INTEGRATION OF CULTURAL PRACTICES AND HERBICIDE-RESISTANT CROP TECHNOLOGIES FOR THE MANAGEMENT OF GLYPHOSATE-RESISTANT WATERHEMP IN SOYBEAN

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INTEGRATION OF CULTURAL PRACTICES AND HERBICIDE-RESISTANT CROP TECHNOLOGIES FOR THE MANAGEMENT OF GLYPHOSATE-RESISTANT WATERHEMP IN SOYBEAN

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A candidate for the degree of

Master of Science

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

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CHAPTER I

Justification

Common waterhemp (*Amaranthus rudis* Sauer) is the most common and troublesome weed in agronomic crops in Missouri and throughout most of the Midwest (Rosenbaum and Bradley 2013; Waggoner and Bradley 2011; Legleiter and Bradley 2008; Bradley et al. 2007). Waggoner and Bradley (2011) reported waterhemp was found in 87% of Missouri soybean fields surveyed at an average density of 22 plants per m^2, resulting in yield losses up to 545 kg/ha. Bayer CropScience developed a transgenic soybean that is resistant to HPPD-inhibiting herbicides (Matringe et al. 2005). It is expected that this technology will be commercially available within the next five years; therefore it is important to determine how HPPD-resistant soybean reacts to HPPD-inhibiting herbicides, and to provide producers with effective weed management recommendations for use with this technology. The use of HPPD resistant soybean could be a valuable tool for the management of glyphosate-resistant (GR) waterhemp. Waterhemp has evolved resistance to six different modes of action (Heap 2014; Bradley 2013). The need to understand the level of multiple resistances present in waterhemp populations in Missouri is of utmost importance when considering management practices for future seasons (Rosenbaum and Bradley 2013). A survey is needed to determine the scope and extent of multiple herbicide resistance present in Missouri waterhemp populations. Management practices such as row spacing, seeding rate, and herbicide program are all practices that can have a significant effect on weed control (Anderson 1996). Research should be conducted to determine which combination(s) of row spacing, seeding rate, and type of herbicide program are most effective for the management of GR waterhemp. The objectives of this research are to: 1) determine the effects of various HPPD-inhibiting herbicide programs on GR waterhemp control in FG72 soybean, 2) determine the effects of row spacing, seeding rate, and type of herbicide program on the control of GR waterhemp, and 3) determine the level of multiple herbicide resistance present among waterhemp populations in Missouri.

Introduction

No-tillage cropping systems offer environmental benefits such as reduced soil erosion and water conservation, and economic benefits such as reduced labor, fuel, and machinery costs (Doran et al. 1984; Gebhardt et al. 1985). Adoption of no-tillage systems changed weed control tactics from a tillage emphasis to the need for non-selective pre-plant (PP) and residual herbicide applications prior to planting in order to plant into a weed free field (Krausz et al. 1993). In 1997, only 17 percent of U.S. soybean acres were planted with herbicide resistant varieties (USDA 2013). By 2013, 93 percent of soybean acres were planted with herbicide resistant varieties and the vast majority of these are glyphosate-resistant (USDA 2013). Since their release in 1996, glyphosate-resistant (GR) crops have been rapidly adopted allowing producers to simplify their weed management programs through glyphosate applications that controlled a broad spectrum of common agronomic weeds without causing injury to the existing crop (Fernandez-Cornejo and McBride 2002). Continuous use of glyphosate on millions of hectares has led to the selection of GR weed populations throughout the world (Heap 2014). The United States currently has 14 glyphosate resistant species including Palmer amaranth (*Amaranthus palmeri* S. Watson), spiny amaranth (*Amaranthus spinosus* L.), common waterhemp (*Amaranthus rudis* Sauer), common ragweed

(*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), hairy fleabane (*Conyza bonariensis* L. Cronq.), horseweed (*Conyza Canadensis* L. Cronq.), junglerice (*Echinochloa colona* L. Link.), goosegrass (*Eleusine indica* L. Gaertn.), kochia (*Kochia scoparia* L. Schrad.), Italian ryegrass (*Lolium multiflorum* Lam. Husnot), rigid ryegrass (*Lolium rigidum* Gaudin.), annual bluegrass (*Poa annua* L.), and johnsongrass (*Sorghum halepense* L. Pers.) (Heap 2014). Among these GR weeds, common waterhemp is the most problematic weed in Missouri cropping systems (Legleiter and Bradley 2008; Heap 2014; Waggoner and Bradley 2011; Bradley 2013). With waterhemp being present in the majority of Missouri soybean fields, research should be conducted to better understand ways to control waterhemp and exploit its weaknesses.

Literature Review

Waterhemp

Common waterhemp is native to the north-central United States (Hager et al. 2000), but did not emerge as a problematic weed in corn (*Zea mays* L.) or soybean production until the 1980s. The introduction of no-tillage cropping systems has likely contributed to the rise of waterhemp as a problematic weed, and waterhemp is now one of the most problematic weeds Midwest farmers must contend with (Bradley et al. 2007; Legleiter and Bradley 2008; Bradley 2013; Waggoner and Bradley 2011). Corn and soybean can suffer substantial yield losses due to waterhemp. Hager et al. (2002) found that 10 weeks of waterhemp interference at densities from 89 to 362 plants per m^2 resulted in a 43% soybean yield loss. Cordes et al. (2004) reported waterhemp densities from 82 to 445 plants per m^2 resulted in a 10 to 36% corn yield reduction.

Since its introduction in 1996, the adoption of GR crops has increased dramatically (USDA 2013). GR soybean hectares increased from 13% in 1997 to 88% in 2005 in the United States alone (Sankula 2006). Following the rapid adoption of GR crop technologies was an equivalent increase in glyphosate use and a decrease in the total number of active ingredients used in U.S. soybean production. In 1995, 11 herbicidal active ingredients were applied on 10% of the soybean hectares in the United States (Young 2006). Seven years later in 2002, the number of active ingredients declined to only one, contributing to the selection of GR weeds (Young 2006). In 1993, the first reported herbicide resistance incident in waterhemp was to ALS-inhibiting herbicides in Iowa (Heap 2014). By 2013, waterhemp populations with resistance to glyphosate, acetolactate synthase (ALS)-, protoporphyrinogen (PPO)-, photosystem II-, and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides have been reported throughout the Midwest (Heap 2014). Some waterhemp populations in Illinois have also evolved multiple resistances to as many as four modes of action (Bell et al. 2013), while populations in Kansas, Iowa, and Missouri have evolved multiple resistances to two or more herbicide modes of action (Legleiter and Bradley 2008; Heap 2014). Determining weed resistance and quantifying the size, scope, and extent of a resistance case requires effective and efficient screening tests (Beckie et al. 2000). Surveys are a practical tool to gather important information to assist in educating producers on the current weed resistance distribution among a certain geographic area (Givens et al. 2009; Johnson and Gibson 2006). The most common survey parameters include location, field and/or farm size, tillage type, crop rotation history, herbicide application history and frequency, weed species present, and weed frequency and density. These parameters have proven to be potential indicators of weed resistance and offer a better understanding of the factors that lead to resistance among weed populations (Rosenbaum and Bradley 2013).

Cultural Practices for Weed Control

Cultural practices can be used to control weeds through good crop, water, and land management (Anderson 1996). Utilizing cultural practices such as the manipulation of crop row-spacing, crop cultivars, and crop populations can have a significant impact on the level of weed control achieved (Anderson 1996). Previous research has found that soybean planted in narrow rows can result in greater season-long weed control than wide-row soybean (Burnside and Colville 1964; Legere and Schreiber 1989; Yelverton and Coble 1991; Nelson and Renner 1998; Buehring et al. 2002; Puricelli et al. 2003; Steckel and Sprague 2004; Dalley et al. 2004; Bradley 2006; Rich and Renner 2007). Legere and Schreiber (1989) reported that soybean yield increased by 18% and redroot pigweed (*Amaranthus retroflexus* L.) biomass was reduced by 20% when soybean row spacing was reduced from 76- to 25-cm. Buehring et al. (2002) found that sicklepod (*Senna obtusifolia* L.) control was 18% higher and yield was 32% greater in narrow compared to wide row soybean. As row spacing decreases, photosynthetically active radiation (PAR) that reaches the soil surface is simultaneously reduced (Steckel and Sprague 2004; Yelverton and Coble 1991). Steckel and Sprague (2004) reported that by the V2 stage of soybean, the amount of PAR that reached the soil surface in narrow-row soybeans was 50% less than that of wide-row soybean. Enhanced germination and emergence rates of *Amaranthus* spp. have been reported under conditions where there is available light (Gallagher and Cardina 1998). Yelverton and Coble (1991) reported as row spacing increased, weed resurgence increased. While narrow row spacing offers weed control benefits, it does not always offer a yield advantage over wide-row soybean (Buehring et al. 2002; Burnside 1979; Pedersen and Lauer 2003).

Another potential cultural weed management practice is optimum soybean plant populations. For weed control, soybean populations of 688,000 plant/ha resulted in 92% control of sicklepod compared to only 29% control of sicklepod (*Senna ovtusifolia* L.) with soybean populations of 269,000 plants/ha (Buehring et al. 2002). Norsworthy and Oliver (2002) observed greater hemp sesbania (*Sesbania exaltata* Raf.) control with soybean populations of 521,000 plants/ha compared to 217,000 and 371,000 plants/ha. Harder et al. (2007) reported that there were no differences in weed biomass in response to four populations of soybean after an effective POST herbicide application. However, in the non-treated control, 300,000 plants/ha provided the greatest suppression of weeds and was not different than 445,000 plants/ha (Harder et al. 2007). While soybean populations greater than 450,000 plants/ha can provide higher weed control due to increased competition, the seed cost associated with them often exceeds the benefit (Norsworthy and Oliver 2001).

HPPD-resistant Soybean

In plants, the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD) catalyzes the formation of homogentisate and carbon dioxide from p-hydroxyphenylpyruvate (HPP) and molecular oxygen (Lindblad et al. 1970). HPPD is essential in plastoquinone biosynthesis. HPPD-inhibiting herbicides were discovered in 1977, and were not commercially released until 2001 (Beaudegnies et al. 2009). This makes HPPD inhibiting herbicides the newest mode of action available on the herbicide market.

Mitchell et al. (2001) reported that HPPD-inhibiting herbicides offer a broad spectrum of weed control and that uptake of mesotrione, a common HPPD-inhibiting herbicide, is rapid (Mitchell et al. 2001). Within 24 hours after application, 55 to 90% of the mesotrione applied to giant foxtail (*Setaria faberi* Herrm.), common lambsquarters (*Chenopodium album* L.), and ivyleaf morningglory (*Ipomoea hederacea* Jacq.) had been absorbed. Corn exhibits much lower levels of foliar uptake compared to weeds which contributes to the selectivity of these herbicides in corn. Corn is also able to metabolize mesotrione at a greater rate than most weed species. For example, seven days after treatment, 42% of the mesotrione that translocated outside of the treated leaf was still found as the parent molecule in common lambsquarters while none of the mesotrione that translocated outside of the treated leaf in corn occurred as the parent molecule (Mitchell et al. 2001). While mesotrione is herbicidally active at the time of application, isoxaflutole, a common isoxazole, is in essence herbicidally inactive when applied. When isoxaflutole is applied, it is readily taken up and degraded to a more polar open-chain diketonitrile derivative of isoxaflutole and transported through the xylem and phloem (Pallett et al. 1998). The diketonitrile is further degraded to a benzoic acid derivative that is herbicidally inactive. The susceptibility of each species is dependent on each species' ability to degrade the diketonitrile to the herbicidally inactive benzoic acid (Pallett et al. 1998). Corn is able to rapidly metabolize the diketonitrile while velvetleaf is not (Pallett et al. 1998).

While corn's ability to metabolize HPPD-inhibiting herbicides allows the use of these herbicides in-crop, soybean is extremely sensitive to HPPD-inhibiting herbicides like mesotrione. Bleaching symptoms have been observed with as low as 4 g mesotrione per hectare when applied to soybean (Mitchell et al. 2001). However, in the mid 2000's, Bayer CropScience began to develop strategies to expand the use of isoxaflutole and other HPPD-inhibiting herbicides in soybean (Matringe et al. 2005). The *HPPD W336* gene was discovered to confer resistance to isoxaflutole (USDA-APHIS 2013) and stable introduction of the gene to soybean occurred by direct gene transfer (S van Wert, personal communication). Glyphosate tolerance was also introduced by the same method through the *2mEPSPS* gene (USDA-APHIS 2013). Soybean resistant to HPPD-inhibiting herbicides and glyphosate is now referred to as the FG72 event.

With a variety of herbicide resistant weeds present in agronomic fields throughout the United States and other countries, the need for new and multiple modes of action is growing in order to combat the continued growth and spread of these species (Heap 2014). The FG72 soybean event will allow producers to utilize a mode of action in soybean weed management programs that was not previously available.

Summary and Objectives

Rapid adoption of GR crops and no-till practices among producers over the past two decades has placed an increasing amount of pressure on POST applications of glyphosate that has resulted in shifts to GR weed species. Common waterhemp is one of the most common and problematic weeds in the Midwestern United States that has evolved resistance to glyphosate, and in many instances, multiple resistance to herbicides with other modes of action. GR and multiple resistant waterhemp is a difficult species to manage, especially in GR soybean systems. The use of HPPD-inhibiting herbicides in soybean will allow a supplemental mode of action to combat GR waterhemp. Exploiting the weaknesses of waterhemp through cultural practices and understanding the extent of waterhemp resistance in Missouri will benefit producers as they move forward in the battle against herbicide resistant weeds. The objectives of this research are to: 1) determine the effects of various HPPD-inhibiting herbicide programs on GR waterhemp control in FG72 soybean, 2) determine the effects of row spacing, seeding rate, and type of herbicide program on the control of GR waterhemp, and 3) determine the level of multiple herbicide resistance present among waterhemp populations in Missouri.

