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WATERHEMP (AMARANTHUS TUBERCULATUS) IN SOYBEAN IN KENTUCKY CONDITIONS

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WATERHEMP (*AMARANTHUS TUBERCULATUS*) IN SOYBEAN IN KENTUCKY CONDITIONS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Plant and Soil Science
at the University of Kentucky

By

Blake Paul Patton

Lexington, Kentucky

Director: Dr. W. W. Witt, Professor of Crop Science

2013

ABSTRACT OF THESIS

WATERHEMP (*AMARANTHUS TUBERCULATUS*) IN SOYBEAN IN KENTUCKY CONDITIONS

Waterhemp was a sporadic weed in Kentucky soybean production since the 1970's. Waterhemp's presence was not significant until the 1990's after a widespread adoption of imazaquin and imazethapyr herbicides in the late 1980's by Kentucky farmers which resulted in ALS-resistant waterhemp in some Kentucky areas. The introduction of glyphosate resistant soybeans in 1996 resulted in glyphosate-containing products being widely used by Kentucky farmers. Waterhemp populations resistant to glyphosate have occurred in Kentucky in the past few years. The majority of Kentucky soybeans are produced in some type of conservation tillage system, primarily to conserve soil and water, which is advantageous on Kentucky's rolling topography.
controls a wide range of weeds and popular with farmers because of this characteristic. However, waterhemp resistant to glyphosate developed in some fields with the continuous glyphosate usage. Waterhemp control research trials were conducted in Union and Hancock Counties in Western Kentucky in an attempt to find herbicide combinations to provide season-long control. Waterhemp populations in these studies were resistant and susceptible to glyphosate but the resistant populations were great enough to cause soybean yield loss if not controlled.

KEYWORDS: *Amaranthus tuberculatus,* Herbicide Resistance, EPSPS, PPO, ALS

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January 29, 2013
(Date)

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Chapter 1

Introduction

Over the past decade, many soybean growers in the midwestern United States has concerned with controlling waterhemp (*Amaranthus tuberculatus* Moq. Sauer). Research conducted in the midwestern United States attempted to find a solution for this troublesome weed. Until recently, Kentucky did not have widespread infestations of waterhemp. Waterhemp area of infestation and the population of waterhemp in infested fields have both increased in the western part of Kentucky. Previous research demonstrated that soil active herbicides provide the greatest control of waterhemp as well as other noxious weeds. Soil-applied herbicide treatment soon before or right after soybean planting can extend waterhemp control into the growing season (Hager et al. 2001). Hager et al. (2002) reported that for effective management of waterhemp throughout the growing season with glyphosate resistant soybeans, a multiple herbicide application strategy should be used. For this reason, research was conducted in this thesis was to manage waterhemp in already infested areas that will also preventing the spread of this weed. A variety of soil residual treatments were evaluated with and without a post foliar treatment of fomesafen or glyphosate for the control of late emerging waterhemp.

Chapter 2
Literature Review

Waterhemp

Waterhemp is a member of the Amaranthus family and include grain amaranths and the weedy species Palmer amaranth, smooth pigweed, redroot pigweed, spiny amaranth and common waterhemp. Identification of these species is difficult due to the phenotypic similarities as well as genetic variation within each species. Key features allow correct identification (Nordby 2007.) Immature pigweed plants are most difficult to separate due to similar cotyledon shape and size. Waterhemp has an egg shaped cotyledon with a slightly longer petiole than the other Amaranthus species. As waterhemp matures, the leaves average 2 to 12 centimeters in length while maintaining a slender 1 to 8 centimeter width giving a lanceolate appearance. Leaf size and shape is variable throughout the growth stages even in similar sites (Horak et al. 1994). Waterhemp has glabrous stems and leaves giving it a smooth surface and a shiny appearance. Stem and branch colors range from green to a deep purple. Plant height and width varies from 0.5 to 3 meters tall with erect to prostrate branching. Waterhemp is a dioecious plant with female and male flowers on separate plants. Flowering structures are mainly located at the ends of the branches, but often appear from the node to the tip of the branch (Horak et al. 1994). Waterhemp is a prolific seed producer with an average seed production in a field environment of about 290,000 seeds per female plant (Sellers et al. 2003).

J. L. Riddell was the first to describe the plant in 1835 in western Ohio (Pratt and Clark 2001.) Two specimens were found, a female plant and a young male plant. Riddell went on to name the female plant *Amaranthus miamiensis* and the male plant

Amaranthus altissimus. Since Riddell stated that these were just temporary names until further investigation, Moquin and Tandon were the first to give the plant a true name with *Amaranthus altissimus* which then changed to *Acnida tuberculata*. In 1972, Sauer found specimens that had a distinct dividing point on the seed capsule and a well-developed sepal differentiating it from another biotype that had no distinct breaking point and it was named *Amaranthus rudis*. *Amaranthus rudis and Amaranthus tuberculatus* were similar morphologically. Hybrid waterhemp plants were found in an area of sympatry in the states of Iowa, Illinois, and Missouri. Distinct *A. rudis* plants were found to the south and west of the area of sympatry, while *A. tuberculatus* to the north and east. The first hypothesis was developed by Uline and Bray in 1895 who stated "there appears to be but one polymorphous species," stating that *A. rudis* and *A. tuberculatus* should be the same species. Pratt and Clark 2001 studied the different biotypes from the south to the north, as well as west to east to determine if this hypothesis was valid using isozyme testing. Neither the morphological nor molecular data supported the decision to have two separate species. With that finding, Pratt and Clark proposed that a single, yet highly variable plant species of waterhemp would be recognized. This single species was to be named *Amaranthus tuberculatus* (Pratt and Clark 2001.)

Waterhemp in the midwestern United States has adapted to thrive in a crop rotation that includes soybean. Prolific seed production, emergence and maturity dates, and growth rates allowed waterhemp to be a competitive, persistent, and difficult to control. After the adoption of conservative tillage and no-tillage systems, waterhemp began to adapt to the cultural changes as well as to foliarly applied herbicides (Sellers et al. 2003.) Seed production can exceed 290,000 seeds per plant when growing in competition among other crop plants, making waterhemp the most prolific seed producer per gram of plant dry matter than any of the pigweed species (Sellers et al. 2003.) Waterhemp emergence varies upon weather conditions within the growing season with soil temperature and rainfall being the most important. Knowledge and understanding of when these waterhemp emerge in a soybean field may be critical to management strategies for control (Hartzler et al. 1999.) After pollination, newly forming seeds may mature in 9 days and maximum maturity occurs about 26 days after pollination (Bell and Tranel 2010). Reducing the amount of seed production through the means of herbicide control is of great importance (Bell and Tranel 2010.) Buhler and Hartzler (2001) reported waterhemp seeds in a controlled environment remained viable after 17 years. Field experiments documented an 11% viability rating after four years (Buhler and Hartzler 2001). Waterhemp, once established, is difficult to control regardless of the level of weed control in the following years due to the seed bank persistence in soil. Understanding the growth characteristics and optimum growth periods will allow for an advanced system of management tactics through planting dates, pre-emergence and post emergence herbicide treatments for a weed-free growing season (Buhler and Hartzler 2001).

Waterhemp's capability to out-produce other pigweeds in seed production can be related to the seed size of waterhemp being the smallest of all of the *Amaranthus* species. Although the size of waterhemp is smaller than Palmer amaranth, redroot pigweed, and smooth pigweed, it can produce 28% or more seeds per gram of plant dry matter at 3,670 seeds/g plant dry matter compared to smooth pigweed at 2,780 seeds/g plant dry matter (Sellers et al. 2003). Palmer amaranth was reported to be the tallest species accumulating more plant dry matter than any of the pigweed family. However, Palmer amaranth produced 49% (2,060 seeds/g plant dry matter) of the amount of seeds produced by waterhemp (Seller 2003). Competition with other plants may decrease the size of waterhemp, but due to waterhemp's survival mechanisms, compensation with an increase in seed production occurs suggesting waterhemp may exerts more energy towards increasing seed population in the soil seed bank than overall vegetative growth (Seller 2003).

Kentucky no-till practices allow the abundance of waterhemp seed to lie close to the soil surface. Small-seeded broadleaf seeds are subjected to conditions that allow for ease of germination while also having the presence of predation and degradation more so than in a tillage system with the burial of seed (Murdock and Ellis 1992). Tillage may allow aeration, scarification of waterhemp seed, and exposure to sunlight. Therefore tillage systems may allow for a higher rate of germination of these small seeded broadleaf weeds (Lueschen et al. 1993). However, Egley and Williams (1991) reported that tillage applications do not affect the germination of waterhemp. According to Steckel et al. (2007), no-till may aid in the depletion of waterhemp in the soil seed bank. Enhanced germination in no-till situations along with continuous control of waterhemp throughout the soybean season allows for waterhemp's short-lived seed persistence to deplete the seed bank (Steckel et al. 2007).

Environmental conditions were the driving factor to produce flushes of waterhemp emergence according to Buhler and Hartzler (1999) in which the number of waterhemp seedlings emerged were related with the amount of precipitation in 1994 to 1996. Emergence started in late May all years while the majority of the plants emerged after a rainfall in late June to mid-July (Hartzler et al 1999). Buhler and Hartzler (2001) reported 5% seedling emergence in the first year after burial while declining to 2% after four years in a waterhemp soil seed bank of 2,000 seeds planted in the upper 5 cm of soil. Annual emergence of waterhemp never exceeded 7%, while cumulative percentages reached up to 15% with the majority emerging in the 4 year study. Although a low percentage of the waterhemp seeds emerged, recovery of the waterhemp seed from the soil each year had a 70% or greater viability in the first three years. This percentage increased to 95% after the fourth year. The high percentage of viability and prolonged emergence of waterhemp allows for survival and establishment of resistant populations of waterhemp. A No-till system with the lack of soil-residual that relies on post treatments of the same modes of action catalyzes herbicide resistance (Buhler and Hartzler 2001).

Annual weeds may develop resistance to herbicides more quickly than plants with longer life cycles. Reasons for this include the rapid growth and turnover of the next generation of weeds (Foes et al. 1998). This allows for more plants to be exposed to the herbicide being applied. The genetic diversity of these annual weeds also provides an edge towards gaining resistance (Foes et al. 1998). Adaptation to produce herbicide resistance shifts occurs more rapidly for waterhemp because of its annual life cycle, dioecious nature through continuous out-crossing, and prolific seed production (Jasieniuk et al. 1996). Pollination among waterhemp plants allow for new gene transfer of resistant alleles which increases resistance within a population and expresses the importance of genetic variation (Jasieniuk et al. 1996). Waterhemp will also cross with *Amaranthus palmeri,* and *Amaranthus hybridus* (Frenssen et al. 2001; Trucco et al. 2005; Wetzel et al. 1999).

Weed Resistance

A shift toward weed resistant biotypes depends on production practices, herbicide use, and genetic diversity and growth habits of waterhemp. However, the introduction and adoption of a new herbicide for effective weed management often results in a population shift toward resistance (Culpepper 2006). Since the introduction of glyphosate resistant soybeans in 1996, a 2012 survey revealed 93% of the United States soybeans were reported to be herbicide resistant (USDA 2012). Many reasons push the adoption of glyphosate resistant soybeans. Glyphosate herbicide is an economical, effective post-emergence herbicide with little to no foliar damage to soybeans (Reddy and Norsworthy 2010). Post-emergence control also allows for ease of weed control without or with little tillage (Vengessel 2001). With the decrease in tillage and reliance on glyphosate, an increase in troublesome weeds occurred. Waterhemp is now a model weed for resistance pest management due to its weedy characterization and rapid invasion of U.S soybeans (Chao et al. 2005). Additionally, since the mid 1990's waterhemp populations resistant to other modes of action were reported. These include acetolactate synthase (ALS) (Horak and Peterson 1995; Hinz and Owen 1997; Sprague et al.1997), photosystem II (PSII) (Anderson et al. 1996), protoporphyrinogen oxidase (PPO) (Shoup et al. 2003), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Owen and Zelaya 2005), 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Hausman et al 2011), and most recently, 2,4-D (Synthetic Auxin) (Bernards 2012). Multiple herbicide resistance was first reported in Illinois with ALS and PS II inhibitors in 1996 (Foes et al. 1998). Biotypes of waterhemp that have multiple herbicide resistances have been reported as well. Pratzoldt and Hager (2005) reported a waterhemp biotype resistant to PSII, PPO, and ALS resistance in Adam's county Illinois while multiple resistance of ALS, EPSPS, and PPO was confirmed in Missouri in 2006 and 2007 (Legleiter and Bradley 2008).

Research has been conducted on limited tillage since the late 1800's (Shear 1985). New York State reported few benefits to cultivation besides weed control in that yields were relatively the same in each tillage system applied. Other states including Illinois, Missouri, and South Carolina had similar results (Shear 1985). No-tillage can be defined as the insertion of seed into the soil without the practice of tillage by opening a narrow slot of sufficient width and depth for optimum soil/seed contact (Phillips 1981). Limited tillage decreases the number of methods one has to control weed species. Before the development of herbicides, cultivation and mulch were the two main mechanisms for weed removal (Shear 1985). This limited the advancement and adoption of limited tillage. Developing plant growth regulators during World War II gave new hope to no-tillage (Phillips 1981). One of the first successful no-till operations was reported by Barrons and Fizgerald in 1952 (Shear 1985.) Soybeans were planted in sod after chemical application of 3.08 kg/ha (0.5 lb/A) of DNBP or dinoseb (2-*sec*-butyl-4,6-dinitropenol). Weed control was acceptable while yields amounted to 1334 kg/ha (20 bu/A) (Shear 1985). Cultivation and pre-plant incorporated herbicides are two methods often observed in the tillage operations which cannot be used in no-tillage systems. This directly increases the amount of herbicides used as burndowns, soil residuals, and selective post treatments (Lewis 1985).

The introduction of chlorimuron and imazethapyr in the 1980's provided selective, residual, and a broad spectrum control of weed species and a wide window of application for no-till late season weed control (Tranel and Wright 2002).

