INTERACTIONS BETWEEN GLYPHOSATE, *FUSARIUM* INFECTION OF WATERHEMP, AND SOIL MICROORGANISMS

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ABSTRACT

In recent years, an increasing number of weed populations have been characterized with resistance to the herbicide glyphosate. In particular, waterhemp has evolved glyphosate resistance (GR) across numerous soybean fields in Missouri. Therefore research is needed to determine best management practices for GR weed biotypes. The objectives of these experiments were to determine the frequency and distribution of GR waterhemp in Missouri and identify any in-field parameters which could serve as indicators of GR in future crop production systems; determine the effects of various pre-emergence (PRE) and post-emergence (POST) herbicide programs on palmer amaranth and waterhemp control, soybean yield, and net income in conventional, glyphosate-resistant, and glufosinate-resistant soybean production systems; determine the effects of soil microbial and phytopathogen populations on GR and susceptible (GS) waterhemp survival and *Fusarium* infection; and determine the soil microbial abundance and diversity in soils collected from soybean fields with differences in waterhemp biotypes and herbicide and crop rotation histories. Results from these experiments indicate herbicide programs that contain PRE herbicide treatments provide the best opportunity for season-long control of waterhemp and palmer amaranth, highest grain yields, and highest net returns in the soybean systems evaluated. GR was confirmed in 69% of the total waterhemp populations sampled in Missouri. Additionally, the in-field parameters evaluated suggest that soybean fields containing GR waterhemp were more likely to be free of other weed species, occur where soybeans were continuously cropped, occur where glyphosate was the only herbicide applied for several seasons consecutively, and where waterhemp exhibited signs of surviving herbicide treatment compared to fields characterized with GS waterhemp. Results of the soil study indicate plants are more sensitive to glyphosate in soils with microbial populations compared to those without and that glyphosate may predispose plants to soilborne phytopathogens. The results also suggest continuous use of glyphosate does not significantly affect soil microbial abundance or diversity.

Chapter I

Literature Review

Identification

Palmer amaranth and common waterhemp are summer annual C4 weed species. Identification of either species can be quite difficult, especially in the early stages of seedling growth as many species within the *Amaranthus* or "pigweed" genus look the same (Horak et al. 1994). Once members of the pigweed family are mature, plant identification becomes less difficult (Horak et al. 1994). Conversely, some pigweedspecies may cross to produce hybrids; therefore hybrid plants may exhibit characteristics of both parents which leads to improper identification (Horak et al. 1994; Trucco et al. 2005).

Common waterhemp (*Amaranthus rudis* Sauer) is a summer annual with seedlings that have egg-shaped cotyledons and first true leaves that are long, narrow, without hairs, and often have a waxy or glossy appearance (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). During all growth stages, waterhemp stems are hairless and stem and leaf color tend to be shades of green or sometimes stems in particular can be distinctly red (Nordby et al. 2007). Mature common waterhemp plants may reach as much as 3.6 m in height with long and narrow leaves that are waxy or glossy in appearance (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). Common waterhemp is dioecious, meaning that the male and female flowers occur on separate plants (Bradley et al. 2009a; Nordby et al. 2007). The male and female seedheads of waterhemp differ in that the male waterhemp seedhead is more compact than the female (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). Another distinguishing characteristic between male and female plants is that only the female produces seed (Nordby et al. 2007).

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is also a summer annual with seedlings that have cotyledons that are narrow and green to reddish in color. The first true leaves are ovate in shape, with few or no hairs present. Leaves are slightly notched at the tip and have petioles often as long as or longer than the leaf blades (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). Mature palmer amaranth has leaves that are without hairs, and are 5 to 20 cm long and egg-shaped in outline. The leaves of palmer amaranth take on a poinsettia-like appearance with a symmetrical leaf arrangement and occasional v-shaped variegation on the topside of the leaf (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). The stems of palmer amaranth are also hairless, with occasional small lateral inflorescences occurring between the stem and leaf petioles. Mature plants can grow to more than 2 m in height (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). Palmer amaranth is also dioecious, with the male plants producing only pollen and the female plants only producing seed. The terminal inflorescences of male and female plants are generally unbranched, with thick stalks that can reach 15 to 45 cm in length (Bradley et al. 2009a; Horak et al. 1994; Steckel 2007). The inflorescence of a mature female palmer amaranth plant produces bracts that are very stiff and sharp.

Distribution and Habitat

Most members of the pigweed family are found predominately in agricultural-based settings and thrive in wet areas of fields, but can readily adapt to a variety of conditions. Within the pigweed family there are ten species which can be encountered in the Midwestern United States: redroot pigweed (*Amaranthus retroflexus* L.), smooth pigweed (*Amaranthus hybridus L.*), Powell amaranth (*Amaranthus powellii* S. Wats.), tumble pigweed (*Amaranthus albus* L.), prostrate pigweed (*Amaranthus blitoides* S.

Wats.), spiny amaranth (*Amaranthus spinosus* L.), tall waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer.], sandhills waterhemp (*Amaranthus arenicola* I.M. Johnst.), common waterhemp and palmer amaranth (Great Plains Flora Association 1986; McGregor et al. 1977; Steckel 2007). In Missouri, the most common pigweed species encountered in corn and soybean production are common or tall waterhemp. Due to the high degree of genetic similarity and hybridization between these two, many botanists now group them into one “waterhemp” species (Pratt and Clark 2001).

Waterhemp is one of the most common weeds with which Midwest farmers must contend (Nordby et al. 2007). In a survey conducted in Illinois, *Amaranthus* species was listed as the number one weed encountered in corn and soybean fields with 75% of those responses specifically listing waterhemp (Hager and Sprague 2002). Waterhemp is native to the Midwest with a distribution ranging from Texas to Maine (Nordby et al. 2007). Palmer amaranth is native to southwestern United States and Mexico and has spread from southern California to Texas and is now a major weed pest of soybean and cotton production in the midsouth and southeastern United States (Steckel 2007). Although palmer amaranth is predominately found in the southern United States, recently we have discovered sporadic infestations of palmer amaranth in corn and soybean fields in central and northwestern Missouri (Payne and Bradley 2010).

The success of both of these weeds is attributed to their extended period of emergence, abundant growth at high light intensities and temperatures, and prolific seed production (Hartzler et al. 1999; Jha et al. 2008; Massinga et al. 2003; Sauer 1957). If the proper photoperiod is available, these species have a remarkable ability to produce flowers at almost any size and maturity level (Sauer 1957). Plants that have germinated early in the growing season have the ability to produce at least 250,000 seeds within a single plant (Sellers et al. 2003). In addition, late germinating pigweeds can still produce a small crop of 5,000 seeds or less in a short period of time (Knezevic et al. 1994).

Impact on row-crop production

Germination rate, plant height, seed production, dry matter, and leaf area index are all components that describe the relative size, productivity, and competitive ability of a plant. Palmer amaranth typically germinates 3 to 8 days sooner than other pigweed species and palmer amaranth and waterhemp are both prolific seed producers capable of producing 140,000 to 514,000 seeds m^{-2} (Massinga et at. 2001; Sauer 1957; Sellers et al. 2003; Steckel 2007). Palmer amaranth has the greatest plant volume (13,154 to 4,768,843 cm^3), dry weight (606 to 1,578 g), leaf area (149 to 261 cm^2 g^{-1}), growth rate (0.32 g g^{-1} day^{-1}) and plant height (0.18 to 0.21 cm per growing degree day) of any species in the pigweed family and is closely followed by waterhemp for these previously described characteristics (Bensch et al. 2003; Horak and Loughin 2000; Sellers et al. 2003). Therefore, palmer amaranth is perhaps the most “aggressive” *Amaranthus* species with respect to growth rate and competitive ability.

Palmer amaranth and waterhemp can cause significant yield reductions in corn (*Zea mays* L.), soybean (*Glycine max* L.) and cotton (*Gossypium hirsutum* L.) (Klingamen and Oliver 1994; Massinga et al. 2003; Murphy et al. 1996). Palmer amaranth has reduced corn yields from 11 to 91% at densities of 0.5 to 8 plants per meter of row, reduced soybean yield from 17 to 79% at densities of 0.33 to 10 plants per meter of row, and reduced cotton lint yield by 6 to 28% at densities of 1 plant per 1 to 3 meter of row (Bensch et al. 2003; Klingamen and Oliver 1994; Massinga and Currie 2002;

Massinga et al. 2003; Smith et al. 2000). Further, Monks and Oliver (1988) determined soybean biomass and yield were reduced when growing within 50 and 25 cm of palmer amaranth, respectively. Klingaman and Oliver (1994) observed that soybean yield reductions are correlated to palmer amaranth biomass and density; as palmer amaranth densities and biomass increase, soybean yield will reduce linearly.

Steckel and Sprague (2004a) found that common waterhemp that emerged with the crop and was allowed to compete season-long reduced soybean yields by 37 to 44%. Hartzler et al. (2004) also found that common waterhemp that emerges before the V4-V5 stage of soybeans needs to be controlled in order to reduce soybean yield loss (10 to 45% loss if not controlled) and common waterhemp seed production (Hartzler et al. 2004; Steckel and Sprague 2004a). Similar work has been conducted with common waterhemp populations in corn. Cordes et al. (2004) found that season long interference at densities of 362 or more waterhemp plants per m^2 reduced corn yields up to 36%. Cordes et al. (2004) also found that if high densities of waterhemp were not controlled by the time they reached 15 cm in height, corn yield reductions of up to 15% would occur. A 10% corn yield loss was observed when lower waterhemp densities of 35 to 82 plants per m^2 were allowed to compete season-long (Cordes et al. 2004). Season long waterhemp interference reduced corn yield by 11 to 74% over three growing seasons (Steckel and Sprague 2004b).

Herbicide Resistance

The most common method of weed removal in agronomic cropping systems is the application of a herbicide. Biotypes of palmer amaranth and waterhemp have developed resistance to at most six herbicide modes of action, respectively, that once effectively controlled these species in row crop agricultural settings (Heap 2013). Waterhemp and palmer amaranth, among other weed species, have a history of a adapting to a variety of herbicide classes and developing resistance to herbicides. Worldwide, there are currently 397 resistant biotypes within 217 separate weed species that are resistant to one or more herbicides (Heap 2013). As defined by the Weed Science Society of America, resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA 2010).

One of the most effective ways to decrease the frequency of resistant biotypes is by implementing crop and herbicide mode of action rotation with cultural practices over multiple years (Jasieniuk et al. 1996; Schuster and Smeda 2006; Wise et al. 2009). The high persistence of weed seed in the soil seed bank and rapid growth rate of palmer amaranth and waterhemp are two of the most important reasons for the control of these resistant weed species (Buhler and Hartzler 2001; Sellers et al. 2003).

Herbicide resistance can either develop *de novo* (neither parent contributed) or be acquired through gene flow (Jasieniuk et al. 1996). A major source observed with resistant biotypes is that of genetic variation or outcrossing; both the resistant and susceptible genotypes will be present within the field and therefore there will never be 100% resistant progeny within the progeny of the parent biotypes. Resistance therefore likely occurs because of gene mutations and is believed to evolve spontaneously (Jasieniuk et al. 1996). It has been hypothesized that interspecific hybridization contributes to the evolution of herbicide resistance (Trucco et al. 2005).

Glyphosate and Glyphosate Resistant Soybeans

Glyphosate-resistant soybean (*Glycine max* L.) was the first glyphosate-resistant crop to be released in the United States in 1996. Glyphosate resistance in soybean was obtained through the insertion of an EPSPS enzyme that occurs naturally in the microorganism *Agrobacterium* sp. strain CP4. The CP4-EPSPS enzyme is highly tolerant to glyphosate as compared to the susceptible EPSPS (Devine et al. 1993). Glyphosate is responsible for the inhibition of the enzyme 5-enolpyruvoylshikimate 3-phosphate synthase (EPSPS) which is a key enzyme in the shikimate pathway producing the aromatic amino acids phenylalanine, tyrosine and tryptophan. These amino acids are three of the most important amino acids produced by the shikimate pathway (Franz et al. 1997) and are used for the synthesis of proteins, which are used in a variety of plant products. Though the exact events responsible for plant death due to glyphosate application are unknown, it is understood that the inhibition of EPSPS and the lack of production of the aromatic amino acids play a major role in the death of susceptible plants (Franz et al. 1997).

After their introduction in 1996, glyphosate-resistant soybeans were adopted quite rapidly. In 1997 the percent of herbicide tolerant soybean acreage in the United States was 17% and this percentage has increased over the past 15 years to 93 percent (USDA 2012). The increase in glyphosate-resistant soybean acreage has been attributed to the fact that glyphosate is an economical and convenient broad spectrum herbicide when compared to other herbicide options (Dill 2005). Glyphosate-resistant crops offer several advantages compared to non-transgenic cultivars with respect to herbicide programs. Applications of glyphosate to glyphosate-resistant crops allows growers to reduce or eliminate soil-applied herbicides and often results in a reduction in total herbicide use

(Culpepper and York 1998). Glyphosate-resistant crops also provide effective weed management options in conservation tillage systems, greater rotational crop flexibility, the ability to control previously uncontrollable weeds, and economical, broad spectrum weed control without crop injury (Bradley et al. 2001; Byrd 1995; Rogers et al. 1986; Wilcut et al. 1996; Young 2006).

Reddy and Whiting (2000) compared the economics of a glyphosate-resistant soybean system in which glyphosate was used as the only herbicide for weed control to a comparable sulfonylurea-tolerant and non-transgenic, or “conventional” soybean system. Results from this study showed that the net returns from the glyphosate-resistant system was \$407/ha as compared to \$271/ha and \$317/ha in the sulfonylurea-tolerant and conventional soybean systems, respectively.

Glyphosate Resistance in Weeds

With the rapid adoption of glyphosate-resistant crops across the United States has come a concurrent increase in the number of glyphosate-resistant weeds due to the extensive use of glyphosate. Currently, 24 weed species are confirmed to be resistant to glyphosate: palmer amaranth, spiny amaranth, tall waterhemp/common waterhemp, common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), ripgut brome (*Bromus diandrus* Roth), Australian fingergrass (*Chloris truncate* R. Br.), hairy fleabane (*Conyza bonariensis* (L.) Cronq.), horseweed, sumatran fleabane (*Conyza sumatrensis* (Retz.) E. Walker), gramilla mansa *(Cynodon hirsutus*) sourgrass (*Digitaria insularis* (L.) Mez ex Ekman), junglerice (*Echinochloa colona* L.), goosegrass, kochia (*Kochia scoparia* (L.) Schrad.), tropical sprangletop (*Leptochloa virgate*)**,** Italian ryegrass, perennial ryegrass (*Lolium perenne* L.), rigid ryegrass (*Lolium rigidum*

Gaudin), ragweed parthenium (*Parthenium hysterophorus* L.), buckhorn plantain (*Plantago lanceolata* L.), annual bluegrass (*Poa annua* L.), johnsongrass (*Sorghum halepense* (L.) Pers.), and liverseedgrass (*Urochloa panicoides* Beauv.) Within the U.S. alone, glyphosate-resistant palmer amaranth has been documented in 17 states while 12 states have documented glyphosate resistance in waterhemp (Heap 2013). As stated, glyphosate resistant palmer amaranth and waterhemp exist in the U.S., and research is necessary to determine the factors which heavily influence resistant boitypes. Surveys serve to educate producers about the current distribution of a resistant species within that area and also allow weed scientists to predict the likelihood of spread of this resistant species in the future (Givens et al. 2009; Johnson and Gibson 2006; Preston et al. 2009; Werth et al. 2008). This education is necessary for producers as the development of herbicide resistant weeds may not be a new phenomenon, but there is a perception that until resistance happens on their personal field it is of low importance (Nice et al. 2007). Therefore a second objective of this research is to conduct a survey of weedy soybean fields at harvest to determine the distribution of glyphosate-resistant waterhemp in Missouri and determine if there are any factors that may serve as “predictors” for glyphosate resistance in future crop production systems.

Glufosinate and Glufosinate Resistant Soybeans

Glufosinate-resistant soybeans were first introduced in 1999 on a commercialized limited basis (Wiesbrook et al. 2001) and then fully commercialized and released in 2009 (Fortune 2010). Glufosinate-resistant soybeans contain a glufosinate resistance gene from *Streptomyces viridochromogenes*, which encodes for phosphinothricin acetyltransferase (Devine et al. 1993). Phosphinothricin, the active form of the glufosinate molecule, inhibits glutamine synthetase which is the initial enzyme that assimilates inorganic nitrogen into organic compounds, converting glutamic acid and ammonia into glutamine (Devine et al. 1993). Inhibition of glutamine synthetase leads to a rapid accumulation of high levels of ammonia that allow a reduction in photosynthesis and/or conditions appropriate for photorespiration to occur (Devine et al. 1993).

The use of herbicide-resistant crops for weed management offers several advantages when compared to conventional weed management systems (Wiesbrook et al. 2001). Glufosinate and glyphosate are non-selective herbicides for postemergence programs and offer good control of weeds and flexibility to treat weeds on an as-needed basis. Application timing is very important for either glyphosate or glufosinate when targeting hard-to-control weeds, and neither herbicide offers any residual effects for multiple flushes throughout the growing season (Shaw et al. 2001). Non-transgenic soybean systems, however, are more likely to provide soil residual effects but crop injury is also more likely to be observed (Shaw et al. 2001).

Few studies have compared weed management systems in glufosinate-resistant, glyphosate-resistant, and non-transgenic soybean cropping systems. In one study, sequential applications of glufosinate improved control over single applications, while in comparison sequential applications of glyphosate generally provided no advantages over single applications (Holshouser et al. 2009; Wiesbrook et al. 2001). Several non-transgenic soybean herbicide programs can be comparable to transgenic programs, however conventional herbicides must be applied to small weeds (2 cm to 7 cm in height) to obtain acceptable control (Holshouser et al. 2009). Similar to non-transgenic soybeans, it is necessary to apply glufosinate-based herbicides when weeds are small (7 cm to 8 cm in height) to obtain the best control (Holshouser et al. 2009). Due to the wide range of weed management practices and strategies currently available for soybean producers, a final objective of this research is to evaluate the use of herbicide programs for the management of waterhemp and palmer amaranth in non-transgenic, glufosinate- and glyphosate- resistant soybean systems.