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CHAPTER II

Influence of Soybean Seeding Rate, Row Spacing and Herbicide Programs on the Control of Resistant Waterhemp in Glufosinate-resistant Soybean

John L. Schultz and Kevin W. Bradley

ABSTRACT

A field experiment was conducted in Randolph County, Missouri in 2012 and 2013 to determine the effects of row spacing, seeding rate, and herbicide programs on multiple-resistant waterhemp control and yield in glufosinate-resistant soybean. The two herbicide programs evaluated were: 1) a PRE application of fomesafen plus *S*-metolachlor followed by an early POST application of glufosinate plus acetochlor, referred to as the PRE fb POST w/RES herbicide program, and 2) an early POST application of glufosinate followed by a late POST application of glufosinate, referred to as the two-pass POST herbicide program. Results from this research indicate that the PRE fb POST w/RES program will provide greater control of multiple resistant waterhemp compared to the two-pass POST herbicide program. In 2012, the PRE fb POST w/RES program resulted in a 99% waterhemp density reduction and 156 kg ha^{-1} increase in soybean yield compared to the 72% density reduction by two-pass POST program. In 2013, the two-pass POST program was equally as effective on density reduction and soybean yield as the PRE fb POST w/RES program, likely due to reduced waterhemp densities caused by delayed planting. Waterhemp control and density reduction was always greatest with 19- and 38-cm rows compared to 76-cm rows. In 2012, the PRE fb POST w/RES program provided at least 95% control and greater than

98% waterhemp density reduction across all row spacings while the two-pass POST program provided 95%, 95%, and 85% control and 87%, 80%, and 50% waterhemp density reduction in 19-, 38-, and 76-cm rows, respectively. Soybean seeding rate did not affect waterhemp control or density in either year. In both years, 165,000 seeds ha^{-1} yielded lower than the three higher seeding rates. In 2012, the highest yields were achieved with seeding rates of 315,000 and 390,000 seeds ha^{-1}, but no yield differences were observed among the three highest seeding rates in 2013. Overall, results from this experiment indicate that the use of the PRE fb POST w/RES program, narrow-row spacing, and seeding rates of 240,000 to 315,000 seeds ha^{-1} or greater will provide the greatest waterhemp control, density reduction, and soybean yield when troublesome herbicide resistant weed species such as multiple resistant waterhemp are present.

INTRODUCTION

The adoption of glyphosate-resistant (GR) soybean and conservation practices like no-till over the last several decades has resulted in an increased reliance on herbicides as one of the primary methods of weed control (Krausz et al. 1993; Culpepper et al. 2000; Young 2006). As production was simplified with GR soybean, many producers relied on glyphosate as their primary and often sole method of weed control (Powles 2008; Young 2006). The continuous use of glyphosate over multiple years has led to increased selection pressure for weeds to evolve resistance to glyphosate (Powles 2008; Young 2006). The evolution of glyphosate resistance in weeds like waterhemp (*Amaranthus rudis* Sauer) has complicated weed management and increased production costs in corn, cotton, and soybean production systems dramatically.

Currently, waterhemp is the most common and troublesome weed found in Missouri, Iowa, and Illinois (Rosenbaum and Bradley 2013; Waggoner and Bradley 2011; Legleiter and Bradley 2008; Bradley et al. 2007; Hager et al. 2000). In recent years, the number of waterhemp populations with multiple herbicide resistances in Missouri and throughout the Midwest has risen. Waterhemp in Missouri, Iowa and Illinois has evolved resistance to EPSPS-, PPO-, HPPD-, photosystem II-, and ALS-inhibitors with one population in Missouri being resistant to all five modes of action (Schultz et al. 2014; Heap 2014). One population from Illinois and one population from Iowa have been reported with resistance to four modes of action (Heap 2014). Kansas has waterhemp resistant to EPSPS-, PPO-, photosystem II-, and ALS-inhibitors (Heap 2014). Waterhemp from Nebraska has been documented with resistance to synthetic auxins, EPSPS-, HPPD-, and photosystem II-inhibitors (Heap 2014).

The evolution of waterhemp or other weeds with multiple herbicide resistances will require producers to diversify their production systems and integrate optimum cultural and herbicidal control methods (Bradley 2013; Schultz et al. 2014; Heap 2014; Norsworthy et al. 2012). Cultural practices, such as row spacing and seeding rate, can significantly impact weed control (Grichar et al. 2004; O'Donovan et al. 2001; Anderson 1996). The majority of research studies conducted to date have shown that soybean planted in narrow rows can provide greater season-long weed control than wide-row soybean (Burnside and Colville 1964; Burnside 1979; Legere and Schreiber 1989; Yelverton and Coble 1991; Nelson and Renner 1998; Buehring et al. 2002; Puricelli et al. 2003; Steckel and Sprague 2004; Dalley et al. 2004; Bradley 2006; Rich and Renner 2007). Harder et al. (2007) observed lower weed density and biomass following an effective postemergence (POST) application in 19-cm compared to 76-cm rows. Buehring et al. (2002) found that sicklepod (*Senna obtusifolia* L.) control was 29% higher in 19- compared to 76-cm soybean. Steckel and Sprague (2004) reported a 57% waterhemp biomass reduction in 19- compared to 76-cm soybean when waterhemp emergence occurred at the V2-V3 soybean growth stage. Weed resurgence increases as row spacing increases due to the amount of light penetrating to the soil surface (Steckel and Sprague 2004; Yelverton and Coble 1991). As a result, soybean planted in 76-cm rows or greater requires earlier weed management programs to prevent yield loss than soybean planted in narrower rows (Knezevic et al. 2003; Mulugeta and Boerboom 2000). Early soybean growth in 19- to 38-cm soybean also exceeds that of 76-cm soybean resulting in lower radiation transmitted through the canopy and partially accounts for the greater competitiveness of narrow- versus wide-row soybean (Puricelli et al. 2003; Steckel and Sprague 2004).

Soybean plant population is another cultural practice that can be manipulated for optimum weed management. Buehring et al. (2002) found that a soybean population of 688,000 plants ha^{-1} resulted in 92% control of sicklepod compared to only 29% control with a soybean population of 269,000 plants ha^{-1}. Norsworthy and Oliver (2002) observed greater hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A. W. Hill) biomass reduction with soybean populations of 521,000 plants ha^{-1} compared to 217,000 and 371,000 plants ha^{-1}. Harder et al. (2007) also found that weed biomass was not suppressed by soybean populations of 124,000 to 198,000 plants ha^{-1} across 19-, 38-, and 76-cm rows, but was suppressed by soybean populations of 300,000 to 445,000 plants ha^{-1}. At 300,000 plants ha^{-1}, the biomass reduction was greater in 19-cm rows than 38- and

76-cm rows. While soybean populations greater than 450,000 plants ha^{-1} can provide higher weed control, the seed cost associated with these seeding rates often exceeds the benefit (Norsworthy and Oliver 2001).

The simplicity of the GR cropping system led many producers to rely solely on POST herbicide applications for weed control in soybean (Powles 2008; Young 2006). Waterhemp has evolved resistance to many POST herbicide modes of action; however, glufosinate remains an effective POST herbicide mode of action (Heap 2014). Producers can utilize glufosinate applications in-crop if they choose to plant glufosinate resistant soybean. Craigmyle et al. (2013) observed up to 90% waterhemp control with one POST glufosinate application in glufosinate-resistant soybean. However, POST-only herbicide applications can result in herbicide failures and the evolution of herbicide resistance in weed biotypes (Bradley 2013; Powles 2008). The addition of preemergence (PRE) and POST residual herbicide applications has been proven to reduce weed density, improve season-long weed control, and reduce the opportunity for the evolution of herbicide resistance (Legleiter et al. 2009; Bradley 2013; Craigmyle et al. 2013; Spaunhorst et al. 2014). The use of multiple, effective herbicide modes of action in both PRE and POST applications is critical to the management of multiple-resistant waterhemp (Bradley 2013). It is also critical to prevent the development of resistance to glufosinate by maintaining effective control of weeds with multiple herbicide resistances. The influence of row spacing and seeding rate on the management of a glyphosate-resistant weed in glufosinate resistant soybean has not been researched extensively.

The objectives of this research were to determine the effect of row spacing, seeding rate, and herbicide programs on multiple-resistant waterhemp control and yield in glufosinate-resistant soybean.

MATERIALS AND METHODS

Site Description. An experiment was conducted in 2012 and repeated in 2013 at a field site in Randolph County, Missouri (39°18'10.29"N, 92°22'14.42"W) that contained a dense infestation of waterhemp that exhibited resistance to glyphosate, ALS-, and PPO-inhibiting herbicides. This site has been in continuous soybean production for at least 10 years and was an upland area with a clay pan soil. The soil type at this location was a Putnam silt loam (Fine, smetitic, mesic Vertic Albaqualfs) with 2.2% organic matter and pH of 6.3 in 2012 and 1.9% organic matter and pH of 6.3 in 2013. Truman 938 LL soybean containing a glufosinate-resistance trait (maturity group 3.8, Merschman Seeds, West Point, IA) was seeded at 165,000, 240,000, 315,000, and 390,000 seeds ha^{-1} in rows spaced 19-, 38-, and 76-cm apart into a conventional-tilled seedbed. Tillage consisted of two passes with a field cultivator. The various populations were planted in 38- and 76-cm rows using a variable rate planter (Almaco®, Nevada, IA) and in 19-cm rows using a variable rate grain drill (Wintersteiger®, Salt Lake City, UT). Dates of major field operations for each experiment are provided in Table 2.1. Monthly rainfall totals and average monthly temperatures are presented in Table 2.2.

The experiment was a split-plot with row spacing as the main plot and herbicide program and seeding rate as subplots arranged in a randomized complete block design. The experiment was conducted with six replications in 2012 and four replications in 2013. Individual plots measured 3 by 9 m in size. Two herbicide programs were evaluated: 1) a PRE application of fomesafen plus *S*-metolachlor (0.27 + 1.12 kg ai ha^{-1}) followed by an early POST application of glufosinate plus acetochlor (0.60 kg ae + 1.26 kg ai ha^{-1}), referred to as the PRE fb POST w/RES herbicide program, and 2) an early POST application of glufosinate (0.60 kg ae ha^{-1}) followed by a late POST application of glufosinate (0.60 kg ae ha^{-1}), referred to as the two-pass POST herbicide program. The specific herbicide formulations utilized are listed in Table 2.3. All treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR8002 flat-fan nozzle tips (TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL. 60187) calibrated to deliver 140 L ha^{-1} at 117 kPa. Treatments were applied at a constant speed of 5 km hr^{-1}. A non-treated control for each row spacing and seeding rate was included for comparison. PRE treatments were applied to a conventional-tilled seedbed prior to planting. Early POST applications were made once weeds reached 10-cm in height. Late POST applications were made when weed regrowth reached 10-cm in height.

Treatment Evaluation and Data Collection. Visible weed control and crop injury evaluations were assessed at regular intervals after application on a scale of 0 to 100 percent, where 0 represented no plant death or crop injury and 100 was equivalent to complete plant death. Late-season waterhemp density was determined at the R4 reproductive stage each year by counting individual plants within the center 1- by 9-m^2 area of each plot. Soybean plant densities for each seeding rate were determined at the R4 reproductive stage each year by counting two random 1-m subsamples of the 38- and 76-cm rows and two 0.5-m^2 areas within plots that were planted in 19-cm rows (Table 2.4). Soybean were harvested from the center 1.5- by 9-m^2 within each plot with a small plot combine (Kincaid®, Haven, KS) and yield was adjusted to 13% moisture content.

Statistical Analysis. Late-season visible waterhemp control, waterhemp density, and soybean yield data were analyzed using the PROC GLM procedure in SAS (SAS 9.3, SAS® Institute Inc. Cary, NC). Replications, herbicide program, row spacing, and seeding rate were considered fixed effects. Significant interactions were present between years; therefore, late-season visible waterhemp control, density, and soybean yield were separated by year (Table 2.5). Individual treatment differences were separated using Fisher's protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Herbicide Programs. In both years, the PRE fb POST w/RES program provided greater visible waterhemp control and in 2012 provided greater density reduction than the two-pass POST program (Table 2.5). In 2012, late-season waterhemp density was reduced 99% with the PRE fb POST w/RES program and 72% with the two-pass POST program compared to the non-treated control. Legleiter et al. (2009) reported 97% and 98% GR waterhemp density reductions with PRE fb POST programs compared to less than 40% waterhemp density reduction with POST-only programs. In 2013, no density differences were observed between the PRE fb POST w/RES and the two-pass POST program. This was likely due to the later soybean planting date induced by above-average spring rainfall (Table 2.2) that resulted in lower waterhemp densities. In 2012, there were differences in soybean yield as a result of herbicide programs, but not in 2013. In 2012, the PRE fb POST w/RES program resulted in an average soybean yield of 1716 kg ha^{-1} compared to the two-pass POST program at 1560 kg ha^{-1}. The use of PRE herbicides in soybean will often result in increased yields in environments with high waterhemp densities, as waterhemp emergence can usually be delayed through the V4-V5 critical weed-free stage

(Legleiter et al. 2009; Steckel and Sprague 2004). It is also important to note that in both years, both herbicide treatments yielded greater than the non-treated control. Visible crop injury did not exceed 2% at any time interval after application in either year.