From 1988 to 1996, the percent of no-tillage acreage reported in Kentucky increased by 30% to a total of 49% of the total full season soybean acreage. No-till in double crop soybeans consisted of 94% of the total double crop acreage (Thomas et al. 1996). Due to the popularity, extensive use, and propensity to select resistant weed populations, by the year 2002, ALS type herbicides accumulated at least 22 monocot and 48 dicot reported to have resistance to this class of herbicides (Tranel and Wright 2002). By 1993, Illinois reported between two to five million acres infested with ALS resistant waterhemp (Weedscience.org 2011). By 1997, Illinois no longer recommended ALS materials for the control of waterhemp (Tranel and Wright 2002). According to weedscience.org survey of resistant weeds in 2011, 116 weed species were documented as ALS resistant.

Although ALS herbicides helped increase soybean production through late season weed control in of no-till farming, the introduction of glyphosate resistant soybeans in 1996 revolutionized weed management and control tactics in no-tillage situations. Soybean growers quickly reaped the benefits glyphosate resistant soybeans had to offer: Improved late season weed control, ease of management, less tillage, decreased input costs and labor, and most of all, the economical return. Glyphosate resistant soybeans dramatically altered production in the United States (Young 2006).

For over two decades, glyphosate resistance was not considered to be a problem. During that time period, it was speculated that the evolution of glyphosate resistance was unlikely (Powles 2008). However, rigid ryegrass (*Lolium rigidum* Gaudin) and Italian ryegrass (*Lolium multiflorum* Lam) were recorded resistant in 1996 after 14 years of continuous use in orchards (Perez-Jones et al. 2005; Simarmata et al. 2005). Glyphosate resistant horseweed (*Conyza Canadensis* (L.)) and common ragweed (*Ambrosia artemisiifolia* (L.)) were identified after three and six years of continuous glyphosate resistant soybeans (Pollard et al. 2004; VanGessel 2001). By 2007 eight species with glyphosate resistance were known. Waterhemp from Missouri, Iowa, and Illinois were collected in 2005 and rate responses of glyphosate treatments within the waterhemp populations were confirmed (Smith and Hallett 2006). They hypothesized that the evolution of resistance had begun in waterhemp and if the trends of overuse of glyphosate were not controlled, resistance would spread and intensify with the selection pressure that glyphosate imposed (Smith and Hallett 2006). Legleiter and Bradley (2008) included waterhemp to the list of resistance to glyphosate in Missouri in 2008. The biotype located in Missouri was also resistant to PPO and ALS inhibitors. The field was put in continuous soybeans for six years with one or more treatments of glyphosate each year (Legleiter and Bradley 2008).

Waterhemp Management

Legleiter et al. 2009 documented less than 23% control of waterhemp with glyphosate alone treatments in 2006-2007. Herbicide treatments consisting of pre-emergence (PRE) and post-emergence (POST) treatments significantly decreased waterhemp population compared to POST only treatment of either acifluorfen or lactofen. A common treatment for the control of waterhemp in non-glyphosate resistant waterhemp populations was sulfentrazone followed by a POST of glyphosate which provided 99% control 56 days after treatment (DAT) (Krausz and Young 2003). Control of glyphosate resistant waterhemp was obtained with a residual PRE herbicide followed by a POST mixture of glyphosate plus a PPO treatment of fomesafen, acifluorfen, or lactofen (Mueller et al. 2005).

Pre-emergence herbicides are considered one of the most important and effective management strategies for waterhemp control. Many have been evaluated for percent waterhemp control or population reduction from soybean planting to late in the soybean growing season. Hager et al. (2002) reported 80% or less control with dimethenamid, linuron, metolachlor, metribuzin, or pendimethalin 4 WAT in 1996 and 1997. S-metolachlor and metribuzin retained a 70 and 60% control rating, respectively, 6 WAT. In other research, the combination of S-metolachlor plus metribuzin or fomesafen applied at soybean planting provided 99% control 6 WAT (Moody et al. 2005). Duff et al. (2008) recorded 95% or greater control of waterhemp 8 WAT of a pre-emergence treatment of S-metolachlor plus fomesafen while S-metolachlor plus metribuzin achieved 91% control. At another site, waterhemp control using S-metolachlor plus fomesafen or metribuzin provided 60 and 59% control 8 WAT (Duff et al. 2008). Krausz and Young

(2003) observed a 97% control of waterhemp 56 DAT with a PRE treatment of sulfentrazone. They also noted that the addition of either chlorimuron or cloransulam delayed the POST treatment by an average of 12 days providing more time for an optimum application to 10 cm waterhemp. Schuster and Smeda (2007) reported 77-100 % season long control with a PRE or early POST followed by a mid-POST treatment. They noted a 15% increase in control when the PRE was sulfentrazone. Niekamp and Johnson (2001) reported a PRE treatment of flumioxazin controlled 91-96% of waterhemp. They also note that flumioxazin alone can provide a 78% control rating in high density situations.

Pre-emergence herbicide treatments are influenced by soil, climate, and spray conditions. Due to this variation, POST treatments may be necessary for optimum waterhemp control (Hager et al. 2002). Resistance to multiple herbicide sites of action makes the selection of the treatments limited. Knowing waterhemp resistance issues in areas of application allows for the right decision to be made. Within non-ALS resistant waterhemp populations, chlorimuron can provide 90% control 3 weeks after application (Mayo et al. 1995). Within ALS resistant populations, ALS herbicides such as imazethapyr showed no control at 28 DAT in a Kansas study (Hoss et al. 2003). Hager et al. 2003 showed slight control with imazethapyr with a 42% control 21 DAT. Hager reported 66, 69, and 81 percent control of waterhemp with acifluorfen, fomesafen, and lactofen, respectively (Hager et al. 2003). Waterhemp control was about 9% greater at early post-emergence compared to late post-applications.

Chapter 3
Materials and Methods

Union County 2010

Site Description

A field study was conducted in Union County during the summer of 2010 in a field adjacent to the Ohio River 1 km north of Uniontown Kentucky.

Soil types within the field include Huntington silt loam and a Robinsonville silt loam. These soils are predominantly found in stream terraces and within flood plains. Plot location had flooding from the Ohio River in 2008 and 2009 and also in previous years. This explains why soybeans had been planted for the past 3 years with a strict glyphosate weed management plan.

Experimental Design

This preliminary study consisted of a randomized complete block design with eight treatments and 4 replications. Waterhemp population previously not controlled by glyphosate was variable in different areas of the field. Plots were established in areas where consistent waterhemp populations were observed. Replications one and two were grouped together while replications 3 and 4 were non-adjacent. Layout of replications one and two consisted of a rectangular block measuring 12 meters by 40 meters with stacked plots (4 plots per tier) ascending left to right, front to back in order of 101-104, 105-108, 201-204, 205-208 one tier each. Whereas, replications 3 and 4 measured 3 meters by 10 meters in total area with 8 plots per tier.

Equipment and Treatments

Soybeans were planted by the farmer in late May with a direct seed planter on 76 cm row spacing. All herbicide treatments were applied using a hand-held CO_2 plot sprayer with water as the carrier. TeeJet 8003 DG flat fan nozzle tips and 50 cm nozzle spacing were used to deliver 187 l/ha. Treatments were applied July 8, 2010 (Table 3.1) with specific treatment information provided in Table 3.2. Soybean stage of growth on spray date was reproductive (R1) stage one and averaged 45 cm in height (Table 3.3). Waterhemp size averaged 50-60 cm but ranged from 30-120 cm in height. Weed stages ranged from vegetative to reproductive flowering (Table 3.4). The farmer had previously applied Roundup PowerMax at 1.6 liters per hectare (22 oz. per acre) two times. One treatment was applied at planting and again at V3 crop stage.

Data Collection and Analysis

All waterhemp plant within the four rows of every plot was counted on the day of treatment on July 8. Waterhemp plants were counted again 18 DAT on July 26 in between the four rows of each plot. Waterhemp plants were only counted if they survived the treatment. Survival was based on newly emerged growth from the meristems and/or majority green leaf area (evidence of survival when new growth was evident and easily recognized). Plants with no newly emerged growth and/or total chlorotic/necrotic leaf tissue were pronounced dead, therefore not counted. On July 26 newly emerged plants that came up after the July 8 spray date were also counted with the plants which had survived treatment.

Plant counts were analyzed using SAS PROC GLM. Statistical differences among treatments were assessed at Fisher's 0.05 LSD. Transformations of data were conducted but not documented in this research.

Waterhemp seeds were collected before soybean harvest on October 8th 2010. At least five plants were stripped of their seed producing structures in each plot and combined according to treatment number. Waterhemp seeds were allowed to air dry until February 8th 2011.

Table 3.1 - Herbicide treatments used for experimentation in 2010 in Union County

Trt No.	Active Ingredient	Product Name	Form Conc	Form Unit	Form Type	Rate	Rate Unit
1	Glyphosate	Roundup Powermax	4.5	LBAE/GAL	SL	870	g ae/ha
	Amonium Sulfate	Activator 90	100	%	SL	3.7	% v/v
2	Glyphosate	Roundup Powermax	4.5	LBAE/GAL	SL	1740	g ae/ha
	Amonium Sulfate	Activator 90	100	%	SL	3.7	% v/v
3	Chlorimuron	Classic	25	%	DF	13.1	g ai/ha
	Crop Oil Concentrate	COC	100	%	SL	0.5	% v/v
4	Imazethapyr	Pursuit	2	LBA/GAL	EC	70	g ai/ha
	Crop Oil Concentrate	COC	100	%	SL	0.5	% v/v
5	Acifluorfen	Ultra Blazer	2	LBA/GAL	EC	420	g ai/ha
	Non-Ionic Surfactant	Activator 90	100	%	SL	0.25	% v/v
6	Fomesafen	Flexstar	1.88	LBA/GAL	EC	395	g ai/ha
	Non-Ionic Surfactant	Activator 90	100	%	SL	0.25	% v/v
7	Untreated Check	Untreated Check					

Table 3.2 – Application Information

Application Date:	July-8-2010
Time of Day:	Afternoon
Application Method:	SPRAY
Application Timing:	POSPOS
Application Placement:	BROFOL
Applied By:	BlakePatton
Air Temperature, Unit:	94 f
% Relative Humidity:	46
Wind Velocity, Unit:	0 mph
Wind Direction:	NA
Dew Presence (Y/N):	N no
% Cloud Cover:	15

Table 3.3 – Crop Stage at Treatment Application Timing

Crop Stage:	
Stage Majority, Percent:	R1 80
Stage Minimum, Percent:	V6-7 20
Stage Maximum, Percent:	R1 80
Height, Unit:	45 cm

Table 3.4 – Weed Stage at Treatment Application Timing

Waterhemp Stage:	
Stage Majority, Percent:	Vegetative 90
Stage Maximum, Percent:	Flowering 10
Height, Unit:	30-120 cm

Greenhouse

Preparation

After waterhemp seed heads dried, they were thrashed by hand. A seed cleaning blower was used to separate unwanted trash from the seed. The separation process was duplicated to ensure a clean seed collection. After the seeds were separated, they were placed in a paper envelop and stored at 4 C.

Waterhemp seeds were removed from storage on February 8 2011and seeds were treated with 20% sulfuric acid 80% deionized water for two minutes. After two minutes in the solution, the seeds were then submerged in a beaker of distilled water for two minutes. Seeds were then dipped in a 50/50 bleach water solution for two minutes and rinsed with distilled water. Seeds were allowed to dry, placed in a plastic Petri dish and stored in a cooler at 4 C.

Waterhemp seeds were planted in 10 x 10 cm pots into a 50/50 sand soil (Maury silt loam) mixture on March 5, 2011. The soil was watered before sprinkling the seeds on top of the soil. A thin layer of soil was place on the seeds and then misted with water to ensure adequate moisture for germination.

Continuous thinning ensured 2 to 3 plants per pot at similar heights and growth stages.

Treatments

Treatments in the greenhouse study included no herbicide, glyphosate (870, 3480, 6960 g/Ha), chlorimuron (13, 26, 52 g/Ha), and fomesafen (384, 1580, 3160g/Ha). Each treatment was applied at a spray volume of 190 l/Ha with H_2O as a carrier in a research spray booth. Liquid ammonium sulfate (AMS) was added to glyphosate at 2.5% total volume. Crop oil concentrate (COC) was added to chlorimuron at 0.25% of the total spray volume. Nonionic surfactant (NIS) was added to fomesafen at 0.25% of the total spray volume. Each treatment was applied to 10-16 cm waterhemp (Table 3.6) and replicated 16 times. Glyphosate greenhouse treatments were applied to waterhemp that had survived glyphosate applications at Union County 2010. Chlorimuron greenhouse treatments were applied to waterhemp that survived either chlorimuron or imazethapyr treatments in Union County. Fomesafen greenhouse treatments were applied to waterhemp that survived either fomesafen or acifluorfen in Union County.

Data Collection and Analysis

Evaluation of plants living or dead were taken at 21 and 34 days after treatment with ratings of 1 (plant dead), 2 (plant visually injured but still remained alive), and 3 (plant was visually healthy and remained unaffected by the treatment).

Surviving plants were transplanted into a 3.78 liter pot to ensure normal growth. A structure was built for plant pollination to decrease cross pollination between plants surviving chlorimuron and glyphosate treatments. After pollination and plant maturity, seeds were collected and placed in a paper bag marked chlorimuron, or glyphosate to recognize which treatment they survived for future research.