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Chapter II

A Distribution Survey of Glyphosate-Resistant Waterhemp in Missouri Soybean Fields With a Focus on In-Field Prediction of Future Resistance

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Abstract

A survey of weedy soybean fields in Missouri with waterhemp infestations was conducted just prior to harvest in 2008 and 2009 to determine the frequency and distribution of glyphosate-resistant waterhemp, and determine if there is any in-field parameters that may serve as indicators of glyphosate-resistance in future crop production systems. Glyphosate-resistance was confirmed in 99 out of 144 (69%) of the total waterhemp populations sampled. These resistant populations occurred across 41 counties. Based on the data collected from each sampling site, soybean fields with confirmed glyphosate-resistant waterhemp were 83% more likely to be free of other weed species, were 89% more likely to occur where soybeans were continuously cropped, were 87% more likely to occur where glyphosate was the only herbicide applied for several seasons consecutively, and were 80% more likely to show obvious signs of surviving herbicide treatment compared to fields characterized with glyphosate-susceptible waterhemp. Therefore we suggest that these four site parameters, and certain combinations of these parameters, can serve as indicators of glyphosate-resistance in future waterhemp populations.

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Introduction

In Missouri, the most common pigweed species encountered in corn and soybean production systems are common waterhemp *(Amaranthus rudis* Sauer) or tall waterhemp *(Amaranthus tuberculatus* (Moq.) Sauer.) Due to the high degree of genetic similarity and hybridization between these two, many botanists now group them into one species, known collectively as “waterhemp” (Pratt and Clark 2001). Waterhemp is dioecious, with male and female flowers occuring on separate plants (Sauer 1957). Waterhemp is a summer annual that is native to the central United States with a distribution ranging from Texas north to Canada and east to Maine (Sauer 1957; USDA 2013). Waterhemp is also one of the most common weeds with which Midwest farmers must contend (Nordby et al. 2007).

In a survey conducted in Illinois, the *Amaranthus* species was listed as the number one weed encountered in corn and soybean fields, with 75% of those responses specifically listing waterhemp (Hager and Sprague 2002). Furthermore, the Hager and Sprague (2002) survey identified waterhemp as one of the three most common weeds to escape herbicide treatments in corn and soybeans. Separate field surveys have identified waterhemp as the most common weed observed in Missouri soybean fields (Bradley et al. 2007; Waggoner and Bradley 2011).

The success of this weed can be attributed to its extended period of emergence, rapid growth at high light intensities and temperatures, and prolific seed production (Hartzler et al. 1999; Jha et al. 2008; Massinga et al. 2003; Sauer 1957). Additionally, Steckel and Sprague (2004a) found that waterhemp plants emerging with the crop and allowed to compete season-long reduced soybean yields by 37 to 44%. Hartzler et al. (2004) also found that waterhemp emerging prior to the V4-V5 stage of soybean growth must be controlled in order to prevent soybean yield loss and waterhemp seed production (Hartzler et al. 2004; Steckel and Sprague 2004a). Similar work has been conducted with waterhemp in corn; Cordes et al. (2004) found that season-long interference at densities of 362 plants per m^2 or higher reduced corn yields up to 36 percent. A 10% corn yield loss was observed when lower waterhemp densities of 35 to 82 plants per m^2 were allowed to compete season-long. Steckel and Sprague (2004b) also reported that season long waterhemp interference reduced corn yield by an average of 11 to 74% over three growing seasons.

Worldwide, there are currently 397 resistant weed biotypes within 217 distinct species that are resistant to one or more herbicides (Heap 2013). Biotypes of waterhemp have evolved resistance to six different classes of herbicides that once effectively controlled this species in agricultural row crop settings (Heap 2013). Concurrent with the rapid adoption of glyphosate-resistant crops in the United States has been a corresponding increase in the evolution of glyphosate-resistance in weeds due to the extensive and repeated use of glyphosate. Currently, 24 weed species have been characterized with resistance to glyphosate in the United States; 12 states have documented glyphosate-resistance in waterhemp (Heap 2013).

Diagnosing weed resistance and quantifying the nature, distribution, and extent of resistance requires efficient and effective screening tests (Beckie et al. 2000). Green foxtail (*Setaria viridis* (L.) Beauv.), wild oat (*Avena fatua* L.), horseweed (*Conyza canadensis* (L.) Cronq.) and annual ryegrass (*Lolium rigidum*) are just a few examples of weed species that have been evaluated for herbicide resistance via field surveys (Beckie et al. 2004; Beckie et al. 1999a; Beckie et al. 1999b; Bourgeois et al. 1997; Davis et al. 2008; Owen et al. 2007). Surveys serve to educate producers about the current distribution of a resistant species within a given geographic area and allow for the prediction of the spread of resistant species in future crop production systems (Givens et al. 2009; Johnson and Gibson 2006; Preston et al. 2009; Werth et al. 2008). For example, Davis et al. (2008) determined that 55% of the horseweed populations in Indiana exhitited visual mean control ratings less than 60% and were considered resistant to glyphosate (Davis et al. 2008). In addition, the highest frequencies of glyphosate-resistant horseweed populations were distributed in the southeastern regions of Indiana (Davis et al. 2008). Preemptive educational efforts targeted to producers are necessary as the development of herbicide resistance in weeds may not be a new phenomenon, but there is a perception that resistance is of little importance until it develops on their personal field (Beckie et al. 2008; Nice et al. 2007).

Perhaps more important than confirmation of herbicide-resistance in a weed population is an understanding of the parameters which may lead to the selection of a resistant weed biotype. Within the weed science literature, parameters such as field distribution, the presence of other weed species, tillage type, weed density, crop rotation, field and/or farm size, frequency of herbicide applications, application rate, application timing, location, and site history have been investigated as potential indicators of weed resistance and may provide a better understanding as to the practices more likely to lead to weed resistance (Beckie 2009; Beckie and Reboud 2009; Beckie et al. 2008; Beckie et al. 2004; Bourgeois et al. 1997; Davis et al. 2009; Givens et al. 2009; Johnson and Gibson 2006; Stephenson et al. 1990; Werth et al. 2008). In a multi-year survey of wild oat populations in Canada, resistance to ALS-inhibitors was associated with overuse of the same herbicide mode of action over time (Beckie et al. 2004). Continuous use of herbicides with the same mechanism of action year after year is a leading cause of herbicide resistance evolution (Jasieniuk et al. 1996; Neve et al. 2011). Beckie (2009) and Johnson and Gibson (2006) also determined that large farms (greater than 400 hectares) have a greater likelihood of developing resistant weed species than smaller farms while Stephenson et al. (1990) found that triazine resistance in weed biotypes in Canada was associated with reduced tillage practices and crop monocultures. In Indiana, the most important indicator for predicting glyphosate-resistance in horseweed was an altered plant phenotype as a result of injury from post-emergent glyphosate applications (Davis et al. 2009).

However, all of the existing surveys that have attempted to correlate in-field crop management parameters with the frequency of herbicide resistance were conducted with species other than waterhemp. Therefore, the objectives of this research were to conduct a survey of weedy soybean fields at harvest to determine the distribution of glyphosate-resistant waterhemp in Missouri and determine possible in-field parameters that may serve as indicators of glyphosate-resistance in future crop production systems.

Materials and Methods

Seed collection. Waterhemp seed samples were collected from weedy soybean fields located across the primary corn and soybean production areas in Missouri during 2008 and 2009 (Figure 2.1). Samples were collected for an approximate four-week period prior to soybean harvest each year. At each survey location, approximately 10 to 15 female waterhemp seedheads were harvested from the selected field. The GPS coordinate of the survey location was also recorded, along with the tillage practice, field size, soybean row spacing, previous crop (determined by the residue remaining in the field), visual estimate of waterhemp infestation level, presence of other weed species, and whether the waterhemp population in question showed obvious signs of surviving herbicide treatment (Table 2.1 and 2.2). This was determined by looking for signs of stunting and/or branching in the upper portions of the plants as a result of the loss of the apical meristem. Soon after the field-based component of the survey was conducted, landowners from each survey location were contacted via a phone-based survey to determine the crop rotation and herbicide use history for five years prior to the date of waterhemp sampling. A continuous crop represented soybeans as the only crop in the history of the field for three or more continuous years, whereas a crop rotation represented a corn to soybean, corn to wheat to soybeans, or fallow to soybean rotation at least once during the five-year time period (Table 2.1 and 2.2). Herbicide use history was determined in a similar manner with 'glyphosate-only' representing glyphosate as the sole herbicide utilized in the field for three or more years and 'rotate modes of action' representing rotating to at least one other herbicide mode of action other than glyphosate between crop years (Table 2.1 and 2.2).

Glyphosate screening. Mature seed were gleaned from the waterhemp seedheads and combined into a collective sample representative of the waterhemp population from each field in question and designated with a code according to the location harvested. A known susceptible population from Missouri was also included for comparison. Approximately 0.25 g of seed from each population was broadcast into 25- by 50-cm greenhouse flats containing a 3:1 mixture of commercial potting medium to sand. This same mixture was used to cover the seedbed at a thickness of approximately 6 mm. After emergence, waterhemp seedlings were thinned to 20 plants per flat. All plants were maintained in a greenhouse at 25 to 30 C, watered and fertilized as needed, and provided with artificial lighting from metal halide lamps (600 µmol photon m^{-2} s^{-1}) simulating a 16-h-photoperiod. Experiments consisted of a completely randomized, 2-factor design (biotypes by treatments) with three replications and were repeated once in time. Treatments consisted of 1.7 kg ae ha^{-1} of glyphosate (represents twice the labeled rate) plus ammonium sulfate (AMS) at 2.9 kg ha^{-1}. Glyphosate was applied to all waterhemp biotypes when plants grew to 12- to 15-cm in height. Applications were made with a compressed air, laboratory spray chamber equipped with a TeeJet 8001EVS nozzle (Teejet Spraying Systems Co, Wheaton, IL) delivering 220 L/ha at 234 kPa. Data collection and analysis consisted of visual counts of surviving plants taken at 3 weeks after treatment (WAT). For the purposes of this research, a waterhemp population was classified as glyphosate-resistant when total plant survival was equal to or greater than 60 percent (Figure 2.2 and 2.3).

Statistical analysis. Once a waterhemp population was characterized as either glyphosate-resistant or susceptible, an analysis of the site parameters leading to the selection of resistant biotypes was conducted using the PROC GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC) using a logit link function and a binomial distribution of the data. To initially determine if a site parameter was an essential indicator for predicting glyphosate resistance, a P-value less than 0.05 for the parameter least square means represented a good predictor of future resistance in a waterhemp population. A site parameter was recognized as a non-essential indicator if the P-value of the least square means was greater than 0.05. After each site parameter was evaluated, the odds (logits) of a waterhemp population being glyphosate-resistant were converted to the percentage of resistance in the population for each indicator within a site parameter. If significant differences occurred in the odds then the probability of resistance was different from each logit ($P \leq 0.05$). If significant differences were not observed, the ratio of glyphosate-susceptible to glyphosate-resistant waterhemp was essentially 1:1 ($P > 0.05$). A good indicator of glyphosate resistance in a future waterhemp population occurred if there was not a 1:1 ratio ($P \leq 0.05$; Table 2.3). However, if both indicators within one site parameter were good indicators ($P \leq 0.05$), then results reverted back to the initial site parameter determination and were evaluated for differences between the least square means to determine if the site parameter on an overall scale was a good indicator of future resistance (Table 2.3).

Results and Discussion

Distribution of Glyphosate-resistant Waterhemp in Missouri. Across both years and all locations, 99 out of 144, or 69% of the total waterhemp populations were classified as resistant to glyphosate (Figures 2.2-2.3). Davis et al. (2008) classified horseweed populations as glyphosate-resistant if visual control ratings were less than 60%. Similarly, in this research, a waterhemp population was classified as glyphosate-resistant when 60% or more of the total number of plants treated with a 2X rate of glyphosate survived treatment. Glyphosate-resistance is characterized in any plant that survives a discriminating dose of glyphosate and are capable of growth and reproduction (Beckie et al. 2000). As illustrated in Figures 2.2 and 2.3, there was a considerable amount of variability in the survival to glyphosate within a given waterhemp population. Much of this variability can be attributed to seed sampling techniques utilized in this survey (Beckie et al. 2000); in this case 10 to 15 female waterhemp plants were sampled per survey location. As waterhemp is dioecious and requires cross-pollination for seed production, a higher level of variability within a given population should be expected (Sauer 1957). This inconsistency in the level of herbicide resistance in waterhemp has also been noted in previous research (Foes et al. 1998; Horak and Peterson 1995).

In this survey, glyphosate-resistant waterhemp populations were distributed across 41 counties in Missouri (Figures 2.1, 2. 2 and 2.3). Few trends were observed when evaluating the distribution of glyphosate-resistant waterhemp within each county across the state of Missouri. The distribution of glyphosate-resistant waterhemp primarily occurred in counties where greater than 4000 hectares were planted in row crops (USDA 2011), but other than this no significant trends in the distribution of glyphosate-resistant waterhemp occurred (Figure 2.1). In contrast to the results of this survey, a state-wide survey of Indiana fields found the greatest percentage of glyphosate resistant horseweed in the southern portion of the state (Davis et al. 2008).

Indicators of Resistance. *Tillage Practices.* Conventional and conservation tillage practices are individually both good indicators of glyphosate resistance in waterhemp, because the likelihood of occurrence of a resistant population is 75 and 64%, respectively, (Table 2.3). Although conventional and conservation tillage practices are good indicators of future resistance, resistant or susceptible populations did not significantly differ between the two tillage methods (P=0.16; Table 2.3). Therefore, as tillage practices are evaluated on an overall scale, tillage is not a good indicator of future resistance as both tillage methods resulted in about the same amount of glyphosate-resistant waterhemp. Although we observed few differences between tillage methods, triazine resistance in broadleaf weed biotypes including common lambsquarters (*Chenopodium album* L.) and Powell amaranth (*Amaranthus powellii* S. Wats.) in Canada was associated with reduced tillage more than conventional tillage systems (Stephenson et al. 1990). In addition, ALS-resistant wild oat was associated with conservation-tillage systems (Beckie et al. 2004). In both surveys, these results suggest that conservation tillage practices may increase the abundance of certain weed species and therefore require greater herbicide use. However, in this research there were no differences in the likelihood of glyphosate resistance in waterhemp as a result of the use of conservation or conventional tillage.

Field Size. In this research, field size was not a good indicator of future glyphosate-resistance in waterhemp (Table 2.3). Although there are differences in the likelihood of resistance when evaluating fields less than 10 and greater than 30 ha, overall the size of the field is not a good indicator of future resistance in waterhemp (P=0.44; Table 2.3). While Johnson and Gibson (2006) indicated that producers farming 800 ha or greater are typically more knowledgeable about herbicide-resistant weeds and their impact towards production costs, a Canadian survey found that producers with field sizes greater than 400 ha have an increased risk of wild oat resistance compared to those whose farm size is smaller (Beckie 2009). Therefore, based on the results of our survey and those of the Canadian and Indiana surveys, it is possible that larger field sizes could be at more risk for herbicide resistance. This risk may result because farmers who manage larger acreages have greater time management pressures that may lead to less adoption of best management practices for herbicide-resistant weeds.

Soybean Row Spacing. Although the 38- and 76-cm soybean row spacings were more likely to contain a glyphosate-resistant waterhemp population, there was only a 9% margin of difference between 19-, 38- and 76-cm row spacings, indicating that this is not a good parameter for predicting future resistance in waterhemp (P=0.66; Table 2.3). However, the wider soybean row spacing may play a small role in the survival of resistant biotypes as other studies suggest weed control is greater in narrow- than in wide-row spacings (Chandler et al. 2001; Knezevic et al. 2003; Légére and Schreiber 1989; Nelson and Renner 1999; Nelson and Renner 1998; Nice et al. 2001; Norris et al. 2002; Patterson et al. 1988; Wax and Pendleton 1968; Yelverton and Coble 1991; Young et al. 2001).

Waterhemp Infestation Level. Waterhemp infestation level within a field was not a good indicator of future glyphosate resistance in waterhemp (P=0.55; Table 2.3). Davis et al. (2009) found that a greater amount of glyphosate-resistant horseweed was more likely as infestation levels surpassed 10% coverage throughout the field. The lack of differences between glyphosate resistance and the infestation levels reported in this survey may be attributed to the wide range of infestation levels encountered in the surveyed locations. For example, five fields contained a 0-1% waterhemp infestation while 41 fields contained a 20-49% waterhemp infestation level (Table 2.1 and 2.2). The distribution of waterhemp throughout the field may have served as a better indicator of future glyphosate resistance than the overall infestation level in the field, but “patchiness” or waterhemp infestation levels in different portions of the fields was not evaluated in this survey. Davis et al. (2009) predicted that if the horseweed distribution was patchy, glyphosate-resistant biotypes were less likely to occur; conversely, when horseweed escapes were more evenly distributed across a field no differences were observed and the level of resistant and susceptible horseweed were predicted at a 1:1 ratio within the field.

Cropping History. Cropping sequence was a good indicator of glyphosate-resistance in waterhemp (P=0.04; Table 2.3). When soybean was grown continuously for at least two years, 89% of the weedy soybean fields surveyed at harvest contained glyphosate-resistant waterhemp (P=0.01; Table 2.3). Alternatively, when some form of crop rotation was utilized, soybean fields that contained waterhemp at harvest were almost equally as likely to contain glyphosate-resistant (45%) or glyphosate-susceptible (55%) waterhemp and therefore a crop rotation, such as a corn-soybean rotation, was not a good indicator of future glyphosate-resistance in waterhemp (P=0.59; Table 2.3). Davis et al. (2009) also found horseweed escapes were more frequent in continuous soybeans than in corn-soybean rotations. Furthermore, triazine resistance in broadleaf weed biotypes including Powell amaranth, common lambsquarters, and common ragweed (*Ambrosia artemisiifolia* L.) in Canada was associated with continuous years of planting the same crop (Stephenson et al. 1990). In addition, previous research has linked ALS-inhibitor resistance in wild oats to a lack of crop rotation diversity (Beckie et al. 2004; Stephenson et al. 1990). In grower perception surveys, failure to rotate crops was also identified as a key factor contributing to the development of herbicide-resistant weed populations (Bourgeois et al. 1997; Johnson and Gibson 2006).

