST applications of PPO-inhibiting herbicides provided yields similar to most PRE fb POST and PRE herbicide programs while glyphosate alone still resulted in lower yields than any of the other treatments evaluated (Table 4.10).

Although a true untreated weed-free control was not included in these experiments, we observed a 23 to 34% soybean yield reduction with the POST glyphosate treatment when compared to the highest-yielding treatment in each year. Yield losses from the POST glyphosate treatment were not as severe as the 56% yield loss reported by Bensch et al. (2003) due to season-long waterhemp interference, but were similar to yield losses reported by Hager et al. (2002b) from 6 to 8 weeks of waterhemp interference.

Net Income. Herbicide treatments resulted in a net income of -\$23 to \$336/ha in 2006 and \$138 to \$409/ha in 2007. The POST glyphosate treatment, despite being the cheapest in cost (data not shown), returned a negative income in 2006 and ranked as one of the lowest in net income in 2007 due to ineffective waterhemp control, increased waterhemp competition, and lower soybean yields (Tables 4.11 and 4.12). In 2006, POST applications of lactofen and acifluorfen resulted in net incomes of \$137 and \$117/ha, respectively. In 2007, however, net income from lactofen and acifluorfen treatments was \$338 and \$232/ha, which were some of the highest net incomes reported during this year (Tables 4.11 and 4.12). These results illustrate that high net incomes can be obtained when treating glyphosate-resistant waterhemp populations with PPO-inhibiting herbicides, but that net income will be significantly reduced if waterhemp exhibits multiple resistance to both modes of action.

Because of only minor differences in soybean yield between PRE fb POST herbicide programs, net income was influenced to a much greater extent by the cost of the individual herbicide treatments within these programs. Programs containing sulfentrazone, which was relatively high in cost, resulted in lower net incomes than flumioxazin that was much lower in cost. During both years, PRE-only applications of *S*-metolachlor plus metribuzin resulted in the highest net income of all PRE treatments and also provided the highest and second highest net income of all herbicide programs in 2007 and 2006, respectively (Tables 4.11 and 4.12).

These results indicate that the occurrence of glyphosate-resistant common waterhemp will have a significant economic impact in soybeans. Other researchers have found that a sequential glyphosate program in glyphosate-resistant soybeans resulted in $90/ha more in net income compared to sulfentrazone fb glyphosate in a field infested with waterhemp and giant foxtail (Dirks et al. 2000). In our research, sulfentrazone fb glyphosate provided $292/ha and $91/ha greater net income in 2006 and 2007, respectively, than the glyphosate-only treatment. Additionally, *S*-metolachlor plus metribuzin, which resulted in one of the highest net incomes in both years of this research, provided $271/ha and $340/ha greater net income than the glyphosate-only treatment.

Collectively, the results from this research reveal the necessity of a PRE herbicide application for the management of glyphosate- and specifically multiple-resistant waterhemp in glyphosate-resistant soybeans. Programs containing PRE herbicide applications resulted in the greatest control of glyphosate-resistant waterhemp, reduced the amount of glyphosate-resistant waterhemp seed production, and provided the highest soybean yields and net incomes. POST herbicide applications following the initial PRE treatment typically increased the control of glyphosate-resistant waterhemp, but the overall value of a PRE fb POST program was dependent on the PRE herbicide in question.

LITERATURE CITED

Bensch, C.N., M.J. Horak, and D. Peterson. 2003. Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci. 51:37-43.

Bradley, K.W., T. Legleiter, L. Hunter, C. Nichols, C. Foresman. 2007. The status of glyphosate-resistant waterhemp in Missouri. Proc N. Cent. Weed Sci. Soc. 62:192.

Bradley, K.W., J. Li, and N.H. Monnig. 2006. Greenhouse investigations of suspected glyphosate-resistant common waterhemp populations form Missouri. Proc Weed Sci. Soc Amer. 46.