Table 3.5 – Herbicide Treatments for Greenhouse Resistance Screening

Trt No.	Active Ingredient	Product Name	Form Conc	Form Unit	Form Type	Rate	Rate Unit
1	Glyphosate Ammonium Sulfate	Roundup Powermax Ammonium Sulfate	4.5 100	LBAE/GAL %	SL SL	870 3.7	g ae/ha % v/v
2	Glyphosate Ammonium Sulfate	Roundup Powermax Ammonium Sulfate	4.5 100	LBAE/GAL %	SL SL	3480 3.7	g ae/ha % v/v
3	Glyphosate Ammonium Sulfate	Roundup Powermax Ammonium Sulfate	4.5 100	LBAE/GAL %	SL SL	6960 3.7	g ae/ha % v/v
4	Chlorimuron Crop Oil Concentrate	Classic COC	25 100	% %	DF SL	13.1 0.5	g ai/ha % v/v
5	Chlorimuron Crop Oil Concentrate	Classic COC	25 100	% %	DF SL	52.4 0.5	g ai/ha % v/v
6	Chlorimuron Crop Oil Concentrate	Classic COC	25 100	% %	DF SL	104.8 0.5	g ai/ha % v/v
7	Fomesafen Non-Ionic Surfactant	Flexstar Activator 90	1.88 100	LBA/GAL %	EC SL	395 0.25	g ai/ha % v/v
8	Fomesafen Non-Ionic Surfactant	Flexstar Activator 90	1.88 100	LBA/GAL %	EC SL	1580 0.25	g ai/ha % v/v
9	Fomesafen Non-Ionic Surfactant	Flexstar Activator 90	1.88 100	LBA/GAL %	EC SL	3160 0.25	g ai/ha % v/v
10	CHECK	Untreated					

Table 3.6 – Waterhemp stage at time of treatment application

Waterhemp Stage and Size:	
Stage Majority, Percent:	6 Leaf 80
Stage Minimum, Percent:	5 Leaf 10
Stage Maximum, Percent:	7 Leaf 10
Diameter, Unit:	4-6 cm
Height, Unit:	13 cm
Height Minimum, Maximum:	10 14

Hancock County 2011: Study One

Site Description

Field Study 1 was conducted during the summer of 2011 approximately 2 miles northwest of Hawesville, Kentucky in Hancock County. Study one lies in the river bottoms where excessive flooding has occurred numerous occasions in the past. Plot location for this study had flooding in 2009 and 2010 and in years past due to high water levels in the Ohio River.

According to Web Soil Survey, the soil type in this study includes an Elk silt loam. Elk silt loam is predominantly found in stream terraces. Soil tests show a pH of 6.0, cation exchange capacity of 14.95 meq/100g and an organic matter content of 2.6%. Phosphorus and potassium levels were 180 MehP(lb/ac), 327 MehK(lb/ac).

Experimental Design

A randomized complete block design with 13 treatments and 3 replications were used for this study. Individual plot size was 3 meters wide and 10 meters in length. Plot location was in the area of the field with a history of waterhemp which has not been previously controlled by glyphosate. Soybeans were planted on June 1st with a direct seeding planter on 38 cm row spacing at roughly 329,000 plants per ha. Soybeans were a Northup King variety with a 3.9 maturity rating. Prior to planting, a harrow was used to knock down any weeds that were present. Paraquat (840 g/ha) was applied over the entire plot area three days after planting but before soybean emergence to ensure a weed-free plot prior to the study.

Equipment and Treatments

All treatments were applied using a hand held CO_2 plot sprayer with water as the carrier. TeeJet 8003 DG flat fan nozzle tips and 50 cm nozzle spacing were used to deliver 233.8 l/ha. Treatments consisted of applications pre-emergence only, pre-emergence plus post emergence applications, or a V3 followed by a V5 post application of glyphosate or chlorimuron for testing glyphosate and ALS resistance within the plot. Pre-emergence only treatments were applied to examine the duration of residual control. Pre-emergence treatments plus post-emergence treatments of glyphosate and fomesafen were applied to determine if a weed-free growing season was possible to obtain.

Soybeans in each treatment were harvested October 25th 2011. Three meters of the middle 4 rows were hand harvested and allowed to air dry. Soybean seeds were removed with a portable thrasher and collected.

Data Collection and Analysis

All treatments were rated as percent biomass reduction of waterhemp compared to the untreated check. Visual ratings were collected on June 24 (mid POST spray date, 21 days after PRE treatment), July 14th (late POST spray date, 41 days after PRE treatment), July 28th (55 days after PRE treatment), and August 9th (66 days after PRE treatment). Data from visual ratings were analyzed using PROC GLM of SAS to determine statistical differences between treatments. The untreated check was not analyzed with the herbicide treated plots. Treatments were also segregated into two groups based on application method. These two groups were pre-treatments only and pre-emergence plus post-emergence treatments. The purpose of the grouping was to determine if any significant differences occurred by applying a post application of glyphosate plus fomesafen. PROC

CORR was used to identify any relationships between the two groups. Differences within treatments were determined using Fisher's α value of 0.05 as the LSD within all treatment comparisons.

After harvesting, the soybeans samples were cleaned and weighed to determine kg/ha for each treatment. Total sample area of 4.65 m^2 was converted to hectares to obtain kg/ha for each sample harvested.

$$\frac{Sample\ (oz) *\ 0.028349523}{Plot\ area\ 4.65\ (m^2) * 0.0001} = Sample\ (\frac{kg}{Ha})$$

Soybean yields were analyzed by PROC GLM of SAS to identify any statistical differences between treatments. Differences within treatments were verified using Fisher's protected LSD at the 0.05 α level.

Table 3.7 - Herbicide treatments used for experimentation in 2011 in Study 1

Trt No.	Active Ingredient	Treatment Name	Rate	Rate Unit	Other Rate	Other Rate Unit	Appl Code
1	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	B
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	B
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	C
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	C
2	Chlorimuon	Classic	0.75	oz wt/a	13.1	g ai/ha	B
	Crop Oil Concentrate		0.5	% v/v	1.17	l/ha	B
	Chlorimuon	Classic	0.75	oz wt/a	13.1	g ai/ha	C
	Crop Oil Concentrate		0.5	% v/v	1.17	l/ha	C
3	Fomesafen, Metolachlor	Prefix	36	fl oz/a	1370	g ai/ha	A
4	Fomesafen, Metolachlor	Prefix	24	fl oz/a	910	g ai/ha	A
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	B
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	B
	Fomesafen	Flexstar	13.276	fl oz/a	219	g ai/ha	B
	Non-ionic Surfactant	Activator 90	0.25	% v/v	0.584	l/ha	B
5	Metribuzin, Metolachlor	Boundary	48	fl oz/a	2730	g ai/ha	A
6	Metribuzin, Metolachlor	Boundary	48	fl oz/a	2730	g ai/ha	A
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	B
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	B
	Fomesafen	Flexstar	24	fl oz/a	396	g ai/ha	B
	Non-ionic Surfactant	Activator 90	0.25	% v/v	0.584	l/ha	B
7	Sulfentrazone	Spartan	10.1	fl oz/a	354	g ai/ha	A
8	Sulfentrazone	Spartan	10.1	fl oz/a	354	g ai/ha	A
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	B
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	B
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	B
	Non-ionic Surfactant	Activator 90	0.25	% v/v	0.584	l/ha	B
9	Saflufenacil	Sharpen	1	fl oz/a	25	g ai/ha	A
10	Saflufenacil	Sharpen	1	fl oz/a	25	g ai/ha	A
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	B
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	B
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	B
	Non-ionic Surfactant	Activator 90	0.25	% v/v	0.584	l/ha	B
11	Sulfentrazone, Metribuzin	Authority MTZ	18	oz wt/a	570	g ai/ha	A
12	Sulfentrazone, Metribuzin	Authority MTZ	18	oz wt/a	570	g ai/ha	A
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	B
	Ammonium Sulfate		3.7	% v/v	8.65	l/ha	B
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	B
	Non-ionic Surfactant	Activator 90	0.25	% v/v	0.584	l/ha	B
13	CHK						

Table 3.8 - Hancock Co. Spray date and weather information for Study 1

	A	B	C
Application Date:	Jun-3-2011	Jun-24-2011	Jul-14-2011
Time of Day:	3:00 PM	12:00 PM	12:00 PM
Application Method:	SPRAY	SPRAY	SPRAY
Application Timing:	PREMCR	POSPOS	POSPOS
Application Placement:	BROSOI	BROFOL	BROFOL
Applied By:	BlakePatton	BlakePatton	BlakePatton
Air Temperature, Unit:	92 f	85 F	84 F
% Relative Humidity:	32	58	64
Wind Velocity, Unit:	1.5 MPH	7 MPH	8 MPH
Wind Direction:	SE	NE	ENE
Dew Presence (Y/N):	N no	N no	N no
% Cloud Cover:	0	60	30

Table 3.9 - Crop Stage at spray timings

	A	B	C
Crop 1 Code, BBCH Scale:	GLXMA BSOY	GLXMA BSOY	GLXMA BSOY
Stage Scale Used:	BBCH	BBCH	BBCH
Stage Majority, Percent:	Pre-emergence	V3 70	V5 65
Stage Minimum, Percent:		V2 30	V4 25
Stage Maximum, Percent:		V3 70	R1 10
Height, Unit:		20 cm	75 cm
Height Minimum, Maximum:		15 25	60 95

Table 3.10 - Waterhemp description at spray timings

	A	B	C
Pest 1 Code, Type, Scale:	AMATU W	AMATU W	AMATU W
Stage Majority, Percent:	4 LS 65	4 LS 65	8 LS 65
Stage Minimum, Percent:	2 LS 20	2 LS 20	4 LS 25
Stage Maximum, Percent:	6 LS 15	6 LS 15	10 LS 10
Diameter, Unit:	5 cm	7 cm	15 cm
Height, Unit:	5 cm	12 cm	25 cm
Height Minimum, Maximum:	5 15	5 15	12 50
Density, Unit:	110 M2	50 M2	10 M2

Hancock County 2011: Study Two

Site Description

Another field Study was conducted during the summer of 2011 approximately 2 miles northwest of Hawesville Kentucky in Hancock County. This site lies in the river bottoms where excessive flooding frequently occurred in the past. Plot location for this study had flooding in 2009 and 2010 and in years past due to high water levels in the Ohio River.

According to Web Soil Survey, the soil type in this study includes an Elk silt loam. Elk silt loam is predominantly found in stream terraces. Soil tests show a pH of 6.0, cation exchange capacity of 14.95 meq/100g and an organic matter content of 2.6%. Phosphorus and potassium levels were 180 MehP(lb/ac), 327 MehK(lb/ac).

Experimental Design

Field study two consisted of a randomized complete block design with 16 treatments and 3 replications. Individual plot size was 3 meters wide and 10 meters in length. Plot location was in a field with a history of waterhemp not previously controlled by glyphosate. Soybeans were planted on June 1st with a direct seeding planter on 38 cm row spacing at roughly 329,000 plants per ha. Soybeans were a Northup King variety with a 3.9 maturity rating. Prior to planting, a harrow was used to knock down weeds that were present. Three days after planting, before soybean emergence, a treatment of paraquat (840 g/ha) was applied over the entire plot area to ensure a weed-free environment prior to the study.

Equipment and Treatments

All treatments were applied using a hand held CO_2 plot sprayer with water as the carrier. TeeJet 8003 DG flat fan nozzle tips and 50 cm nozzle spacing were used to deliver 233.8 l/ha.

Treatments were examined to determine if the pre-emergence residual herbicide applications plus glyphosate or glyphosate plus fomesafen increased weed control throughout the growing season (3.11.) The addition of acetochlor was applied to determine if antagonism or synergism would occur toward herbicide efficacy. Glyphosate, glyphosate plus fomesafen, and glyphosate plus fomesafen plus acetochlor were applied at mid POST (V-3), late POST (V-5), or both to determine the appropriate timing. Prowl was added to treatment 6 (Table 3.11) to determine if any extended or enhanced control of grasses and small seeded broadleaves could be obtained.

Soybeans in each treatment were harvested October 25th 2011. Three meters of the middle 4 of 8 rows were hand harvested and allowed to air dry until all treatments were harvested. Soybean seeds were collected by a portable thrasher.

Data Collection and Analysis

All treatments were visually rated as percent biomass reduction of waterhemp compared to the untreated check. Phytotoxicity was also assessed to determine if soybean plant tissue damage was evident or significant after post-application treatments of acetochlor. Visual ratings occurred on June 24 (mid POST spray date, 21 days after PRE treatment), July 14th (late POST spray date, 41 days after PRE treatment), July 28th (55 days after PRE treatment), and August 9th (66 days after PRE treatment). Data from ratings were analyzed by PROC GLM of SAS to determine statistical differences among treatments. Differences within treatments were verified using Fisher's protected LSD at α 0.05 level.

After soybean harvest, samples were cleaned and weighed to determine kg/ha for each treatment. Total sample area of 4.65 m^2 was converted to hectares to obtain kg/Ha for each sample harvested.

$$\frac{Sample\ (oz) * \ 0.028349523}{Plot\ area\ 4.65\ (\mathrm{m}^2) * 0.0001} = Sample\ (\frac{kg}{Ha})$$

Soybean yields were analyzed by PROC GLM of SAS to identify statistical differences between treatments. Differences within treatments were verified using Fisher's α value of 0.05 as the LSD.