Row Spacing. Visible waterhemp control was greater in 19- and 38-cm rows than 76-cm rows in both years (Table 2.5). In 2013, 98% or greater visible waterhemp control was observed for all soybean row spacings. Additionally, in both years waterhemp density was lower in 19- and 38-cm compared to 76-cm row spacings. Waterhemp density was reduced by 71 to 75% in 38-cm rows and by 57 to 93% in 19-cm rows compared to 76-cm row spacings. Rich and Renner (2007) observed similar effects with eastern black nightshade (*Solanum ptycanthum* Dun.), where late-season densities were 1 plant m^2 in 19-cm rows and 12 plants m^2 in 76-cm rows. Harder et al. (2007) also reported that weed density 3, 4, and 5 weeks after herbicide treatment was reduced in 38-cm compared to 76-cm rows. In 2012, the PRE fb POST w/RES program provided at least 95% control of waterhemp for all row spacings evaluated, while the two-pass POST program provided 95% control of waterhemp in 19- and 38-cm row spacings, but only 85% waterhemp control in 76-cm row spacing (data not shown). In 2012, the PRE fb POST w/RES program also provided greater than 98% waterhemp density reduction across all row spacings while the two-pass POST program provided 87%, 80%, and 50% waterhemp density reduction in 19-, 38-, and 76-cm rows, respectively (data not shown). However in 2013, all herbicide program and row spacing combinations resulted in greater than 97% visible waterhemp control and greater than 98% density reduction (data not shown). While 19-cm soybean row spacings provided better waterhemp control than 76-cm, they yielded lower than 38- and 76-cm rows in 2012. This yield reduction may be due to inconsistent seed placement provided by the grain drill. Bracy and Parish (2001) reported that grain drills can provide poor seeding precision due to multiple seed drops and large skips between seed drops. In 2013, no yield differences were observed between soybean row spacings. The response of soybean to row spacing has been inconsistent within the literature. Taylor (1980) reported that in years where rainfall was below average there were no yield differences between 25-, 50-, 75-, and 100-cm soybean row spacings. However, when rainfall was ample during the growing season and seed fill, 25-cm rows yielded higher than 100-cm rows (Taylor 1980). Buehring et al. (2002) observed a similar response and suggested that interactions between row spacing and environment may cause variability in yield response.

Seeding Rate. No differences in visible waterhemp control or density reduction were observed in response to soybean seeding rate. Arce et al. (2009) reported no differences in weed density at two of three sites in response to soybean seeding rates ranging from 240,000 to 420,000 seeds ha^{-1}. Harder et al. (2007) observed no differences in weed control among four soybean plant populations following an effective POST application. In 2012, soybean yield was greatest in response to the two highest seeding rates of 315,000 and 390,000 seeds ha^{-1}. In terms of yield, the seeding rates of 240,000 and 315,000 seeds ha^{-1} were not different but were higher than that provided by the 165,000 seeds ha^{-1} seeding rate. In 2013, the three highest seeding rates yielded 180 to 216 kg ha^{-1} higher than the 165,000 seeds ha^{-1} seeding rate. Pedersen (2008) recommended a final plant population of 247,000 plants ha^{-1} in order to maximize the yield per plant while maintaining overall yield levels.

In summary, based on the results of this research PRE fb POST w/RES programs are more likely to provide higher levels of waterhemp control and density reduction when compared to two-pass POST glufosinate programs. Craigmyle et al. (2013) reported similar results in soybean with resistance to glufosinate and 2,4-D. Additionally, this research indicates that in fields with high waterhemp densities, PRE fb POST w/RES programs are more likely to provide higher soybean yields than two-pass POST programs. Legleiter et al. (2009) also observed that PRE herbicide applications resulted in the greatest GR waterhemp control, density reduction, and provided the highest soybean yield. While two-pass POST programs of glufosinate are more likely to provide control of waterhemp in fields with low densities, adding residual herbicides to the overall weed management program allows for longer periods of control under a broad range of environmental conditions that POST programs alone cannot provide. The results from this research also indicate that soybean row spacings of 38-cm or less provide greater waterhemp control and density reduction than rows spaced 76-cm apart. Based on the results of this research and previous research, soybean seeding rates of 240,000 to 315,000 seeds ha^{-1} or greater will result in optimum soybean yield (Pedersen 2008, Arce et al. 2009). However, increasing the soybean seeding rate will likely have only minimal impacts on waterhemp control, especially in fields where an effective PRE or POST herbicide application has been made. Glufosinate is an effective herbicide mode of action that can be utilized to manage GR and multiple resistant weed species like waterhemp in glufosinate resistant soybean. As GR weed species become more prevalent in soybean production systems throughout the U.S., the integration of cultural practices such as narrow-row spacings and optimum soybean plant populations with herbicide programs that include multiple, effective herbicide modes of action will be critical to the success of GR weed management.

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Table 2.1. Dates of major field operations and waterhemp size at the time of the herbicide applications.

	Year	
	2012	2013
Seeding date	6/1	6/20
Dates of herbicide application		
PRE fb POST w/RES[a]	6/1 fb 8/1	6/19 fb 7/19
2-Pass POST	7/5 fb 8/1	7/19 fb 8/4
Soybean growth stage at application		
PRE fb POST w/RES	--- fb R1	--- fb V4
2-Pass POST	V3 fb R1	V4 fb R2
Average waterhemp size (cm) at application		
PRE fb POST w/RES	--- fb 15	--- fb 10
2-Pass POST	10 fb 15	10 fb 12

[a] Abbreviations: fb, followed by; PRE, pre-emergence; POST, post-emergence; RES, residual.

Table 2.2. Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr averages from April through October in 2012 and 2013 at the Randolph County research location.

	Rainfall			Temperature		
Month	2012	2013	30 year Avg.[a]	2012	2013	30 year Avg.[a]
	---------------- mm ----------------			------------------ C ------------------		
April	126	136	103	13.1	11.7	13.0
May	77	202	126	20.0	17.2	18.2
June	73	37	126	23.4	23.3	22.9
July	49	25	113	28.1	24.4	25.5
August	4	39	109	23.9	24.4	24.6
September	125	76	109	19.7	21.7	19.9
October	78	87	81	12.5	13.3	13.7

[a] 30 year averages (1982-2011) obtained from National Climatic Data Center (2014).

Table 2.3. Source of materials used in the experiments.

Herbicide[a]	Trade name	Formulation	Rate	Manufacturer	Address[a]
			kg ai or ae ha^{-1}		
Acetochlor	Warrant	3 L	1.26	Monsanto	St. Louis, MO
Fome + *S*. metol.	Prefix	4.34+0.95 EC	0.27 + 1.12	Syngenta	Wilmington, DE
Glufosinate	Liberty	280 SL	0.60	Bayer CropScience	Research Triangle Park, NC
Ammonium Sulfate	N-Pak AMS	3.4 L	2.9	Winfield Solutions	St. Paul, MN

[a] Abbreviations: fome., fomesafen; *S*-metol., *S*-metolachlor; RTP, Research Triangle Park.

Table 2.4 Influence of soybean seeding rate on final soybean density.

	Soybean Plant Density[abc]	
Seeding rate	2012	2013
Seed/ha	--plants/ha--	
165,000	139,578 ± 6,581	153,186 ± 4,649
240,000	217,266 ± 10,171	192,732 ± 4,691
315,000	268,911 ± 10,916	238,584 ± 7,172
390,000	328,028 ± 13,166	261,530 ± 7,688

[a] Data summarized across all herbicide programs and soybean row spacings.
[b] Data are means ± standard errors.
[c] No significant interaction was present between row spacings.

Table 2.5. Late-season[h] visible waterhemp control and density, and soybean yield as affected by herbicide program, row spacing, and seeding rate.

	Visible Control		Density		Yield	
Factor	2012	2013	2012	2013	2012	2013
	------- (%) -------		------ (# 9m^2) ------		----- (kg ha^{-1}) -----	
Herbicide program[abcd]						
PRE fb POST w/RES	95	99	5	0.3	1716	2216
two-pass POST	92	98	113	0.1	1560	2205
Non-treated	---	---	397	121	662	1505
LSD $_{0.05}$ [g]	2.5	0.25	38	17	78	78
Row spacing[e]						
19-cm	95	99	26	0.13	1540	2209
38-cm	96	99	46	0.03	1715	2247
76-cm	90	98	106	0.45	1658	2174
LSD $_{0.05}$ [g]	3	0.3	34	0.26	100	NS
Seeding rate[f] (seed/ha)						
165,000	91	99	57	0.2	1477	2063
240,000	95	99	44	0.3	1617	2259
315,000	93	99	67	0.1	1700	2279
390,000	94	99	69	0.2	1758	2243
LSD $_{0.05}$ [g]	NS	NS	NS	NS	116	100
ANOVA[g]	---------------------------------P > *F*---------------------------------					
Herbicide program (H)	0.0037	0.0041	<0.0001	0.0146	0.0002	<0.0001
Row spacing (R)	0.0003	0.0050	<0.0001	0.0052	0.0027	NS
Seeding rate (S)	NS	NS	NS	NS	<0.0001	0.0001
H X R	0.0010	0.0203	<0.0001	0.0087	NS	NS
H X S	0.0360	NS	NS	NS	NS	NS
R X S	NS	NS	NS	NS	NS	NS
H X R X S	NS	NS	NS	NS	NS	0.0385

[a] All POST treatment included ammonium sulfate at 2.9 kg ai ha^{-1}.

[b] Abbreviations: fb, followed by; PRE, pre-emergence; POST, post-emergence; RES, residual.

[c] Application timing: PRE, at planting; early POST, 10-cm waterhemp; late POST, 10-cm waterhemp regrowth.

[d] Data combined across all row spacings and seeding rates.

[e] Data combined across all seeding rates and plots with herbicide applications.

[f] Data combined across all row spacings and plots with herbicide applications.

[g] NS, not significant at the α=0.05 level.

[h] Late season waterhemp control and density taken at R4 soybean growth stage.

CHAPTER III

Evaluation of Weed Management Programs and Response of FG72 Soybean to HPPD-inhibiting Herbicides

John L. Schultz, Michael Weber, Jayla Allen, and Kevin W. Bradley

ABSTRACT

Field experiments were conducted near Columbia and Moberly, Missouri in 2012 and 2013 to evaluate herbicide programs for use in HPPD-resistant soybean, referred to as FG72 soybean, and their tolerance to four HPPD-inhibiting herbicides. In the weed management experiment at Columbia, all preemergence followed by postemergence (PRE fb POST) and two-pass POST treatments provided 97% or greater control of all weeds in both years except ivyleaf morningglory. Metribuzin plus *S*-metolachlor fb glyphosate provided the lowest weed biomass reduction (BR) of 76% in 2012, but BR was increased to 89% when isoxaflutole (low) was added to the POST glyphosate treatment. At Moberly, PRE fb POST treatments provided 95% or greater control and 100% BR of glyphosate-resistant (GR) waterhemp in 2012 with the exception of the low rate combination. In 2013, all PRE fb POST treatments provided greater than 89% control and 93% BR. Two-pass POST treatments of isoxaflutole plus glyphosate fb isoxaflutole plus glyphosate always provided greater visible control and BR of GR waterhemp compared to glyphosate fb glyphosate; however, at Columbia where glyphosate susceptible weeds were present, there were no differences in visible control or BR between the two-pass POST treatments. One-pass POST treatments provided lower control of giant foxtail at Columbia in 2012 and GR waterhemp at Moberly in 2012 and

2013 compared to isoxaflutole plus glyphosate fb isoxaflutole plus glyphosate and the PRE fb POST treatments. In the soybean tolerance experiment, isoxaflutole provided the lowest levels of visual injury. Visible injury induced by mesotrione was greater than isoxaflutole 7 days after application (DAA) but was similar 28 DAA in 2012 and 2013. Applications of tembotrione resulted in the highest visible injury, height reduction (HR), and BR. Topramezone always provided less visible injury than tembotrione, but was often similar in BR. Visible injury and HR was always greater with the 2X rate compared to the 1X rate, but 28DAA in 2013 the 1X rate provided a 6% greater BR than the 2X rate. PRE applications caused the least amount of visible injury compared to V3 and R1 applications. V3 applications were generally most injurious to soybean. V3 and R1 applications of isoxaflutole and mesotrione were always similar in visible injury, HR, and BR 28 DAA in 2012 and 2013. Topramezone generally provided less visible injury to V3 soybean than tembotrione, but BR 28 DAA in 2013 was similar. Overall results indicate that FG72 soybean could allow the use of HPPD-inhibiting herbicides such as mesotrione PRE along with isoxaflutole PRE and POST to provide an additional herbicide mode of action to producers that was not previously available in soybean.

INTRODUCTION

In 2013, soybean varieties containing herbicide resistance traits comprised 93% of all soybean hectares in the United States, and the vast majority of these were glyphosate-resistant (GR) (USDA 2013). Adoption of GR crops was rapid following their introduction in 1996, mostly due to the simplicity associated with weed management with glyphosate (Fernandez-Cornejo and McBride 2002). Applications of glyphosate can be made with no crop injury yet it provides broad spectrum, post-emergence (POST) weed control (Carpenter and Gianessi 1999). Prior to the introduction of GR crops, as many as 11 herbicidal active ingredients were applied to the soybean hectares in the United States (Young 2006). Young (2006) noted that seven years after the introduction of GR soybean, the number of active ingredients applied over the same area had declined to only glyphosate. This reduction in the total number of active ingredients and herbicide modes of action has led to increased selection pressure for weeds to evolve resistance to glyphosate (Young 2006). As of 2014, 14 weed species in the United States were reported with resistance to glyphosate, including Palmer amaranth (*Amaranthus palmeri* S. Watson), spiny amaranth (*Amaranthus spinosus* L.), common waterhemp (*Amaranthus rudis* Sauer), common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), hairy fleabane (*Conyza bonariensis* L. Cronq.), horseweed (*Conyza Canadensis* L. Cronq.), junglerice (*Echinochloa colona* L. Link.), goosegrass (*Eleusine indica* L. Gaertn.), kochia (*Kochia scoparia* L. Schrad.), Italian ryegrass (*Lolium multiflorum* Lam. Husnot), rigid ryegrass (*Lolium rigidum* Gaudin.), annual bluegrass (*Poa annua* L.), and johnsongrass (*Sorghum halepense* L. Pers.) (Heap 2014). However, glyphosate continues to provide effective control of many weed species where sufficient diversity of weed control practices has been implemented and there has not been an overreliance on glyphosate alone (Powles 2008).