Dirks, J. T., W. G. Johnson, R. J. Smeda, W. J. Wiebold and R. E. Massey. 2000. Use of preplant sulfentrazone in no-till, narrow-row, glyphosate-resistant *Glycine max*. Weed Sci. 48:628-639.

[FAPRI] Food and Agriculture Policy Research Institute. 2008a.US Baseline Briefing Book March 2008. Columbia, MO: Food and Agriculture Policy Research Institute at the University of Missouri. p27.

[FAPRI] Food and Agriculture Policy Research Institute. 2008b. Missouri Crop Budget for 2008. Available at http://www.fapri.missouri.edu/farmers_corner/budgets/2008/BudgetUpdate_March08.pdf. Accessed April 23, 2008.

Foes, M.J., L. Liu, P.J. Tranel, L.M. Wax, E.W. Stoller. 1998. A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci. 46:514-520.

Hager, A. and C. Sprague. 2002. Weeds on the horizon. *In* The Bulletin, Issue no. 6. Champaign, IL: University of Illinois.

Hager, A., L. Wax, and M. McGlamery. 1997. Waterhemp-Biology of a troublesome species. *In* The Bulletin, Issue no. 4. Champaign, IL: University of Illinois.

Hager, A. G., L. M. Wax, G. A. Bollero and F. W. Simmons. 2002a. Common waterhemp (*Amaranthus rudis* Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.). Crop Prot. 21:277-283.

Hager A.G., L.M. Wax, E.W. Stoller, and G.A. Bollero. 2002b. Common waterhemp (*Amaranthus rudis)* interference in soybean. Weed Sci. 50: 607-610.

Hartzler, R.G. and B.A. Battles. 2004. Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci. 52:242-245.

Hartzler, R. G., D. D. Buhler and D. E. Stoltenberg. 1999. Emergence characteristics of four annual weed species. Weed Sci. 47:578-584.

Heap, I. 2008. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/in.asp. Accessed March 26, 2008.

Horak, M.J. and D.E. Peterson. 1995. Biotypes of palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol. 9:192-195.

Johnson, W.G., P.R. Bradley, S.E. Hart, M.L. Buesinger, and R.E. Massey. 2000. Efficacy and economics of weed management in glyphosate-resistant corn (*Zea mays*). Weed Technol. 14:57-65.

Krausz, R. F., G. Kapusta and J. L. Matthews. 1998. Sulfentrazone for weed control in soybean (*Glycine max*). Weed Technol. 12:684-689.

Legleiter T.R. and K.W. Bradley. *In press*. Glyphosate and multiple herbicide resistance in waterhemp (*Amaranthus rudis)* populations from Missouri. Weed Sci.

Lovell S.T., L.M. Wax, M.J. Horak, and D.E. Peterson. 1996. Imidazolinone and sulfonylurea resistance in a biotype of common waterhemp (*Amaranthus rudis*). Weed Sci. 44:789-794.

Patzoldt, W.L., P.J. Tranel, and A.G. Hager. 2005. A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci. 53:30-36.

Reddy, K.N. and K. Whiting. 2000. Weed control and economic comparisons of glyphosate-resistant, sulfonylurea-resistant, and conventional soybean (*Glycine max*) systems. Weed Technol. 14:204-211.

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

Steckel, L.E., C.L. Sprague, F.W. Simmons, G. Bollero, A. Hager, E.W. Stoller, and L.M. Wax. 2001. Tillage and cropping effects on common waterhemp (*Amaranthus rudis*) emergence and seed bank distribution over four years. Weed Sci. Soc. of Amer. Abstract. 41:321.

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

Sweat, J.K., M.J. Horak, D.E. Peterson, R.W. Lloyd, and J.E. Boyer. 1998. Herbicide efficacy on four *Amaranthus* species in soybean (*Glycine max*). Weed Technol. 12:315-321.

[USDA] United States Department of Agriculture. 2008. National Agriculture Statistics Service. Agriculture Chemical Use Database: Web page: http//www.pestmanagement.info/nass/app_useage.cfm. Accessed: March 26, 2008.

