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Glyphosate-Resistant Palmer Amaranth (Amaranthus Palmeri) in Arkansas: Resistance Mechanisms and Management Strategies

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GLYPHOSATE-RESISTANT PALMER AMARANTH (AMARANTHUS PALMERI) IN ARKANSAS: RESISTANCE MECHANISMS AND MANAGEMENT STRATEGIES
GLYPHOSATE-RESISTANT PALMER AMARANTH (AMARANTHUS PALMERI) IN ARKANSAS: RESISTANCE MECHANISMS AND MANAGEMENT STRATEGIES

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Crop, Soil, and Environmental Sciences

By

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ABSTRACT

Since 2000, there have been thirteen weed species confirmed resistant (R) to glyphosate in the United States, six of those resistant species are present in Arkansas. The goal of this research was to confirm and to determine the level of resistance in two R Palmer amaranth biotypes from Mississippi (MC-R) and Lincoln (LC-R) Counties, Arkansas, and one susceptible (S) biotype from Clarendon County, South Carolina, which had never been exposed to glyphosate. Shikimic acid concentration over time was significantly greater in the S biotype than both the MC-R and LC-R biotypes. The lethal dose required to kill 50% (LD$_{50}$) of the population was 2,255 and 3,223 g ae ha$^{-1}$ for the MC-R and LC-R biotypes, respectively, and it was hypothesized that the two Arkansas biotypes each had a different resistance mechanism. Results indicate metabolism of glyphosate to its major metabolite, aminomethylphosphonic acid (AMPA), was not responsible for resistance in any biotype. Reduced absorption in the LC-R and limited translocation from the treated leaf in the MC-R were at least partially responsible for the observed resistance to glyphosate. The LC-R biotype effectively colonized a field within two years of a single resistant female producing ~20,000 seed. Cotton lint yield was reduced over 100 kg ha$^{-1}$ by some densities of LC-R Palmer, depending on the soil and relative elevation in that region. Several resistant management options exist in cotton; however, results indicate that timely herbicide applications based off of Palmer amaranth size are required for effective season-long control and management of the soil seedbank.
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DEDICATION

Dedicated to my parents
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INTRODUCTION AND LITERATURE REVIEW
INTRODUCTION

Palmer Amaranth Biology

The genus *Amaranthus* comprises several important weed species, approximately 50 of which are native to the Americas (Kigel 1994; Sauer 1976). Palmer amaranth is an erect, branched, dioecious, summer annual broadleaf native to the Southern Great Plains (Bryson and DeFelice 2009). Season-long emergence, rapid growth, prolific seed production, and genetic diversity make Palmer amaranth highly competitive (Foes et al. 1998; Jha et al. 2009; Morgan et al. 2001; Sellers et al. 2003). Known to many as careless weed or pigweed, Palmer amaranth can grow over 2 m tall and produce over 600,000 seed from a single plant (Keeley et al. 1987). Palmer amaranth exhibits extended emergence, increasing the chance of yield reductions throughout the season (Jha et al. 2009; Keeley et al. 1987). Palmer amaranth leaves are rhombic-lanceolate, with petioles as long or longer than the leaf blades. The inflorescence of Palmer amaranth is a terminal spike that may reach 0.5 m in length (Horak and Peterson 1995). Male and female inflorescences are distinguishable from one another by touch. The female inflorescence has stiff, pointy bracts that make it prickly to touch. The frequency of Palmer amaranth infestations has increased in recent years because of conversion to reduced-tillage systems, reduced reliance on soil-applied residual herbicides, evolution of herbicide resistance, and differential susceptibility within the species to some postemergence herbicides (Horak and Loughin 2000).

Escapes of Palmer amaranth populations may cause mechanical interference during harvest. Palmer amaranth densities of 650 to 3,260 plants ha\(^{-1}\) increased harvest times (stoppages for biomass removal) from 155 to 274 min ha\(^{-1}\) (Smith et al. 2000). If not controlled, this species
can cause significant yield losses and impede harvest in cultivated crops, particularly with slower-growing crops such as cotton (*Gossypium hirsutum* L.).