Herbicide Use History. Herbicide use history was also a good indicator of glyphosate resistance (P=0.01; Table 2.3). Glyphosate-resistant waterhemp occurred in 87% of the fields where glyphosate was the only herbicide applied for several seasons consecutively (P=0.01; Table 2.3). Otherwise, when a herbicide rotation with more than one mode of action was utilized, 38% of the weedy soybean fields surveyed at harvest contained glyphosate-resistant waterhemp (P=0.23; Table 2.3). In other words, glyphosate-resistance in waterhemp was 49% more likely to occur in fields where glyphosate was the only herbicide utilized for the previous three or more years. This is supported by a review of the literature which suggests one of the most effective ways to decrease the frequency of resistant biotypes is by implementing herbicide mode of action rotation (Beckie 2011; Jasieniuk et al. 1996; Neve et al. 2011; Norsworthy et al. 2012; Schuster and Smeda 2006; Wise et al. 2009). Additionally, ALS-inhibitor resistance in wild oats was linked to continuous use of herbicides with the same mode of action (Beckie et al. 2004; Stephenson et al. 1990). Similarly, separate grower surveys have identified the long-term use of the same herbicide as an indicator contributing to herbicide resistance (Bourgeois et al. 1997; Johnson and Gibson 2006). Therefore, the continuous use of the same or similar herbicide will select for resistant plants at a much faster rate compared to using herbicide mode of action rotation (Jasieniuk et al. 1996).

Presence of Other Weed Species at Harvest. When waterhemp was the only weed species present in the field, 83% of the weedy soybean fields surveyed at harvest contained glyphosate-resistant waterhemp (P=0.01; Table 2.3). Heavy infestations of a single weed species surviving herbicide treatment provide one of the best indicators of resistance (Beckie et al. 2000). Davis et al. (2009) observed a 3:1 ratio of glyphosate-resistant to glyphosate-susceptible horseweed in Indiana when no additional weeds were present. If a wide variety of weed species are present in the field including waterhemp, the probability of future glyphosate-resistance is not significant (P=0.17; Table 2.3). Similar to our results, Davis et al. (2009) and Westhoven et al. (2008) found that the presence of other weed species was not correlated as an indicator for the evolution of herbicide resistance. Collectively, the absence or presence of other weed species as a site parameter is a good indicator of future glyphosate-resistance (P=0.01; Table 2.3).

This research suggests that if a monoculture of waterhemp develops in the field, a greater likelihood of selection for a resistant waterhemp biotype exists at that location. Herbicide resistance, such as glyphosate-resistance, can either develop *de novo* (neither parent contributed) or be acquired through gene flow (Jasieniuk et al. 1996). A major source observed with resistant biotypes is that of genetic variation or outcrossing; both the resistant and susceptible genotypes will be present within the field and therefore will never be 100% resistant progeny within the progeny of the parent biotypes. Resistance therefore likely occurs in heavy infestations of waterhemp monocultures because of gene mutations and is believed to evolve spontaneously (Jasieniuk et al. 1996; Vencill et al. 2012).

Signs of Herbicide Survival. If the waterhemp showed obvious signs of surviving the herbicide application, such as stunting or significant branching of the upper portions of the plant as a result of the loss of the apical meristem, then 80% of the soybean fields surveyed at harvest contained glyphosate-resistant waterhemp (P=0.01; Table 2.3). Davis et al. (2009) found that horseweed plants showing signs of herbicide injury resulted in a glyphosate-resistant to glyphosate-susceptible horseweed prediction ratio of 2:3. Conversely, if escaped plants were not injured, the horseweed population was 16 times more likely to be a glyphosate-susceptible population. Similarly, in this research, if waterhemp did not show signs of surviving a herbicide application, a low probability of glyphosate resistance likely occurs in future waterhemp populations (P=0.35; Table 2.3).

Overall in this research, the site parameter of waterhemp with signs of herbicide survival was a good indicator of future glyphosate resistance (P=0.01; Table 2.3).

Multi-Factor Comparison. As cropping history, herbicide-use history, and signs of herbicide survival were site parameters that were significant for glyphosate resistance prediction, a three-way analysis of these parameters was also conducted (Table 2.3). Ninety-four percent of the waterhemp populations in weedy soybean fields at harvest were resistant to glyphosate if the field was a) continuously cropped in soybeans for a period of three or more years; b) treated with glyphosate as the sole herbicide utilized for weed control for a period of three or more years; and c) contained waterhemp that showed signs of surviving the previous herbicide application (P=0.01; Table 2.3). If waterhemp does not survive herbicide application and crop and herbicide rotation is utilized, the probability of glyphosate-resistant waterhemp in this survey is reduced by 85% (P=0.03; Table 2.3). The remaining combinations within this multi-factor comparison were not different (P>0.28; Table 2.3). Therefore, this three-way interaction is the best indicator to determine the likelihood of future glyphosate-resistance in waterhemp.

Overall, the results of this survey indicate that 69% of the waterhemp populations sampled in 41 counties in Missouri were confirmed to be resistant to glyphosate. Results also indicate that the in-field parameters of tillage practices, field size, soybean row spacing, and waterhemp infestation level are not good indicators of glyphosate-resistance in waterhemp. Site parameters that will serve as the best indicators of glyphosate-resistance in future waterhemp populations include cropping history, herbicide use history, presence of other weed species, and signs of herbicide survival.

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Table 2.1 Site information for 2008 glyphosate-resistant waterhemp screening.[X]

Sample ID	Size of Field	Waterhemp Biotype	Tillage Practice	Row Spacing	Previous Crop	Waterhemp Survival	Other Weed Species Present at Harvest	Percent Waterhemp in Field	Herbicide History[Y]	Crop History[Y]
Adair 1	16.2	S	Conv	19	Soybean	No	No	20-49%	N/A	Continuous Soybeans
Andrew1	40.5	R	Cons	76	Corn	Yes	Yes	0-1%	N/A	N/A
Andrew2	81	R	Cons	19	Soybean	Yes	No	10-20%	Glyphosate-only	Continuous Soybeans
Atchison1	12.1	R	Cons	38	Soybean	Yes	No	0-1%	Rotate Modes	Crop Rotation
Atchison2	32.4	R	Cons	N/A	Corn	Yes	No	10-20%	Glyphosate-only	Crop Rotation
Audrain1	26.3	S	Cons	38	Corn	No	No	10-20%	Rotate Modes	Crop Rotation
Audrain2	6.1	R	Cons	38	Soybean	Yes	No	20-49%	N/A	N/A
Audrain3	18.2	R	Cons	38	Soybean	Yes	No	50-74%	N/A	N/A
Bates1	40.5	R	Cons	38	Soybean	Yes	Yes	20-49%	N/A	N/A
Boone1	16.2	S	Cons	19	Corn	No	Yes	2-10%	Rotate Modes	Crop Rotation
Boone2	8.9	S	Cons	19	Soybean	No	Yes	20-49%	Glyphosate-only	Continuous Soybeans
Boone3	30.4	S	Cons	38	Corn	Yes	Yes	20-49%	Glyphosate-only	Continuous Soybeans
Buchanan 3c	30.4	R	Cons	38	Soybean	Yes	Yes	2-10%	N/A	N/A
Buchanan1	2	R	Cons	76	Soybean	Yes	Yes	0-1%	N/A	N/A
Buchanan2	18.2	R	Cons	38	Soybean	Yes	Yes	20-49%	N/A	N/A
Callaway1	60.7	R	Conv	38	Soybean	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Callaway2	34.4	R	Conv	38	Soybean	Yes	Yes	20-49%	Glyphosate-only	Continuous Soybeans
Callaway4	56.7	R	Conv	38	Corn	Yes	No	>75%	Rotate Modes	Crop Rotation
Callaway5	26.3	R	Conv	19	Soybean	Yes	Yes	50-74%	Glyphosate-only	Continuous Soybeans
Callaway8	16.2	S	Conv	19	Soybean	Yes	Yes	50-74%	Rotate Modes	Crop Rotation
Carroll1	30.4	R	Conv	38	Soybean	Yes	No	10-20%	Glyphosate-only	Continuous Soybeans
Carroll2	30.4	S	Cons	76	Soybean	Yes	No	20-49%	Rotate Modes	Crop Rotation
Carroll3b	22.3	R	Cons	38	Soybean	No	Yes	0-1%	Glyphosate-only	Continuous Soybeans
Carroll4	16.2	S	Conv	38	Soybean	No	No	>75%	Rotate Modes	Crop Rotation

Carroll5a	27.5	S	Cons	38	Corn	No	Yes	2-10%	Rotate Modes	Crop Rotation
Carroll7	18.2	R	Cons	38	Soybean	Yes	No	10-20%	Glyphosate-only	Continuous Soybeans
Chariton1	28.3	R	Cons	38	Soybean	Yes	Yes	20-49%	N/A	Crop Rotation
Carroll6a	52.6	S	Cons	38	Soybean	No	Yes	20-49%	Rotate Modes	Crop Rotation
Carroll7	18.2	R	Cons	38	Soybean	Yes	No	10-20%	Glyphosate-only	Continuous Soybeans
Chariton2	22.3	R	Cons	76	Soybean	Yes	Yes	50-74%	N/A	Continuous Soybeans
Clark1	28.3	S	Cons	19	Soybean	No	No	20-49%	Rotate Modes	Crop Rotation
Clark2	22.3	R	Conv	38	Soybean	No	Yes	10-20%	N/A	Continuous Soybeans
Clay1a	16.2	S	Cons	19	Wheat	Yes	Yes	20-49%	N/A	N/A
Clinton1	32.4	S	Cons	38	Corn	Yes	No	0-1%	N/A	N/A
Cooper1	27.5	R	Cons	38	Soybean	Yes	No	20-49%	N/A	N/A
Daviess1	24.3	R	Conv	76	Soybean	Yes	Yes	>75%	Glyphosate-only	Crop Rotation
Dekalb1	20.2	R	Cons	76	Soybean	Yes	No	0-1%	Glyphosate-only	Continuous Soybeans
Dekalb2	8.9	R	Cons	76	Soybean	Yes	No	>75%	Glyphosate-only	Continuous Soybeans
Dekalb3	8.1	R	Cons	76	Soybean	Yes	No	>75%	Glyphosate-only	Continuous Soybeans
Gentry1	28.3	R	Cons	19	Soybean	Yes	Yes	20-49%	Rotate Modes	Crop Rotation
Gentry2	30.4	R	Conv	38	Soybean	Yes	Yes	>75%	Glyphosate-only	Continuous Soybeans
Grundy 3a	13.4	S	Cons	76	Soybean	Yes	Yes	20-49%	Rotate Modes	Crop Rotation
Grundy4	26.3	S	Cons	19	Soybean	Yes	Yes	50-74%	N/A	Continuous Soybeans
Grundy5	18.2	S	Cons	76	Corn	Yes	Yes	20-49%	N/A	Crop Rotation
Grundy6	13.4	S	Cons	38	Wheat	No	Yes	50-74%	Rotate Modes	Crop Rotation
Harrison2	18.2	S	Cons	38	Soybean	No	Yes	20-49%	Glyphosate-only	Crop Rotation
Holt1	12.1	R	Cons	76	Soybean	Yes	No	2-10%	Glyphosate-only	Continuous Soybeans
Jackson	8.1	S	Cons	38	Wheat	Yes	Yes	50-74%	Rotate Modes	Crop Rotation
Knox2	76.9	R	Cons	76	Soybean	Yes	Yes	50-74%	N/A	Continuous Soybeans

Knox3a	12.1	R	Conv	38	Soybean	Yes	Yes	50-74%	N/A	N/A
Lafayette1	16.2	S	Cons	51	Wheat	Yes	Yes	20-49%	Rotate Modes	Crop Rotation
Lewis2	20.2	S	Conv	19	Corn	No	Yes	50-74%	Rotate Modes	Crop Rotation
Lewis3	28.3	R	Cons	38	Soybean	Yes	No	50-74%	Glyphosate-only	Continuous Soybeans
Lincoln1	19.4	R	Cons	76	Soybean	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Lincoln2	32.4	R	Conv	76	Soybean	Yes	No	>75%	N/A	N/A
Linn1	24.3	S	Cons	38	Corn	No	Yes	20-49%	Glyphosate-only	Crop Rotation
Linn2	24.3	S	Cons	38	Soybean	Yes	Yes	50-74%	Rotate Modes	Crop Rotation
Livingston6a	32.4	S	Conv	76	Soybean	Yes	Yes	20-49%	Rotate Modes	Crop Rotation
Livingston7	10.1	S	Cons	19	Soybean	No	No	50-74%	Rotate Modes	Crop Rotation
Macon1	32.4	S	Conv	76	Soybean	No	Yes	50-74%	Glyphosate-only	Continuous Soybeans
Macon2	28.3	S	Conv	76	Soybean	No	Yes	50-74%	Rotate Modes	Crop Rotation
Macon3	52.6	S	Conv	38	Soybean	No	No	20-49%	N/A	Continuous Soybeans
Marion1	19.4	S	Cons	76	Corn	No	Yes	20-49%	N/A	Crop Rotation
Mercer1	16.2	S	Cons	76	Soybean	Yes	Yes	20-49%	N/A	N/A
Monroe1	18.2	S	Cons	38	Soybean	Yes	No	10-20%	Glyphosate-only	Continuous Soybeans
Monroe2	26.3	S	Cons	38	Soybean	No	Yes	20-49%	N/A	N/A
Montgomery1	26.3	R	Cons	19	Corn	Yes	Yes	50-74%	Rotate Modes	Crop Rotation
Nodaway1	32.4	R	Cons	76	Soybean	Yes	No	2-10%	Glyphosate-only	Continuous Soybeans
Pettis1	N/A	R	N/A	N/A	N/A	N/A	No	N/A	N/A	N/A
Platte1	26.3	R	Cons	38	Soybean	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Randolph1	28.3	R	Conv	76	Soybean	Yes	Yes	10-20%	Glyphosate-only	Crop Rotation
Randolph2	32.4	S	Cons	36	Wheat	No	Yes	50-74%	Rotate Modes	Crop Rotation
Ray1	0.4	R	Conv	38	Soybean	Yes	No	>75%	Glyphosate-only	Crop Rotation
Ray3a	32.4	R	Cons	38	Wheat	No	Yes	2-10%	N/A	N/A
Ray4	14.2	R	Conv	38	Soybean	Yes	No	50-74%	Glyphosate-only	Continuous Soybeans

Schuyler2	16.2	S	Conv	76	Soybean	No	Yes	2-10%	N/A	Continuous Soybeans
Scotland1	60.7	R	Conv	76	Soybean	Yes	Yes	50-74%	Glyphosate-only	Continuous Soybeans
Scotland2	38.5	S	Cons	19	Soybean	No	Yes	>75%	N/A	N/A
Scott1	16.2	R	Cons	76	Soybean	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Scott2a	32.4	S	Cons	38	Soybean	Yes	Yes	50-74%	N/A	Continuous Soybeans
Scott5b	64.8	R	Cons	38	Soybean	Yes	Yes	50-74%	N/A	N/A
St Charles1	12.1	R	Conv	19	Soybean	Yes	No	50-74%	Glyphosate-only	Continuous Soybeans
St. Clair	24.3	S	Conv	91	Corn	Yes	Yes	50-74%	N/A	Crop Rotation
Warren1	10.1	R	Cons	38	Wheat	Yes	No	2-10%	Rotate Modes	Crop Rotation

[X] Abbreviations within each column: waterhemp biotype-R (Resistant), S (Susceptible); tillage practice-Conv (Conventional), Cons (Conservation); and across all columns-N/A (Not Available).

[Y] Data based on phone based survey, crop and herbicide-use history for five years prior to waterhemp sampling was conducted with continuous soybeans representing soybeans as the only crop for three or more years, and glyphosate-only representing glyphosate as the sole herbicide utilized in the field for three or more years.

Table 2.2 Site information for 2009 glyphosate-resistant waterhemp screening.^{X}

Sample ID	Size of Field	Waterhemp Biotype	Tillage Practice	Row Spacing	Previous Crop	Waterhemp Survival	Other Weed Species Present at Harvest	Percent Waterhemp in Field	Herbicide History^{Y}	Crop History ^{Y}
Andrew 3	60.7	R	N/A	N/A	N/A	N/A	No	10-20%	Glyphosate-only	Continuous Soybeans
Audrain 1	15.8	R	Conv	38	Soybean	Yes	No	20-49%	Glyphosate-only	Crop Rotation
Audrain 2	2.8	R	Cons	38	Soybean	Yes	No	>75%	N/A	N/A
Audrain 3	2.4	R	Cons	38	Soybean	Yes	No	20-49%	Rotate Modes	Crop Rotation
Audrain 4	8.5	R	Cons	38	Soybean	N/A	No	50-74%	Glyphosate-only	Continuous Soybeans
Audrain 5	50.2	R	Conv	38	Soybean	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Barton 1	32.4	R	Conv	76	Wheat	No	No	20-49%	N/A	N/A
Bates 1	8.1	R	Cons	76	Soybean	Yes	Yes	10-20%	Glyphosate-only	Continuous Soybeans
Bates 2	6.1	R	Cons	25	Soybean	Yes	No	50-74%	Glyphosate-only	Continuous Soybeans
Buchanan 1	34.4	R	Conv	38	Soybean	Yes	Yes	50-74%	N/A	N/A
Buchanan 2	20.2	R	Conv	19	Soybean	No	Yes	50-74%	Glyphosate-only	Continuous Soybeans
Cape Girardeau 1	16.2	R	Cons	76	Corn	No	No	20-49%	N/A	N/A
Cape Girardeau 2	28.3	R	Conv	38	N/A	Yes	No	50-74%	N/A	Continuous Soybeans
Cass 2	36.4	R	Conv	19	N/A	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Chariton 1	8.1	R	Cons	76	Corn	N/A	No	N/A	Rotate Modes	Crop Rotation
Chariton 2	37.2	R	Cons	19	Corn	N/A	Yes	N/A	N/A	N/A
Clark 1	32.4	R	Cons	76	Soybean	No	No	20-49%	N/A	Continuous Soybeans
Clark 2	16.2	R	Cons	38	Corn	Yes	No	20-49%	Rotate Modes	Crop Rotation
Clark 3	36.4	R	Conv	38	Corn	No	No	50-74%	N/A	Crop Rotation
Cole 1	68	R	Cons	19	Corn	No	Yes	0-1%	N/A	N/A
Cooper 1	19	R	Cons	38	Soybean	Yes	Yes	0-1%	Glyphosate-only	Continuous Soybeans
Cooper 2	12.1	R	Cons	38	Corn	Yes	Yes	0-1%	Rotate Modes	Crop Rotation
Gentry 1	28.3	R	Cons	76	Corn	No	No	20-49%	Rotate Modes	Crop Rotation