Table 3.11- Herbicide treatments used for experimentation in 2011 in Study 2

Trt No.	Treatment Active Ingredient	Treatment Trade Name	Rate	Rate Unit	Other Rate	Other Rate Unit	App Timing
1	Flumioxazin, Chlorimuron	Valor XLT	4	oz wt/a	113	g ai/ha	PRE
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
2	Flumioxazin, Chlorimuron	Valor XLT	4	oz wt/a	113	g ai/ha	PRE
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
3	Flumioxazin, Chlorimuron	Valor XLT	4	oz wt/a	113	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V3
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
4	Flumioxazin, Chlorimuron	Valor XLT	4	oz wt/a	113	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V5
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V5
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V5
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V5
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V5
5	Flumioxazin, Chlorimuron	Valor XLT	4	oz wt/a	113	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V3
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
	HERB	Warrant	48	fl oz/a	1260	g ai/ha	V5
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V5
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V5
6	Flumioxazin, Chlorimuron	Valor XLT	4	oz wt/a	113	g ai/ha	PRE
	HERB	Prowl H20	32	fl oz/a	1060	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
7	Sulfentrazone, Chlorimuron	Authority XL	6.5	oz wt/a	320	g ai/ha	PRE
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	20	fl oz/a	330	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
8	Sulfentrazone, Chlorimuron	Authority XL	6.5	oz wt/a	320	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V3
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
9	Sulfentrazone, Chlorimuron	Authority XL	6.5	oz wt/a	320	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V5

	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V5
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V5
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V5
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V5
10	Sulfentrazone, Chloransulam	Authority First	6.5	oz wt/a	320	g ai/ha	PRE
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
11	Sulfentrazone, Chloransulam	Authority First	6.5	oz wt/a	320	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V3
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
12	Sulfentrazone, Chloransulam	Authority First	6.5	oz wt/a	320	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V5
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V5
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V5
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V5
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V5
13	Flumioxazin, Pyroxasulfone	Fierce	4.5	oz wt/a	240	g ai/ha	PRE
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
14	Flumioxazin, Pyroxasulfone	Fierce	4.5	oz wt/a	240	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V3
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V3
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V3
	Fomesafen	Flexstar	24	fl oz/a	1.75	l/ha	V3
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V3
15	Flumioxazin, Pyroxasulfone	Fierce	4.5	oz wt/a	240	g ai/ha	PRE
	Acetochlor	Warrant	48	fl oz/a	1260	g ai/ha	V5
	Glyphosate	Roundup Powermax	22	fl oz/a	870	g ai/ha	V5
	Ammonium Sulfate	Ammonium Sulfate	3.7	% v/v	8.65	l/ha	V5
	Fomesafen	Flexstar	24	fl oz/a	395	g ai/ha	V5
	Non-ionic Surfactant	NIS	0.25	% v/v	0.584	l/ha	V5
16	CHK	-	-	-	-	-	-

Table 3.12 - Hancock Co. Spray date and weather information for Study 2

	A	B	C
Application Date:	Jun-3-2011	Jun-24-2011	Jul-14-2011
Time of Day:	3:00 PM	12:00 PM	12:00 PM
Application Method:	SPRAY	SPRAY	SPRAY
Application Timing:	Pre-emergence	Post crop/Post Weed	Post crop/Post Weed
Application Placement:	Broadcast soil	Broadcast Foliar	Broadcast Foliar
Applied By:	Blake Patton	Blake Patton	Blake Patton
Air Temperature, Unit:	92 f	85 F	84 F
% Relative Humidity:	32	58	64
Wind Velocity, Unit:	1.5 MPH	7 MPH	8 MPH
Wind Direction:	SE	NE	ENE
Dew Presence (Y/N):	N no	N no	N no
% Cloud Cover:	0	60	30

Table 3.13 - Crop Stage at spray timings

	A	**B**	**C**
Crop 1 Code, BBCH Scale:	GLXMA BSOY	GLXMA BSOY	GLXMA BSOY
Stage Scale Used:	BBCH	BBCH	BBCH
Stage Majority, Percent:	Pre-emergence	V3 70	V5 65
Stage Minimum, Percent:		V2 30	V4 25
Stage Maximum, Percent:		V3 70	R1 10
Height, Unit:		20 cm	75 cm
Height Minimum, Maximum:		15 25	60 95

Table 3.14 - Waterhemp description at spray timings

	A	B	C
Pest 1 Code, Type, Scale:	AMATU W	AMATU W	AMATU W
Stage Majority, Percent:	4 LS 65	4 LS 65	8 LS 65
Stage Minimum, Percent:	2 LS 20	2 LS 20	4 LS 25
Stage Maximum, Percent:	6 LS 15	6 LS 15	10 LS 10
Diameter, Unit:	5 cm	7 cm	15 cm
Height, Unit:	5 cm	12 cm	25 cm
Height Minimum, Maximum:	5 15	5 15	12 50
Density, Unit:	110 M2	50 M2	10 M2

Chapter 4
Results and Discussion

Union County 2010

Herbicide treatments gave a generally low control percentage across all treatments. This was attributed to the size of waterhemp sprayed as well as resistant biotypes not being affected by the treatment. Noticeable differences were obtained by each mode of action. Percentages of controlled waterhemp within the population in each treatment were calculated by:

$$1-\left[\frac{July\ 26th\ population}{July\ 8th\ population}\right]x\ 100$$

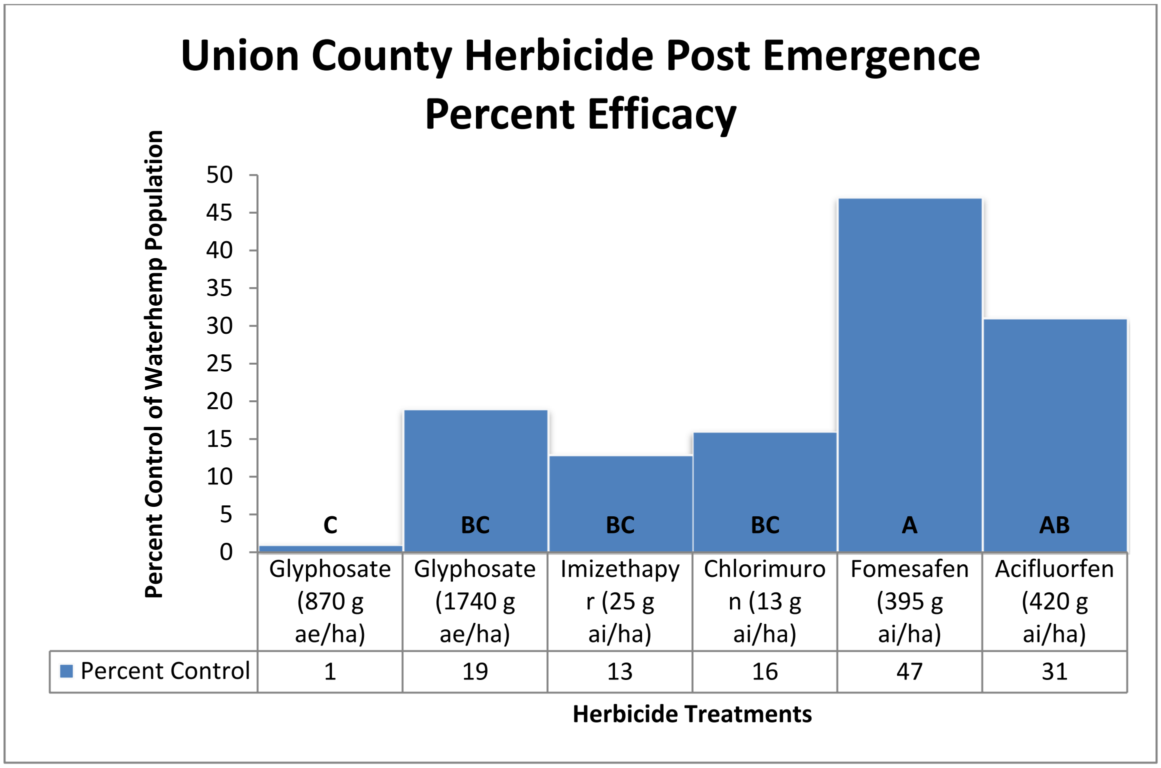
Glyphosate at the 870 g ae/ha (1x rate) produced less than 5 percent control of waterhemp; whereas glyphosate at 1740 g/ha (2x rate) provided19% control (Figure 4.1.) Resistant plants were observed in plots of both treatments. These plants were identified by the meristematic top growth decay, followed by new branching directly underneath the necrotic tissue. Resistant plants were more prevalent in the 1x treatment plots showing a rate response with the 2x rate. In addition to the resistant plants, new plant emergence occurred after the herbicide treatment due to the lack of residual characteristics of glyphosate. This decreased the overall percent control for the second counting date on July 26 for both treatments.

Acetolactate synthesis (ALS) inhibitors including chlorimuron and imazethapyr obtained a 13 and a 16% control average. Some surviving plants were suppressed while others remained unaffected by the treatment. Unaffected plants were assumed to be ALS resistant. Fomesafen and acifluorfen, both Protoporphyrinogen Oxidase (PPO) inhibitors provided the highest average control. Acifluorfen provided 31% average control while fomesafen controlled 47% of the total population of waterhemp counted on July 8 2010

(Figure 4.1). Plants within the population not controlled by the two PPO inhibitors showed the greatest amount of necrotic plant tissue and overall damage to the waterhemp plants. Some necrotic speckling was observed on the soybeans, but plants soon recovered within a couple weeks (data not shown.)

Plant population reduction percentages were run through SAS using proc glm. Significant differences were present when using Fisher's α value of 0.05 as the LSD.

Figure 4.1 – Percent control of waterhemp from July 8 population count



Greenhouse

Chemical modes of actions (MOA's) were very different among the treatments which allowed a wide span of plant responses. Fomesafen caused chlorotic and necrotic speckling about two hours after treatment. Chlorotic and necrotic leaves were rolled up and at a downward angle after 24 hours, but the waterhemp were not completely dead. Glyphosate and chlorimuron treated plants showed some injury symptoms one day after treatment, especially at the higher concentrations among the susceptible plants. Limp petioles and water-soaked leaves were symptoms associated with these treatments.

Final visual ratings were collected 34 days after treatment. These observations showed which plants survived treatment and proceeded to live a full lifecycle. Fomesafen treated waterhemp yielded only one survived plant with applications of at a 1x rate of 395 g ai/ha (data not shown.) Comparing the glyphosate 1, 4, and 8x rate treatments (870, 1740, 3480 g/Ha), 18, 7, and 2 waterhemp plants survived (Figure 4.4.) Of the chlorimuron 1, 4, and 8x rates (13.1, 26.2, 52.4 g/Ha), 19, 8, and 8 waterhemp plants survived (Figure 4.7.)

Both glyphosate and chlorimuron treatments showed a rate response. Higher rates of each herbicide provided a larger number of dead plants. Glyphosate showed a gradual decrease in the total survived pants with a 47% survival at the 1x rate, 21% survival at the 4x rate, and a 5% survival rate at the 8x rate (Figure 4.3.) Combining all glyphosate treatments, a 25% survival rating was recorded (Figure 4.2.) Total chlorimuron treated plants survived with the 1, 4, and 8x rates equaled 41% (Figure 4.5.) Rate response with the treatments exhibited a more dramatic response. 4 and 8x rates provided 73 and 71% control, while a 1x treatment only provided 32% control (figure 4.5.)

Resistance was statistically tested using a Chi squared method. Chi squared null hypothesis states that 50% or more of the population tested will be susceptible to the 1x, 4x, and 8x rates of either glyphosate or chlorimuron. When testing glyphosate resistance, we rejected the null hypothesis at a 50/50 ratio with one degree of freedom at the 1 and 4x rates. The waterhemp population proved to be not susceptible to a glyphosate rate of 1740 g ae/ha and lower. We accepted the Chi squared null hypothesis with one degree of freedom at the 8x rate stating that the waterhemp population is still susceptible to glyphosate at a high rate of 3480 g ae/ha. Chlorimuron showed the same results by rejecting the null hypothesis at the 1 and 4x rate, and accepting the null hypothesis at the 8x rate of chlorimuron.

Figure 4.2 - Total Percent Resistant and Percent Susceptible waterhemp plants with 1, 4, and 8x rates of glyphosate

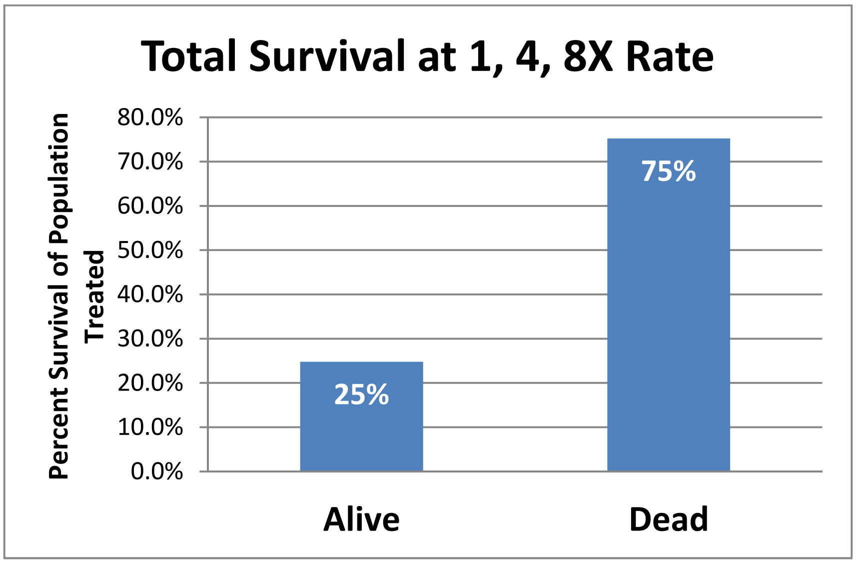


Figure 4.3 - Percent waterhemp survival with a 1, 4, and 8x rate of glyphosate

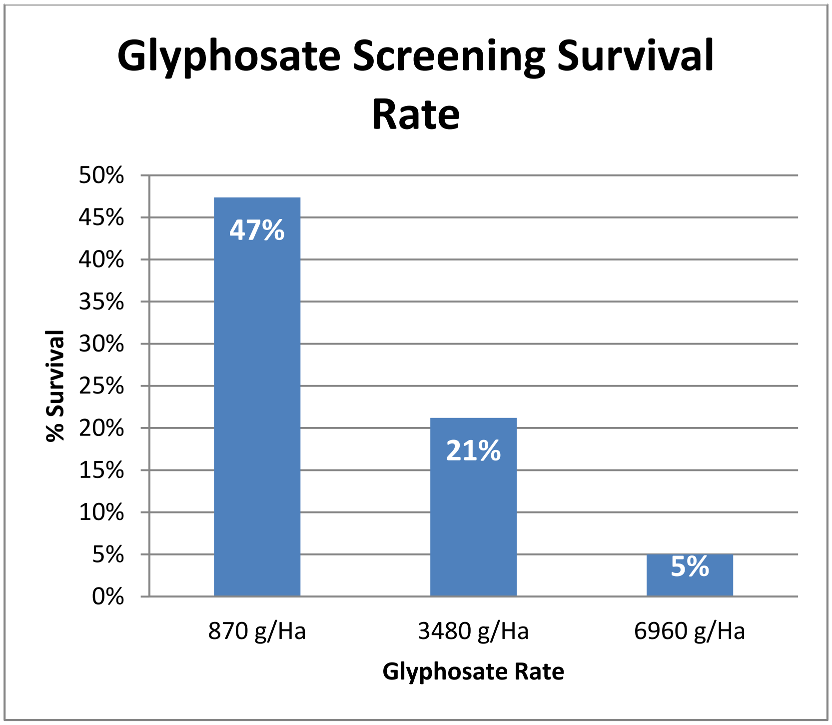
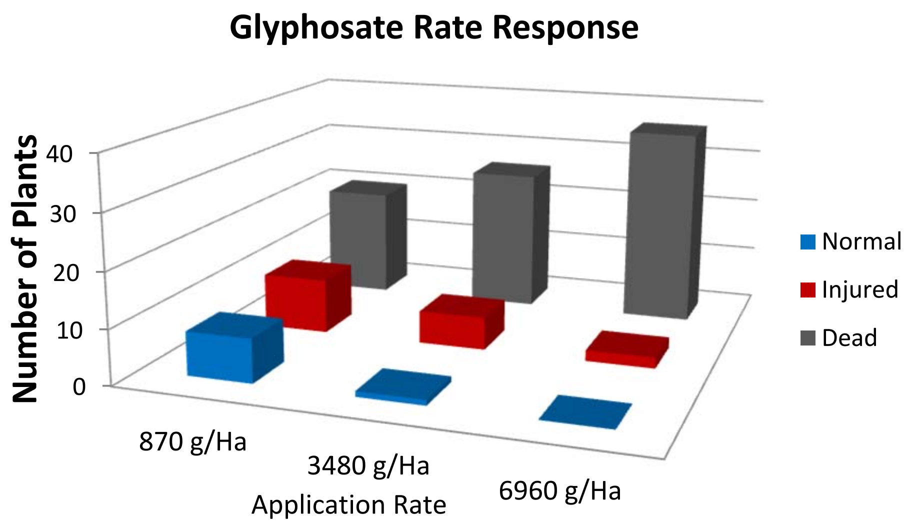