Herbicides that inhibit 4-hydroxyphenylpyruvate dioxygenase (HPPD) are commonly used in corn production systems across the United States. Corn exhibits a natural tolerance to HPPD-inhibitors by rapidly metabolizing these herbicides to inactive compounds (Mitchell et al. 2001). Beaudegnies et al. (2009) noted that HPPD-inhibiting herbicides offer broad spectrum weed control which contributed to their widespread integration into corn production systems. However, Hausman et al. (2011) reported that continuous applications of HPPD-inhibiting herbicides over multiple growing seasons led to the selection of HPPD-resistant waterhemp. Currently, waterhemp in Iowa, Illinois, and Nebraska, and Palmer amaranth in Kansas and Nebraska are the only weed species that have been reported with resistance to this class of herbicides (Heap 2014). Evolution of HPPD-resistance in *Amaranthus* species is especially concerning due to the inherent likelihood of this species to evolve multiple herbicide resistances (Legleiter and Bradley 2008; Bradley 2013; Hausman et al. 2011).

Isoxaflutole is an HPPD-inhibiting herbicide that provides residual control of some common summer annual weeds such as velvetleaf (*Abutilon theophrasti*), Pennsylvania smartweed (*Polygonum pensylvanicum*), smooth pigweed (*Amaranthus hybridus*), barnyardgrass (*Echinochloa crus-galli*), large crabgrass(*Digitaria sanguinalis*), and common lambsquarters (*Chenopodium album* L.) in corn (Young et al. 1999; Knezevic et al. 1998; Bhowmik et al. 1999). Young et al. (1999) noted that isoxaflutole alone at 105 g ha^{-1} applied preemergence (PRE) provided 93% control of smooth pigweed, 85% control of waterhemp, and 68% control of common lambsquarters 60 days after treatment (DAT). Bhowmik et al. (1999) observed complete control of large crabgrass at 79 g ha^{-1}. Yellow foxtail was effectively controlled at rates of 105 to 132 g ha^{-1} (Bhowmik et al. 1996). Effective residual control of a range of weed species can be achieved with isoxaflutole, but it should be combined with other effective residual herbicides to provide complete broad spectrum residual control of broadleaf and grass species (Anonymous 2013; Young et al. 1999). POST weed control with isoxaflutole is limited and is not recommended for weeds greater than one true leaf in growth (Anonymous 2013).

Bayer CropScience, in partnership with M.S. Technologies and Mertec, has developed a soybean cultivar with resistance to isoxaflutole and glyphosate to provide producers with additional tools to combat GR weed species (Matringe et al. 2005). The *HPPD W336* gene confers resistance to isoxaflutole (USDA-APHIS 2013) and stable introduction of the gene to soybean occurred by direct gene transfer (S. van Wert, personal communication). Glyphosate resistance was also introduced by the same method through the *2mEPSPS* gene (USDA-APHIS 2013). Soybean with resistance to HPPD-inhibiting herbicides and glyphosate is now referred to as the FG72 event. HPPD-inhibitors applied POST to corn result in little crop injury, but applications of HPPD-inhibiting herbicides to soybean that do not contain the *HPPD W336 gene* will cause severe bleaching symptoms and necrosis (Mitchell et al. 2001; Bradley et al. 2009). For example, Mitchell et al. (2001) observed severe soybean bleaching symptoms with applications of mesotrione as low as 4 g ha^{-1}. Little is known about the tolerance of the FG72 soybean to various HPPD-inhibiting herbicides.

The objectives of this research were to 1) determine the effects of isoxaflutole- and mesotrione-based herbicide programs on weed control in FG72 soybean, and 2) to determine the tolerance of FG72 to PRE and POST applications of isoxaflutole, mesotrione, tembotrione, and topramezone.

MATERIALS AND METHODS

Site Descriptions

Field experiments were conducted in 2012 and repeated in 2013 in Boone County at the University of Missouri Bradford Research and Education Center near Columbia, Missouri (38°53'53.22"N, 92°13'6.86"W) and in Randolph County near Moberly, Missouri (39°18'10.29"N, 92°22'14.42"W). Site selection was based upon the presence of a variety of common summer annual grass and broadleaf weed species at the Columbia site and the presence of dense infestations of waterhemp that exhibited resistance to glyphosate, ALS-, and PPO- inhibiting herbicides at the Moberly site. The soil type at the Columbia site was a Mexico silt loam (fine, smectic, mesic Aeric Vertic Epiaqualfs) with 2.3% organic matter and a pH of 6.5 in 2012 and 2.1% organic matter and pH of 6.4 in 2013. At the Moberly site, the soil was a Putnam silt loam (Fine, smetitic, mesic Vertic Albaqualfs) with 2.2% organic matter and pH of 6.3 in 2012 and 1.9% organic matter and pH of 6.3 in 2013 and has been in continuous soybean production for at least 10 years. Both sites were upland areas with a clay pan soil. At each location, FG72 soybean (maturity group 3.6) containing HPPD- and glyphosate-resistance traits provided by Bayer CropScience (Research Triangle Park, NC) were planted at a seeding rate of 338,000 seeds ha^{-1} in rows spaced 76-cm apart into a conventional-tilled seedbed. Tillage consisted of two passes with a field cultivator. Dates of major field operations for each experiment are provided in Table 3.1. Monthly rainfall totals and average monthly temperatures are presented in Table 3.2. The herbicide formulations evaluated in all experiments are listed in Table 3.3.

General Materials and Methods for All Experiments

Experiments were arranged in a randomized complete block design with six replications. Individual plots measured 3 by 9 m in size. In all experiments, treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR8002 flat-fan nozzle tips (TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) calibrated to deliver 140 L ha^{-1} at 117 kPa. Treatments were applied at a constant speed of 5 km hr^{-1}. Due to USDA regulations, soybean was required to be destroyed prior to the R4 stage of growth therefore yield was not determined in any experiment.

Weed Management Experiment

This experiment was conducted at both the Columbia and Moberly locations. The herbicide treatments, timings, and rates evaluated are listed in Table 3.5. A non-treated control was included for comparison. PRE treatments were applied to a recently-tilled conventional seedbed prior to soybean emergence. Early postemergence (EPOST) applications were made once the average size of all weeds present reached 10-cm in height. Late postemergence (LPOST) applications were made once the average size of weed regrowth of all weeds present reached 10-cm in height.

Visible weed control and crop injury evaluations were assessed at regular intervals after application on a scale of 0 to 100 percent, where 0 represents no plant death or crop injury and 100 was equal to complete plant death. Late-season biomass samples were taken from two random 0.5-m^2 areas between the center two rows of each plot once soybean reached the R3 stage of growth. Weeds were clipped at the soil surface, dried at 49 C for five days, and weights recorded.

Soybean Tolerance Experiment

This experiment was conducted at the Columbia location in 2012 and 2013. The herbicide treatments, timings, and rates evaluated are listed in tables 3.7. The herbicide rates evaluated represent the recommended labeled use rate (1X) and twice the recommended labeled use rate (2X) for each HPPD-inhibiting herbicide. This experiment was maintained weed-free by applying a PRE application of *S*-metolachlor, sulfentrazone, and cloransulam (1.39 + 0.26 + 0.034 kg ha^{-1}) and hand weeding as necessary throughout the season. A non-treated control for each application timing was included for comparison. PRE treatments were applied to a recently-tilled conventional seedbed prior to soybean emergence. V3 applications were made once soybean reached the third trifoliate. R1 applications were made when soybean reached the first reproductive stage.

Visible soybean injury evaluations, soybean height, and soybean biomass was assessed 7 and 28 days after each respective application (DAA). Visual soybean injury was assessed on a scale of 0 to 100 percent, where 0 represents no plant death or crop injury and 100 is equivalent to complete plant death. Soybean height reduction (HR) and biomass reduction (BR) were determined by measuring five plants from each plot from the soil surface to the base of the uppermost trifoliate and clipping the soybean plant at the soil surface. Plants were dried at 49 C for seven days and weights were recorded.

Statistical Analysis

The PROC GLM procedure in SAS (SAS 9.3, SAS® Institute Inc. Cary, NC) was used to analyze all data. For the weed management experiment, weed control and BR were analyzed with replication and treatment considered as fixed effects. Comparisons were made between herbicide treatments to determine the impact of treatments used. Significant interactions were present between years; therefore weed control and BR were separated by year (Tables 3.4 and 3.5). Comparisons between herbicide programs were made to determine whether differences exist between PRE fb POST, two-pass POST, and one-pass POST programs (Table 3.6). For the soybean tolerance experiment, visual soybean injury, HR, and BR were analyzed with replication and treatment considered as fixed effects. Comparisons were made between herbicides, rates, and application timing to determine the effect of each factor on soybean injury, HR, and BR. Significant interactions were present between years; therefore visible soybean injury, HR, and BR were separated by year (Tables 3.7 and 3.8). Individual treatment differences were separated using Fisher's protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Weed Management Experiment. Few differences in weed control and BR were observed among herbicide treatments at Columbia (Table 3.4 and 3.5). In both years, all PRE fb POST treatments provided 97% or greater control of all weeds at Columbia except ivyleaf morningglory. There were no differences in ivyleaf morningglory control between any of the one- and two-pass POST treatments and there were few differences between the PRE fb POST treatments. Within the PRE fb POST treatments, mesotrione plus *S*-metolachlor fb glyphosate or mesotrione plus *S*-metolachlor fb isoxaflutole plus glyphosate provided some of the highest levels of ivyleaf morningglory control and also greater weed BR than all one-pass POST and several PRE fb POST treatments in 2012. Ivyleaf morningglory control and BR was lowest in response to metribuzin plus *S*-metolachlor fb glyphosate in 2012 but BR was not different in response to any PRE fb POST or two-pass POST treatment in 2013. PRE treatments of *S*-metolachlor plus metribuzin plus isoxaflutole (low, mid, and high rate combinations) fb glyphosate improved ivyleaf morningglory control and BR in 2012, but visible weed control and BR were similar to *S*-metolachlor plus metribuzin fb glyphosate in 2013. Giant foxtail control was at least 6% lower with one-pass POST treatments compared to PRE fb POST and two-pass POST treatments in 2012 but control was similar among all treatments in 2013. No differences in large crabgrass, common cocklebur, or common sunflower control were observed between treatments in either year.

In 2013, isoxaflutole (low) plus *S*-metolachlor plus glyphosate provided 94% weed BR which was less than all other treatments evaluated (Table 3.5). Weed BR provided by all other treatments was similar and was 98% or greater. With the exception of the lower giant foxtail control provided by the one-pass POST program in 2012, there were no visible control or BR differences between PRE fb POST, two-pass POST, and one-pass POST programs (data not shown). At Moberly in 2012, the low rate combination of isoxaflutole plus *S*-metolachlor plus metribuzin fb glyphosate provided at least 21% lower waterhemp control and 17% lower waterhemp BR than the higher isoxaflutole plus *S*-metolachlor plus metribuzin combinations and all other PRE fb POST treatments, but in 2013 there were no differences in control or BR provided by any of the PRE fb POST treatments (Tables 3.4 and 3.5). Knezevic et al. (1998) reported that 0.52 g ha^{-1} of isoxaflutole provided 65% BR of redroot pigweed, and that 100 g ha^{-1} was needed to achieve at least 90% BR of this species. The addition of isoxaflutole (low) or isoxaflutole (low) and pyroxasulfone to POST glyphosate applications of the mid-rate combination treatments did not improve visible weed control or biomass reduction at either site in either year, but lower ivyleaf morningglory control was provided by the mid-rate combination followed by isoxaflutole (low) plus glyphosate in 2012. The addition of isoxaflutole (low) to glyphosate in the POST application following metribuzin plus *S*-metolachlor did not improve control or BR at Columbia in 2013 or at Moberly in

2012 or 2013; however, it did improve ivyleaf morningglory control by 22% and BR by 13% over a POST application of glyphosate alone in 2012 at Columbia. At Moberly, glyphosate followed by glyphosate provided 28 and 38% control and 45 and 71% waterhemp BR in 2012 and 2013, respectively, which was one of the lowest levels of waterhemp BR observed in either year and can be explained by the presence of glyphosate-resistant waterhemp at this location. Similarly, fomesafen plus *S*-metolachlor plus glyphosate provided less than 54% waterhemp BR and 23% control in either year, also due to the presence of waterhemp with resistance to glyphosate and PPO-inhibiting herbicides like fomesafen.