Cotton canopy volume was decreased 35 to 45% for Palmer amaranth densities of 1 to 10 plants 9.1 m⁻¹ of row (Morgan et al. 2001). Smith et al. (2000) reported that Palmer amaranth densities of 3,260 plants ha⁻¹ decreased cotton seed yields up to 210 kg ha⁻¹ [1,050 kg ha⁻¹ (check) to 840 kg ha⁻¹]. Morgan et al. (1997) reported that each Palmer amaranth plant added to a 10-m row of cotton reduced lint yield 62 kg ha⁻¹. Cotton fiber characteristics are also important in determining cotton quality. Rowland et al. (1999) reported that in Oklahoma, cotton lint yield was reduced 5.9 to 11.5% for an increase of one Palmer amaranth 10 m⁻¹ row, and that lint micronaire was also influenced by Palmer amaranth densities.

Yield reductions have also been seen in other crops such as soybean (*Glycine max* (L.) Merr.), corn (*Zea mays* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench). In soybean, an increase of Palmer amaranth density from 0.33 to 10 plants m⁻¹ of row reduced yields linearly from 17 to 68% (Klingaman and Oliver 1994). Palmer amaranth densities of 8 plants m⁻¹ of row reduced yields up to 79% in Kansas soybean (Bensch et al. 2003). Massinga et al. (2001) reported that Palmer amaranth that emerged with corn reduced yield from 11 to 91% for densities of 0.5 to 8 plants m⁻¹ of row. If Palmer amaranth emerged after corn, yields were reduced 7 to 35% at the same densities. The leaf area index (LAI) for corn at silking was also reduced for increasing Palmer amaranth densities (Massinga et al. 2001). Other research has shown that corn’s early exposure to weeds reduced the rate of seedling growth and development, contributing to the onset of the critical period of weed control (Page et al. 2009). Grain sorghum yields were reduced from 1.8 to 3.5% Palmer amaranth⁻¹ in Oklahoma for an increase of 1
Palmer amaranth 15 m⁻¹ of row, and grain sorghum seed loss through the combine increased 11 kg ha⁻¹ (Moore et al. 2004).

**Palmer Amaranth Control**

Identification of Palmer amaranth in the seedling stage is difficult, and it is often confused with other *Amaranthus* species, making herbicide selection an important factor in controlling this species. Herbicide recommendations vary in application timing, depending on which species is present; however, most labels recommend application when the target species is very small. Mayo et al. (1995) reported that thifensulfuron and imazethapyr applied postemergence (POST) provided >80% Palmer amaranth control in soybean, while imazaquin, chlorimuron, and acifluorfen provided <75% Palmer amaranth control. Furthermore, Horak and Peterson (1995) reported that Palmer amaranth and common waterhemp [*Amaranthus rudis* (Sauer)] have evolved resistance up to eight times the labeled use rates of thifensulfuron and imazethapyr.

Palmer amaranth control in soybean is often achieved with herbicides applied either preplant incorporated (PPI), preemergence (PRE), or POST. Examples of PRE herbicides used in soybean are acetochlor, alachlor, metolachlor, linuron, metribuzin, pendimethalin, trifluralin, imazaquin, imazethapyr, and sulfentrazone. Sweat et al. (1998) reported that at 21d after treatment (DAT), Palmer amaranth was controlled at least 94% by all PRE-applied soybean herbicides except for the two dinitroanilines, pendimethalin and trifluralin. Pendimethalin and trifluralin had differential efficacy among *Amaranthus* species and provided only 67 to 88% Palmer amaranth control. Gossett et al. (1992) reported that Palmer amaranth in South Carolina evolved resistance up to 5 to 6 times the normal use rate of the dinitroanilines. Furthermore, Heap (2012) reported that Palmer amaranth resistance to the acetolactate synthase (ALS)
herbicides has now been confirmed in over 10 states, rendering herbicides targeting this mode of action unreliable for weed control.