Figure 4.4 - Waterhemp rate response towards glyphosate



	870 g/Ha	3480 g/Ha	6960 g/Ha
🟦 Normal	8	1	0
🟥 Injured	10	6	2
■ Dead	20	26	36

Figure 4.5 - Total Percent Resistant and Percent Susceptible waterhemp plants towards 1, 4, and 8x Rates of chlorimuron

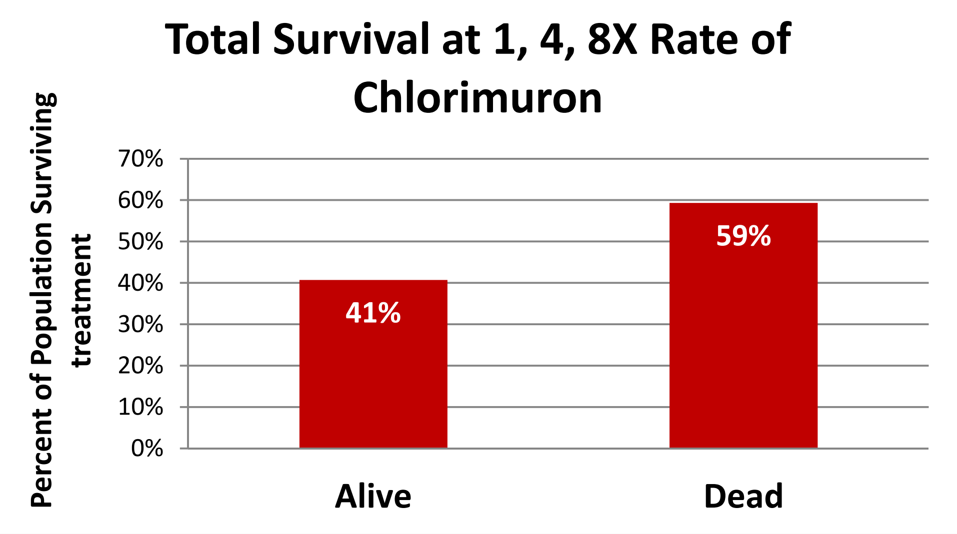


Figure 4.6 - Percent of waterhemp surviving a 1, 4, and 8x rate treatment of chlorimuron

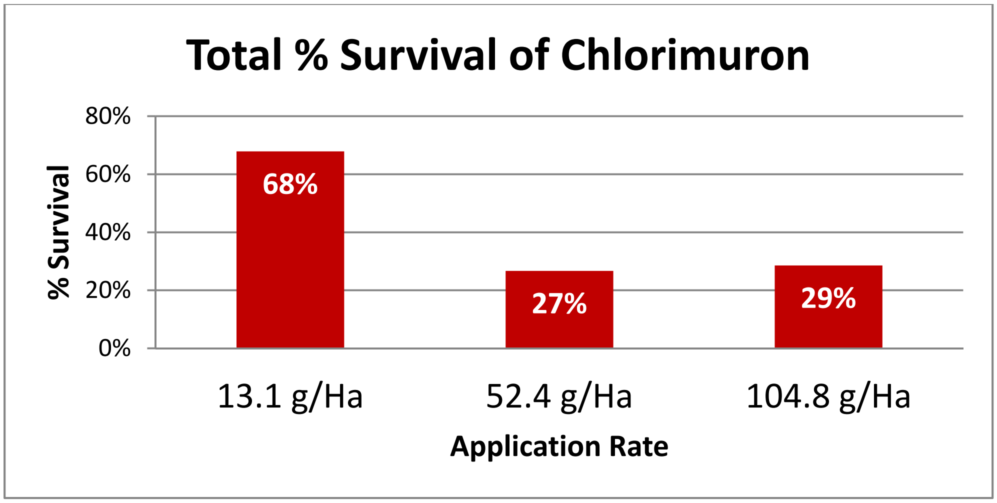
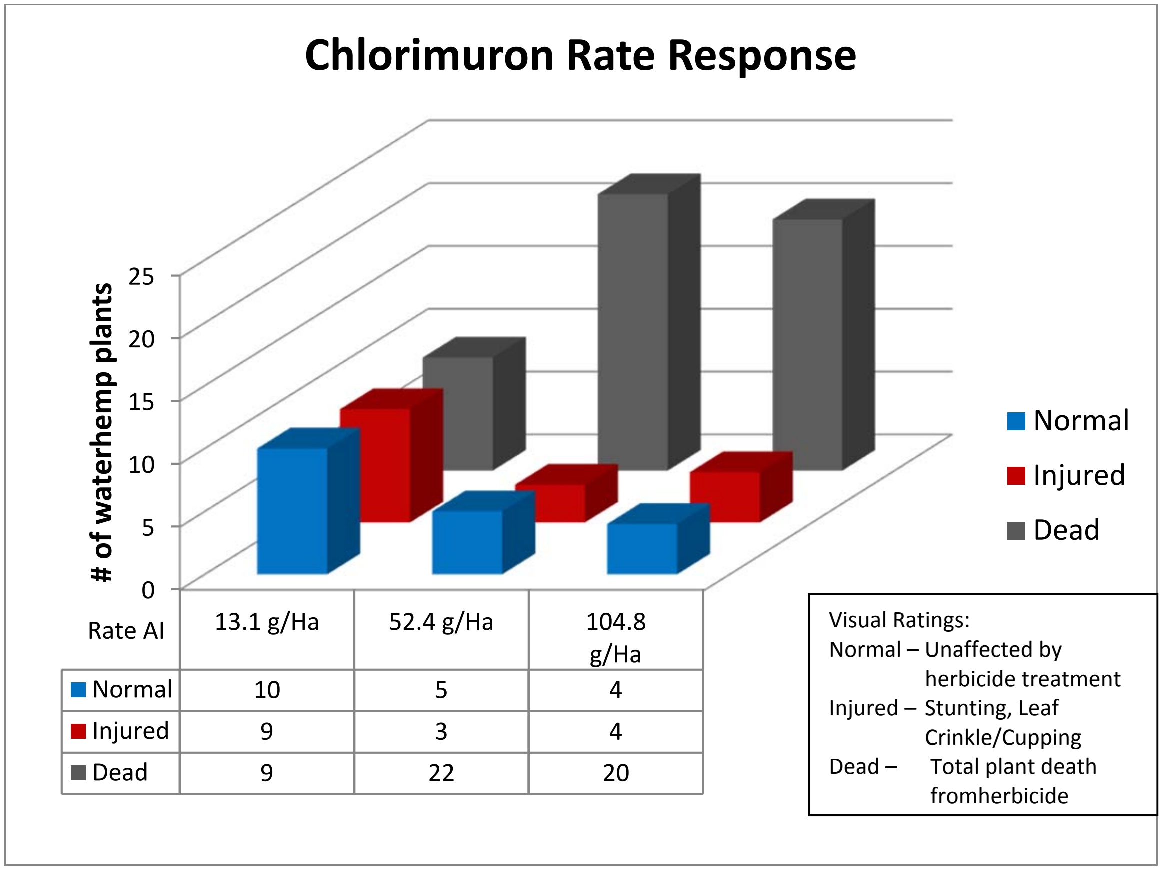


Figure 4.7 - Waterhemp rate responses to chlorimuron applications



Rate AI	13.1 g/Ha	52.4 g/Ha	104.8 g/Ha
🟦 Normal	10	5	4
🟥 Injured	9	3	4
⬛ Dead	9	22	20

Visual Ratings:
Normal – Unaffected by herbicide treatment
Injured – Stunting, Leaf Crinkle/Cupping
Dead – Total plant death fromherbicide

Hancock County 2011: Study One

Evaluation of crop injury was assessed 41 days after treatment on July 14, 2011 (Table 4.3). This visual evaluation was based on the total soybean biomass reduction from herbicide injury compared to the untreated check. Stunting was evident within some treatments, as well as necrosis of the older leaf tissue on some soybean plants. Herbicide injury may have been instigated by a substantial rainfall of 40 centimeters starting one week after treatment in the month of June. Herbicide may have splashed on newly emerged soybean tissue causing added plant stress.

Statistical analysis of crop injury was evaluated using SAS proc glm which indicated no significant injury at the 0.05 α level among herbicide treatments. However treatments of Metribuzin, sulfentrazone, and a combination of sulfentrazone and metribuzin were all significantly different to the untreated check with an average reduction of biomass of 12, 12, and 10% respectively within the plot area (Table 4.3.)

Final visual control evaluations were taken on August 9 of 2011. Across all treatments, there were no significant differences with a p value of 0.0687. However, when contrasting pre-emergence treatments only to PRE plus POST treatments, a significant difference was revealed with a p value of 0.0035 and an F value of 11.29 (Table 4.1.) PRE plus POST treatments had a significantly higher control average with an average increase of 23% control compared to pre-treatments alone (Figure 4.10.) Average control percentage of PRE plus POST treatments were 97% indicating good waterhemp control throughout the soybean growing season (Figure 4.10.)

Resistance of glyphosate and ALS materials was evident with an average control percentage of 86 % and 55% two glyphosate or two chlorimuron broadcast applications, respectively (Figure 4.8.) Noticeable damage and recovery from glyphosate was observed after treatment. Observations included meristematic burndown with auxiliary bud stem extension underneath the glyphosate damage. Chlorimuron treated waterhemp showed little injury. Injury that was noticeable included leaf puckering and stunted growth compared to the untreated check.

Soybean yield data were analyzed through SAS proc glm to determine if there were any treatment differences relative to control of waterhemp. With a P-value of 0.4168 shows no significant differences observed among all treatments (Table 4.3.) Proc Corr analysis indicated no treatment differences when comparing control to yield.

Crop response data was also analyzed through Proc Corr to determine if early crop damage was correlated to soybean yield. No correlation was found within any treatment.

Figure 4.8 - Glyphosate and chlorimuron treatment applications determining herbicide resistance

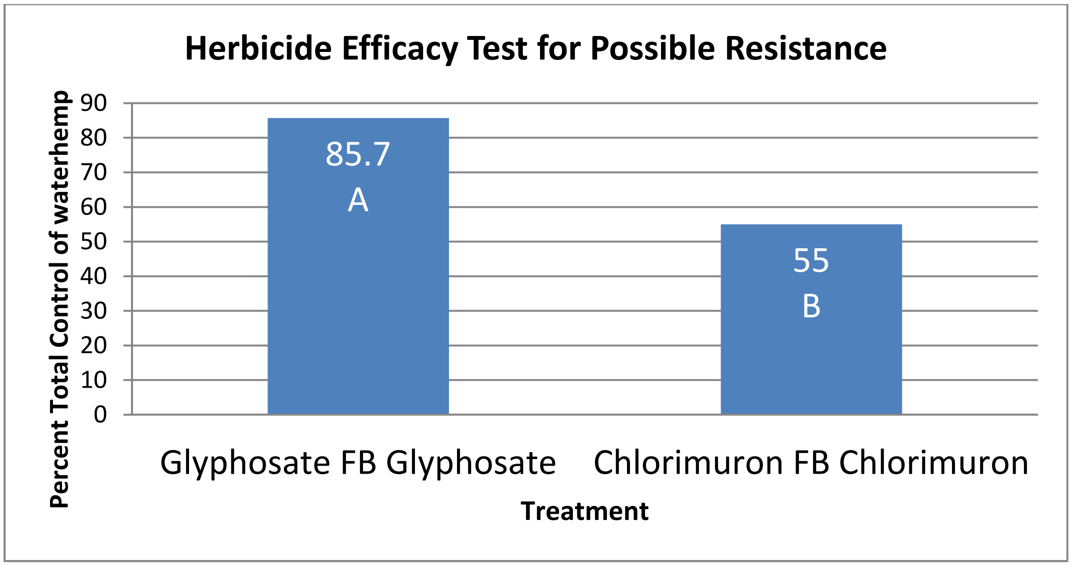


Figure 4.9 - Pre-emergence soil residual treatment vs. Pre-emergence soil residual plus Post-emergence fomesafen + glyphosate

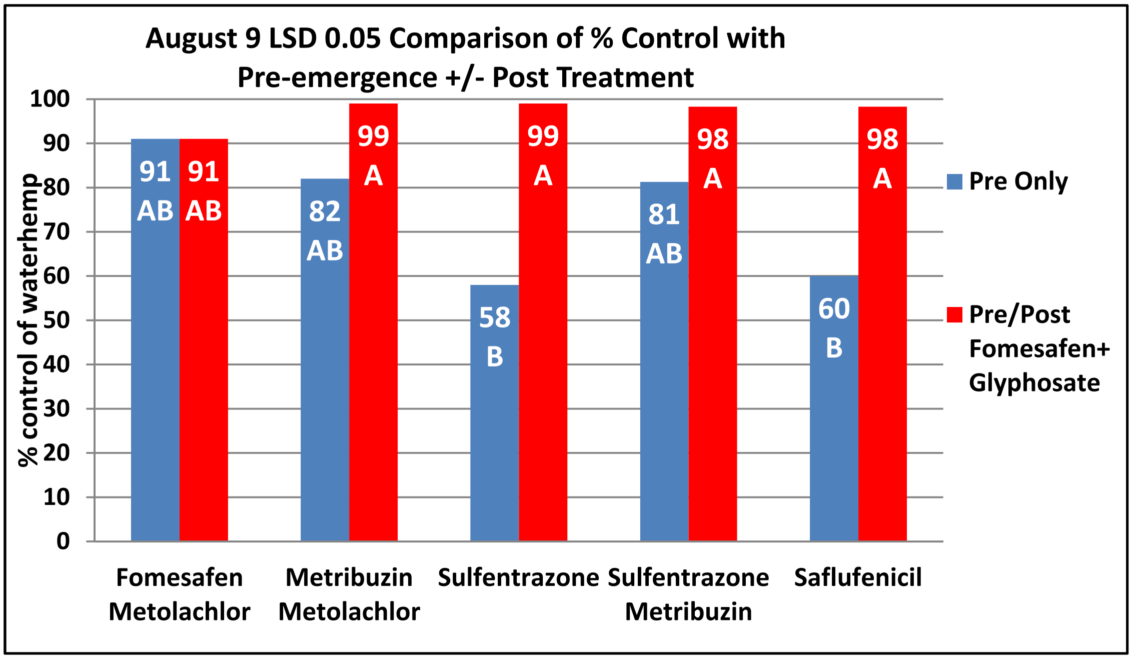


Figure 4.10 - Contrast between Pre-emergence treatment only and Pre-emergence plus Post-emergence treatment of fomesafen plus glyphosate