In both years, two-pass POST treatments of isoxaflutole plus glyphosate fb isoxaflutole plus glyphosate resulted in higher control and BR than all one-pass POST treatments and glyphosate fb glyphosate. In 2013, no differences in BR were observed between the PRE fb POST treatments and the two-pass POST treatments containing isoxaflutole, but the PRE fb POST treatments provided at least 12% higher visible control than the isoxaflutole plus glyphosate fb isoxaflutole plus glyphosate treatments (Table 3.5). One-pass POST treatments of isoxaflutole (low and mid) plus *S*-metolachlor plus glyphosate resulted in 80 and 85% BR in 2013 which was lower than all PRE fb POST treatments and isoxaflutole plus glyphosate fb isoxaflutole plus glyphosate treatments. Contrary to Columbia, visible control and BR differences between herbicide programs were present in both years at Moberly. This may be due to the weed species differences between the two locations, and to the presence of multiple-resistant waterhemp at the Moberly location.

At Moberly, PRE fb POST programs resulted in 29 to 58% greater visible waterhemp control and 8 to 31% greater waterhemp BR than any other herbicide program evaluated (Table 3.6). Two-pass POST herbicide programs also provided greater visible waterhemp control and BR than one-pass POST programs. One-pass POST herbicide programs provided less than 25% waterhemp control and less than 72% BR in both years. Legleiter et al. (2009) and Craigmyle et al. (2013) also observed that PRE fb POST programs provided higher waterhemp control than POST only programs.

Soybean Tolerance Experiment. Isoxaflutole was generally least injurious to soybean when compared to all other herbicides (Table 3.7). Weber and Allen (2012) and Hinz et al. (2013) also reported that FG72 soybean exhibits the best tolerance to isoxaflutole. Mesotrione was similar to isoxaflutole with respect to visible injury, HR, and BR 28 days after application (DAA) in 2013. However, mesotrione caused 3 and 9% more visible injury than isoxaflutole 7 DAA in 2012 and 2013. Visible soybean injury from topramezone 28 DAA was 2 and 11% less than tembotrione in 2012 and 2013, but BR was similar. Tembotrione generally resulted in the highest visible soybean injury and usually the greatest HR and BR. In 2013, visible soybean injury was generally greater than that which was observed in 2012. Variable rainfall and temperature conditions between years likely contributed to the contrast in the injury recorded (Table 3.2).

Applications for all herbicides at the 2X rate always resulted in higher visible injury than the 1X rate (Table 3.7). No differences between rates were observed 7 DAA for HR or BR. HR was 3% greater with the 2X rate 28DAA in 2013, but not in 2012. Differences in BR 28 DAA were not present in 2012; however, in 2013 the 1X rate resulted in a 6% greater BR 28 DAA than the 2X rate.

Visible injury never exceeded 2% from the PRE application timing (Table 3.7). In 2013, visible soybean injury, HR, and BR were always greater in response to V3 applications of HPPD-inhibiting herbicides compared to PRE or R1 applications. In 2012, the PRE application timing resulted in the greatest HR while in 2013 the V3 timing resulted in the greatest HR at 7 and 28 DAA. In 2012, the R1 timing reduced biomass 6% more than the V3 timing 7 DAA, but there were no differences between the three timings 28 DAA. In 2013, the V3 application reduced biomass 20% and 24% more than the R1 application at 7 and 28 DAA.

When comparing PRE applications of isoxaflutole, tembotrione, mesotrione, and topramezone, few differences were present between visible soybean injury, HR, and BR (Table 3.8). Weber and Allen (2012) and Hinz et al. (2013) reported that FG72 soybean is tolerant to PRE applications of isoxaflutole and mesotrione. Isoxaflutole reduced soybean biomass 12% more than mesotrione 28 days after the PRE application in 2013, but there were no differences in BR in 2012. When differences were present for the V3 timing, tembotrione resulted in the highest visible soybean injury and HR compared to isoxaflutole, mesotrione, and topramezone. However, BR 28 days after the V3 application in 2013 was greater in response to tembotrione and topramezone compared to isoxaflutole and mesotrione, but in 2012 no BR differences were present. In both years, mesotrione resulted in higher visible soybean injury than isoxaflutole 7 days after the V3 application, but visible injury was similar 28 DAA. Height reduction and BR were similar between V3 applications of isoxaflutole and mesotrione in both years except for HR 7 DAA in 2013. Topramezone was similar or less injurious than mesotrione 7 DAA when applied at V3 and R1 in both 2012 and 2013. However in 2013, visible injury, HR, and BR 28 DAA of topramezone always exceeded that caused by mesotrione. With the exception of visible injury 7 DAA, no differences in visible injury, HR, or BR were observed between mesotrione and topramezone in 2012 for any application timing. In 2013, visible injury 28 days after the tembotrione application was 30% less for the R1 versus V3 timing. In both years, isoxaflutole and mesotrione applied at R1 resulted in similar BR 7 and 28 DAA. BR from topramezone and tembotrione were similar with all application timings in both years 7 and 28 DAA. Weber and Allen (2012) and Hinz et al. (2013) also reported that this soybean event has reduced tolerance to POST applications of mesotrione, topramezone, and tembotrione.

Based on the results of this research, PRE fb POST programs are necessary to provide adequate control of GR weeds like waterhemp in FG72 soybean. While the addition of isoxaflutole to PRE treatments was not always necessary to provide high levels of weed control and BR of either glyphosate-susceptible or GR weed species, utilizing PRE fb POST programs that incorporate isoxaflutole or mesotrione in FG72 soybean can provide extended residual control of a broad range of weed species and will provide a greater diversity of effective herbicide modes of action. Two-pass POST herbicide treatments of isoxaflutole plus glypohsate fb isoxaflutole plus glypohsate will likely improve GR weed control and BR compared to glyphosate fb glyphosate or any one-pass treatment. However, when weed species were glyphosate-susceptible, isoxaflutole did not improve weed control when added to glyphosate in two-pass POST treatments. PRE applications resulted in the lowest visible soybean injury of the three application timings. V3 applications will likely induce the most visible soybean injury, HR, and BR. Applications of tembotrione and topramezone made at V3 and R1 are most likely to cause unacceptable levels of visible injury and BR to FG72 soybean. Isoxaflutole and mesotrione generally provide the least visible injury, HR, and BR 28DAA for both the V3 and R1 application timings. FG72 soybean could allow the use of HPPD-inhibiting herbicides such as mesotrione PRE along with isoxaflutole PRE and POST to provide an additional mode of action to producers that was not previously available in soybean.

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Table 3.1. Dates of major field operations and soybean growth stages and weed sizes at the time of application in the weed management and soybean tolerance experiments in 2012 and 2013.

	Research location			
	Columbia		Moberly	
	2012	2013	2012	2013
Weed Management Experiment[a]				
Seeding date	6/7	6/19	6/6	6/20
Dates of herbicide application				
PRE fb EPOST	6/8 fb 7/13	6/21 fb 7/15	6/6 fb 7/19	6/19 fb 7/19
EPOST fb LPOST	7/2 fb 7/27	7/15 fb 7/30	7/5 fb 8/1	7/12 fb 7/30
Soybean growth stage at application				
PRE fb EPOST	--- fb V5	--- fb V4	--- fb V5	--- fb V4
EPOST fb LPOST	V3 fb R1	V4 fb R1	V3 fb R2	V3 fb R1
Average weed size (cm) at application				
PRE fb EPOST	--- fb 12	--- fb 9	--- fb 20	--- fb 10
EPOST fb LPOST	10 fb 10	15 fb 12	10 fb 20	10 fb 15
Soybean Tolerance Experiment[a]				
Seeding date	6/7	6/19		
Dates of herbicide application				
PRE	6/7	6/21		
V3	7/3	7/11		
R1	7/24	7/30		
Soybean growth stage at application				
PRE	---	---		
V3	V3	V3		
R1	R1	R1		

[a] Abbreviations: PRE, pre-emergence; fb, followed by; EPOST, early post-emergence; LPOST, late post-emergence; V3, three trifoliate; R1, reproductive stage one.

Table 3.2. Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr average from April through October in 2012 and 2013 at Moberly, Missouri and Columbia, Missouri.

		Rainfall			Temperature		
Location	Month	2012	2013	30 year Avg.[a]	2012	2013	30 year Avg.[a]
		---------- mm ----------			------------ C ------------		
Moberly	April	126	136	103	13.1	11.7	13.0
	May	77	202	126	20.0	17.2	18.2
	June	73	37	126	23.4	23.3	22.9
	July	49	25	113	28.1	24.4	25.5
	August	4	39	109	23.9	24.4	24.6
	September	125	76	109	19.7	21.7	19.9
	October	78	87	81	12.5	13.3	13.7
Columbia	April	204	188	114	14.9	11.9	12.8
	May	33	249	126	21.4	17.8	17.8
	June	79	52	113	24.9	23.0	22.7
	July	116	100	111	29.6	24.0	25.2
	August	64	48	110	25.9	24.4	24.6
	September	57	61	98	19.9	21.7	19.8
	October	78	72	84	12.5	13.5	13.3

[a] 30 year averages (1982-2011) obtained from National Climatic Data Center (2014).

Table 3.3. Sources of materials used in the experiments.

Herbicide[a]	Trade name	Formulation[a]	Rate	Manufacturer	Address
			kg ai or ae Ha^{-1}		
Isoxaflutole	Balance Pro	4 SC	0.035 (low), 0.07 (mid), 0.105 (high), 0.140	Bayer CropScience	Research Triangle Park, NC
Mesotrione	Callisto	4 SC	0.105, 0.210	Syngenta	Wilmington, DE
Tembotrione	Laudis	3.5 SC	0.092, 0.184	Bayer CropScience	Research Triangle Park, NC
Topramezone	Impact	2.8 SC	0.018, 0.037	AMVAC	Newport Beach, CA
S-metolachlor	Dual II Magnum	7.64 EC	0.6 (low), 1.4 (mid), 1.5, 2.1(high)	Syngenta	Wilmington, DE
Fome. + *S*-met.	Prefix		1.52 + 0.33	Syngenta	Wilmington, DE
Pyroxasulfone	Zidua	85 WG	0.09	BASF	Research Triangle Park, NC
Metribuzin	Sencor	75 WG	0.2 (low), 0.3 (mid), 0.4 (high)	Bayer CropScience	Research Triangle Park, NC
Glyphosate	Rndup. WMax.	4.5 SC	0.86	Monsanto	St. Louis, MO
Metrib. + *S*-met.	Boundary	6.5 EC	0.42 + 1.77	Syngenta	Wilmington, DE
Meso. + *S*-met.	Zemax	3.67 EC	0.185 + 1.87	Syngenta	Wilmington, DE
Non-Ionic Surfactant	Astute	100 L	0.35 L ha^{-1}	MFA	Columbia, MO
Ammonium Sulfate	N-Pak AMS	3.4 L	2.9	Winfield Solutions	St. Paul, MN

[a] Abbreviations: fome., fomesafen; *S*-met., *S*-metolachlor; Meso., mesotrione; Metrib., metribuzin; Rndup. WMax., Roundup WeatherMax; SC, soluble concentrate; EC, emulsifiable concentrate, WG, water-dispersable granule; L, liquid.

Table 3.4. Influence of herbicide treatments on late-season[e] visible weed control across four site-years in Missouri.

			Visual Control[c]								
			Columbia							Moberly	
Herbicide	Application		IPOHE	SETFA		DIGSA	XANST		HELAN	AMATA	
Treatment[ac]	Timing[bc]	Rate	2012	2012	2013	2012	2012	2013	2012	2012	2013
		kg ai or ae Ha^{-1}	(%)								
Isox+*S*-met+metr Glyphosate	PRE EPOST	0.035+0.6+0.2 0.86	52	99	99	99	98	97	99	74	91
Isox+*S*-met+metr Glyphosate	PRE EPOST	0.07+1.4+0.3 0.86	77	99	99	99	99	97	99	95	92
Isox+*S*-met+metr Glyphosate	PRE EPOST	0.105+2.1+0.4 0.86	78	99	99	99	99	97	99	98	95
Isox+*S*-met+metr Isox+glyphosate	PRE EPOST	0.07+1.4+0.3 0.035+0.86	61	99	99	99	99	97	99	96	96
Isox+*S*-met+metr Isox+pyrox+gly	PRE EPOST	0.07+1.4+0.3 0.035+0.09+0.86	76	99	99	99	99	97	99	96	94
Metrib+*S*-met Glyphosate	PRE EPOST	0.42 + 1.77 0.86	31	99	99	99	99	97	99	95	92
Metrib+*S*-met Isox+glyphosate	PRE EPOST	0.42 + 1.77 0.035+0.86	53	99	99	99	99	97	99	96	92

Meso+*S*-met Glyphosate	PRE EPOST	0.185+1.87 0.86	90	99	99	99	99	97	99	96	90
Meso+*S*-met Isox+glyphosate	PRE EPOST	0.185+1.87 0.035+0.86	83	99	99	99	99	97	99	97	92
Glyphosate Glyphosate	EPOST LPOST	0.86 0.86	71	99	99	99	99	97	99	28	38
Isox+glyphosate Isox+glyphosate	EPOST LPOST	0.035+0.86 0.070+0.86	71	99	98	99	99	97	99	39	77
Isox+glyphosate Isox+glyphosate	EPOST LPOST	0.070+0.86 0.035+0.86	73	99	98	99	99	97	99	42	78
Fome+*S*-met+gly	EPOST	1.52+0.33+0.86	68	93	97	95	88	96	98	22	18
Isox+*S*-met+gly	EPOST	0.035+1.5+0.86	73	92	97	93	85	96	98	23	22
Isox+*S*-met+gly	EPOST	0.07+1.5+0.86	71	93	99	93	87	97	98	28	24
Non-treated			0	0	0	0	0	0	0	0	0
LSD$_{0.05}$[d]			11	3	NS	NS	NS	NS	NS	6	7

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[a] All POST treatment included ammonium sulfate at 2.9 kg ai Ha^{-1}.