Postemergence herbicides often lack the residual control that some PRE-applied herbicides have, decreasing Palmer amaranth control and increasing the number of applications required for acceptable control; however, some POST herbicides do have residual activity and can provide some extent of residual control. Imazamox, imazethapyr, and thifensulfuron all provided 94 to 100% control of susceptible Palmer amaranth through 21 DAT (Sweat et al. 1998).

Crop rotations are still used to help control some problematic weeds that escape herbicide applications. This is one way producers can change the herbicide and mode of action (MOA) they are using, decreasing the chance of resistance. Although different herbicides are available in soybean, corn, and cotton, glyphosate is still the primary herbicide used in all three cropping systems (NASS 2006). Glyphosate has a broad spectrum that includes many troublesome weeds, making it a convenient herbicide. Convenience, cost, and crop safety make glyphosate the most popular herbicide in today’s market.

Delayed glyphosate applications have been used in corn to control late-emerging weeds; however, PRE herbicides may also be required for full-season weed control. Nurse et al. (2006) reported that flufenacet plus metribuzin applied PRE followed by (fb) one in-season glyphosate application provided greater control than glyphosate applications alone in soybean. Hartzler et al. (2006) reported that in a soybean-corn rotation in chisel and no-tillage systems there were acceptable levels of weed control for all treatments. The herbicide programs used over this 4-yr study had no impact on the density of any weed species upon completion of the study; however, Amaranthus spp. were more prevalent in the chisel-plow system than in the no-tillage system.
Palmer amaranth control in cotton can be more difficult than in other crops. Not many POST over-the-top herbicides are available to the cotton producer, leaving glyphosate as the primary herbicide for weed control. Prior to 2006, when glyphosate-resistant cotton was introduced, glyphosate could be applied POST only until the fourth true cotton leaf and then had to be post directed (PD) to avoid fruit abortion (Viator et al. 2004). Edenfield et al. (2005) reported yield losses of 14 to 19% when glyphosate was applied POST over-the-top of 12-leaf cotton. Although glyphosate provides control of many problematic weeds in cotton, it lacks residual activity and requires multiple applications throughout the season for acceptable weed control (Culpepper and York 1998).

Glyphosate used in conjunction with a residual herbicide may provide excellent weed control without decreasing yields (Brecke and Colvin 1997; Isgett et al. 1997; Keeling and Dotray 1997). Askew and Wilcut (1999) reported when soil-applied herbicides were followed by glyphosate, cotton yield increased 210 to 220 kg ha\(^{-1}\) compared to glyphosate alone. However, other research suggests that cotton yield from sequential applications of glyphosate alone is equal or superior to soil-applied herbicides with or without glyphosate (Groves et al. 2001).

**Resistance Evolution**

In recent years, agricultural practices have shifted from conventional tillage systems to reduced-tillage or no-tillage systems. The shift in cultural practices has been accompanied by increased cropping intensity and heavy reliance on herbicides for weed control (Llewellyn et al. 2002). The importance of glyphosate in weed management programs has been invaluable over the last decade; however, overreliance on a single herbicide increases the risk of resistance.
evolution (Llewellyn et al. 2007; Maxwell et al. 1990; Neve et al. 2003; Richter et al. 2002). Young (2006) reported that glyphosate use increased over the last decade with the introduction of glyphosate-resistant crops. Glyphosate use increased from 2.5 to 30 million kg yr$^{-1}$ from 1996 to 2002. The average number of glyphosate applications increased from one year$^{-1}$ in 1996 to 1.8 year$^{-1}$ by 2002 (Young 2006). By 2005, glyphosate was applied to over 95% of the total cotton acreage in the United States (NASS 2006). It has been suggested in recent years that producers should adopt management practices to prevent or delay glyphosate resistance (Beckie 2006; Johnson and Gibson 2006; Llewellyn et al. 2007). Although attempts have been made to keep glyphosate at its original level of effectiveness by rotating with other herbicides, most have been unsuccessful, because they require large-scale adoption from producers.