Holt 1	9.7	R	Conv	76	Soybean	Yes	Yes	20-49%	N/A	N/A
Jasper 1	14.2	R	Conv	38	N/A	Yes	Yes	50-74%	N/A	N/A
Jasper 2	40.5	S	Conv	38	N/A	Yes	Yes	50-74%	N/A	N/A
Johnson 1	8.9	R	Cons	76	Soybean	Yes	Yes	20-49%	Rotate Modes	Crop Rotation
Johnson 3	10.1	R	Cons	76	Soybean	No	No	20-49%	N/A	N/A
Johnson 4	24.3	R	Cons	76	Soybean	Yes	Yes	20-49%	Glyphosate-only	Continuous Soybeans
Knox 1	40.5	R	Conv	38	N/A	Yes	Yes	>75%	N/A	Continuous Soybeans
Layfayette 1	32.4	R	Conv	19	Wheat	Yes	Yes	50-74%	Rotate Modes	Crop Rotation
Lewis 1	16.2	R	Conv	38	Soybean	No	No	20-49%	N/A	Crop Rotation
Lincoln 2	4	R	Conv	38	N/A	Yes	Yes	>75%	Rotate Modes	Crop Rotation
Monroe 1	20.2	R	Cons	38	Corn	Yes	No	50-74%	N/A	N/A
Montgomery 1	N/A	R	N/A	N/A	N/A	N/A	No	N/A	Rotate Modes	Crop Rotation
Montgomery 2	N/A	R	N/A	N/A	N/A	N/A	No	N/A	Glyphosate-only	Continuous Soybeans
Nodaway 1	30.4	R	Conv	76	Soybean	No	No	10-20%	Glyphosate-only	Continuous Soybeans
Nodaway 2	16.2	R	Conv	76	Soybean	Yes	No	20-49%	Glyphosate-only	Continuous Soybeans
Nodaway 3	26.3	R	Cons	38	Soybean	Yes	Yes	20-49%	Glyphosate-only	Continuous Soybeans
Perry 1	32.4	R	Conv	38	N/A	Yes	No	20-49%	N/A	N/A
Perry 2	40.5	R	Conv	38	N/A	Yes	Yes	50-74%	N/A	N/A
Perry 3	32.4	R	Conv	38	N/A	No	No	50-74%	N/A	Continuous Soybeans
Perry 4	24.3	S	Conv	38	N/A	No	Yes	>75%	N/A	Crop Rotation
Pike 1	2	S	Conv	38	N/A	Yes	No	>75%	Rotate Modes	Crop Rotation
Platte 1	16.2	R	Conv	38	Soybean	No	Yes	20-49%	Glyphosate-only	Continuous Soybeans
Ralls 1	16.2	R	Conv	38	N/A	Yes	Yes	50-74%	Rotate Modes	Crop Rotation
Randolph 2	20.2	S	Cons	76	Soybean	N/A	Yes	N/A	Rotate Modes	Crop Rotation
Scott 1	32.4	S	Conv	76	N/A	No	No	20-49%	Rotate Modes	Crop Rotation

Shelby 1	16.2	R	Conv	18	N/A	Yes	No	50-74%	Glyphosate-only	Continuous Soybeans
Shelby 2	4.9	R	Conv	18	N/A	Yes	No	50-74%	N/A	Continuous Soybeans
Shelby 3	20.2	R	Conv	38	N/A	Yes	Yes	>75%	N/A	Crop Rotation
St. Clair 1	20.2	R	Conv	38	N/A	No	No	10-20%	N/A	N/A
St. Clair 2	28.3	R	Cons	19	Wheat	Yes	Yes	10-20%	N/A	N/A
Warren2	34.4	R	Conv	76	Soybean	No	Yes	50-74%	Glyphosate-only	Continuous Soybeans

[X] Abbreviations within each column: waterhemp biotype-R (Resistant), S (Susceptible); tillage practice-Conv (Conventional), Cons (Conservation); and across all columns-N/A (Not Available).

[Y] Data based on phone based survey, crop and herbicide-use history for five years prior to waterhemp sampling was conducted with continuous soybeans representing soybeans as the only crop for three or more years, and glyphosate-only representing glyphosate as the sole herbicide utilized in the field for three or more years.

Table 2.3 Influence of various factors to indicate the probability of future glyphosate-resistant waterhemp (AMATA) in soybeans.

	AMATA Population	
Site parameter	Resistant [a]	P-Value [b]
	---% likely---	
Tillage practices		0.16
Conventional	75	**0.01**
Conservation	64	**0.01**
Field size		0.44
0 to 10 ha	79	**0.01**
10 to 20 ha	63	0.09
20 to 30 ha	64	0.12
>30 ha	74	**0.01**
Soybean row spacing		0.66
19 cm	63	0.15
38 cm	72	**0.01**
76 cm	70	**0.01**
Waterhemp infestation level		0.55
0-1 %	88	**0.05**
2-10 %	67	0.33
10-20 %	86	**0.02**
20-49 %	63	0.08
50-74 %	66	**0.05**
>75 %	69	0.15
Cropping History		**0.04**
Continuous soybean	89	**0.01**
Crop rotation	45	0.59
Herbicide Use History		**0.01**
Glyphosate-only	87	**0.01**
Rotate modes of action	38	0.23
Presence of other weed species at harvest		**0.01**
No	83	**0.01**
Yes	58	0.17

Signs of herbicide survival		**0.01**
No	42	0.35
Yes	80	**0.01**
Multi-factor comparison		-
No survival, no crop and herbicide rotation	71	0.28
No survival, yes crop and no herbicide rotation	0	0.98
No survival, yes crop and herbicide rotation	7	**0.02**
Yes survival, no crop and herbicide rotation	94	**0.01**
Yes survival, yes crop and no herbicide rotation	99	0.97
Yes survival, yes crop and herbicide rotation	60	0.37

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[a] Probability of resistance=odds/(1+odds); all odds converted to percentage of resistance in population; if an analysis is significantly different in the logits then probability of resistance to susceptible were different from each other.

[b] Probability values are reported for the indicators and in bold when significant effects were observed; P-value= if $P>0.05$ the site parameter is not an essential indicator or the ratio of resistance to susceptible is equal to a 1:1 ratio; if $P<0.05$ then ratio is not equal to a 1:1 ratio.

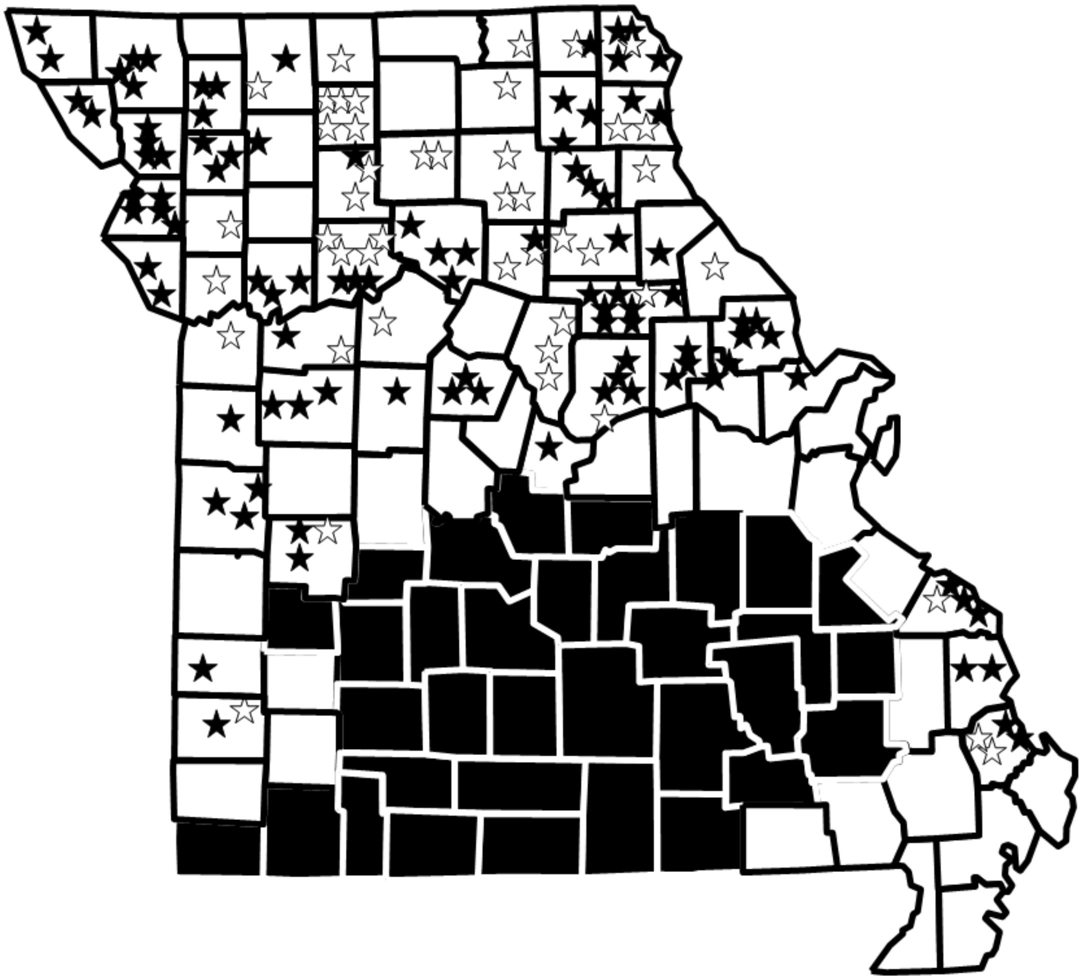

Figure 2.1 Distribution of glyphosate-resistant waterhemp in the 2008 and 2009 Missouri soybean survey. Black stars represent glyphosate-resistant populations and white stars represent glyphosate-susceptible populations. Counties highlighted in black represent counties with less than 4000 ha reported annually in row crops and were not surveyed.

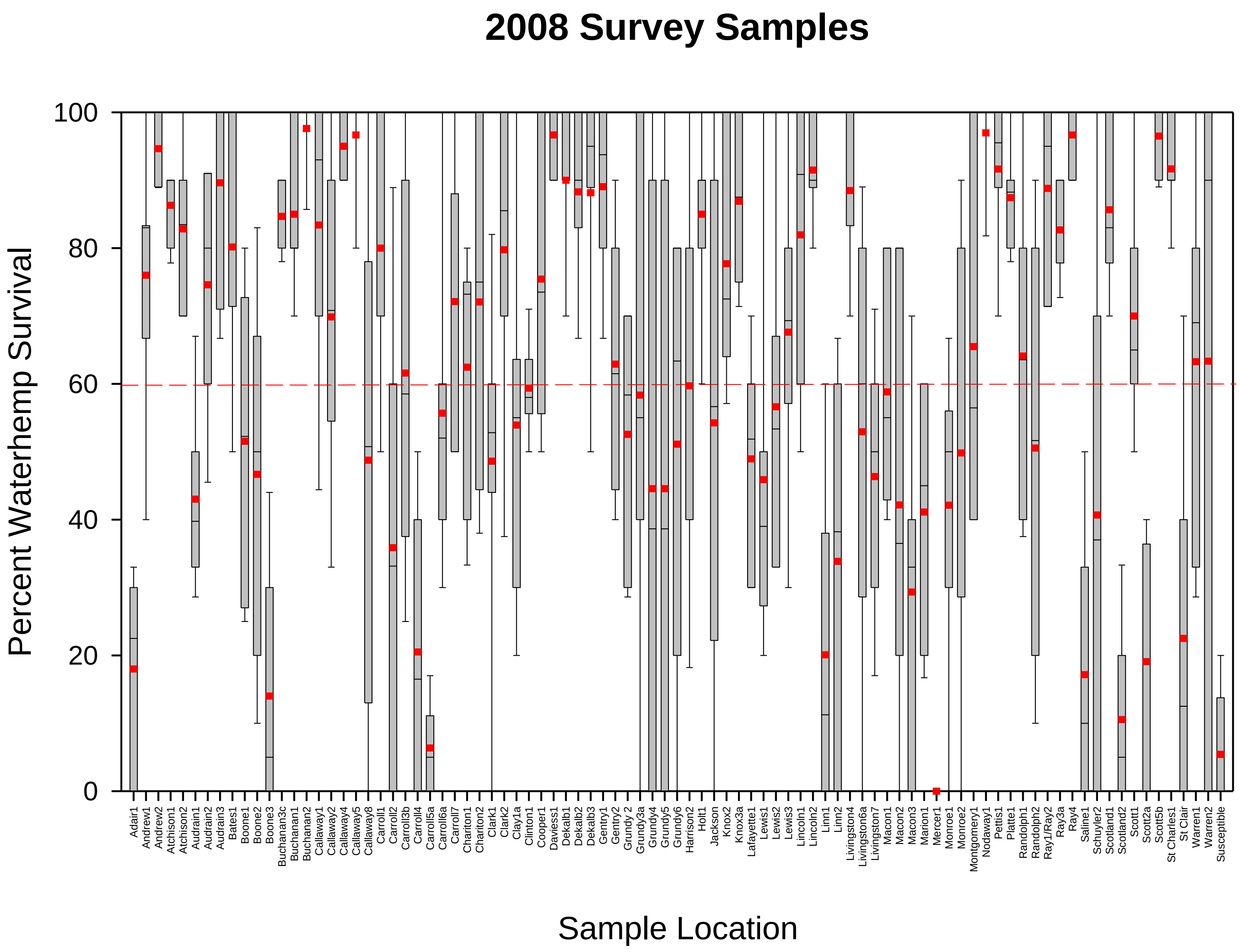

Figure 2.2 Percentage of waterhemp plants surviving 3 weeks after glyphosate application for the 2008 distribution survey. Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines extend to the maxima, median, mean, and minima of the data with the thickest vertical line representing the mean. The dashed line across the figure at 60% is the dividing point used to classify glyphosate resistant (≥60% survival) from glyphosate susceptible (<60% survival) populations. X axis represents waterhemp sample locations across the state of Missouri.

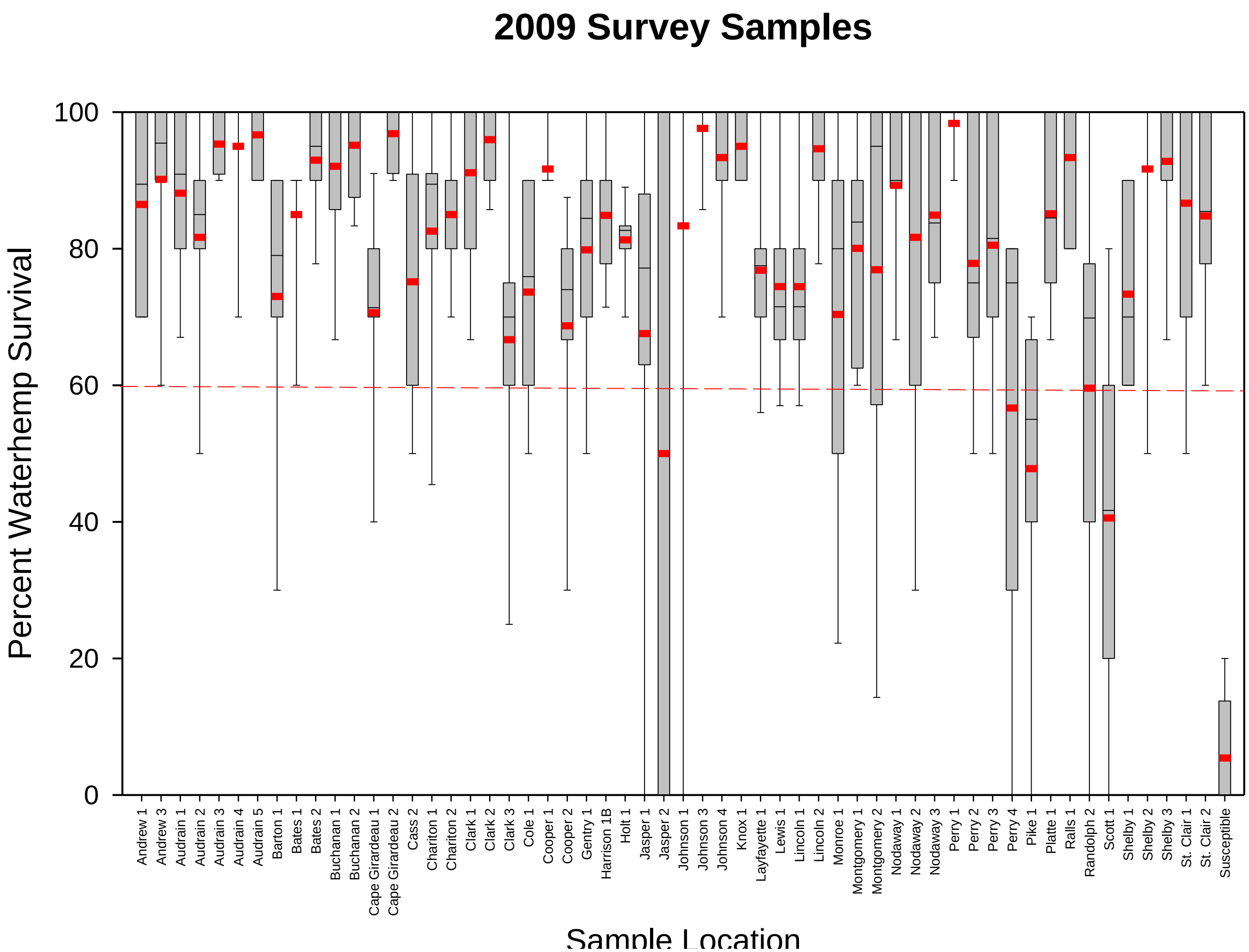

Figure 2.3 Percentage of waterhemp plants surviving 3 weeks after glyphosate application for the 2009 distribution survey. Lower and upper boxes represent the second and third quartiles, respectively. Vertical lines extend to the maxima, median, mean, and minima of the data with the thickest vertical line representing the mean. The dashed line across the figure at 60% survival is the dividing point used to classify glyphosate resistant (≥60% survival) from glyphosate susceptible (<60% survival) populations. X axis represents waterhemp sample locations across the state of Missouri.