[b] Application timing: PRE, at planting; EPOST, 10-cm weeds; LPOST, 10-cm weed regrowth.

[c] Abbreviations: PRE, pre-emergence; EPOST, early post-emergence; LPOST, late post-emergence; Isox, isoxaflutole; Fome, fomesafen; *S*-met, *S*-metolachlor; Meso, mesotrione; Metr, metribuzin; Gly, glyphosate; Pyrox, pyroxasulfone; SETFA, giant foxtail; DIGSA, large crabgrass; XANST, common cocklebur; IPOHE, ivyleaf morningglory; HELAN, common sunflower; AMATA, common waterhemp.

[d] NS, not significant at the α=0.05 level.

[e] Late-season weed control determined at R3 soybean.

Table 3.5. Influence of herbicide treatments on late-season[e] weed biomass reduction across four site-years in Missouri.

Herbicide Treatement[ac]	Application Timing[bc]	Rate	Biomass Reduction Columbia 2012	Biomass Reduction Columbia 2013	Biomass Reduction Moberly 2012	Biomass Reduction Moberly 2013
		kg ai or ae Ha^{-1}	---------------------------- (%)			----------------------------
Isox+*S*-met+metr Glyphosate	PRE EPOST	0.035+0.6+0.2 0.86	89	99	83	97
Isox+*S*-met+metr Glyphosate	PRE EPOST	0.07+1.4+0.3 0.86	94	98	100	96
Isox+*S*-met+metr Glyphosate	PRE EPOST	0.105+2.1+0.4 0.86	97	100	100	97
Isox+*S*-met+metr Isox+glyphosate	PRE EPOST	0.07+1.4+0.3 0.035+0.86	95	100	100	100
Isox+*S*-met+metr Isox+pyrox+gly	PRE EPOST	0.07+1.4+0.3 0.035+0.09+0.86	97	99	100	100
Metrib+*S*-met Glyphosate	PRE EPOST	0.42 + 1.77 0.86	76	99	100	94
Metrib+*S*-met Isox+glyphosate	PRE EPOST	0.42 + 1.77 0.035+0.86	89	99	100	99
Meso+*S*-met Glyphosate	PRE EPOST	0.185+1.87 0.86	99	100	100	98
Meso+*S*-met	PRE	0.185+1.87	99	99	100	98

Isox+glyphosate	EPOST	0.035+0.86				
Glyphosate	EPOST	0.86	96	99	45	71
Glyphosate	LPOST	0.86				
Isox+glyphosate	EPOST	0.035+0.86	96	99	79	99
Isox+glyphosate	LPOST	0.070+0.86				
Isox+glyphosate	EPOST	0.070+0.86	96	100	78	99
Isox+glyphosate	LPOST	0.035+0.86				
Fome+*S*-met+gly	EPOST	1.52+0.33+0.86	93	99	49	53
Isox+*S*-met+gly	EPOST	0.035+1.5+0.86	95	94	58	80
Isox+*S*-met+gly	EPOST	0.07+1.5+0.86	95	98	70	85
Non-treated			0	0	0	0
$LSD_{0.05}$[d]			3	3	7	8

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[a] All POST treatment included ammonium sulfate at 2.9 kg ai Ha^{-1}.

[b] Application timing: PRE, at planting; EPOST, 10-cm weeds; LPOST, 10-cm weed regrowth.

[c] Abbreviations: PRE, pre-emergence; EPOST, early post-emergence; LPOST, late post-emergence; Isox, isoxaflutole; Fome, fomesafen; *S*-met, *S*-metolachlor; Meso, mesotrione; Metr, metribuzin; Gly, glyphosate; Pyrox, pyroxasulfone.

[d] NS, not significant at the α=0.05 level.

[e] Late-season weed control determined at R3 soybean.

Table 3.6. Influence of herbicide program on late season[a] visible weed control and biomass reduction at Moberly, Missouri.

	Visible Control		Biomass Reduction	
Herbicide Program	2012	2013	2012	2013
	------------------------------	(%)	--------------------------------	
PRE fb POST	94	93	98	97
Two-Pass Post	36	64	67	89
One-Pass Post	24	21	59	71
$LSD_{0.05}$	3	4	4	4

[a] Late-season weed control and biomass reduction determined at R3 soybean.

Table 3.7. Influence of herbicide, rate, and application timing on soybean injury and height and biomass reduction at Columbia, Missouri.

	Soybean Injury				Height Reduction				Biomass Reduction			
	7 DAA		28 DAA		7 DAA		28 DAA		7 DAA		28 DAA	
Factor	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
	--------------------- (%) ---------------------				--------------------- (%) ----------------------				--------------------- (%) ----------------------			
Herbicide[ab]												
Isoxaflutole	1	1	0	4	13	8	6	8	17	18	20	23
Tembotrione	9	19	2	27	13	13	8	18	12	26	15	36
Mesotrione	4	10	0	6	15	10	8	9	11	19	18	20
Topramezone	3	7	0	16	13	11	7	15	15	21	22	33
LSD $_{0.05}$ [f]	1.2	1.4	0.4	2	NS	1.5	1	1	NS	NS	NS	6
Rate[c]												
1X	4	7	0.4	10	13	10	8	12	13	19	20	31
2X	5	11	1	17	13	11	8	15	14	23	18	25
LSD $_{0.05}$ [f]	0.9	1	0.3	1.4	NS	NS	NS	1	NS	NS	NS	4
Timing[de]												
PRE	2	0	0.6	0	23	10	10	9	10	7	23	12
V3	6.5	22	0	29	10	14	6	22	13	38	18	48
R1	5	5	1.4	10	7	8	7	10	19	18	16	24
LSD $_{0.05}$ [f]	1	1	0.3	1.7	2	1.3	1	1	5	5	NS	5
ANOVA[f]	---P > *F*---											
Herbicide (H)	<0.0001	<0.0001	<0.0001	<0.0001	NS	<0.0001	0.0459	<0.0001	NS	NS	NS	<0.0001
Rate (R)	0.0283	<0.0001	0.0032	<0.0001	NS	NS	NS	0.0053	NS	NS	NS	0.0020
Timing (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0010	<0.0001	NS	<0.0001

H X R	NS	<0.0001	NS	<0.0001	NS	NS	NS	0.0001	NS	NS	NS	NS
H X T	<0.0001	<0.0001	<0.0001	<0.0001	NS	<0.0001	NS	<0.0001	NS	NS	<0.0001	<0.0001
R X T	NS	<0.0001	0.0097	<0.0001	NS	0.0265	NS	<0.0001	NS	NS	NS	0.0073
H X R X T	NS	<0.0001	<0.0001	<0.0001	NS	NS	NS	<0.0001	NS	NS	NS	NS

[a] All POST treatment included ammonium sulfate at 2.9 kg ai Ha^{-1} and non-ionic surfactant at 0.35 L Ha^{-1}.

[b] Data combined across all rates and timings.

[c] Data combined across all herbicides and timings.

[d] Data combined across all herbicides and rates.

[e] Application timing: PRE, at planting; V3, three trifoliate; R1, reproductive stage one.

[f] NS, not significant at the $\alpha=0.05$ level.

Table 3.8. Influence of the timing of HPPD-inhibiting herbicides on soybean injury and height and biomass reduction at Columbia, Missouri.

		Soybean Injury				Height Reduction				Biomass Reduction			
Application		7 DAA		28 DAA		7 DAA		28 DAA		7 DAA		28 DAA	
Timing^{ab}	Herbicide^{b}	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
		--------------- (%) --------------				--------------- (%) ---------------				--------------- (%) ----------------			
PRE	Isoxaflutole	2	0	1	0	23	11	9	8	15	11	24	19
	Tembotrione	2	0	1	0	22	9	10	10	9	7	18	13
	Mesotrione	2	0	1	0	24	9	12	8	6	4	20	7
	Topramezone	2	0	1	0	22	10	10	9	9	7	29	10
V3	Isoxaflutole	2	2	0	9	9	8	5	11	17	28	21	41
	Tembotrione	13	50	0	55	10	19	7	30	15	50	14	61
	Mesotrione	5	21	0	14	12	12	5	12	8	35	18	34
	Topramezone	6	16	0	38	10	14	7	26	11	40	18	55
R1	Isoxaflutole	0	0	0	2	6	5	6	6	19	15	14	9
	Tembotrione	14	7	5	25	8	10	8	13	13	20	14	32
	Mesotrione	5	8	0	3	5	9	8	8	19	19	17	20
	Topramezone	2	5	0	11	6	8	6	11	15	18	18	34
$\text{LSD}_{0.05}^{c}$		2	4	0.8	6	NS	3	NS	2.5	NS	NS	NS	11

[a] All POST treatment included ammonium sulfate at 2.9 kg ai Ha^{-1} and non-ionic surfactant at 0.35 L Ha^{-1}.

[b] Data summarized across all herbicide rates.

[c] NS, not significant at the $\alpha=0.05$ level.

CHAPTER IV

Investigations of the Distribution of Herbicide Resistances and Molecular Mechanisms Conferring Resistance in Missouri Waterhemp Populations

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ABSTRACT

A survey of soybean fields containing waterhemp was conducted just prior to harvest in 2012 to determine the scope and extent of herbicide resistance and multiple herbicide resistances among a subsample of Missouri waterhemp populations. Resistance was confirmed to glyphosate, ALS-, PPO-, PSII-, and HPPD-inhibitors but not to 2,4-D. Of the 187 populations tested, 186 exhibited resistance to chlorimuron. The proportion of populations with atrazine and glyphosate resistance was similar with 30 and 29% of the populations surviving the 3X rate. Lactofen resistance was observed in 5% of the populations while mesotrione resistance was only found in 1.6% of the populations. All populations tested were susceptible to 2,4-D at the 3X rate. At least 52% of the waterhemp populations tested exhibited resistance to two herbicide modes of action. Resistance to atrazine plus chlorimuron as well as glyphosate plus chlorimuron was present in 29% of the populations. Three-way resistance was present in 11% of the populations with resistance to atrazine plus chlorimuron plus glyphosate present in 10% of the populations. Resistance to four-modes of action was shown in 2% of the populations while one population exhibited resistance to five modes of action. DNA analysis of a subsample of plants revealed that previously documented mechanisms of resistance in waterhemp such as the ΔG210 deletion conferring PPO resistance, the

Trp574Leu amino acid substitution determining ALS resistance, and elevated EPSPS copy numbers and the Pro106Ser amino acid substitution resulting in glyphosate resistance explained survival in the majority of instances. However, there are indications that resistance to glyphosate due to alternate unknown mechanisms may be present as well. Overall, results from these experiments indicate that Missouri soybean fields contain waterhemp populations with resistance to glyphosate, ALS-, PPO-, PSII-, and HPPD-inhibiting herbicides, which are some of the most common modes of action currently utilized for the control of this species in corn and soybean production systems. Additionally, these results indicate that slightly more than half of the populations tested exhibit resistance to more than one herbicide mode of action. Managing the current resistance levels in existing populations is of utmost importance. The use of multiple, effective herbicide modes of action, both preemergence and postemergence, and the integration of optimum cultural control practices will be vital to controlling Missouri waterhemp populations in the future.

INTRODUCTION

Common waterhemp (*Amaranthus rudis* Sauer) is the most prominent and troublesome weed in agronomic crops in Missouri, Iowa, and Illinois (Rosenbaum and Bradley 2013; Bradley 2013; Waggoner and Bradley 2011; Legleiter and Bradley 2008; Bradley et al. 2007; Hager et al. 2000). Its extended period of germination, rapid growth habit, and prolific seed production are all characteristics that have enabled this weed to thrive in current corn and soybean production systems (Sauer 1957; Hartzler et al. 1999; Hartzler et al. 2004). Waggoner and Bradley (2011) reported that waterhemp was found in 87% of the Missouri soybean fields that were surveyed at an average density of 22 plants per m^2, and resulted in yield losses up to 545 kg/ha. Corn and soybean can suffer substantial yield losses due to waterhemp. Hager et al. (2002) found that 10 weeks of waterhemp interference at densities from 89 to 362 plants per m^2 resulted in a 43% soybean yield loss. Cordes et al. (2004) reported waterhemp densities from 82 to 445 plants per m^2 resulted in a 10 to 36% corn yield reduction. Many producers rely primarily and often solely on herbicides for weed control; therefore, increasing selection pressure is placed on weed populations to evolve resistance (Powles 2008; Young 2006). As waterhemp is dioecious, it can transfer its genes easily from male to female plants via pollen (Costea et al. 2005; Steckel 2007; Hausman et al. 2011; Tranel et al. 2011).

As of 2014, numerous waterhemp populations within the United States have been reported to be resistant to one or more of the following herbicide modes of action: growth regulators, EPSPS-, acetolactate synthase (ALS)-, protoporphyrinogen (PPO)-, photosystem II (PSII)-, and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Heap 2014). One population of waterhemp from Illinois and one from Iowa evolved multiple resistances to four modes of action (Bell et al. 2013; Heap 2014). Populations of waterhemp throughout Missouri, Iowa, and Kansas have evolved multiple resistances to two or more modes of action (Legleiter and Bradley 2008; Heap 2014). In Missouri, waterhemp with resistance to glyphosate, ALS-, PPO-, or PSII-inhibiting herbicides has been documented (Legleiter and Bradley 2008; Heap 2014). In 2005, a waterhemp biotype that exhibited three-way resistance to glyphosate, ALS-, and PPO-inhibitors was confirmed in Platte County, Missouri (Legleiter and Bradley 2008; Heap 2014).