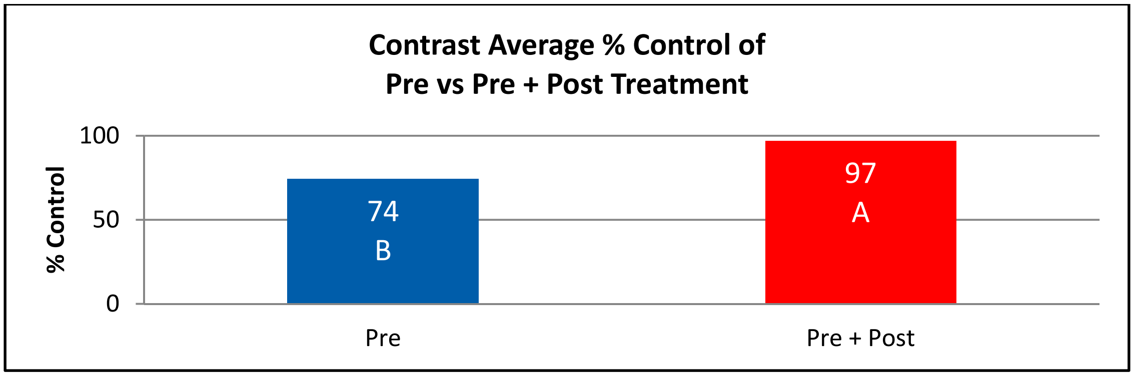


Table 4.1 - Contrast Values of Pre-emergence treatment only and Pre-emergence plus Post-emergence treatment of fomesafen plus glyphosate on August 9, 2011

Contrast	DF	F Value	Pr > F
Pre Vs. Pre/Post	1	11.29	0.0035

Figure 4.11 – Soybean yield Comparison between Pre-emergence treatment only and Pre-emergence plus Post-emergence treatment of fomesafen plus glyphosate. No significant differences were recorded.

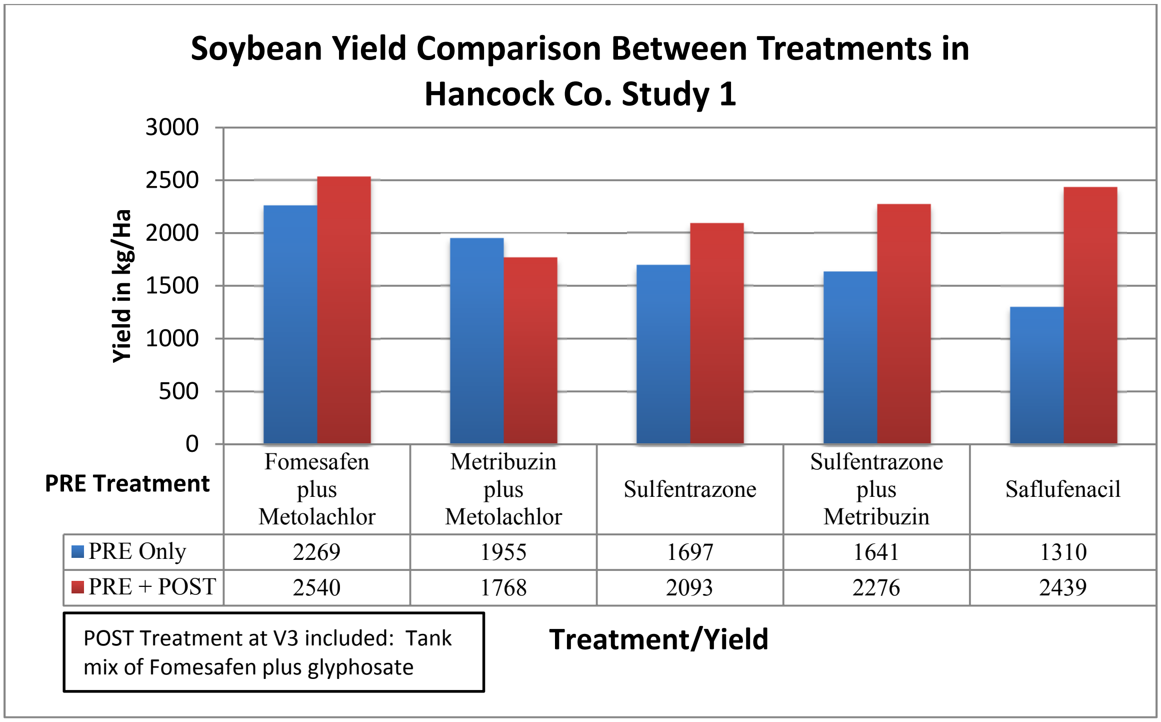


Figure 4.12 - Contrast Values of soybean yields of Pre-emergence treatments only and Pre-emergence plus Post-emergence treatments of fomesafen plus glyphosate. No significant differences were recorded.

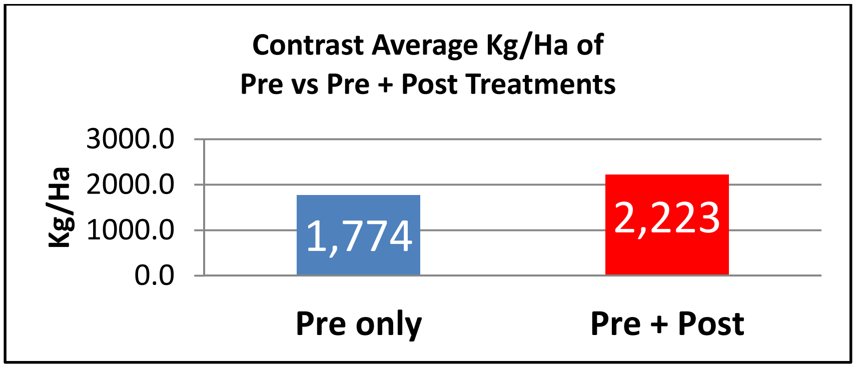


Table 4.2- Contrast Values of soybean yield of Pre-emergence treatments only and Pre-emergence plus Post-emergence treatments of fomesafen plus glyphosate

Contrast	DF	F Value	Pr > F
Pre Vs. Pre/Post	1	1.78	0.1983

Table 4.3 - Study one rating dates, total percent general phytotoxicity to soybean tissue, and percent control of total waterhemp biomass

Trt No.	Active Ingredient	Treatment Name	App. Timing	Jul-14-2011 %PHYTO	Jul-14-2011 % Control	Jul-28-2011 % Control	Aug-9-2011 % Control
1	Glyphosate	Roundup Powermax	V3	0 c	87 ab	88 ab	83 ab
	Ammonium Sulfate						
	Glyphosate	Roundup Powermax	V5				
	Ammonium Sulfate						
2	Chlorimuon	Classic	V3	2 bc	57 c	63 b	45 c
	Crop Oil Concentrate						
	Chlorimuon	Classic	V5				
	Crop Oil Concentrate						
3	Fomesafen, Metolachlor	Prefix	PRE	5 abc	92 a	83 ab	91 ab
4	Fomesafen, Metolachlor	Prefix	PRE	5 abc	96 a	93 a	91 ab
	Glyphosate	Roundup Powermax	V3				
	Ammonium Sulfate		V3				
	Fomesafen	Flexstar	V3				
	Non-ionic Surfactant	Activator 90	V3				
5	Metribuzin, Metolachlor	Boundary	PRE	3 abc	91 a	83 ab	82 ab
6	Metribuzin, Metolachlor	Boundary	PRE	7 abc	99	99 a	99 a
	Glyphosate	Roundup Powermax	V3				
	Ammonium Sulfate		V3				
	Fomesafen	Flexstar	V3				
	Non-ionic Surfactant	Activator 90	V3				
7	Sulfentrazone	Spartan	PRE	0 c	70 bc	65 b	58 bc
8	Sulfentrazone	Spartan	PRE	12 a	99	99 a	99 a
	Glyphosate	Roundup Powermax	V3				
	Ammonium Sulfate		V3				
	Fomesafen	Flexstar	V3				
	Non-ionic Surfactant	Activator 90	V3				
9	Saflufenacil	Sharpen	PRE	3abc	81 ab	68 ab	60 bc
10	Saflufenacil	Sharpen	PRE	3 abc	99 a	98 a	98 a
	Glyphosate	Roundup Powermax	V3				
	Ammonium Sulfate		V3				
	Fomesafen	Flexstar	V3				
	Non-ionic Surfactant	Activator 90	V3				
11	Sulfentrazone, Metribuzin	Authority MTZ	PRE	7 abc	85 ab	67 b	81 ab
12	Sulfentrazone, Metribuzin	Authority MTZ	PRE	10 ab	99	99 a	98 a
	Glyphosate	Roundup Powermax	V3				
	Ammonium Sulfate		V3				
	Fomesafen	Flexstar	V3				
	Non-ionic Surfactant	Activator 90	V3				
	LSD (0.05) Value			8.5	18.4	30.9	36.7

Hancock County 2011: Study Two

Evaluation of crop injury was assessed on July 14, July 30, and August 9 of 2011. This evaluation was based on the total biomass reduction as well as leaf puckering and cupping from herbicide injury compared to the untreated check. Some stunting was evident within the treatments as well in the July 14 rating. Herbicide injury may have been instigated by a substantial rainfall of 40 centimeters starting one week after the PRE treatment in the month of June (Table 3.11.) Leaf puckering on the newer leaf tissue resulted from the foliar broadcast treatment of acetochlor on June 24. July 30 and August 9 crop injury ratings show statistical differences within treatments after the first and second post application of acetochlor in four of the five treatments applied at the V-3 and V-5 soybean cropping stage.

July 14 visual crop response rating revealed significance within treatments with a P-value of 0.0034. Flumioxazin plus pyroxasulfone with a V3 timing of glyphosate plus fomesafen and acetochlor at V3received the highest crop injury rating of 22%, but was not statistically different as the same post treatment at V5. Treatment of flumioxazin plus chlorimuron pre-emergence followed by glyphosate plus fomesafen at V3 and also with acetochlor added to glyphosate and fomesafen at V5 showed the same level of crop biomass reduction. Early ratings show Sulfentrazone plus chlorimuron applied pre-emergence had the lowest overall crop damage. However, after the V5 treatment of glyphosate plus fomesafen and acetochlor, damage was significantly higher than all other treatments with a 20% average rating on July 30. Treatments including flumioxazin plus chlorimuron, flumioxazin plus pyroxasulfone, and sulfentrazone plus cloransulam all received a 17% crop damage rating after the V5 tank mix application of glyphosate, fomesafen and acetochlor which was less than sulfentrazone plus chlorimuron followed by the same post treatment, but significantly higher than all other treatments with noticeable leaf cupping and puckering. August 9 visual ratings were with some cupping and puckering still prevalent. Total crop damage to treatment significance was at the <0.001 significance level for both July 30 and August 9 PHYTO ratings when ran through proc glm having the same damage to treatment rating percentages.

Final waterhemp efficacy ratings were taken on August 9 of 2011. Across all treatments, there were no significant differences with a P-value of 0.1075 when run through proc glm.

Soybean yield data was analyzed with SAS proc glm to determine if there were any treatment differences relative to control of waterhemp. A P-value of 0.9398 shows no significant differences among individual treatments.

No correlation with SAS procedure Proc Corr was found within any treatment when comparing crop injury response with soybean yield.

Figure 4.13 - Percent control of waterhemp with Pre-emergence treatment plus Post-emergence treatments at V3 and V5 timings. No significant differences were recorded.

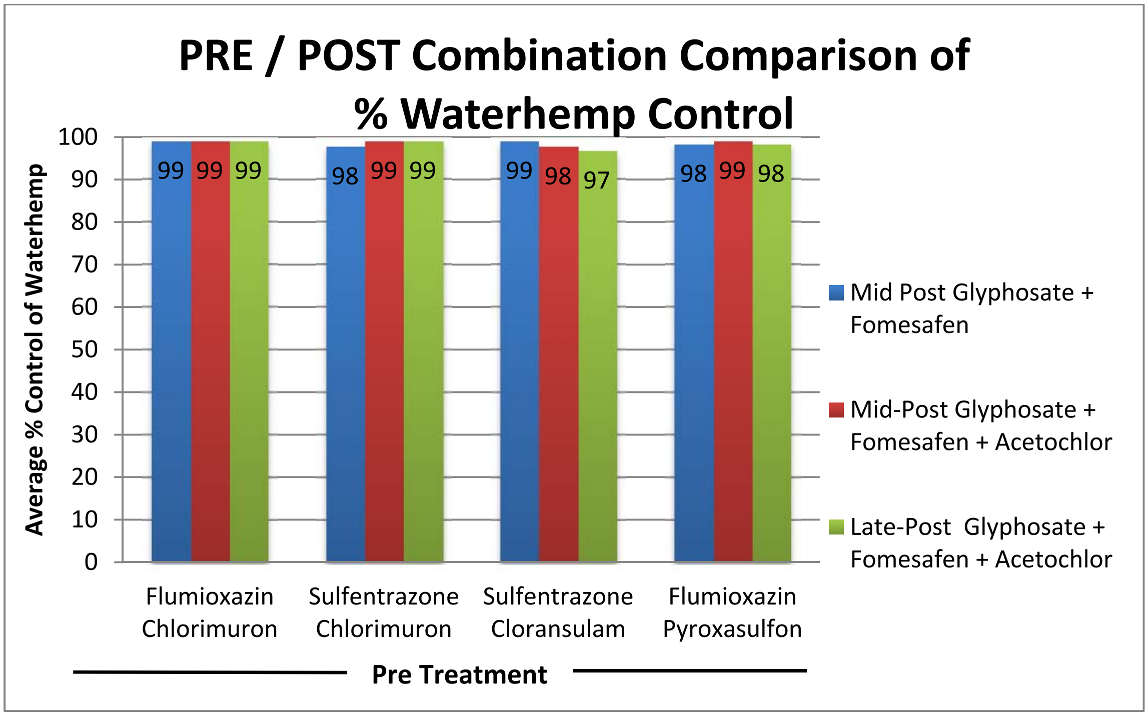


Figure 4.14 - Soybean Yield comparison with Pre-emergence treatments plus Post-emergence treatments at V3 and V5 timings. No significant differences were recorded.