Chapter III

Comparison of Weed Control, Yield and Net Income in Conventional, Glyphosate-Resistant, and Glufosinate-Resistant Soybean

Kristin K. Rosenbaum, Raymond E. Massey and Kevin W. Bradley.[1]

Abstract

Separate field experiments were conducted in central and southeast Missouri during 2009 and 2010 to evaluate the effect of preemergence (PRE) and postemergence (POST) herbicide programs on palmer amaranth (*Amaranthus palmeri* S. Wats.) and waterhemp (*Amaranthus rudis* Sauer) control, soybean yield, and net income in conventional, glyphosate-resistant, and glufosinate-resistant soybean (*Glycine max*) production systems. Visual control evaluations ten weeks after emergence (WAE) at the waterhemp site revealed that all PRE-only and PRE fb POST applications provided greater than 92% waterhemp control in either soybean system and at the palmer amaranth site, all PRE-only applications provided greater than 83% palmer amaranth control across soybean systems. Averaged across all herbicide programs at both locations, glufosinate-resistant soybeans provided the highest grain yield and net return followed by glyphosate-resistant and conventional soybean systems. Furthermore, with the exception of the conventional PRE-only program at the waterhemp site, all glyphosate-resistant soybean herbicide programs provided greater net return than all conventional soybean system herbicide programs. Collectively, the results from both trials indicate that programs

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containing PRE herbicide treatments provide the best opportunity for season-long control of waterhemp and palmer amaranth and highest grain yields and net returns in conventional, glyphosate-resistant, or glufosinate-resistant soybean systems. The results from these experiments also suggest that palmer amaranth is a more competitive and difficult species to control than waterhemp, regardless of the soybean system or herbicide program.

Introduction

Palmer amaranth and waterhemp, both members of the *Amaranthus* genus, are two of the most troublesome agronomic weeds in the United States due to their extended period of emergence, rapid growth at high light intensities and temperatures, and prolific seed production (Hartzler et al. 1999, Jha et al. 2008, Massinga et al. 2003, Sauer 1957). In Missouri, the most common pigweed species encountered in corn and soybean production are common or tall waterhemp. Due to the high degree of genetic similarity and hybridization between these two, many botanists now group them into one species, now referred to simply as “waterhemp” (Pratt and Clark 2001). Palmer amaranth and waterhemp can cause significant yield reductions in corn (*Zea mays* L.), soybean (*Glycine max* [L.] Merr.) and cotton (*Gossypium hirsutum* L.) (Klingamen and Oliver 1994, Massinga et al. 2003, Murphy et al. 1996). Palmer amaranth has reduced corn yields from 11 to 91% at densities of 0.5 to 8 plants per meter of row, reduced soybean yield from 17 to 79% at densities of 0.33 to 10 plants per meter of row, and reduced cotton lint yield by 6 to 28% at densities of 1 plant per 1 to 3 meter of row (Bensch et al. 2003, Klingamen and Oliver 1994, Massinga and Currie 2002, Massinga et al. 2003, Smith et al. 2000). Additionally, soybean biomass and yield has been reduced when growing within 50- and 25-cm of palmer amaranth, respectively (Monks and Oliver 1988). Soybean yield reductions are correlated to palmer amaranth biomass and density; as palmer amaranth densities and biomass increase, soybean yield will reduce linearly (Klingamen et al. 1994). Waterhemp plants that emerged with the crop and were allowed to compete season-long reduced soybean yield by 37 to 44% (Steckel and Sprague 2004a). Waterhemp that emerges prior to the V4-V5 stage of soybean growth must be controlled in order to prevent soybean yield loss and waterhemp seed production (Hartzler et al. 2004, Steckel and Sprague 2004a). Similar work has been conducted with waterhemp populations in corn; season long interference at densities of 362 or more waterhemp plants per m^2 reduced corn yield up to 36% (Cordes et al. 2004). In addition, if high densities of waterhemp were not controlled by the time plants reached 15-cm in height, corn yield reductions of up to 15% occurred (Cordes et al. 2004). A 10% corn yield loss was observed when lower waterhemp densities of 35 to 82 plants per m^2 were allowed to compete season-long (Cordes et al. 2004). Steckel and Sprague (2004b) found that season-long waterhemp interference reduced corn yield by 11 to 74% over three growing seasons.

The most common method of weed removal in agronomic cropping systems is the application of single or mixtures of selective herbicides. However, sole dependency on any one herbicide and/or herbicide-resistant crop technology has led to the selection of palmer amaranth and waterhemp biotypes with resistance to herbicides that once effectively controlled these species (Heap 2013). One of the most effective ways to reduce the selection of herbicide-resistant biotypes is by implementing crop and herbicide mode-of-action rotation with cultural practices over multiple years (Jasieniuk et al. 1996, Schuster and Smeda 2006, Wise et al. 2009). There are three soybean systems currently available in the marketplace that could be integrated into a crop rotation over multiple years; these include glyphosate-resistant (Roundup Ready), glufosinate-resistant (Liberty Link) and non-transgenic, or conventional soybean cultivars. Glyphosate-resistant soybean was the first glyphosate-resistant crop to be commercially introduced in the United States in 1996. After their introduction, glyphosate-resistant soybeans were rapidly adopted. In 1997, 17% of the soybean acreage in the U.S. was planted with glyphosate-resistant varieties; by 2012, the adoption of herbicide-resistant soybean in the U.S. had increased to 93%, the vast availability of which were glyphosate-resistant varieties (USDA 2012). The increase in glyphosate-resistant soybean acreage has been attributed to the fact that glyphosate is an economical and convenient broad spectrum herbicide when compared to other herbicide options (Dill 2005). Glufosinate-resistant soybeans were first introduced in 1999 on a limited basis (Wiesbrook et al. 2001) and were then fully commercialized and released in 2009 (Fortune 2010). Few studies have been conducted to evaluate the economics of herbicide-resistant and conventional soybean systems. Reddy and Whiting (2000) evaluated the economics of a glyphosate-resistant soybean system in which glyphosate was used as the only herbicide for weed control compared to a sulfonylurea-resistant and conventional soybean system. Results from this study indicated that net returns from the glyphosate-resistant system were $136 and $91/ha higher than the sulfonylurea-resistant and conventional soybean systems, respectively. However, the cost of glyphosate-resistant soybean seed has increased and glyphosate herbicide has decreased in recent years and a more recent comparison of net returns across the different herbicide programs and soybean systems is needed. In addition, few studies have compared weed management systems in glyphosate-resistant, glufosinate-resistant, and conventional soybean cropping systems. In one study, sequential applications of glufosinate improved control over single applications, while in comparison sequential applications of glyphosate generally provided no advantages over single applications (Holshouser et al. 2009, Wiesbrook et al. 2001). Several conventional soybean herbicide programs can be comparable to transgenic programs, however conventional herbicides must be applied to small weeds (2 to 7 cm in height) to obtain acceptable control (Holshouser et al. 2009). Similar to conventional soybeans, it is necessary to apply glufosinate-based herbicides when weeds are small (7 to 8 cm in height) to obtain optimum control (Holshouser et al. 2009).

The objectives of this study were to: (i) determine which herbicide program provides the most effective, season-long weed control and maximize soybean seed yield in conventional, glyphosate-resistant, and glufosinate-resistant soybean systems; and (ii) compare net income in response to common herbicide programs and determine which soybean system and herbicide program provide the greatest income to soybean producers.

Materials and Methods

Herbicide Programs for the Management of Waterhemp and Palmer Amaranth in Soybeans Systems.

Field experiments were conducted at two locations during the summers of 2009 and 2010. The sites were located in Callaway County in central Missouri (38°39’ 57.02”N, 91°52’ 28.51”W; ‘waterhemp site’) and at the Delta Research Center in Pemiscot County in southeast Missouri (36°23’ 26.49”N, 89°36’ 32.31”W; ‘palmer amaranth site’). Both sites were selected based on the presence of dense infestations of waterhemp at the central location (184 plants per m^2) and palmer amaranth at the southeast location (199 plants per m^2). The soil type at the waterhemp site was a Hodge fine sand (loamy substratum, Mixed, mesic Typic Udipsamments) and contained 1.4% organic matter with a pH of 6.8. The soil type at the southeast location was a Dundee silt (loam Fine-silty, mixed, active, thermic Typic Endoaqualfs) and contained 1.3% organic matter with a pH of 5.6. At both locations, 'Schillinger 388TC'-conventional, 'Asgrow AG3803' glyphosate-resistant, and 'MBS Genetics ML3963N' glufosinate-resistant soybeans were planted into a conventionally-tilled seedbed in rows spaced 76-cm apart at a seeding rate of 370,500 seeds ha^{-1}. Planting dates were on May 12, 2009 and May 6, 2010 at the waterhemp site and May 20, 2009 and May 25, 2010 at the palmer amaranth site. In all experiments, the experimental design was a randomized complete block with 18 treatments. All plots were 2 by 9 m and replicated 6 times.

The herbicide programs evaluated in these experiments consisted of a preemergence only (PRE), a preemergence followed by a postemergence (PRE fb POST), a two-pass postemergence application (2-pass POST), a one-pass postemergence application with a residual herbicide (1-pass POST W/Res), and a one-pass postemergence application (1-pass POST). A non-treated control was included for comparison. POST applications in the 1-pass POST herbicide program were made when waterhemp and palmer amaranth averaged 25-cm in height, while POST applications in the remaining herbicide programs were made when the average height of waterhemp or palmer amaranth was between 10- and 15-cm. Individual treatments within each soybean system are listed in Table 3.1, herbicide application dates are listed in Tables 3.2, and monthly rainfall and average monthly temperatures are listed in Table 3.3. All herbicide applications were made with a CO_2 pressured backpack sprayer set to deliver 140 L ha^{-1} with XR8002 flat fan nozzles at a speed of 5 km h^{-1}. To prevent herbicide drift across the different soybean systems, 1 x 2 m tarps were held on each side of the spray boom during herbicide application.

Treatment Evaluation and Data Collection

Visual waterhemp or palmer amaranth control and soybean injury were evaluated in response to each herbicide treatment. Visual evaluations were taken at two week intervals up to 10 weeks after soybean emergence. Visual ratings were based on a scale of 0 to 100%, with 0 equal to the plant vigor and ground cover observed in the non-treated control or no soybean injury, and 100 equal to complete weed control or complete soybean death (Table 3.4). In both years, soybeans were harvested from the center two rows in each plot with a small plot combine and yields adjusted to 13% moisture content.

Economic and Statistical Analysis

The net return in response to each herbicide program and soybean system was calculated by subtracting the estimated treatment costs from gross income. Gross income was determined in \$/ha by multiplying the soybean yield from each treatment by an average soybean price of \$0.42/kg. This is the average soybean price for the 2010-2011 crop year provided by the Food and Agricultural Research Policy Institute (University of Missouri, Columbia, MO). The cost of each herbicide treatment was calculated from the 2009 wholesale price sheet of herbicides and adjuvants provided by a major agricultural retailer in the Midwest and also included technology fees and seed costs associated with each soybean variety. A custom application fee of \$12.35/ha was also included for each herbicide application made within a herbicide program (Plain et al. 2009). A partial budget was conducted to evaluate specific changes in the producers operation including soybean systems and herbicide programs. The partial budget system analyzes alternatives to an industry standard and determines if a change from the industry standard will increase, decrease, or not change the net income. Therefore, a partial budget compares the positive and negative effects of the proposed change (alternative) on net income. In this type of budgeting system, only the affected costs and revenues are included in the analysis and non-affected operating costs are presumed as fixed costs and are not included in the final net return. In this study, glyphosate-resistant soybeans with a 2-pass POST glyphosate program were utilized as the industry standard. This standard was selected based upon USDA-ERS data, which indicate 93% of all soybeans planted in 2009-2010 were genetically modified and the total number of times a field was treated with a herbicide was 2.1 (USDA 2012). Visual weed control, soybean injury and soybean yield data were subjected to analysis of variance using SAS PROC MIXED (SAS Institute Inc., Cary, NC). Fixed effects included site, year, soybean system, and herbicide program, and replication within year was utilized as the random effect. Data were combined over years but not site due to different weed species responses; site means are presented separately. Means were separated using Fisher's Protected LSD at $P \leq 0.05$. Data were reported with non-transformed means.

Results and Discussion

Soybean Injury in Response to Various Herbicide Programs in Three Soybeans Systems

Across all sites, the highest level of soybean injury was observed in the conventional soybean system where at least one POST herbicide application occurred

(Table 3.4). Soybean injury 6WAE ranged from 7 to 25% with all conventional herbicide programs that contained a POST treatment and was greater than the injury observed in the glufosinate- and glyphosate-resistant soybean systems in almost every instance (Table 3.4). The soybean injury observed in the POST herbicide programs within the conventional soybean system is consistent with previous studies, as the soil residual with many conventional POST herbicide products will lead to elevated crop injury (Nolte and Young 2002, Shaw et al. 2001). Although glyphosate- and glufosinate-resistant soybeans have high levels of tolerance to glyphosate and glufosinate, respectively, Nolte and Young (2002) also reported that the inclusion of a residual herbicide with POST herbicide applications in these systems could result in a higher level of soybean injury with some varieties (Nolte and Young 2002). Soon after application, higher levels of soybean injury were observed in response to the 1-pass POST w/Res herbicide programs but in all instances the soybeans recovered within a 2-week time period (data not shown). Less than 15% soybean injury was reported across all other soybean systems and herbicide treatments evaluated at both locations. PRE fb POST and 2-pass POST herbicide programs in both the glyphosate- and glufosinate-resistant soybean systems resulted in the lowest levels of visual soybean injury at the waterhemp site while few trends were observed at the palmer amaranth site (Table 3.4).

Waterhemp and Palmer Amaranth Control with Herbicide Programs and Soybean Systems

Across both locations, herbicide programs that included PRE herbicide applications generally provided highest levels of waterhemp and palmer amaranth control 10 WAE (Table 3.5). At the waterhemp site, other herbicide programs such as the 2-pass

POST program with glufosinate-resistant soybeans provided similar waterhemp control as herbicide programs that included PRE-only and PRE fb POST for all three soybean systems 10 WAE (Table 3.5). Additionally, palmer amaranth control with the 2-pass POST program for either glufosinate-resistant or glyphosate-resistant soybeans at the palmer amaranth site was similar to the palmer amaranth control observed in the PRE-only herbicide program. Greater control of weed species including waterhemp and ivyleaf morningglory (*Ipomoea hederacea* Jacq.) with sequential applications of glufosinate or glyphosate compared to single applications has also been reported in previous research (Bradley et al. 1998, Johnson et al. 1998, Nolte and Young 2002, Young and Kapusta 1998). It is also important to note that neither of these research locations contained glyphosate-resistant populations of waterhemp or palmer amaranth. The most notable weed with glyphosate resistance in Missouri is waterhemp (Rosenbaum et al. 2012). Therefore, the presence of glyphosate-resistant weed biotypes at these research locations would have influenced the results of the 2-pass POST program considerably. The absence of glyphosate resistant weed biotypes is specifically noted at the palmer amaranth site in the glyphosate-resistant soybean system as all herbicide programs provided similar levels (>82%) of palmer amaranth control (Table 3.5).

Within each soybean system at the waterhemp site, a lower level of waterhemp control was observed with the 1-pass POST program and these results are supported by previous research (Nolte and Young 2002) (Table 3.5). At the palmer amaranth site, the 1-pass POST and 1-pass POST W/Res program provided similar palmer amaranth control in the glufosinate-resistant soybean system. In conventional soybeans, a 1-pass POST and 2-pass POST program provided similar and poor levels of palmer amaranth control.

With the exception of conventional soybeans at the palmer amaranth site, the 1-pass POST herbicide program at both research locations provided the lowest levels of weed control for each soybean system (Table 3.5). These data correspond with previous research suggesting the most successful weed control is achieved when smaller weeds (10 cm or less) are targeted with herbicides (Holshouser et al. 2009). Furthermore, conventional soybean systems that included a POST herbicide provided less than 70% palmer amaranth control (Table 3.5). The results from this research indicate that palmer amaranth is harder to control than waterhemp, and particularly difficult to control in conventional soybean systems.

Soybean Yield Response to Herbicide Programs and Soybeans Systems

Averaged across all herbicide programs and research locations, glufosinate-resistant soybeans yielded higher (2544 kg/ha) than glyphosate-resistant (2370 kg/ha) and conventional soybeans (1961 kg/ha), respectively (P=0.001; Table 3.6). Within each soybean system at the waterhemp site, all herbicide programs resulted in similar soybean yields. At the palmer amaranth site, conventional soybeans that included a PRE herbicide in the program yielded highest and were different from the POST-only herbicide programs. In fact, POST-only programs in conventional soybeans resulted in soybean yields similar to the non-treated control at the palmer amaranth site. Regardless of the location, the lower yields in the conventional system are likely due to the poor weed control of waterhemp or palmer amaranth due to the herbicide programs used in this system (Table 3.5). In the glufosinate-resistant soybean system at the palmer amaranth site, soybean yields were similar for all herbicide programs except the 1-pass POST herbicide program which resulted in lower yields. This response was also likely related to the poor control of palmer amaranth provided by this herbicide program in glufosinate-resistant soybean (Table 3.5). As reported previously, if waterhemp or palmer amaranth density and biomass increase throughout the growing season, soybean yield will be reduced (Cordes et al. 2004, Klingamen et al. 1994). Glyphosate-resistant soybean yields were similar at the palmer amaranth site in response to PRE-only, PRE fb POST, 1-pass POST W/Res, and 1 pass-POST herbicide programs (Table 3.6). However, glyphosate-resistant soybean yields were highest with the 2-pass POST herbicide program at the palmer amaranth site, and the PRE-only herbicide program was the only other herbicide program that resulted in similar soybean yield (Table 3.6). Across soybean systems the non-treated control provided similar soybean yield, and in the case of the glufosinate-resistant and glyphosate-resistant soybean systems, soybean yield in the non-treated control were lower than all other herbicide programs. When averaged across soybean systems and locations, PRE-only herbicide programs provided the greatest soybean yield (2532 kg/ha) followed by 2-pass POST (2464 kg/ha), PRE fb POST (2454 kg/ha), 1-pass POST w/ Res (2398 kg/ha), and 1-pass POST (2264 kg/ha) (P=0.001; Table 3.6).

Net Returns in Response to Various Herbicide Programs in Three Soybeans Systems

The positive or negative alternative treatment differences calculated from the industry standard net return is reported for each soybean system and herbicide program in Table 3.7. The net return of the industry standard was $1,026.80 per ha at the waterhemp site (Table 3.7). At the waterhemp site, the partial budget analysis net return gain or loss ranged from $(-300.72) in the 2-pass POST conventional soybean to $183.60 in the 1-pass POST glufosinate-resistant soybean when compared to the industry standard net income (Table 3.7). At the waterhemp site, all conventional soybean herbicide programs provided a negative net return which is likely due to lower grain yield compared to other soybean systems (Table 3.6) and/or reduced waterhemp control (Table 3.5). Positive net gains were observed in response to all glufosinate-resistant soybean system herbicide programs at the waterhemp site. In addition, the PRE-only herbicide program was the only program that resulted in a negative net return in the glyphosate-resistant soybean system. Across these two soybean systems, greater net return was observed in the glufosinate-resistant soybean system as compared to the glyphosate-resistant system. This can be explained by the fact that glufosinate-resistant soybean seed cost less than glyphosate-resistant seed, and that glufosinate-resistant soybean yielded higher than the glyphosate-resistant soybean (Table 3.6).