VanGessel, M.J. 2001. Glyphosate-resistant horseweed in Delaware. Weed Sci. 49:703-705.

Table 4.1. Preemergence and postemergence herbicide treatments and rates and representative herbicide programs used to evaluate waterhemp densities.

Preemergence treatment		Postemergence Treatment		
Herbicide	Rate	Herbicide[a]	Rate[b]	Herbicide Program[c]
	--kg ai/ha--		--kg ai/ha--	
flumioxazin	0.09	glyphosate	0.86	PRE fb glyphosate
		lactofen	0.14	PRE fb PPO
		acifluorfen	0.42	PRE fb PPO
		glyphosate + lactofen	0.86 + 0.14	PRE fb glyphosate + PPO
		glyphosate + acifluorfen	0.86 + 0.42	PRE fb glyphosate + PPO
		None	----	PRE only
sulfentrazone	0.28	glyphosate	0.86	PRE fb glyphosate
		lactofen	0.14	PRE fb PPO
		acifluorfen	0.42	PRE fb PPO
		glyphosate + lactofen	0.86 + 0.14	PRE fb glyphosate + PPO
		glyphosate + acifluorfen	0.86 + 0.42	PRE fb glyphosate + PPO
		None	----	PRE only
alachlor	2.8	glyphosate	0.86	PRE fb glyphosate
		lactofen	0.14	PRE fb PPO
		acifluorfen	0.42	PRE fb PPO
		glyphosate + lactofen	0.86 + 0.14	PRE fb glyphosate + PPO
		glyphosate + acifluorfen	0.86 + 0.42	PRE fb glyphosate + PPO
		None	----	PRE only
S-metolachlor + metribuzin	1.54 + 0.36	glyphosate	0.86	PRE fb glyphosate
		lactofen	0.14	PRE fb PPO

		acifluorfen	0.42	PRE fb PPO
		glyphosate + lactofen	0.86 + 0.14	PRE fb glyphosate + PPO
		glyphosate + acifluorfen	0.86 + 0.42	PRE fb glyphosate + PPO
		None	----	PRE only
None	----	glyphosate	0.86	POST glyphosate
		lactofen	0.14	POST PPO
		acifluorfen	0.42	POST PPO
		glyphosate + lactofen	0.86 + 0.14	POST glyphosate + PPO
		glyphosate + acifluorfen	0.86 + 0.42	POST glyphosate + PPO
		None	----	Untreated

[a] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatment when applied alone.

[b] glyphosate rates presented in acid equivalent per hectare, all other herbicides presented in active ingredient per hectare.

[c] PRE= preemergence herbicide application; fb= followed by; PPO=protoporphyrinogen oxidase inhibiting herbicide; POST= postemergence herbicide application.

Table 4.2. Average temperature and precipitation totals for the months of April through October for 2006 and 2007.

	Temperature		Precipitation	
Month	2006	2007	2006	2007
	-----------------C-----------------		----------------mm---------------	
April	17	11	105	78
May	20	20	42	151
June	25	23	32	106
July	28	25	81	25
August	27	28	195	43
September	19	22	56	63
October	14	15	84	164
Total	-	-	596	63

Table 4.3. Application information for glyphosate-resistant waterhemp (AMATA) and soybean in the 2006 and 2007 field experiments.

			Average Height				Average Density			
Application	Application Date		AMATA[a]		Soybean		AMATA[a]		Soybean	
Timing	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
			---------- cm ----------				---------- m^2 ----------			
Preemergence	May 18	May 22	--	--	--	--	--	--	--	--
Postemergence[b]	June 21	June 20	8	8	28	15	101	54	210	168
Postemergence[c]	June 28	July 5	10	8	41	28	16	6	180	162

[a] AMATA= waterhemp

[b] Postemergence herbicide applications to plots that did not receive a preemergence herbicide.