Several mechanisms have been discovered to explain these herbicide resistances in waterhemp. ALS resistance has been confirmed mostly through a Trp574Leu amino acid substitution in the ALS enzyme, but also through a non-target site mechanism that appears to be metabolism-based (Warwick et al. 2010; Guo et al. 2013). Glyphosate resistance has been conferred through EPSPS gene amplification, a Pro106Ser amino acid substitution in the EPSPS enzyme, and a non-target site mechanism (Chatham et al. 2013; Guo et al. 2013; Nandula et al. 2013). Resistance to PPO-inhibiting herbicides is highly conserved and is conferred by a codon deletion at amino acid 210 in the *PPX2L* gene (Patzoldt et al. 2006). PSII herbicide resistance is mainly confirmed by a Ser264Gly amino acid mutation in the psbA enzyme (Foes et al. 1998, Mechant et al. 2008). Ma et al. (2013) reported that, contrary to the aforementioned resistance mechanisms, there were no alterations in the *HPPD* sequence or *HPPD* expression in HPPD-resistant waterhemp. The mechanism of resistance to HPPD-inhibitors in an Illinois waterhemp population was determined to be enhanced oxidative metabolism (Ma et al. 2013).

Surveys are a practical tool to gather important information to assist in educating producers on the current weed resistance status and distribution within a given geographic area (Rosenbaum and Bradley 2013; Beckie et al. 2000; Givens et al. 2009; Johnson and Gibson 2006). Rosenbaum and Bradley (2013) conducted a survey of weedy soybean fields at harvest in 2008 and 2009 to determine the extent and distribution of glyphosate resistance in Missouri waterhemp populations. They found that 69% of the Missouri waterhemp populations sampled were resistant to glyphosate across 54 counties in the state (Rosenbaum and Bradley 2013). However, currently there is no information as to the extent of multiple herbicides resistances in Missouri waterhemp populations.

The objectives of this research were: 1) to determine the level of herbicide resistance and multiple herbicide resistances among a subsample of Missouri waterhemp populations to six modes of action, and 2) to determine the specific mechanisms responsible for resistance in a subset of these waterhemp plants.

MATERIALS AND METHODS

Plant materials and growth conditions. In 2012, waterhemp seed samples were collected from 187 randomly-chosen soybean fields across 57 counties in the primary corn and soybean production areas in Missouri. Sites for seed collection were chosen at random, but based on the presence of waterhemp in soybean fields just prior to soybean harvest. At each survey location, approximately 5 to 10 female waterhemp seedheads were harvested and the global positioning system coordinate was recorded (Figure 1). Mature seed were gleaned from waterhemp seedheads and combined into a collective sample representative of that waterhemp population. Seed were treated with a 50/50 water and Clorox mixture for 10 minutes, washed with water, suspended in a 0.15% agarose solution, and stored at 4°C for 6 weeks prior to the start of the experiments to improve germination. Seed from each population were broadcast into 25- by 50-cm greenhouse flats containing commercial potting medium (Premier Tech Horticulture, Quakertown, PA), which was used to cover the seedbed to a thickness of approximately 6 mm following planting. After emergence, 1-2 leaf seedling waterhemp plants were transplanted into cones 4-cm in diameter (‘Cone-tainers’, Stewe and Sons Inc., Tangent, OR) containing a 4:1 mixture of commercial potting medium to sand. 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.

Experimental design. The trial design was a factorial arrangement of treatments in a randomized complete block with six replications, where the factors were population, herbicide, and rate. The experiment was repeated once.

Treatment, evaluation and data collection. The herbicides and rates evaluated are listed in Table 4.1. Herbicide rates included the standard labeled rate (1X) and three times the standard labeled rate (3X) for each respective herbicide. A non-treated control was included from each population for comparison. Treatments were applied when plants reached 8- to 12-cm in height, using a compressed air, laboratory spray chamber equipped with an 8001EVS nozzle (Teejet Spraying Systems, Wheaton, IL) delivering 220 L ha^{-1} at 234 kPa. Resistance was characterized based on the number of plants that survived the 3X rate and were capable of growth and reproduction 21 days after application (DAA) as suggested by Beckie et al. (2000) and Rosenbaum and Bradley (2013). Rosenbaum and Bradley (2013) classified waterhemp populations resistant to glyphosate if survival was 60% or more at the 2X rate. In this experiment, waterhemp populations were considered resistant if 50% or more of the plants survived the 3X herbicide application and were capable of growth and reproduction. Survivorship in response to each treatment was determined and recorded for each plant 21 DAA.

Tissue collection and DNA extraction. Prior to herbicide application, tissue samples were taken from the new growth of six plants within 26 potential PPO-resistant populations to determine if the codon deletion in the *PPX2L* gene was present. These same plants were tracked through the remainder of the experiment to determine survival or death following the application of the 3X rate of lactofen. Three weeks after the herbicide applications, additional tissue samples were randomly collected from the new growth of 92 plants from 41 populations that survived the 3X rate of chlorimuron, and from 93 plants from 36 populations that survived the 3X rate of glyphosate to confirm the mechanisms of resistance for these respective herbicides. Tissue was stored at -80 C until DNA extraction. All DNA was extracted from frozen leaf tissue using the hexadecyltrimethylammonium bromide method previously described by Doyle and Doyle (1990). Quality and quantity of DNA were examined using a spectrophotometer (NanoDrop 1000 Spectrophotometer, Thermo Fisher Scientific, 81 Wyman St., Waltham, MA 02451), and samples were diluted to either 10 ng µL-1 for qPCR or to 10% of the original concentration for all other downstream applications.

DNA analysis. Relative *EPSPS* copy number was determined using real-time qPCR as described previously (Ma et al. 2013). Detection of the point mutation responsible for the Pro106Ser substitution was carried out using a dCAPS assay designed in the manner described by Delye et al. (2014). A portion of the *EPSPS* gene containing the codon at position 106 was amplified using the forward primer EPSf1 (5'-ATG TTG GAC GCT CTC AGA ACT CTT GGT-3') and reverse primer eps106wt-R3 (5'-CTC CAG CAA CGG CAA CCG CAA CTG TCC ATG-3'), which includes a single mismatch to introduce a *Nco*I restriction site in wild-type alleles. After PCR, resulting amplicons were digested with the enzyme *Nco*I (New England BioLabs Inc., 240 County Road, Ipswich, MA 01938-2723), and products were fractionated on a 2% agarose gel containing 0.5µg mL-1 ethidium bromide and visualized with ultraviolet light.

The mutation in the waterhemp *ALS* gene, resulting in the Trp574Leu amino acid substitution, causes a novel *Mfe*I recognition site. Primers AmALS-F2 (5’- TCC CGG TTA AAA TCA TGC TC-3’) and AmALS-R2 (5’-CTA AAC GAG AGA ACG GCC AG-3’) were used to amplify from DNA, and the amplified product was digested with *Mfe*I, similar to the protocol for kochia (Foes et al. 1999).

Allele-specific primers described previously by Lee et al. (2008) were used to screen samples for the codon deletion in the *PPX2L* gene, which correlates to a ΔG210 amino acid deletion in the enzyme.

RESULTS AND DISCUSSION

Greenhouse results. Resistance was confirmed to five of the six modes of action tested (Table 4.2, Figure 1). Of the 187 populations tested, 186 exhibited resistance to chlorimuron. Atrazine and glyphosate resistance were similar with 30 and 29% of the populations surviving the 3X rates, respectively. Lactofen resistance was observed in 5% of the populations while mesotrione resistance was only found in 1.6% of the populations. All populations tested were susceptible to 2,4-D at the 3X rate.

While the resistance threshold used in this experiment was 50% or greater survival to the 3X rate, data from the 1X rate was collected and included to show the variability in response at 50% survival or greater and potential future resistances in Missouri waterhemp populations (Table 4.2). There were no differences in the percentage of waterhemp populations that survived 1X and 3X rates of chlorimuron. High levels of resistance to chlorimuron and other ALS-inhibitors have been well documented within the literature (Patzoldt and Tranel 2007; Patzoldt et al. 2005; Shoup et al. 2003). However, the number of populations resistant to the 1X rate of atrazine, glyphosate, lactofen, and mesotrione exceeded the level of resistance to the 3X rate of these same herbicides in every instance. Patzoldt et al. (2005) also observed differences in waterhemp survival between high and low application rates of atrazine. They attributed this response to a non-target site mechanism of resistance. Unlike resistance to ALS-inhibitors, current evidence suggests that glyphosate target site mechanisms of resistance do not confer an absolute resistance level. In most cases of glyphosate resistance in *Amaranthus* species to date, overproduction of EPSPS reduces the ability of glyphosate to successfully bind to all EPSPS copies within the plant due to a higher ratio of EPSPS proteins to glyphosate (Powles 2010). Chatham et al. (2013) observed increasingly higher levels of glyphosate-resistant waterhemp control with each incremental increase in glyphosate rate across five separate waterhemp populations in the Midwest. Four of these populations were confirmed to have an elevated EPSPS copy number while one population had the Pro106Ser amino acid substitution. An approximate 20% increase in control was also seen in a Mississippi GR waterhemp population in response to increasing the glyphosate rate from 1X to 2X (Nandula et al. 2013). With regard to lactofen, the greater survival of waterhemp to the 1X compared to the 3X rate may have occurred because the resistance mechanism was overwhelmed and, since lactofen is a cell membrane disruptor, plant tissue may have been damaged to the extent that effective control was achieved. Thinglum et al. (2011) also reported increased control of PPO-resistant waterhemp as the herbicide rate increased. Enhanced oxidative metabolism, as noted by Ma et al. (2013), is the only known mechanism of HPPD resistance in waterhemp to date, and may be responsible for the few HPPD-resistant populations observed in this study.

At least 52% of the waterhemp populations tested exhibited resistance to two herbicide modes of action (Table 4.2, Figure 2). Resistance to atrazine plus chlorimuron as well as glyphosate plus chlorimuron was present in 29% of the populations. Three-way resistance was present in 11% of the populations with resistance to atrazine plus chlorimuron plus glyphosate present in 10% of the populations. Resistance to four-modes of action was shown in 2% of the populations while only one population exhibited resistance to five modes of action. This is the first documented case of a waterhemp population being resistant to five modes of action.

Molecular examination of resistance mechanisms. DNA analysis indicated that all sampled plants resistant to lactofen in the greenhouse experiment contained the ΔG210 deletion in the *PPX2L* gene (Table 4.3). To date, this mutation remains the only known mechanism of PPO resistance in waterhemp. According to results from the greenhouse experiments, the total number of sensitive plants sampled was 135. However, the number of sensitive plants that did not have the ΔG210 deletion was only 98. This indicates that 37 out of 135 plants did have the ΔG210 deletion yet were killed by the 3X rate of lactofen in the greenhouse experiment. The use rates in this study were 0.18 (1X) and 0.53 (3X) kg lactofen ha^{-1}. Thinglum et al. (2011) reported increased control of PPO-resistant waterhemp as the lactofen rate increased. To determine the frequency of PPO-resistant waterhemp in populations from three states, Thinglum et al. (2011) used 0.11 kg of lactofen ha^{-1}. Sampling of plants surviving the 3X rate of lactofen for genetic analysis may have exposed an underestimate of the amount of PPO resistance present in these Missouri waterhemp populations.

Of the 92 plants sampled that exhibited resistance to chlorimuron, 20 plants contained the homozygous substitution conferring the Trp574Leu amino acid substitution, 55 plants were heterozygous for the substitution, and 17 plants were homozygous wild type (no substitution) (Table 4.3). The 17 plants lacking the amino acid substitution most likely have a non-target site mechanism of resistance. Guo et al. (2013) found that a non-target site, metabolism based mechanism was responsible for ALS resistance in a waterhemp population from Illinois.

Ninety-three glyphosate-resistant plants from the greenhouse experiment were sampled to determine potential mechanisms of resistance. An elevated EPSPS copy number (≥ 2-fold) was found in 82 plants (Table 4.3). Four plants had a homozygous mutation leading to a Pro106Ser amino acid substitution, 15 were heterozygous for the substitution, and 74 were wild type (no mutation). Of the plants with an elevated EPSPS copy number, only one was homozygous and 11 were heterozygous for the Pro106Ser amino acid substitution. Of the resistant plants, four had neither an elevated EPSP copy number nor the Pro106Ser substitution. These plants may have a novel non-target site mechanism of resistance or one similar to the GR waterhemp population from Mississippi documented by Nandula et al. (2013). Teaster and Hoagland (2014) also suggest that there are other, unknown, mechanisms of GR in *Amaranthus* species.

Results from these experiments indicate that Missouri soybean fields contain waterhemp populations with resistance to glyphosate, ALS-, PPO-, PSII-, and HPPD-inhibiting herbicides, which are some of the most common modes of action currently utilized for the control of this species in corn and soybean production systems (Figure 1). Additionally, these results indicate that slightly more than half of the populations tested exhibit resistance to more than one herbicide mode of action (Figure 2). ALS-inhibiting herbicides are not effective in controlling many waterhemp populations and will likely continue to be unsuccessful in the future. ALS resistance in waterhemp was first documented in the U.S. in 1993 (Heap 2014). Twenty years later ALS resistance is present in most Missouri waterhemp, and in most other areas in the U.S. where waterhemp occurs (Heap 2014). Glyphosate and atrazine are still effective on some waterhemp populations, but resistance to these herbicides now occurs across such a wide geographical region (Figure 1) that, based on previous experiences with ALS-inhibiting herbicide resistance in waterhemp, resistance in future waterhemp populations may be inevitable. Resistance to lactofen and mesotrione is present in only a small proportion of waterhemp populations in Missouri at this time, while no resistance to 2,4-D has been discovered. The results from the DNA analysis of the subsample of plants that survived applications of glyphosate, PPO-, and ALS-inhibitors revealed that many of the same mechanisms of resistance documented in previous research were present in Missouri waterhemp. However, there are indications that glyphosate resistance due to unknown mechanisms could be present as well. Looking to the future, it is not clear that reversal of herbicide resistance is possible. Managing the current resistance levels in existing populations is likely the most plausible action. The use of multiple, effective herbicide modes of action, both preemergence and postemergence, and the integration of optimum cultural control practices will be vital to managing Missouri waterhemp populations in the future. In order to prevent the spread of multiple herbicide resistances and preserve these herbicide technologies, additional effective mechanisms of actions should be included in the overall weed management program.