The net return of the industry standard was $803.52 per ha at the palmer amaranth site (Table 3.7). At the palmer amaranth site, the partial budget analysis resulted in a negative net return regardless of the soybean system or herbicide program when compared to the 2-pass POST glyphosate-resistant soybean industry standard. Net loss ranged from $-35.07 in the 2-pass POST with glufosinate-resistant soybean to $-496.10 in the 2-pass POST conventional soybean system (Table 3.7). It is important to note that this occurred at a site without glyphosate-resistant palmer amaranth and this would not likely occur in a location where glyphosate-resistant palmer amaranth is present. The net decrease observed across all soybean systems and herbicide programs at the palmer amaranth site can be partially attributed to the differences in soil composition, weed control (Table 3.5), and yield as compared to the industry standard at the waterhemp site (Table 3.6). Soil texture at the waterhemp site was more coarse and sandy compared with the palmer amaranth site which had a higher clay content; therefore the high clay texture at the palmer amaranth site may have a greater potential for herbicide sorptions leading to decreased herbicide activity (Curran 1998). Additionally, the palmer amaranth site received less rainfall during the 2010 growing season (Table 3.3), which contributed to lower soybean yields in both years compared to the waterhemp site (Table 3.6). Rainfall is essential for not only influencing soybean yield but also herbicide activation. Drought-stressed weeds are more difficult to control with POST herbicide applications because of reduced herbicide absorption and low toxicity activity (Menalled et al. 2001).

Summary

The results from this research indicates that the greatest crop injury, poorest control of waterhemp or palmer amaranth, and lowest crop yields were realized in conventional soybeans where a POST-only herbicide programs were utilized. This study also suggests palmer amaranth is a more competitive and difficult species to control than waterhemp. In general, herbicide programs that contained preemergence herbicides provided the highest level of waterhemp control, and similar palmer amaranth control was observed in both preemergence programs and the 2-pass POST program for glyphosate- and glufosinate-resistant soybean systems. The results from these experiments also indicate that higher net returns can occur in glufosinate-resistant soybean systems, primarily due to lower seed costs and acceptable levels of waterhemp or palmer amaranth control with certain herbicide programs. Evaluating differences between herbicide, seed, and other production costs while simultaneously determining weed control and yield responses enables producers to develop a partial budget. That being said, in this research net return differences were separated by weed control and soybean yields more than treatment costs. Regardless of the research results, incorporation of herbicide programs that use two or more multiple modes of action may reduce the shift to herbicide-resistant weed biotypes. Furthermore, weed control and soybean yield may have been significantly altered if resistant weeds were present at the research sites.

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Table 3.1 Specific herbicide treatments and programs evaluated in the experiments.

Herbicide Program	Treatments Applied in Each Soybean System[x]		
	Conventional	Glyphosate-Resistant	Glufosinate-Resistant
PRE-only[y]	0.18 sulfentrazone + 0.03 choransulam + 0.77 S-metolacholor + 0.17 fomesafen	0.18 sulfentrazone + 0.03 choransulam + 0.77 S-metolacholor + 0.17 fomesafen	0.18 sulfentrazone + 0.03 choransulam + 0.77 S-metolacholor + 0.17 fomesafen
PRE fb POST	0.62 S-metolacholor + 0.13 fomesafen fb 0.20 lactofen + 0.07 clethodim	0.62 S-metolacholor + 0.13 fomesafen fb 0.77 glyphosate	0.62 S-metolacholor + 0.13 fomesafen fb 0.40 glufosinate
2-pass POST	0.16/0.20 lactofen + 0.07 clethodim	0.77 glyphosate fb 0.77 glyphosate	0.40 glufosinate fb 0.40 glufosinate
1-pass POST W/Res	0.20 lactofen + 0.07 clethodim + 0.71 S-metolacholor + 0.16 fomesafen	0.77 glyphosate + 0.71 S-metolachlor+ 0.16 fomesafen	0.40 glufosinate + 0.71 S-metolachlor+ 0.16 fomesafen
1-pass POST	0.20 lactofen + 0.08 clethodim + 0.03 flumiclorac	1.55 glyphosate	0.66 glufosinate

[x] All rates in lb ai or ae/A. Surfactant or adjuvant used at labeled rate.
[y] Abbreviations: PRE=preemergence; POST=postemergence; fb=followed by; W/Res=with residual.

Table 3.2 Dates of herbicide applications at the central (AMATA) and southeast (AMAPA) location in 2009 and 2010.

Herbicide Program	Soybean System											
	Conventional				Glyphosate-Resistant				Glufosinate-Resistant			
	AMATA site[x]		AMAPA site		AMATA site		AMAPA site		AMATA site		AMAPA site	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
PRE-only	5/12	5/6	5/20	5/25	5/12	5/6	5/20	5/25	5/12	5/6	5/20	5/25
PRE fb POST	5/12 fb 7/6	5/6 fb 6/24	5/20 fb 7/9	5/25 fb 7/6	5/12 fb 7/6	5/6 fb 6/24	5/20 fb 7/9	5/25 fb 7/6	5/12 fb 7/6	5/6 fb 6/24	5/20 fb 7/9	5/25 fb 7/6
2-pass POST	6/10 fb 6/30	6/10 fb 6/24	6/18 fb 7/2	6/14 fb 7/6	6/10 fb 6/25	6/10 fb 6/24	6/18 fb 7/16	6/14 fb 7/6	6/10 fb 6/25	6/10 fb 6/24	6/18 fb 7/16	6/14 fb 7/6
1-pass POST W/Res	6/18	6/10	6/18	6/14	6/18	6/10	6/18	6/14	6/18	6/10	6/8	6/14
1-pass POST	6/23	6/16	7/2	6/23	6/23	6/16	7/2	6/23	6/23	6/16	7/2	6/23

[x] Abbreviations: AMATA=waterhemp; AMAPA=palmer amaranth; fb=followed by; PRE=preemergence; POST=postemergence; W/Res=with residual.

Table 3.3 Monthly rainfall (cm) and average monthly temperatures (F) from May through October in 2009 and 2010 in comparison to the 30-yr average[x] at the central (AMATA) and southeast (AMAPA) research sites in Missouri.

Month	Average Monthly Rainfall						Average Monthly Temperature					
	AMATA[y] site			AMAPA site			AMATA site			AMAPA site		
	2009	2010	30 year Avg.	2009	2010	30 year Avg.	2009	2010	30 year Avg.	2009	2010	30 year Avg.
	--------------------------cm--------------------------						--------------------------F--------------------------					
May	18.0	18.4	10.7	15.3	17.9	10.0	63.2	63.4	63.0	67.7	71.3	68.5
June	15.9	7.9	9.5	15.8	3.2	8.6	73.9	73.8	71.8	79.0	82.0	76.9
July	20.4	11.2	9.5	22.2	7.3	7.5	71.7	76.3	76.6	76.4	80.8	80.2
August	11.6	4.8	8.9	12.0	1.1	4.6	70.6	76.5	75.4	75.0	80.8	78.6
September	14.4	15.6	8.6	29.0	4.7	7.4	63.5	65.7	66.8	71.3	67.5	71.3
October	41.9	25.1	8.0	2.0	1.3	9.0	49.3	56.9	55.3	56.0	61.3	60.2

[x] 30 year average (1980-2009) obtained from National Climatic Data Center (2012).

[y] Abbreviations: AMATA=waterhemp; AMAPA=palmer amaranth.

Table 3.4 Influence of herbicide programs in conventional, glyphosate-resistant, and glufosinate-resistant soybean systems on soybean crop injury (2009-2010).

Soybean System	Herbicide Program	Waterhemp	Palmer Amaranth
		----------Crop Injury 6WAE^{x}----------	
Conventional	PRE-only	2	0
	PRE fb POST	10	25
	2-pass POST	24	21
	1-pass POST W/Res	15	7
	1-pass Post	23	14
Glufosinate-Resistant	PRE-only	3	1
	PRE fb POST	1	1
	2-pass POST	1	1
	1-pass POST W/Res	7	2
	1-pass POST	3	7
Glyphosate-Resistant	PRE-only	9	3
	PRE fb POST	2	3
	2-pass POST	4	3
	1-pass POSTW/Res	8	4
	1-pass POST	6	14
LSD (0.05)		4	4

[x] Abbreviations: PRE=preemergence; POST=postemergence; WAE=weeks after emergence; W/Res=with residual.

Table 3.5 Influence of herbicide programs in conventional, glyphosate-resistant, and glufosinate-resistant soybean systems on late-season waterhemp or palmer amaranth control (2009-2010).

Soybean System	Herbicide Program	Waterhemp	Palmer Amaranth
		--------% Weed Control 10WAE[x]--------	
Conventional	PRE-only	99	86
	PRE fb POST	92	68
	2-pass POST	89	26
	1-pass POST W/Res	80	18
	1-pass Post	72	30
Glufosinate-Resistant	PRE-only	99	84
	PRE fb POST	97	81
	2-pass POST	93	86
	1-pass POST W/Res	87	68
	1-pass POST	79	63
Glyphosate-Resistant	PRE-only	99	83
	PRE fb POST	96	92
	2-pass POST	78	90
	1-pass POSTW/Res	74	86
	1-pass POST	64	85
LSD (0.05)		7	9

[x] Abbreviations: PRE=preemergence; POST=postemergence; WAE=weeks after emergence; W/Res=with residual.

Table 3.6 Influence of herbicide programs in conventional, glyphosate-resistant, or glufosinate-resistant soybean systems on grain yield (2009-2010).

Soybean System	Herbicide Program	Waterhemp	Palmer Amaranth
		----------------Yield (kg/ha)[x]	----------------
Conventional[y]	PRE-only	2824	2017
	PRE fb POST	2689	1681
	2-pass POST	2286	1277
	1-pass POST W/Res	2555	1210
	1-pass Post	2555	1210
	Non-Treated	2286	941
Glufosinate-Resistant	PRE-only	3227	2219
	PRE fb POST	3362	2017
	2-pass POST	3362	2353
	1-pass POST W/Res	3227	2286
	1-pass POST	3362	1614
	Non-Treated	2488	1009
Glyphosate-Resistant	PRE-only	2757	2151
	PRE fb POST	3026	1950
	2-pass POST	3026	2488
	1-pass POSTW/Res	3026	2084
	1-pass POST	3026	1815
	Non-Treated	2286	807
LSD (0.05)		403	336

[x] Abbreviations: kg/ha= kilogram per hectare; PRE=preemergence; POST=postemergence; W/Res=with residual.

[y] Average yield for each soybean system across locations was 1961 kg/ha-Conventional, 2544 kg/ha-Glufosinate-Resistant, and 2370 kg/ha Glyphosate-Resistant (P=0.001); across herbicide programs and locations: 2532 kg/ha-PRE-only, 2454 kg/ha-PRE fb POST, 2465 kg/ha-2-pass POST, 2398 kg/ha-1-pass POST w/Res, 2264 kg/ha-1-pass POST (P=0.001).

Table 3.7 Net gain or loss of soybean systems and herbicide programs in a partial budget system considering glyphosate-resistant soybeans with a 2-pass POST herbicide program as the industry standard for comparison. (2009-2010).

Soybean System	Herbicide Program	Waterhemp	Palmer Amaranth
Industry Standard		Net Return ($/ha)[y]	
Glyphosate-Resistant	2-pass POST	$1026.80	$803.52
		Gain/Loss on Return	
Conventional	PRE-only	-$39.17	-$150.82
	PRE fb POST	-107.03	-302.40
	2-pass POST	-300.72	-496.10
	1-pass POST W/Res	-155.21	-490.15
	1-pass Post	-136.49	-471.42
Glufosinate-Resistant	PRE-only	94.03	-101.34
	PRE fb POST	169.64	-165.29
	2-pass POST	160.30	-35.07
	1-pass POST W/Res	121.45	-46.02
	1-pass POST	183.56	-318.80
Glyphosate-Resistant	PRE-only	-122.49	-150.40
	PRE fb POST	9.14	-214.15
	2-pass POST	-	-
	1-pass POSTW/Res	16.77	-150.69
	1-pass POST	21.61	-257.50
LSD (0.05)[x]		$167.47	$139.56

[x] Assumes $0.42/kg of soybean.

[y] Abbreviations: ha= hectare; PRE=preemergence; POST=postemergence; W/Res=with residual.

Chapter IV

Interactions Between Glyphosate, *Fusarium* Infection of Waterhemp, and Soil Microbial Abundance & Diversity in Soil Collections from Missouri

Kristin K. Rosenbaum, Gerald L. Miller, Robert J. Kremer and Kevin W. Bradley[†]

Abstact

Greenhouse and laboratory experiments were conducted on waterhemp (*Amaranthus rudis* Sauer) and soil collected from 131 soybean fields in Missouri that contained late-season waterhemp escapes. The objectives of these experiments were to: determine the effects of soil sterilization on glyphosate-resistant (GR) and susceptible (GS) waterhemp survival; determine the effects of soil sterilization and glyphosate treatment on infection of GR and GS waterhemp biotypes by *Fusarium* spp.; and determine the soil microbial abundance and diversity in soils collected from soybean fields with differences in waterhemp biotypes and herbicide and crop rotation histories. Waterhemp biotypes were treated with 1.7 kg glyphosate ae/ha once plants reached approximately 15 cm in height or left untreated. Waterhemp survival was visually assessed at 21 days after glyphosate treatment (21 DAT). To determine *Fusarium* infection frequency, a single intact waterhemp root was harvested from each treatment at 0, 3, 7, 14, and 21 DAT, surface-sterilized, and 10-15 mm waterhemp root sections were plated on Komada culture medium. After 14 days incubation, fungal colonies were

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selected from colonized roots and maintained on potato dextrose agar medium amended with antibiotics before identification. Speciation of *Fusarium* isolates was conducted through microscopic examination of fungal characters and confirmed by sequencing and analysis of ribosomal DNA. Soil samples from 131 different collections were subjected to phospholipid fatty acid (PLFA) analysis and were conducted utilizing gas chromatography to determine the soil microbial community abundance and structure. Waterhemp plants grown in sterile soils had the highest waterhemp survival, regardless of biotype. After treatment with glyphosate, survival of GS waterhemp grown in non-sterile soil was only 10% 21 DAT, while survival of GS waterhemp grown in sterile soil was 29%. Similarly following treatment with glyphosate, GR waterhemp survival was reduced from 83 to 61% when grown in non-sterile compared to sterile soil. *Fusarium* spp. were recovered from only 12% of the assayed roots (223 treatments with *Fusarium* out of a total 1,920 treatments). The greatest occurrence of *Fusarium* root infection in both GR and GS waterhemp occurred non-sterile soil with a glyphosate treatment. Few differences in total PLFA were observed in field soil collected from locations with either GR or GS waterhemp, and regardless of herbicide or crop history. This research supports previous findings that plant species are more sensitive to glyphosate in non-sterile than sterile soils and indicates glyphosate may predispose plants to soil-borne phytopathogens. This research also suggests that continuous use of glyphosate does not significantly affect soil microbial abundance or diversity.

Introduction

Glyphosate is a non-selective, foliar herbicide that is the world's best-selling herbicide, being used in over 130 countries and associated with 150 crops (Baylis et al. 2000; Woodburn 2000). In the soil, glyphosate is quickly inactivated by adsorption on soil colloids and readily degraded by soil microorganisms (Smiley et al. 1992; Zimdahl 2007). In plants, glyphosate is leaf-absorbed and translocated to the roots, where it accumulates and may eventually be released into the rhizosphere (Coupland and Casely 1979). Glyphosate inhibits the enzyme 5-enolpyruvoylshikimate 3-phosphate (EPSP) synthase, a key enzyme in the shikimate pathway. Inhibition of this enzyme prevents the plant from synthesizing the aromatic amino acids phenylalanine, tyrosine and tryptophan. These amino acids are used for the synthesis of plant growth regulating compounds, cell walls, and proteins, including those involved in plant defense (Hammond-Kosack and Jones 2000).

The insertion of the transgene that encodes for glyphosate resistance (GR) in genetically modified crops has greatly expanded glyphosate usage. An extensive reliance on glyphosate for weed control in GR crop production systems has resulted in a concurrent increase in the number of GR weeds. Worldwide, there are currently 397 herbicide-resistant biotypes within 217 separate weed species that are resistant to one or more herbicides; 24 of which are resistant to glyphosate (Heap 2013). Within the U.S., 14 species across 30 states contain GR weed species with 12 states specifically documenting GR in waterhemp (*Amaranthus rudis* Saur) (Heap 2013).

In Missouri, waterhemp is the most common species encountered in corn and soybean production systems (Waggoner and Bradley 2012). Additionally, the incidence of GR in waterhemp is prevalent; 69% of the waterhemp populations remaining in soybean fields at harvest were resistant to glyphosate, and these populations occurred across 41 counties in Missouri (Rosenbaum et al. 2012). Similar results have been reported with GR waterhemp populations in Iowa (Owen 2013) and Illinois (Riggins et al. 2012).

Several studies have been conducted to investigate the effects of glyphosate applications on soil microbial and phytopathogen populations such as *Fusarium* in the soil rhizosphere (Baley et al. 2009; Johal and Huber 2009; Johal and Rahe 1984; Liphadzi et al. 2005; Means and Kremer 2007; Sanogo et al. 2000; Sanogo et al. 2001; Smiley et al. 1992; Tesfamariam et al. 2009). The effect of glyphosate on soil microbial populations has been investigated in crops such as the common bean (*Phaseolus vulgaris* L.) (Johal and Rahe 1984; Lévesque et al. 1992b; Lévesque et al. 1993) and also in certain weed species (Close and Kniss 2011; Kawate et al. 1997; Lévesque et al. 1987; Schafer et al. 2012). This research indicates that soil microorganisms play a key role in the lethal effects of glyphosate on plants, and glyphosate treatment may predispose plants to disease in addition to the direct metabolic depletion of aromatic amino acids. Lévesque et al. (1992b) found that 10 times more glyphosate was needed to kill bean seedlings grown in soil free of microorganisms compared to seedlings grown in non-sterile soil. Further, Johal and Rahe (1984) found that the death of glyphosate-treated bean plants in non-sterile soil was at least partially attributed to parasitization by the fungal root-rot phytopathogens *Fusarium* and *Pythium* in the growth medium. Kawate et al. (1997) determined that glyphosate-treated henbit (*Lamium amplexicale* L.) and downy brome (*Bromus tectorum* L.) served as a reservoir for the plant pathogens

(*Fusarium* and *Pythium*) to proliferate compared to non-treated plants, but the pathogens did not readily infect roots of either weed species. Lévesque et al. (1987) found similar results with common chickweed (*Stellaria media* L. Vill). *Fusarium* infection increased in chickweed after application with glyphosate, but common bean and cucumber (*Cucumis sativus*) sown into the common chickweed residue were not detrimentally affected by either the glyphosate application or *Fusarium* infection (Lévesque et al. 1987). Close and Kniss (2011) found that neither field nor greenhouse soil affected glyphosate efficacy on Canada thistle (*Cirsium arvense* L.) rhizomes. Conversely, Schafer et al. (2012) reported that soil microorganisms increased the activity of glyphosate on glyphosate-susceptible (GS) giant ragweed (*Ambrosia trifida* L.) and GS common lambsquarters (*Chenopodium album* L.), but not on GR horseweed (*Conyza canadensis* L. Cronq.) or glyphosate-tolerant common lambsquarters. Variation in response to glyphosate by multiple plant taxa may be associated with differential uptake, translocation, metabolism of the herbicide, differences in regulatory controls with which glyphosate interferes, and variances in the sensitivity of different plants to fungal root-rot pathogens (Johal and Rahe 1984; Tesfamariam et al. 2009).