[c] Postemergence herbicide applications to plots that received a preemergence herbicide application.

Table 4.4. Influence of preemergence and postemergence herbicides and combinations on glyphosate-resistant waterhemp control three months after planting in 2006.

	Waterhemp Control[a]					
	Postemergence Treatments[b]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
	%					
Flumioxazin	68	81	66	86	85	58
Sulfentrazone	89	94	91	95	95	80
Alachlor	76	85	73	86	88	45
S-metolachlor+metribuzin	88	88	81	95	94	80
None	23	23	0	5	3	0
LSD (0.05):	12					

[a] Percent visual control three months after planting.

[b] Ammonium sulfate added to all glyphosate treatments at 2.8/kg ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.

Table 4.5. Influence of preemergence and postemergence herbicides and combinations on glyphosate-resistant waterhemp control three months after planting in 2007.

	Waterhemp Control[a]					
	Postemergence Treatments[b]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
	%					
Flumioxazin	76	73	70	71	84	48
Sulfentrazone	99	99	99	99	99	94
Alachlor	88	89	89	96	96	72
S-metolachlor+metribuzin	99	99	94	94	99	94
None	64	65	23	59	81	0
LSD (0.05):	14					

[a] Percent visual control three months after planting.

[b] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.

Table 4.6. Influence of soybean herbicide programs on glyphosate-resistant waterhemp densities in 2006.

	Waterhemp Density[ab]				
	Weeks After First Postemergence Treatment				
Herbicide Program[c]	0	2	4	6	8
	Waterhemp Plants/m^2				
PRE only	2 aA	2 aA	2 aA	2 aA	2 aA
PRE fb glyphosate	2 aA	2 aA	2 aA	2 aA	2 aA
PRE fb glyphosate + PPO	3 aA	2 aA	1 aA	1 aA	1 aA
PRE fb PPO	2 aA	2 aA	2 aA	2 aA	1 aA
POST glyphosate	112 bB	97 bB	57 bA	70 bA	51 bA
POST PPO	165 cC	130 cB	76 bA	75 bA	60 bA
POST glyphosate + PPO	190 cC	105 bcB	55 bA	66 bA	50 bA
Untreated	105 bB	101 bcB	58 bA	76 bA	54 bA

[a] lower case letters represent differences (LSD at $P \leq 0.05$) between herbicide programs within a column.
[b] upper case letters represent differences (LSD at $P \leq 0.05$) between weeks within a herbicide program.
[c] PRE=preemergence herbicide application; fb=followed by; PPO=protoporphyrinogen oxidase-inhibiting herbicide; POST postemergence herbicide application.

Table 4.7. Influence of soybean herbicide programs on glyphosate-resistant waterhemp densities in 2007.

	Waterhemp Density[ab]				
	Weeks After First Postemergence Treatment				
Herbicide Program[c]	0	2	4	6	8
	-----Waterhemp Plants/m^2-----				
PRE only	3 aA	5 aA	5 aA	5 aA	4 abA
PRE fb glyphosate	0 aA	7 aB	1 aAB	2 aAB	2 abAB
PRE fb glyphosate + PPO	0 aA	7 aB	1 aA	1 aA	1 aA
PRE fb PPO	1 aA	5 aA	2 aA	2 aA	1 abA
POST glyphosate	66 cC	64 bC	39 bB	38 bB	19 bcA
POST PPO	34 bB	14 aA	9 aA	10 aA	6 abA
POST glyphosate + PPO	58 cC	16 aB	11 aAB	12 aAB	7 abA
Untreated	56 cB	69 bC	49 bAB	50 bAB	39 cA

[a] lower case letters represent differences (LSD at $P \leq 0.05$) between herbicide programs within a column.
[b] upper case letters represent differences (LSD at $P \leq 0.05$) between weeks within a herbicide program.
[c] PRE=preemergence herbicide application; fb=followed by; PPO=protoporphyrinogen oxidase-inhibiting herbicide; POST postemergence herbicide application.