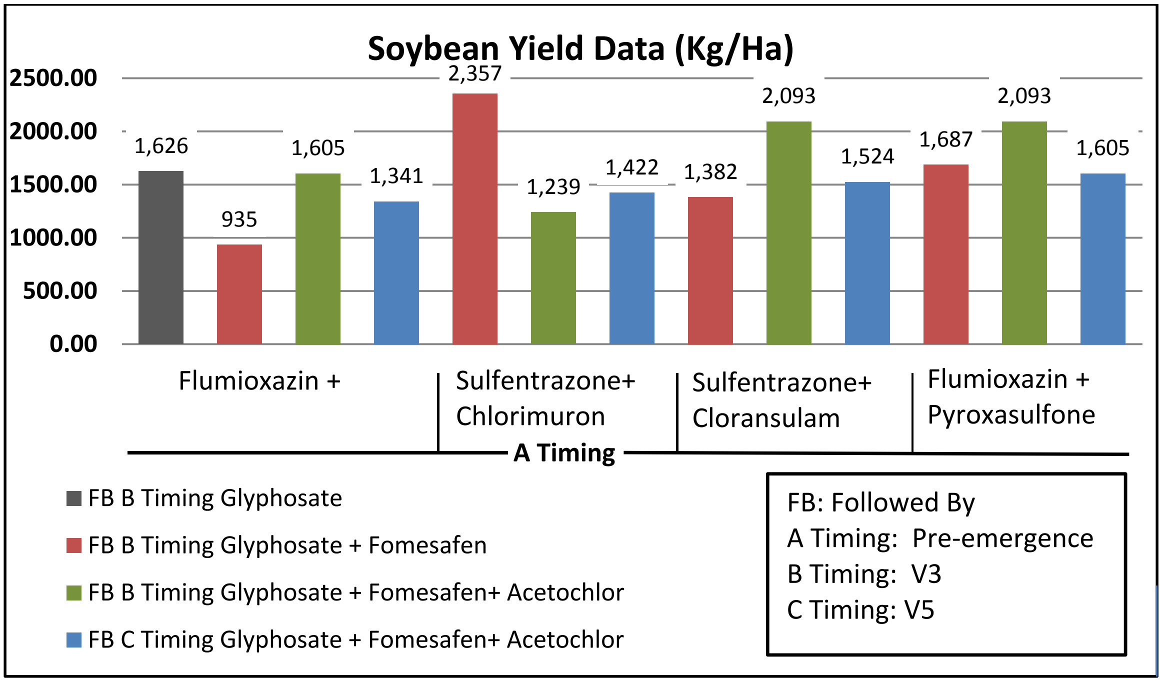


Table 4.4 – Study two rating dates, total percent general phytotoxicity to soybean tissue, and percent control of total waterhemp biomass

Trt No.	Treatment Active Ingredient	Treatment Trade Name	App Time	July 14 2011 % Control	July 14 2011 % PHYTO	July 30 2011 % Control	July 30 2011 % PHYTO	August 9 2011 % Control	August 9 2011 % PHYTO
1	Flumioxazin, Chlorimuron Glyphosate Ammonium Sulfate	Valor XLT Roundup Powermax Ammonium Sulfate	PRE V3 V3	92 a	7 cde	91 a	0 c	91 a	0 b
2	Flumioxazin, Chlorimuron Glyphosate Ammonium Sulfate Fomesafen Non-ionic Surfactant	Valor XLT Roundup Powermax Ammonium Sulfate Flexstar NIS	PRE V3 V3 V3 V3	98 a	13 abc	99 a	0 c	99 a	0 b
3	Flumioxazin, Chlorimuron Acetochlor Glyphosate Ammonium Sulfate Fomesafen Non-ionic Surfactant	Valor XLT Warrant Roundup Powermax Ammonium Sulfate Flexstar NIS	PRE V3 V3 V3 V3 V3	99 a	12 bcd	98 a	0 c	99 a	0 b
4	Flumioxazin, Chlorimuron Acetochlor Glyphosate Ammonium Sulfate Fomesafen Non-ionic Surfactant	Valor XLT Warrant Roundup Powermax Ammonium Sulfate Flexstar NIS	PRE V5 V5 V5 V5 V5	85 a	8 cde	97 a	17 b	99 a	3 a
5	Flumioxazin, Chlorimuron Acetochlor Glyphosate Ammonium Sulfate Fomesafen Non-ionic Surfactant Acetochlor Glyphosate Ammonium Sulfate	Valor XLT Warrant Roundup Powermax Ammonium Sulfate Flexstar NIS Warrant Roundup Powermax Ammonium Sulfate	PRE V3 V3 V3 V3 V3 V5 V5 V5	98 a	10 cd	99 a	0 c	99 a	5 a
6	Flumioxazin, Chlorimuron Pendimethalin Acetochlor Fomesafen Non-ionic Surfactant Glyphosate Ammonium Sulfate	Valor XLT Prowl H20 Warrant Flexstar NIS Roundup Powermax Ammonium Sulfate	PRE PRE V3 V3 V3 V3 V3	99 a	10 cd	99 a	0 c	99 a	0 b
7	Sulfentrazone, Chlorimuron Glyphosate Ammonium Sulfate	Authority XL Roundup Powermax Ammonium Sulfate	PRE V3 V3	99 a	3 de	99 a	0 c	98 a	0 b

	Fomesafen	Flexstar	V3						
	Non-ionic Surfactant	NIS	V3						
8	Sulfentrazone, Chlorimuron	Authority XL	PRE	99 a	10 cd	96 a	0 c	99 a	0
	Acetochlor	Warrant	V3						
	Glyphosate	Roundup Powermax	V3						
	Ammonium Sulfate	Ammonium Sulfate	V3						
	Fomesafen	Flexstar	V3						
	Non-ionic Surfactant	NIS	V3						
9	Sulfentrazone, Chlorimuron	Authority XL	PRE	53 b	0 e	98 a	20 a	99 a	5 a
	Acetochlor	Warrant	V5						
	Glyphosate	Roundup Powermax	V5						
	Ammonium Sulfate	Ammonium Sulfate	V5						
	Fomesafen	Flexstar	V5						
	Non-ionic Surfactant	NIS	V5						
10	Sulfentrazone, Chloransulam	Authority First	PRE	99 a	10 cd	99 a	0 a	99 a	0 b
	Glyphosate	Roundup Powermax	V3						
	Ammonium Sulfate	Ammonium Sulfate	V3						
	Fomesafen	Flexstar	V3						
	Non-ionic Surfactant	NIS	V3						
11	Sulfentrazone, Chloransulam	Authority First	PRE	96 a	7 cde	98 a	0 a	94 a	0 b
	Acetochlor	Warrant	V3						
	Glyphosate	Roundup Powermax	V3						
	Ammonium Sulfate	Ammonium Sulfate	V3						
	Fomesafen	Flexstar	V3						
	Non-ionic Surfactant	NIS	V3						
12	Sulfentrazone, Chloransulam	Authority First	PRE	58 b	0 e	96 a	17 b	96 a	3 a
	Acetochlor	Warrant	V5						
	Glyphosate	Roundup Powermax	V5						
	Ammonium Sulfate	Ammonium Sulfate	V5						
	Fomesafen	Flexstar	V5						
	Non-ionic Surfactant	Activator 90	V5						
13	Flumioxazin, Pyroxasulfone	Fierce	PRE	99 a	20 ab	99 a	0 c	99 a	0 b
	Glyphosate	Roundup Powermax	V3						
	Ammonium Sulfate	Ammonium Sulfate	V3						
	Fomesafen	Flexstar	V3						
	Non-ionic Surfactant	NIS	V3						
14	Flumioxazin, Pyroxasulfone	Fierce	PRE	99 a	22 a	99 a	0 c	99 a	0 b
	Acetochlor	Warrant	V3						
	Glyphosate	Roundup Powermax	V3						
	Ammonium Sulfate	Ammonium Sulfate	V3						
	Fomesafen	Flexstar	V3						
	Non-ionic Surfactant	Activator 90	V3						
15	Flumioxazin, Pyroxasulfone	Fierce	PRE						

	Acetochlor Glyphosate Ammonium Sulfate Fomesafen Non-ionic Surfactant	Warrant Roundup Powermax Ammonium Sulfate Flexstar Activator 90	V5 V5 V5 V5 V5	98 a	12 bcd	98 a	17 b	99 a	5 a
	LSD (0.05) Value			19.7	9.8	22.7	2.16	4	1.8

Chapter 5

Conclusion

Amaranthus tuberculatus was confirmed to be glyphosate and acetolactate synthesis (ALS) resistant at each site during the 2010 and 2011 growing seasons in Union and Hancock counties. Resistance to glyphosate within the populations was believed to be around 25% of the total population while ALS resistance comprised over half of the waterhemp population. Resistance to each of these modes of action has limited post-emergence herbicide efficacy and has pushed farmers to rely more on pre-emergence control tactics to control waterhemp. Union county preliminary results proved that PPO herbicides will provide greater biomass reduction after a POST application. With this knowledge, fomesafen was selected as a tank mix partner with glyphosate for the Hancock county post emergence applications. Hancock county data analysis confirms that there are herbicide programs that will prevent and control waterhemp throughout the soybean growing season in a no-till environment. A two pass PRE + POST system was superior in control providing a 97% average control rating while using just a pre-emergence herbicide gave 74% average control throughout the growing season. Harvest data shows numeric yield loss when no post emergence applications were applied, but no significant loss was recorded. Although no significant yield loss with waterhemp present in PRE only treatments, POST applications of fomesafen plus glyphosate reduced the overall amount of seed produced by the waterhemp allowing for a decline in soil seed bank for following years. This will allow a decline in emergence after several years of complete control.

Summary

With programs currently available for Kentucky soybean farmers, control of herbicide resistant *Amaranthus tuberculatus* may be obtained. Farmers may use this information to allow for a high degree of herbicide efficacy to prevent a major spread of this noxious and damaging weed. Waterhemp control throughout the growing season will provide soybeans with less competition for optimum growing conditions as well as better harvestability at the end of the season. A higher percentage of waterhemp control initiates a lower population of waterhemp in following years. Less overall germination will prevent a growing genetic variation which provides progressive waterhemp evolution. Waterhemp may only be one weed that farmers deal with each year, but given the circumstances of this weed having prolific seed production along with its adaptability far more advanced than other species; this one weed has the chance to provide great damage to a soybean yield. Soybean fields infested with waterhemp decreases crop yields which declines gross income for the farmer. Each growing season varies in weather patterns, planting dates, and a variety of other factors. Given these circumstances, this research will have to continue to provide the best data for Kentucky farmers to continue their battle to control this ever-growing problem throughout the midwestern states.

Apendix

Herbicide Mode of Action and Mechanism of Rsistance

Mode of Action: Acetolactate synthase **(ALS)**

Acetohydroxyacid synthase (AHAS) is also referred to as acetolactate synthase (ALS). Acetolactate synthase is the first enzyme in the process to produce branched-chain amino acids valine, leucine, and isoleucine. ALS catalyzes the formation of both aceto-hydroxybutyrate and acetolactate and is the target site for a large number of herbicides. Sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidine, pyrimidinyl-thiobenzoates, and sulfonyl-aminocarbonyl-triazolinone are all herbicide families in the ALS mode of action. (Powles and Qin Yu 2010).

Mechanism of Resistance:

An insensitive ALS enzyme to the herbicides at normal use rates provides the mechanism of resistance in *Amaranthus tuberculatus* (Sprague et al**.** 1997).

Mode of Action: 5-enolpyruvylshikimic acid-3-phosphate synthase **(EPSPS)**

Glyphosate inhibits the formation of three aromatic amino acids. The herbicide inhibits the incorporation of shikimic acid into phenylalanine, tryptophan, and tyrosine which causes an over-accumulation of shikimic acid in plant tissues and blocks the formation of anthraquinoid pigments derived from chorismic acid. This process is the result of glyphosate inhibiting the enzymatic step between shikimic acid to chorismic acid. 5-enolpyruvylshikimic acid-3-phosphate synthase is the true site of action within the plant to promote plant death (H.C. Steinrücken and N. Amrhein 1980).

Mechanism of Resistance:

Glyphosate resistance occurs in *Amaranthus palmeri* due to the amplification of the EPSPS gene and increased EPSPS expression (Gaines et al. 2010). Change in horseweed (*Conyza canadensis* L.) translocation of glyphosate in resistant biotypes has been documented as a mechanism of resistance (Preston and Wakelin 2008 , Shaner 2009). This change in translocation includes a sequestration of glyphosate in resistant biotypes.

Mode of Action: Protoporphyrinogen IX Oxidase (Protox) Inhibitiors **(PPO)**:

Several classes of commercial herbicides (oxadiazoles, cyclic imides, and diphenyl ethers) inhibit Protox, disrupting the porphyrin pathway and autooxidation of protoporphyrinogen to form destructive singlet oxygen and high levels of Proto. Proto accumulation amounts to almost 200-fold greater in treated than untreated tissues resulting in rapid destruction to the plasmalemma and tonoplast creating bleaching and necrotic plant tissue until plant death (Duke et al. 1991).