It is important to note that similar results have also been observed in response to herbicides other than glyphosate. An increase in disease levels has been observed in response to applications of acifluorfen, imazethapyr, and chlorimuron-ethyl (Sanogo et al. 2001; Zhang et al. 2011). Under continuous applications of chlorimuron-ethyl for five to ten years in a continuously cropped soybean field, Zhang et al. (2011) found that the diversity and evenness of the soil microbial community decreased while the presence of *Fusarium* spp. intensified. Sanogo et al. (2001) also indicated there was an increase in disease levels in response to applications of acifluorfen and imazethapyr and these observations are not limited to glyphosate alone. Conversely, Souza et al. (2013) observed no differences in microbial biomass with conventional and imidazolinone-group herbicide applications that could be attributed to the specific use of imazapyr.

The potential of glyphosate altering the soil environment after application has been evaluated in the literature resulting in variable responses. Zaboloy et al. (2012) evaluated the potential effects of glyphosate treatment on microbial community structure and function of soils and found little to no effect on the microbial community. On the contrary, Lane et al. (2012) reported glyphosate application caused a significant decrease in the total microbial biomass in soybean rhizosphere soil that had no previous exposure to glyphosate, however no significant changes were observed in the overall microbial community structure. A variety of other studies have found no effects of typical use rates of glyphosate on the soil microbial community (Acinelli et al. 2007; Acinelli et al. 2005; Araujo et al. 2003; Gomez et al. 2009; and Weaver et al. 2007). Bacterial soil communities were altered in one study conducted by Ratcliff et al. (2006), but only where glyphosate was applied at 100 times the field use rate. Other studies have shown inconsistent results which suggests additional research is needed to determine the functional consequences of glyphosate on the microbial diversity of treated soils (Gimsing et al. 2004; Lupwayi et al. 2007; Powell et al. 2009).

Previous research suggests a correlation exists between glyphosate tolerance in crops or weeds and root-infecting pathogens, but more work is needed to characterize this interaction. Given the prevalence of GR waterhemp across the Midwestern U.S., more information is needed on the effects of glyphosate application on soil microbial populations, and if this effect is correlated to GR development in waterhemp.The interactions between glyphosate, pathogen infection, and soil microorganisms have been studied in a number of crops and a select number of weed species, but not waterhemp. The objectives of this research were to determine the interactions that occur between glyphosate application, *Fusarium* infection of waterhemp, and soil microbial abundance and diversity in soil collections from Missouri. A greenhouse study was conducted to evaluate if soil microorganisms influence the efficacy of glyphosate on waterhemp biotypes using soil collected from fields with a previous history of GR or GS waterhemp. Laboratory experiments were conducted to identify and determine the abundance of plant phytopathogens present on waterhemp roots from the initial greenhouse experiment, and also to identify the total soil microbial biomass differences in 131 Missouri soil collections with differences in waterhemp biotype, crop-rotation history and herbicide-use history.

Materials and Methods

Soil treatment experiment. Soil was collected from ten fields throughout Missouri; five sites with previously confirmed GR waterhemp populations, and five sites with GS waterhemp populations (Rosenbaum et al. 2012). The 5 GR sites were grown continuously in soybeans and glyphosate was the only herbicide applied for a period of three or more years, whereas the 5 GS had some form or crop and/or herbicide rotation for a period of three or more years. Topsoil was randomly sampled from uniform surfaces within each landscape to a depth of 6- to 10-cm yielding a final 13 to 23 kg sample. One-half of the soil from each of the locations was autoclaved at 120 C for 1 hour to kill all living microorganisms, while the remaining half was left non-sterilized. ‘Weston 2006’

GR and ‘Bradford 2006’ GS waterhemp biotypes (Legleiter and Bradley 2008) were planted (approximately 0.1 to 0.2 g of seed or 300-600 seeds) onto separate 19- by 28-cm greenhouse flats containing sterilized potting soil (General Purpose Potting Media by Premier Tech Horticulture, Hummert Supplies, Earth City, MO) that had been autoclaved at 120 C for 1 hour. Flats 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}) to stimulate a 16-h-photoperiod day. Once seedlings (< 2-cm in height) emerged from the sterilized potting soil, 10 waterhemp plants per biotype were transplanted into 19- by 28-cm greenhouse flats containing a 5 cm depth of either sterile or a non-sterile 3:1 mixture of field soil to sterile vermiculite. Once waterhemp plants reached 10- to 15-cm in height, flats were treated with glyphosate at 1.7 kg ae ha^{-1}, representing twice the labeled use rate for waterhemp control, or left untreated. Glyphosate applications were made with a compressed air, laboratory spray chamber calibrated to deliver 220 L ha^{-1} carrier volume at a pressure of 234 kPa using a 8001EVS nozzle (Teejet Technologies, Wheaton, IL). Ammonium sulfate was added to all treatments at 2.9 kg ha^{-1}. At 21 days after treatment (DAT), the number of plants remaining in each greenhouse flat was counted to determine the percent waterhemp survival in response to each herbicide treatment; plants with green tissue were counted as survivors in this study. Soil and glyphosate treatments were arranged in a completely randomized design with four replications, and the experiment was conducted twice.

***Fusarium* colonization experiment.** From each flat in the soil treatment experiment, a single intact waterhemp plant was collected 0, 3, 7, 14, and 21 DAT. Loosely adhering soil on roots were removed by vigorous shaking. The aboveground portion of the waterhemp plant was severed at the soil line and discarded, and roots were used in subsequent *Fusarium* colonization experiments described. Waterhemp roots were surface sterilized in a 10% NaOCl solution (Clorox Regular Bleach, The Clorox Company, Oakland, CA) for 2-3 minutes, followed by two consecutive, one minute rinse intervals with 50- to 100- mL sterile water. Roots were blotted dry with sterile paper towels and cut into 2-cm segments. Eight root segments from each plant were placed onto a single agar plate containing Komada selective growth medium (Komada 1975). The samples were inspected daily up to 28 days after incubation for mycelium appearance, which usually occurred within 7 to 14 days. After incubation, putative *Fusarium* colonies from root segments were counted (data not shown). Hyphal tips from putative *Fusarium* colonies were transferred aseptically to potato dextrose agar (PDA +++) amended with 50 µg ml^{-1} each of chloramphenicol (Sigma-Aldrich, St. Louis, MO), streptomycin sulfate (Sigma-Aldrich, St. Louis, MO), and tetracycline (Sigma-Aldrich, St. Louis, MO).

Initially, morphology for each species was conducted using the micro- and macroconidia of each sample and were identified by microscopic evaluation of *Fusarium* cultures grown on carnation-leaf agar (Fisher et al. 1982; Nelson et al. 1983). To prepare *Fusarium* colonies for DNA extraction, hyphal tips were transferred onto a sterilized cellophane (Amersham Biosciences, Piscataway, NJ) square overlaid on PDA +++. Mycelia were scraped from the cellophane and placed into a sterile 1.5 ml microcentrifuge tube. The tube was centrifuged at max speed (17,000 rotations per minute) for 5 minutes and stored at -80 C until use.

Fusarium colonies were identified through DNA extraction, amplification, purification and sequencing as described by Gardes and Bruns (1993). Genomic DNA from 408 unknown isolates was extracted using the EasyDNA Kit (Invitrogen Corp.,Carlsbad, CA). PCR amplification of ribosomal (r)DNA regions internal transcribed spacer (ITS) ITS1 and 5.8S rRNA, and ITS2 was performed on 348 DNA extracts from isolates using the universal primers ITS4 and ITS5. PCR reactions were 50 μl in volume and consisted of 35.75 μL of cold DNA H_2O, 10 μL PCR buffer (Bioline Inc., Taunton, MA), 2 μL DNA template, 1 μL of each primer, and 0.25 μL of *Taq* polymerase (Bioline Inc., Taunton, MA). Thermal cycling conditions involved an initial denaturation step at 94 C for 1 min followed by 32 cycles of 94 C for 30 s, 55 C for 1 min, 72 C for 1 min and a final extension step at 72 C for 2 min. Amplicon presence and size were confirmed with gel electrophoresis. Amplification products were purified with a Qiaquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sent to the University of Missouri DNA Sequencing Facility (Columbia, MO) for cleanup, electrophoresis, and fluorometric analysis. The consensus sequence for each isolate was manually aligned from one to two sequencing reactions with each primer. A basic local alignment search tool (BLAST) search of GenBank was performed with each consensus sequence (Zhang et al. 2000). For each sample, one or two of the most similar sequences based on maximal identity percentage were downloaded for comparison. All sequences were aligned using the sequencing alignment tool in BLAST and adjusted by visual examination. In all cases, the molecular analysis confirmed the speciation made based on observed morphological characteristics.

Soil microbial abundance and diversity experiment. Soil samples were collected in the spring of 2011 from 131 field locations. The locations were selected based on the presence of GR or GS waterhemp and known crop- and herbicide-use history

(Rosenbaum et al. 2012). Fields from soil collections were located within 10 Missouri Major Land Resource Areas including: Iowa and Missouri Heavy Till Plain; Central Claypan Areas; Missouri and Iowa Deep Loess Hills; Central Mississippi Valley Wooded Slopes; and Cherokee Prairies. These locations represent major soil groups typically cultivated to corn and soybean in Missouri (USDA-NRCS, 2006). Details of the various soils are presented in Figure 4.1. Soils were sampled from the surface 10-cm depth at each site. For analysis, each soil sample was air-dried and ground to pass through a 2-mm sieve. Carbon:Nitrogen (C:N) ratios were calculated by dry combustion of total nitrogen and total organic carbon (Nelson and Sommers 1996) with a LECO analyzer (TruSpec CN Analyzer, St. Joseph, MI).

All soils were freeze-dried in a production grade freeze drier (Lyph-Lock 12 model, Labconco Corp., Kansas City, MO) at -20 C for 24 h. The soil microbial community was determined in each of the 131 field sites using phospholipid fatty acid (PLFA) analysis as described by Bligh and Dyer (1959) and Peterson and Klug (1994). Gas chromatograph peak responses were translated using internal standards. For example, peaks corresponding to fatty acid carbon chain lengths of 12 to 20 are indicative of microorganisms, bacteria markers correspond to fatty acid carbon lengths of 12 to 19 carbons, fungal markers correspond to fatty acid carbon chain lengths of 16 and 18 carbons, and the ratio of total saturated to total monounsaturated fatty acids use the ratio of the sum of 14 to 20 carbons to the sum of 16 to 17 carbon chain lengths (Unger 2009). For further details regarding markers utilized in this study refer to Unger et al. (2009, 2013). The PLFA analysis determines the amount of living microbial biomass and includes the percentage of total PLFA, bacteria, and fungi within the living microbial biomass.

Statistical analysis. Across all three experiments, data were subjected to analysis of variance using SAS PROC MIXED (SAS 9.3, SAS Institute Inc., Cary, NC) with each treatment combination considered an environment sampled at random. Fixed effects for the greenhouse study included soil collection, soil sterilization treatment, glyphosate treatment, and waterhemp biotype while replication was utilized as the random effect. Data from the greenhouse study was combined across experiment timings (experiment was conducted in time twice) and was specifically evaluated for treatment differences at 21 DAT. The fixed effects for the laboratory experiment evaluating *Fusarium* colonization included soil sterilization, glyphosate treatment, and waterhemp biotype with replication as the random effect. To evaluate treatment differences in the *Fusarium* colonization experiment, due to no differences between experiments, data were combined across experiment timings (experiment was conducted in time twice) and root collection timings (0, 3, 7, 14, and 21 DAT). The soil microbial abundance and diversity experiment effects included site characteristics of waterhemp biotype, herbicide-use history and crop-rotation history and were evaluated for treatment differences within individual site characteristics. Where F-values were significant ($P \leq 0.05$), means were separated using Fisher's protected LSD. Transformations of the data did not improve the model therefore non-transformed means are reported.

Results and Discussion

Soil treatment experiment. No differences in waterhemp survival 21 DAT were observed between any of the ten soils evaluated, regardless of the waterhemp biotype at the soil collection site. These results are derived from an orthogonal contrast which provided a P-value for all treatment variables combined into one contrast to determine treatment differences specifically between soil locations. Therefore all waterhemp survival data were combined across soil locations ($P>0.45$; data not shown). Additionally, there was no significant effect for the specific variable of soil collections on waterhemp survival regardless of the individual or multi-variable interactions evaluated 21 DAT ($P>0.12$; Table 4.1). These findings suggest there are no differences between soils from locations containing GR or GS waterhemp, and that soil microbial-glyphosate interactions are not influenced by origin of soil. With the exception of soil collection, treatment differences were observed for all remaining tested variables including individual and multi-variable interactions for waterhemp biotype, glyphosate application and soil sterilization (Table 4.1).

Treatment differences were evaluated by multi-variable interactions within soil sterilization, glyphosate application, and waterhemp biotype (Table 4.1). Mean waterhemp survival 21 DAT for each biotype in response to soil sterilization and glyphosate treatment is presented in Table 4.2. Regardless of the treatment variable, greater than 95% waterhemp survival occurred 21 DAT where no glyphosate was applied, indicating that soil microbial populations alone cannot solely explain waterhemp death or survival (Table 4.2). If soil microbes were the primary cause of plant death, a significant reduction in waterhemp survival would also have occurred in the non-glyphosate treatments. Therefore, these results suggest that the reduction in survival of waterhemp is dependent upon a combination of both the activity of glyphosate targeting EPSP synthase and the presence of soil microorganisms. When combined across all other factors, 73% of GR waterhemp survived a 2X rate of glyphosate compared to only 25% survival in GS waterhemp (Table 4.2). Although complete death is expected in confirmed GS waterhemp 21 DAT, a low level of the GS biotype survived glyphosate treatment. This can be partially attributed to the dioecious nature of waterhemp and high level of variability within a given population of this species (Bradley et al. 2009a; Chandi et al. 2013; Nordby et al. 2007). This inconsistency in the level of herbicide activity in waterhemp has also been noted in previous research (Foes et al. 1998; Horak and Peterson 1995).

Greater waterhemp survival was observed in sterile soil compared with non-sterile soil; sterilizing soils increased GR and GS waterhemp survival by 5 and 10%, respectively ($P \leq 0.03$; Table 4.1). Overall, the absence of microorganisms in the soil increased the survival of waterhemp following a glyphosate application by 18% when compared to non-sterile soil media in the same treatment. Survival of GS waterhemp grown in non-sterile soil and treated with glyphosate was only 13% by 21 DAT, while survival of GS waterhemp grown in sterile soil was 38%. Similarly, GR waterhemp survival was reduced from 78 to 67% when grown in non-sterile compared to sterile soil.

A similar response to glyphosate treatment and soil sterilization has been reported with giant ragweed, common lambsquarters, and common bean, suggesting that soil microorganisms play a major role in the efficacy of glyphosate (Johal and Rahe 1984; Lévesque and Rahe 1992a; Lévesque et al. 1992b; Lévesque et al. 1993; Schafer et al. 2012). Specifically in weeds, Schafer et al. (2012) reported soil microorganisms play an important role in glyphosate efficacy on giant ragweed by causing root infection by soilbourne pathogens which result in plant death (Schafer et al. 2012). Schafer et al.

(2012) also reported that soil microbes do not influence glyphosate efficacy in glyphosate-tolerant common lambsquarters and GR or GS horseweed biotypes. Lévesque et al. (1992b) suggested the efficacy of glyphosate on common bean can be affected by changes in certain microbial components of the soil.

Overall, the plant-microbe interactions with the rhizosphere can be extremely complex and vary amoung plant species resulting in selection of microbial communities associated with specific plants (Garbeva et al. 2004). In addition, the observed differences suggest that variation based upon the absence or presence of soil microorganisms could provide an insight to the evolution of resistance to glyphosate in weed species. Collectively, all of these findings suggest the cause of death in plants treated with glyphosate involves more than the direct metabolic aromatic amino acid depletion of phenylalanine, tyrosine and tryptophan through the inhibition of EPSP synthase. Therefore the observed differences between plant species and the plant rhizosphere relationship could contribute to the variation in glyphosate efficacy that can occur in certain weed species. Other authors have hypothesized that glyphosate may predispose plants to infection by soilbourne plant pathogens, and this should be considered a secondary mechanism of herbicidal action (Johal and Rahe 1984; Kawate et al. 1997; Lévesque and Rahe 1992a; Lévesque and Rahe 1992b; Lévesque et al. 1987; Schafer et al. 2012).

***Fusarium* colonization experiment.** *Fusarium* root infection of waterhemp plants was evaluated in a total of 1920 treatments. Across all 1920 treatments, *Fusarium* infection was low and *Fusarium* species were recovered from only 12% of the assayed roots (223 treatments). The low incidence of *Fusarium* root infection observed in this study may be attributed to the lower susceptibility of waterhemp to *Fusarium*. Kawate et al. (1997) also observed low *Fusarium* colonization on the roots of downy brome and henbit after glyphosate treatment. Due to this low level of root infection, the results were combined across *Fusarium* species (Table 4.3). Within the infected root samples, the most predominant species identified through molecular analysis included *Fusarium solani* (the group that includes the causal agent of sudden death syndrome in soybean; Li et al. 2008) which comprised 54.9% of the infected samples, *Fusarium oxysporum* (likely the causal agent of vascular wilt; Kistler et al. 1991) which comprised 25.8% of the infected samples, and other miscellaneous species including *F. acuminatum*, *F. avenaceum, F. chlamydosporum, F. culmorum, F. equiseti, F. proliferatum, F. sporotrichioides* and *F. tricinctum,* which comprised 19.3% of the infected samples (Table 4.4).