Table 4.8. Influence of preemergence and postemergence herbicides and combinations on percent glyphosate-resistant waterhemp seed reduction in 2006 and 2007.

	Percent Waterhemp Seed Reduction[a]					
	Postemergence Treatments[b]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
			%			
Flumioxazin	80	81	78	94	99	61
Sulfentrazone	99	100	99	100	100	93
Alachlor	98	96	91	98	99	77
S-metolachlor+metribuzin	98	99	97	99	99	94
None	70	69	21	55	71	0
LSD (0.05):			17			

[a] Percent waterhemp seed reduction per 9.3 m^2 area prior to soybean harvest as compared to the waterhemp seed production in the untreated control.

[b] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.

Table 4.9. Influence of preemergence and postemergence herbicides and combinations on soybean yields at the glyphosate-resistant waterhemp site in Platte County, Missouri in 2006.

	Soybean Yield					
	Postemergence Treatments[a]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
	kg/ha					
Flumioxazin	3,169	2,921	3,207	3,181	3,220	2,754
Sulfentrazone	2,994	3,292	3,265	3,189	3,313	2,858
Alachlor	3,098	3,274	2,980	3,043	3,006	2,484
S-metolachlor+metribuzin	3,152	3,389	3,112	3,120	3,112	3,222
None	2,683	2,643	2,243	2,632	2,691	1,676
LSD (0.05):	413					

[a] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.

Table 4.10. Influence of preemergence and postemergence herbicides and combinations on soybean yields at the glyphosate-resistant waterhemp site in Platte County, Missouri in 2007.

	Soybean Yield					
	Postemergence Treatments[a]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
	kg/ha					
Flumioxazin	2,965	3,176	3,313	3,042	3,268	2,637
Sulfentrazone	3,061	3,187	3,156	2,760	3,114	3,149
Alachlor	3,205	3,300	3,018	2,941	3,114	3,112
S-metolachlor+metribuzin	3,426	3,220	3,124	3,013	3,167	3,470
None	3,222	2,954	2,680	3,189	3,166	1,594
LSD (0.05):	462					

[a] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.

Table 4.11. Influence of preemergence and postemergence herbicides and combinations on NET Income at the glyphosate-resistant waterhemp site in Platte County, Missouri in 2006.

	Net Income[a]					
	Postemergence Treatments[b]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
	---$/ha---					
Flumioxazin	276	178	295	259	269	159
Sulfentrazone	166	273	269	216	259	154
Alachlor	243	303	202	199	181	51
S-metolachlor+metribuzin	253	336	241	218	211	317
None	137	117	-23	95	112	-198

[a] NET Income= [soybean yield (Bu/ha) * five year projected average soybean price ($/Bu)] – [cost of herbicide treatments including adjuvant ($/ha) + $12/ha custom application fee + $826/ha soybean production cost].

[b] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.

Table 4.12. Influence of preemergence and postemergence herbicides and combinations on NET Income at the glyphosate-resistant waterhemp site in Platte County, Missouri in 2007.

	Net Income[a]					
	Postemergence Treatments[b]					
Preemergence Treatments	Lactofen	Acifluorfen	Glyphosate	Glyphosate +Lactofen	Glyphosate +Acifluorfen	None
	---$/ha---					
Flumioxazin	201	274	335	202	287	116
Sulfentrazone	191	233	229	56	184	262
Alachlor	281	313	217	161	221	287
S-metolachlor+metribuzin	353	273	244	178	231	409
None	338	232	138	304	291	-223

[a] NET Income= [soybean yield (Bu/ha) * five year projected average soybean price ($/Bu)] – [cost of herbicide treatments including adjuvant ($/ha) + $12/ha custom application fee + $826/ha soybean production cost].

[b] Ammonium sulfate added to all glyphosate treatments at 2.8 kg/ha. Non-ionic surfactant added at 0.125% v/v to lactofen and acifluorfen treatments when applied alone.