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Table 4.1. Sources of materials and rates used in the experiment.

Herbicide[a]	Trade name	Formulation	Rate (kg ai ha^{-1})	Manufacturer	Address	Website
Mesotrione	Callisto	4 SC	0.11 (1X) 0.31 (3X)	Syngenta	Greensboro, NC	www.syngenta.com
Glyphosate	Buccaneer	3 L	0.84 (1X) 2.53 (3X)	Tenkoz	Alpharetta, GA	www.tenkoz.com
2,4-D Amine	4 Amine	3.8 L	0.53 (1X) 1.59 (3X)	Tenkoz	Alpharetta, GA	www.tenkoz.com
Lactofen	Cobra	2 L	0.18 (1X) 0.53 (3X)	Valent U.S.A.	Walnut Creek, CA	www.valent.com
Chlorimuron	Classic	25 DG	0.012 (1X) 0.035 (3X)	Dupont	Wilmington, DE	www.dupont.com
Atrazine	AAtrex	4 L	1.12 (1X) 3.37 (3X)	Syngenta	Greensboro, NC	www.syngenta.com

[a] Appropriate adjuvants were added based on label recommendations for each respective herbicide.

Table 4.2. Comparison of herbicide resistance in Missouri waterhemp populations.

Factor	Resistance 1X Rate # Resistant populations	 % of populations	 3X Rate # Resistant populations	 % of populations
Herbicide[a]				
Chlorimuron	186	99.5	186	99.5
Atrazine	96	51	56	30
Glyphosate	108	58	55	29
Lactofen	20	11	10	5
Mesotrione	27	14	3	1.6
2,4-D Amine	1	0.5	0	0
2-way Resistances[b]				
2,4-D + Chlorimuron	1	0.5	0	0
2,4-D + Mesotrione	1	0.5	0	0
Atrazine + Chlorimuron	96	51	54	29
Atrazine + Lactofen	11	6	4	2
Atrazine + Glyphosate	53	28	19	10
Atrazine + Mesotrione	18	10	3	1.6
Chlorimuron + Glyphosate	107	57	55	29
Chlorimuron + Lactofen	19	10	9	5
Chlorimuron + Mesotrione	27	14	3	1.6
Glyphosate + Lactofen	15	8	3	1.6
Glyphosate + Mesotrione	17	9	3	1.6
Lactofen + Mesotrione	2	1	1	0.5
Total Populations with 2-way Resistance	157	84	98	52
3-way Resistances[b]				

2,4-D + Chlorimuron + Mesotrione	1	0.5	0	0
Atrazine + Chlorimuron + Glyphosate	53	28	18	10
Atrazine + Chlorimuron + Lactofen	11	6	3	1.6
Atrazine + Chlorimuron + Mesotrione	18	10	3	1.6
Atrazine + Glyphosate + Mesotrione	13	7	3	1.6
Atrazine + Glyphosate + Lactofen	7	4	2	2
Atrazine + Lactofen+ Mesotrione	1	0.5	1	0.5
Chlorimuron + Glyphosate + Lactofen	14	7	3	1.6
Chlorimuron+ Glyphosate+ Mesotrione	17	9	3	1.6
Chlorimuron + Lactofen + Mesotrione	2	1	1	0.5
Glyphosate + Lactofen + Mesotrione	2	1	1	0.5
Total Populations with 3-way Resistance	73	39	20	11
4-way Resistances[b]				
Atra + Chlor + Gly + Meso	13	7	3	1.6
Atra + Chlor + Gly + Lac	7	4	2	1
Atra + Gly + Lac + Meso	1	0.5	1	0.5
Atra + Chlor + Lac + Meso	1	0.5	1	0.5
Chlor + Gly + Lac + Meso	2	1	1	0.5
Total Populations with 4-way Resistance	20	11	4	2
5-way Resistances[b]				
Atra + Chlor + Gly + Lac + Meso	1	0.5	1	0.5
Total Populations with 5-way Resistance	1	0.5	1	0.5

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[a] Appropriate adjuvants were added based on label recommendations for each respective herbicide.

[b] Herbicide combination data is compiled from the single herbicide application data. Herbicides were not tank mixed.

Table 4.3. Analysis of known gene alterations in resistant and susceptible waterhemp plants following 3X rates of selected herbicide treatments.

Herbicide Treatment	Total # of Plants screened	# Resistant plants screened	# Resistant plants with mutation	# Resistant plants heterozygous for mutation	# Sensitive plants with mutation	# Sensitive plants without mutation
Lactofen	155[a]	20[a]	20[a]	n/a[b]	135[a]	98[a]
Chlorimuron	92[c]	92[c]	20[c]	55[c]	n/a[b]	n/a[b]
Glyphosate	93[d]	93[d]	4[d]	16[d]	n/a[b]	n/a[b]
Glyphosate	93[e]	93[e]	82[e]	n/a[b]	n/a[b]	n/a[b]

[a] Plants genotyped for a codon deletion causing a ΔG210 amino acid substitution in the PP2XL enzyme.

[b] Data was not collected for this treatment.

[c] Plants genotyped for the mutation causing a Trp574Leu amino acid substitution in the ALS enzyme.

[d] Plants genotyped for the mutation causing a Pro106Ser amino acid substitution in the EPSP Synthase enzyme.

[e] Plants analyzed for increased copies (≥ 2 fold) of the *EPSPS* gene.

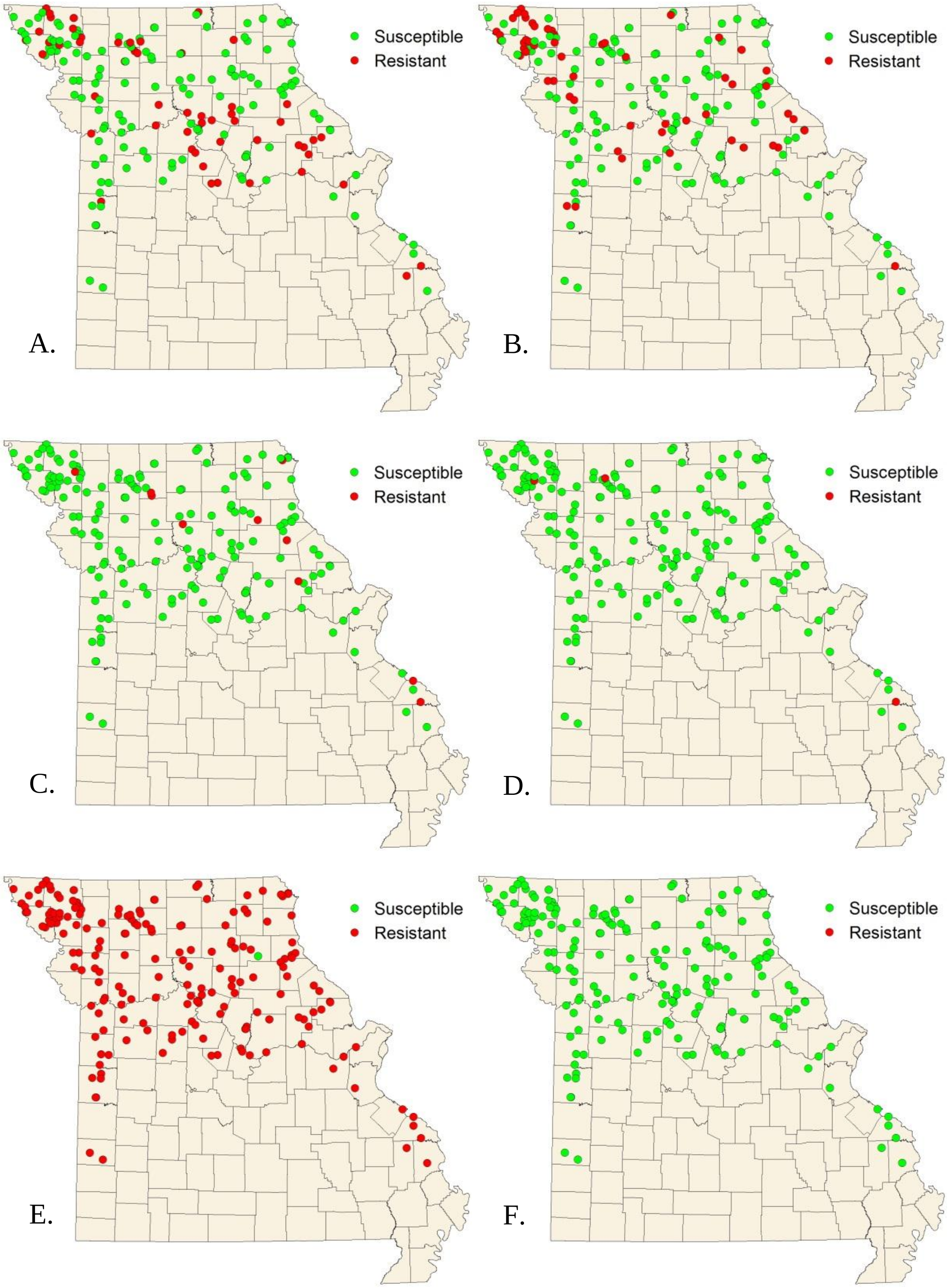

Figure 1. Location of waterhemp samples with resistance to the 3X rate of A) glyphosate, B) atrazine, C) lactofen, D) mesotrione, E) chlorimuron, and F) 2,4-D.

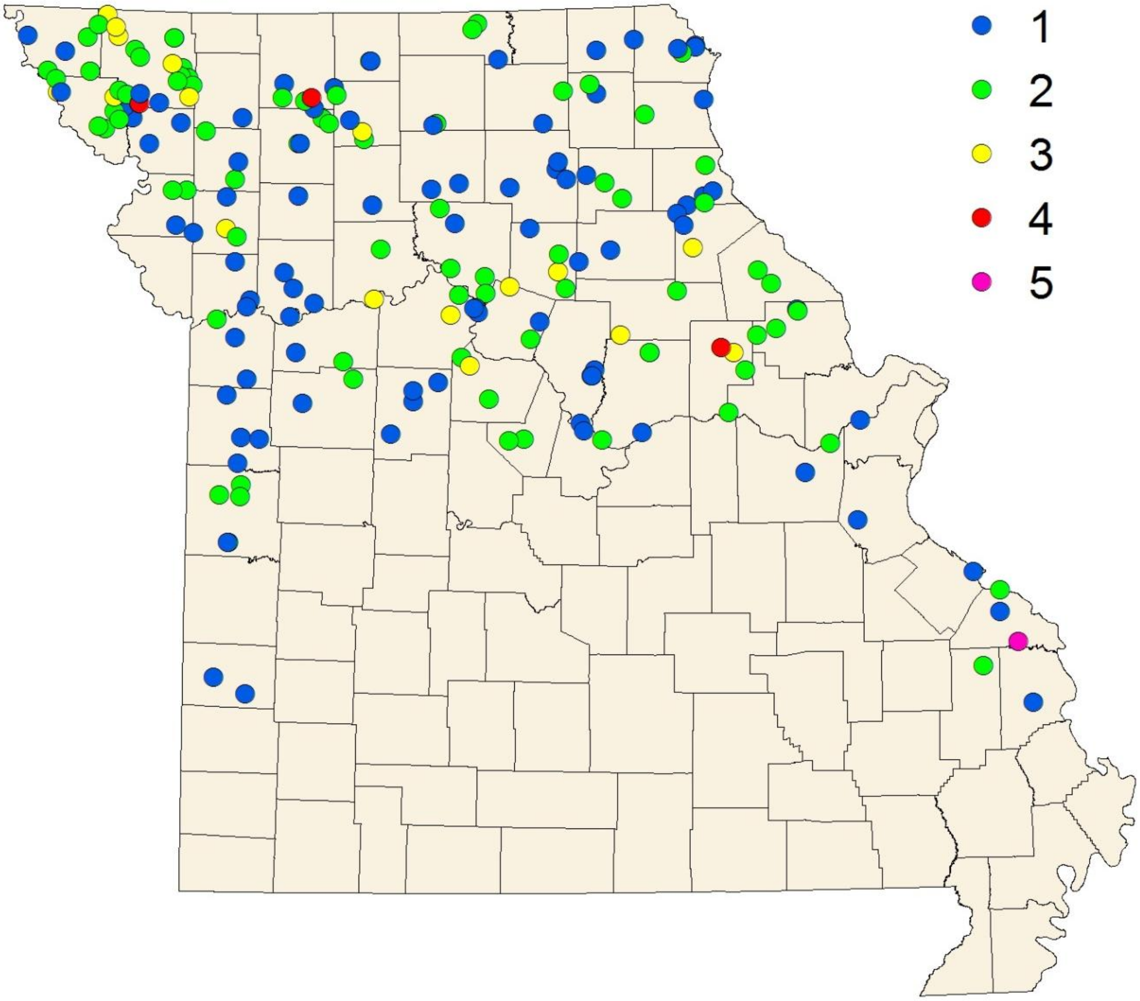

Figure 2. Distribution of waterhemp with multiple herbicide resistances collected in the 2012 survey.