Mechanism of Resistance:

Resistance to Protoporphyrinogen IX Oxidase (Protox) Inhibitors requires great genetic diversity. There are two necessary PPO enzymes in a plant, one in the plastids and one in the mitochondria. In order for the herbicide to effectively kill the plant, the PPO-inhibiting herbicide has to reach both sites of action. For complete resistance towards PPO-inhibiting herbicides, waterhemp must achieve the selection of two mutant allele genes. Waterhemp can achieve this by possessing a PPX gene (PPX2L) that encodes an enzyme which is thought to have the ability to pass as both PPO enzymes (Patzoldt et al. 2006). This mutant allele is the outcome of a deleted amino acid residue

(ΔG210) in the PPO2L enzyme which provides resistance to protoporphyrinogen IX Oxidase (Protox) inhibitors in waterhemp. Evolved resistance by the deletion of a gene (ΔG210) by waterhemp is a new occurrence towards an herbicide treatment (Patzoldt et al. 2006).

Mode of Action: Inhibition of Photosystem II **(PSII)**

PS II herbicides families include the triazines, ureas, nitrophenols, nitriles, pyridazinone, and pyridazinones. Treatment of the herbicide blocks the flow of electrons through PS II which disrupts the transfer of excitation energy from chlorophyll molecules to the PS II reaction center. Excited chlorophyll molecules (singlet chlorophyll) spontaneously form triplet chlorophyll. The triplet chlorophyll reacts with molecular oxygen to form singlet oxygen which results in lipid peroxidation and eventual necrosis of tissue leading to plant death (Fuerst and Norman 1991).

Mechanism of Resistance:

Resistance to photosystem II inhibiting herbicides, to date requires an insensitive enzyme at the D1 target site within the electron transport process. Recent studies towards waterhemp photosystem II resistance by Patzoldt et al. (2003) revealed that waterhemp within the population studied, did not contain the amino acid substitution typically found in a resistant plant. Resistance may be nuclear encoded and not site-of-action mediated. Therefore, more research is required to further determine the exact site of action or mechanism of resistance (Patzoldt et al. 2003).

Mode of Action: Inhibitors of p-HydroxyphenylpyruvateDioxygenase **(HPPD)**

Plant HPPD is part of the biosynthetic pathway leading to the important compounds such as PQ, a critical cofactor for phytoene desaturase (Mayer et al. 1990, Norris et al. 1995). Depletion of these compounds lead to the reduction of carotenoids resulting in bleaching symptoms. Along with the depletion of PQ, an accumulation of tyrosine is present. The mode of action for the Triketone herbicide family is a combination of bleaching symptomology, accumulation of tyrosine, depletion of PQ, and in vitro inhibition of HPPD, but inhibition of HPPD is the primary mechanism (Lee et al. 1997). The combination of symptoms and plant responses are known as the "tri- ketone effect" (Prisbylla et al. 1993).

Mechanism of Resistance:

Future research needs to be implemented to investigate the genetics, inheritance and mechanisms of resistance to HPPD inhibitors. Waterhemp is the first to evolve resistance to HPPD inhibitors. Little research has been done and thus the mechanism of resistance has not been documented for HPPD resistance in weeds (Hausman et al 2011).

Mode of Action: Synthetic Auxins

The synthetic auxin herbicides belong to several chemical classes and include phenoxycarboxylic acids, benzoic acids, pyridinecarboxylic acids, aromatic carboxymethyl derivatives and quinolinecarboxylic acids and mimic the natural auxin indole-3-acetic acid (IAA). These herbicides are more stable in plants than IAA and exhibit systemic mobility and preferential selectivity against dicot weeds in cereal crops. The mechanism of action of these herbicides is complicated and involves derepression of transcriptional active proteins which activates transcription of auxin responsive genes. In shoot tissue, genes of 1-aminocyhclopropane-1-crboxylic acid synthase in ethylene and 9-cis-epoxycarotenoid dioxygenase in abscisic acid biosynthesis are over expressed. The ethylene produced causes the downward curvature of leaves (epinasty) and tissue swelling. At the same time, the auxin cause horizontal curvature of stems and referred to as stem curling. The abscisic acid directly inhibits cell division and expansion, and together with ethylene, promotes foliar senescence with chloroplast damage and destruction of membrane and loss of vascular system integrity. Growth inhibition, tissue desiccation and decay and finally plant death are the results (Grossmann 2009).

Mechanism of Resistance:

Bacterial aryloxyalkanoate dioxygenase enzymes (AADs) can effectively degrade 2,4-D, as well as some other synthetic auxin herbicides. Bacterial AAD-1 cleaves the aryloxyphenoxypropionate family of grass-active herbicides giving grasses resistance to ACCase inhibitors. Bacterial AAD-12 cleaves pyridyloxyacetate auxin herbicides such as triclopyr and fluroxypyr. The 2,4-D monooxygenase geneTfdA, from *Alcaligenes eutrophus* plasmid pJP5, catalyzes the oxygenolytic cleavage of 2,4-D to nonherbicidal dichlorophenol and glyoxylate (Wright 2010).

Table - Appendix - Herbicide Resistance within waterhemp biotypes in North America

HERBICIDE RESISTANT of WATERHEMP GLOBALLY
Amaranthus tuberculatus (syn. rudis)

#	Country	Year	Sites	Acres	Site of Action
1.	**Canada** (Ontario) **Multiple Resistance**	**2002**	1	11-50	**ALS inhibitors (B/2)** **Photosystem II inhibitors (C1/5)**
2.	**Canada** (Ontario)	**2002**	11-50	51-100	**ALS inhibitors (B/2)**
3.	**USA** (Iowa)	**1993**	501-1000	1001-10000	**ALS inhibitors (B/2)**
4.	**USA** (Illinois)	**1993**	10001-100000	2-5 million	**ALS inhibitors (B/2)**
5.	**USA** (Missouri)	**1994**	11-50	101-500	**Photosystem II inhibitors (C1/5)**
6.	**USA** (Missouri)	**1994**	1001-10000	2-5 million	**ALS inhibitors (B/2)**
7.	**USA** (Kansas)	**1995**	1001-10000	2-5 million	**ALS inhibitors (B/2)**
8.	**USA** (Kansas)	**1995**	101-500	100001-1000000	**Photosystem II inhibitors (C1/5)**
9.	**USA** (Nebraska)	**1996**	unknown	unknown	**Photosystem II inhibitors (C1/5)**
10.	**USA** (Iowa)	**1996**	unknown	unknown	**Photosystem II inhibitors (C1/5)**
11.	**USA** (Ohio)	**1996**	101-500	1001-10000	**ALS inhibitors (B/2)**
12.	**USA** (Illinois) **Multiple Resistance**	**1996**	11-50	1001-10000	**ALS inhibitors (B/2)** **Photosystem II inhibitors (C1/5)**
13.	**USA** (Wisconsin)	**1999**	11-50	1001-10000	**ALS inhibitors (B/2)**
14.	**USA** (Michigan)	**2000**	6-10	101-500	**ALS inhibitors (B/2)**
15.	**USA** (Kansas) **Multiple Resistance**	**2001**	11-50	501-1000	**ALS inhibitors (B/2)** **PPO inhibitors (E/14)**
16.	**USA** (Illinois)	**2001**	unknown	unknown	**Photosystem II inhibitors (C1/5)**
17.	**USA** (Oklahoma)	**2002**	101-500	1001-10000	**ALS inhibitors (B/2)**
18.	**USA** (Illinois) **Multiple Resistance**	**2002**	6-10	1001-10000	**ALS inhibitors (B/2)** **Photosystem II inhibitors (C1/5)** **PPO inhibitors (E/14)**
19.	**USA** (Missouri) **Multiple Resistance**	**2005**	101-500	100001-1000000	**ALS inhibitors (B/2)** **PPO inhibitors (E/14)** **Glycines (G/9)**
20.	**USA** (Indiana)	**2005**	2-5	101-500	**ALS inhibitors (B/2)**

21.	**USA** (Kansas)	**2006**	11-50	1001-10000	**Glycines (G/9)**
22.	**USA** (Illinois) **Multiple Resistance**	**2006**	1	51-100	**ALS inhibitors (B/2)** **Glycines (G/9)**
23.	**USA** (Tennessee)	**2007**	6-10	501-1000	**ALS inhibitors (B/2)**
24.	**USA** (Minnesota)	**2007**	501-1000	10001-100000	**Glycines (G/9)**
25.	**USA** (Iowa)	**2009**	11-50	unknown	**PPO inhibitors (E/14)**
26.	**USA** (Iowa)	**2009**	11-50	unknown	**PPO inhibitors (E/14)**
27.	**USA** (Iowa)	**2009**	2-5	unknown	**Glycines (G/9)**
28.	**USA** (Iowa) **Multiple Resistance**	**2009**	1	51-100	**ALS inhibitors (B/2)** **Photosystem II inhibitors (C1/5)** **4-HPPD inhibitors (F2/27)**
29.	**USA** (Illinois) **Multiple Resistance**	**2009**	1	51-100	**ALS inhibitors (B/2)** **Photosystem II inhibitors (C1/5)** **4-HPPD inhibitors (F2/27)**
30.	**USA** (Indiana)	**2009**	2-5	51-100	**Glycines (G/9)**
31.	**USA** (Nebraska)	**2009**	2-5	6-10	**Synthetic Auxins (O/4)**
32.	**USA** (Mississippi)	**2010**	2-5	101-500	**Glycines (G/9)**
33.	**USA** (North Dakota)	**2010**	101-500	1001-10000	**Glycines (G/9)**
34.	**USA** (Iowa) **Multiple Resistance**	**2011**	6-10	101-500	**ALS inhibitors (B/2)** **4-HPPD inhibitors (F2/27)** **Glycines (G/9)**
35.	**USA** (Nebraska)	**2011**	1	51-100	**4-HPPD inhibitors (F2/27)**
					www.weedscience.org 2011

Weather Data

Weather data is from WeatherUnderground.com. Data includes: Temperature (High, Low, and Average), Growing Degree Units (Heating, Cooling, and Growing), Dew Point, and Precipitation.

March:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**81** °F	**57** °F	**37** °F	
Mean Temperature	**70** °F	**49** °F	**34** °F	
Min Temperature	**59** °F	**40** °F	**29** °F	
Degree Days				
Heating Degree Days (base 65)	30	17	0	521
Cooling Degree Days (base 65)	4	0	0	6
Growing Degree Days (base 50)	20	3	0	107
Dew Point				
Dew Point	**61** °F	**37** °F	**15** °F	
Precipitation				
Precipitation	**0.71** in	**0.06** in	**0.00** in	**1.80** in

April:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**86** °F	**71** °F	**52** °F	
Mean Temperature	**76** °F	**61** °F	**45** °F	
Min Temperature	**65** °F	**50** °F	**34** °F	
Degree Days				
Heating Degree Days (base 65)	20	6	0	175
Cooling Degree Days (base 65)	10	1	0	43
Growing Degree Days (base 50)	26	11	0	331
Dew Point				
Dew Point	**70** °F	**50** °F	**22** °F	
Precipitation				
Precipitation	**1.99** in	**0.34** in	**0.00** in	**10.08** in

May:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**95** °F	**75** °F	**56** °F	
Mean Temperature	**84** °F	**67** °F	**50** °F	
Min Temperature	**73** °F	**58** °F	**41** °F	
Degree Days				
Heating Degree Days (base 65)	16	4	0	119
Cooling Degree Days (base 65)	19	5	0	168
Growing Degree Days (base 50)	34	17	0	512
Dew Point				
Dew Point	**75** °F	**59** °F	**33** °F	
Precipitation				
Precipitation	**2.05** in	**0.16** in	**0.00** in	**4.97** in

June:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**97** °F	**88** °F	**73** °F	
Mean Temperature	**84** °F	**78** °F	**69** °F	
Min Temperature	**74** °F	**69** °F	**63** °F	
Degree Days				
Heating Degree Days (base 65)	0	0	0	0
Cooling Degree Days (base 65)	20	14	4	406
Growing Degree Days (base 50)	34	28	19	851
Dew Point				
Dew Point	**85** °F	**68** °F	**53** °F	
Precipitation				
Precipitation	**1.36** in	**0.20** in	**0.00** in	**6.14** in

July:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**99** °F	**91** °F	**81** °F	
Mean Temperature	**87** °F	**82** °F	**74** °F	
Min Temperature	**77** °F	**73** °F	**68** °F	
Degree Days				
Heating Degree Days (base 65)	0	0	0	0
Cooling Degree Days (base 65)	22	17	10	518
Growing Degree Days (base 50)	37	32	24	983
Dew Point				
Dew Point	**83** °F	**73** °F	**63** °F	
Precipitation				
Precipitation	**1.21** in	**0.10** in	**0.00** in	**2.98** in

August:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**96** °F	**88** °F	**79** °F	
Mean Temperature	**84** °F	**78** °F	**70** °F	
Min Temperature	**74** °F	**67** °F	**59** °F	
Degree Days				
Heating Degree Days (base 65)	0	0	0	0
Cooling Degree Days (base 65)	18	13	5	393
Growing Degree Days (base 50)	34	28	20	858
Dew Point				
Dew Point	**81** °F	**66** °F	**47** °F	
Precipitation				
Precipitation	**0.45** in	**0.02** in	**0.00** in	**0.66** in

September:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**99** °F	**76** °F	**61** °F	
Mean Temperature	**84** °F	**67** °F	**58** °F	
Min Temperature	**70** °F	**58** °F	**50** °F	
Degree Days				
Heating Degree Days (base 65)	7	2	0	51
Cooling Degree Days (base 65)	20	4	0	119
Growing Degree Days (base 50)	34	17	8	519
Dew Point				
Dew Point	**72** °F	**56** °F	**34** °F	
Precipitation				
Precipitation	**0.86** in	**0.09** in	**0.00** in	**2.79** in

October:	Max	Avg.	Min	Sum
Temperature				
Max Temperature	**86** °F	**69** °F	**46** °F	
Mean Temperature	**69** °F	**57** °F	**44** °F	
Min Temperature	**56** °F	**44** °F	**32** °F	
Degree Days				
Heating Degree Days (base 65)	22	9	0	274
Cooling Degree Days (base 65)	4	0	0	13
Growing Degree Days (base 50)	19	8	0	236
Dew Point				
Dew Point	**63** °F	**44** °F	**23** °F	
Precipitation				
Precipitation	**0.61** in	**0.05** in	**0.00** in	**1.41** in

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