Regardless of waterhemp biotype, the greatest occurrence of *Fusarium* root infection occurred in waterhemp plants grown in a non-sterile soil treated with glyphosate (Table 4.3). In a non-sterile soil, *Fusarium* root infection was reduced by 11 and 9% for GR and GS waterhemp, respectively, in non-treated compared to glyphosate-treated plants. *Fusarium* infection of waterhemp roots was lower in sterilized (<7%) compared to non-sterilized (12-25%) soil for all treatments. These results suggest increased *Fusarium* infection on the waterhemp roots following a lethal dose of glyphosate is due to the presence of opportunistic phytopathogens within the soil microbial community that occur naturally in non-sterile field soils. These results are similar to responses observed in the literature suggesting a greater level of plant infection occurs in the presence of glyphosate and soil microorganisms (Johal and Rahe 1984; Kawate et al. 1997; Lévesque and Rahe 1992a; Lévesque and Rahe 1992b; Lévesque et al. 1987; Schafer et al. 2012).

Furthermore, infection by soil-borne pathogens such as *Fusarium* or *Pythium* caused by the inability of glyphosate-treated plants to synthesize plant defense compounds may contribute to the overall herbicidal efficacy of glyphosate (Johal and Huber 2009; Schaffer et al. 2012). For example, phenylalanine is a precursor for certain phytoalexins which are important plant defense compounds; therefore the induction of glyphosate activity toward plant roots could be influenced by soil microorganisms (Johal and Huber 2009; Liu et al. 1995).

The results from this study also suggest that allowing GR waterhemp to spread and proliferate could increase *Fusarium* populations in future corn and soybean production systems. Overall, variation in phytopathogen infection will most likely be determined by the interaction of a number of characteristics within an individual crop field such as soil properties, plant properties, environmental conditions, competing organisms, different inoculum levels, initial microbial community, etc. (Garbeva et al. 2004).

Soil microbial abundance and diversity experiment. No differences were observed for soil microbial properties including total PLFA, bacteria, protozoa, and fungi content, or in the bacteria:fungi ratio among sites characterized by waterhemp biotype, glyphosate-use history, and crop-rotation history for all 131 soils evaluated (Table 4.5). In addition, no differences were observed in the saturated to monounsaturated fatty acid ratio in response to the glyphosate-use history or crop-rotation history between any of the 131 soils evaluated (Table 4.5). Similarly, few differences in soil microbial community abundance and structure in response to glyphosate applications have been documented within the literature. In one Missouri study, no shifts were observed in microbial community structure in response to increased antibiotics applied to the soil system (Unger et al. 2013). Therefore this study suggests that soil microbial communities are robust and the use of synthetic organic compounds (xenobiotics) used in agriculture including herbicides or antibiotics may not readily diminish important primary soil functions or properties. A number of other studies revealed no shifts in the soil microbial community abundance and structure occurred after the addition of glyphosate (Accinelli et al. 2005; Gomez et al. 2008; Weaver et al. 2007). Accinelli et al. (2005) observed that incorporated corn residues did not affect or stimulate glyphosate mineralization in sandy or sandy loam soils. Gomez et al. (2008) observed increased dehydrogenase activity and decreased microbial biomass following the application of glyphosate to the soil. However, after incubation, the initial inhibitory effect of the herbicide observed in the Gomez et al. (2008) study had been diluted and was determined that no harmful effects should be expected in the short-term when glyphosate is applied at doses equivalent or higher than those usually applied in the field. No significant differences between the soil and soybean rhizosphere microbial communities due to treatment with glyphosate were observed in a Mississippi silt loam soil (Weaver et al. 2007).

No differences were observed in soil organic matter (SOM), C:N ratio, or nitrogen content among any of the 131 soils evaluated (Table 4.5). These results correspond with previous research suggesting rhizosphere microbial activity, optimum plant growth, soil microbial ecology, and soil quality are not impacted by locations with continuous use of glyphosate, and/or the crop rotation history of the field (Means et al. 2007). Means et al. (2007) observed little effect of glyphosate on general rhizosphere microbial activity measured by enzyme activity and CO2 respiration and was conducted under fairly constant SOM and soil N levels. Therefore, plant-microbe-soil interactions may not be readily affected by specific microbial components or functions within the overall microbial community that were assessed in this study following a history of GR waterhemp, glyphosate usage, and/or lack of crop rotation.

Differences were observed between the saturated to monounsaturated fatty acid ratio. This ratio indicates that under stress such as soil temperature increase or soil moisture decrease, the degree of unsaturated fatty acid decreases and saturated fatty acid increases, thus the ratio will also increase (Peterson and Klug 1994). The ratio is a general indicator for the status of bacterial community in the environment and can provide input regarding whether some impact by an environmental factor (glyphosate treatment and/or glyphosate resistant waterhemp) is shown by the microbes (Peterson and Klug 1994). The soil characteristic of waterhemp biotype caused differences in this ratio but this biomarker was not different for the other site characteristics including glyphosate-use history or crop-rotation history (Table 4.5). A site with GR waterhemp provided a lower saturated to monounsaturated ratio than a site with GS waterhemp. These results suggest that the soil microbial community structure and abundance in locations where a GR waterhemp biotype has evolved may benefit from some growth factor released in the root exudates that influence the soil microbial community. In addition, there may be slight differences between waterhemp biotypes in the amount and/or rate of release of glyphosate from the roots into soil, potentially affecting microbial growth and activity. However, further research into these interactions is necessary to understand the significance of this response within the soil microbial community.

Overall, the plant rhizosphere relationships with soil microorganisms are not completely understood from the standpoint of glyphosate effects on weed-rhizosphere microbial community-interactions. This research supports previous findings with other plant species that indicate a greater sensitivity to glyphosate when grown in non-sterile relative to sterile soils, demonstrating that glyphosate may predispose plants to diseases incited by soil-borne phytopathogens. Few differences were observed across all site characteristics for soil microbial abundance and diversity and therefore suggest crop- and herbicide-use history and waterhemp biotype do not readily alter soil microbial communities in the soils evaluated in our study.

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Table 4.1. Analysis of variance for survival of GR[a] and GS waterhemp biotypes with treatment differences including soil sterilization, herbicide treatment, soil collection and waterhemp biotype, 21DAT.

Variables	AMATA Biotype	Glyphosate Treatment	Soil Treatment	Soil Collection	Soil treatment/glyphosate
	---------% Survival P-value[b]---------				
AMATA Biotype	0.01	0.01	0.03	0.25	0.13
Glyphosate Treatment	0.01	0.01	0.01	0.74	--
Soil Treatment	0.03	0.01	0.01	0.17	--
Soil Collection	0.25	0.74	0.17	0.77	0.32

[a] Abbreviations: GR=glyphosate-resistance; GS=glyphosate-susceptible; DAT=days after treatment; AMATA=waterhemp.

[b] Orthogonal contrast of R vs S soil locations were not different (P>0.45) and were combined with soil collection; P-values ≥ 0.05 are not different.

Table 4.2. Influence of soil sterilization, herbicide treatment, and soil collection on mean waterhemp survival of GR[a] and GS biotypes.

Variables	R AMATA	S AMATA	Glyphosate	No-glyphosate	Sterile	Non-sterile	R soil collection	S soil Collection	Non-sterile/glyphosate	Non-sterile/no-glyphosate	Sterile/glyphosate	Sterile/no glyphosate
	---------------------------% Survival 21DAT---------------------------											
R AMATA	--	--	73	97	87	82	83	86	67	98	78	96
S AMATA	--	--	25	99	66	56	63	62	13	99	38	99
Glyphosate	73	25	--	--	58	40	48	49	--	--	--	--
No-glyphosate	97	99	--	--	98	98	98	98	--	--	--	--
Sterile	87	66	58	98	--	--	79	77	--	--	--	--
Non-sterile	82	56	40	98	--	--	67	71	--	--	--	--
R soil collection	83	63	48	98	79	67	--	--	37	98	59	98
S soil Collection	86	62	49	98	77	71	--	--	43	99	57	98

[a] Abbreviations: GR=glyphosate-resistance; GS=glyphosate-susceptible; DAT=days after treatment; AMATA=waterhemp; R=resistant; S=susceptible; LSD= least significant difference.

[b] Orthogonal contrasts of GR vs GS soil locations were not different (P>0.45). LSD (0.05) for all two-way interactions=5; LSD (0.05) for all three-way interactions=7.

Table 4.3. Influence of soil and glyphosate treatment on the percentage of GR[a] and GS waterhemp roots infected with *Fusarium* species.

		Waterhemp Biotype	
Soil Treatment	Glyphosate Treatment	GR	GS
		----- Infection (%)[b]-----	
Non-sterile	Glyphosate	23 a	25 a
	Non-treated	12 b	16 b
Sterile	Glyphosate	2 c	5 c
	Non-treated	3 c	6 c

[a] Abbreviations: GR=glyphosate-resistance and GS=glyphosate-susceptible.

[b] Means followed by the same letter are not different within individual columns, LSD (0.05).

Table 4.4. Influence of soil and glyphosate treatment on the *Fusarium* isolates recovered from waterhemp roots.

Soil Treatment	Glyphosate Treatment	*Fusarium* Species	Isolates Recovered		
			---# of Isolates---	--- % of Total Isolates ---	
Non-sterile	Glyphosate	*F. acuminatum*	2	1.6	0.9
		F. avenaceum	6	4.8	2.6
		F. equiseti	3	2.4	1.3
		F. oxysporum	30	24.2	12.9
		F. solani	78	62.9	33.5
		F. tricinctum	5	4.0	2.1
Non-sterile	Non-treated	*F. acuminatum*	2	2.9	0.9
		F. avenaceum	2	2.9	0.9
		F. chlamydosporum	1	1.4	0.4
		F. equiseti	2	2.9	0.9
		F. oxysporum	23	32.9	9.9
		F. solani	34	48.6	14.6
		F. tricinctum	6	8.6	2.6
Sterile	Glyphosate	*F. equiseti*	6	35.3	2.6
		F. oxysporum	3	17.6	1.3
		F. poliferatum	1	5.9	0.4

		F. solani	7	41.2	3.0
Sterile	Non-treated	*F. acuminatum*	1	4.5	0.4
		F. culmorum	1	4.5	0.4
		F. equiseti	3	13.6	1.3
		F. oxysporum	4	18.2	1.7
		F. poliferatum	1	4.5	0.4
		F. solani	9	40.9	3.9
		F. sporotrichioides	2	9.1	0.9
		F. tricinctum	1	4.5	0.4

[a] Data includes *Fusarium* incidence recovered from 12% of the assayed roots (233 treatments).

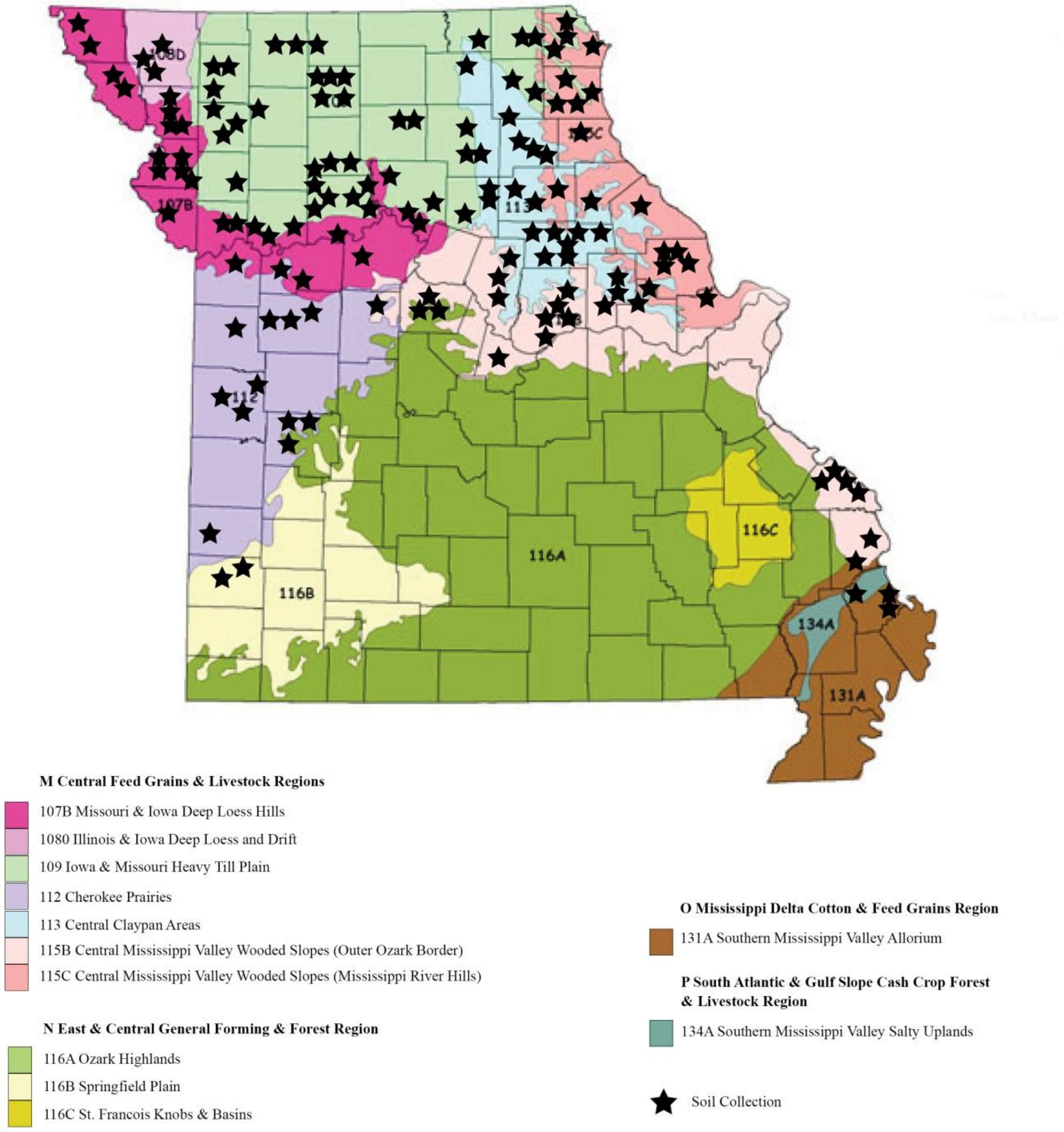
Table 4.5. Analysis of microbial PLFA[a] and carbon and nitrogen content of soil collected from 131 Missouri soybean fields previously characterized with GR or GS waterhemp and varying soil characteristic history varying across waterhemp biotype, herbicide-rotation and crop-rotation.

	Microbial PLFA						Carbon and Nitrogen Content		
Site Characteristic	Total PLFA	Bacteria	Protozoa	Fungi	Bacteria: Fungi	Sat: Monounsat	SOM	TN	C:N
Waterhemp Biotype	---------------ng/g oven-dried soil----------------				-------------------------------%--------------------------------				
Site w/R Waterhemp	1971[b]	1696	9.28	111	18.39	**2.34**	3.5	0.18	11.13
Site w/S Waterhemp	1955	1675	9.05	105	19.45	**2.63**	3.7	0.20	10.64
	(P=0.91)	(P=0.86)	(P=0.89)	(P=0.56)	(P=0.59)	**(P=0.03)**	(P=0.43)	(P=0.15)	(P=0.17)
Glyphosate Use History									
Continuous Glyphosate	1934	1660	9.79	106	19.88	2.37	3.5	0.19	10.90
Some Herbicide Rotation	1825	1574	7.56	97	18.33	2.55	3.4	0.18	11.12
	(P=0.44)	(P=0.48)	(P=0.22)	(P=0.38)	(P=0.53)	(0.21)	(P=0.63)	(P=0.39)	(P=0.59)
Crop Rotation History									
Continuous Soybean	1967	1689	9.28	107	19.86	2.43	3.4	0.18	10.71
Some Corn:Soybean	1993	1716	9.54	112	17.36	2.50	3.5	0.18	11.24
	(P=0.86)	(P=0.84)	(P=0.88)	(P=0.65)	(P=0.23)	(P=0.64)	(P=0.51)	(P=0.97)	(P=0.19)

[a] Abbreviations: PLFA=phospholipid fatty acid analysis; Sat=saturated; Monosat=monounsaturated; SOM=soil organic matter; TN=total nitrogen; C=carbon; GR=glyphosate resistant; GS=glyphosate susceptible; R=resistant; S=susceptible.

[b] Means followed by P-Value ≥ 0.05 are not different.

Figure 4.1. Predominant soil types (USDA-NRCS 2013) and location of soils sampled in Missouri for use in the soil microbial abundance and diversity experiment.



VITA

Kristin Kay (Payne) Rosenbaum was born October 1, 1984 to Kirby and DeLynda Payne. Kristin grew up in rural North Missouri near Gilman City and Jamesport on an 80 acre farm raising sheep, pigs and cattle. Kristin gained an appreciation for agriculture at a very young age, enjoying helping her father Kirby and grandfather, Kenneth Payne with daily farm activities. Kristin and her sisters, Kimberly and Kyla were very active in 4-H and FFA while attending elementary and high school at Gilman City, showing livestock at area, regional, state, and national livestock shows. In addition to working with the livestock, Kristin enjoyed planting and raising a garden. The Payne family was also very involved in other activities in the Gilman City area, including high school sports (basketball, softball, and track) and church. After graduating high school in 2003 from Gilman City, Kristin pursued her associates of arts degree from a neighboring 2-year college North Central Missouri College in Trenton, MO. After receiving her transfer degree, Kristin obtained her Bachelors of Science degree in Agronomy from Northwest Missouri State University in Maryville, MO. Kristin continued her schooling by receiving her Masters of Science degree in Plant, Insect, and Microbial Sciences at the University of Missouri focusing on pasture weed control research, advisor Dr. Kevin Bradley. At the completion of Kristin's Master's degree, Kristin was married to Ryan Rosenbaum on August 7, 2010. Ryan moved to Columbia to support and encourage Kristin while she further expanded her knowledge of the weed science industry focusing her doctorate degree research on glyphosate-resistant waterhemp in soybeans. Kristin's future dreams are to land a job in the industry world pertaining to weed science and utilize her knowledge obtained with all degrees.