Several factors are involved in resistance management decisions. The efficacy of available herbicides, rate of resistance evolution, development of new alternative herbicides, and the cost of managing resistant weed populations are a few of the key factors that affect producers’ management decisions (Llewellyn et al. 2002). Producers have been encouraged to adopt integrated weed management (IWM) or best weed management (BWM) practices to help fight resistance evolution. Llewellyn et al. (2002) reported that many producers fail to adopt these practices because they are not economically feasible. Another resistance management alternative involves incorporating herbicides with different modes of action into weed management programs. Diggle et al. (2003) reported that if two herbicides were used in combination during a single year, as opposed to alternating herbicides each year, it decreases the chance of weeds evolving multiple resistance. Many producers fail to implement these resistance management strategies because they believe new herbicides will become available for
managing resistance or they sense it is not economically feasible to adopt these management strategies (Johnson and Gibson 2006; Llewellyn et al. 2002).

The Weed Science Society of America defines resistance as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (WSSA 1998). Resistance evolution occurs as a phenomenon where preexisting and continued selection for mutant genotypes occur under increasing herbicide pressure (Gressel 2002).

In the past, *Amaranthus* species, like many other weed species, have been noted for their ability to evolve herbicide resistance (Heap 2011). What factors are involved that make Palmer amaranth a favorable species to evolve herbicide resistance? The evolution of pesticide resistance is affected by genetic, biological/ecological, and operational factors (Neve and Powles 2005). Jasieniuk et al. (1996) reported that gene mutations, heritability, gene flow, weed fitness, and reproduction all influence the evolution of herbicide resistance.

The *Amaranthus* species are noted for their high genetic diversity, a characteristic that increases the likelihood of resistance (Foes et al. 1998). The genome size of *Amaranthus* species ranges from 0.95 to 1.4 pg 2C\(^{-1}\) nucleus for Palmer amaranth and tall waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), respectively (Rayburn et al. 2005). This genetic diversity, coupled with prolific seed production, could prove to be detrimental in managing the spread of glyphosate-resistant Palmer amaranth. One of the most critical aspects of resistance evolution is the initial presence of resistant alleles in a population’s genome (Jasieniuk et al. 1996; Neve and Powles 2005). One question regarding the presence of these alleles is whether they are dominant or recessive. Under selection pressure, dominant resistance alleles will increase more rapidly than recessive alleles. This occurs through natural selection, in which susceptible individuals are
controlled and plants with a resistant gene survive and reproduce, increasing the frequency of resistance genes (Tranel et al. 2002).

Point mutations, a type of genetic mutation, are one way that resistant genes evolve in some weed species (Tranel et al. 2002). In species with high genetic diversity, the chance of selecting for a mutation is higher (Jasieniuk et al. 1996). These mutations occur spontaneously and are usually not the result of herbicide applications. The second way that resistant populations may evolve is by transfer of genetic material via introgressive hybridization (Rayburn et al. 2005). Transfer of genetic material usually occurs by either nuclear or cytoplasmic inheritance. Except for the triazines, in which the resistance gene is located in the chloroplast, all documented cases of resistance occur through nuclear inheritance (Jasieniuk et al. 1996). When weed infestations are dense, selecting for resistance can be high, even if gene mutations are relatively low. In the case of Palmer amaranth, an obligate outcrosser, dominate alleles will increase in frequency under intense herbicide selection pressure.

Introgressive hybridization may occur when closely related species, such as the *Amaranthus* species, are present in the same ecosystem. A general question many have asked about hybridization is whether resistant genes can be transferred in this manner (Franssen et al. 2001; Tranel et al. 2002; Trucco et al. 2004; Trucco et al. 2005). Franssen et al. (2001) reported that hybridization could be responsible for the rapid spread of acetolactate synthase (ALS) resistance among and between monoecious and dioecious *Amaranthus* species. When female plants of the dioecious waterhemp were pollinated with male plants of a monoecious smooth pigweed (*Amaranthus hybridus* L.) containing a resistant ALS gene, 85% of the F₁ progeny survived 210 g ae ha⁻¹ of imazethapyr (Tranel et al. 2002). A survival rate of 85% indicates that the ALS resistance gene was paternally inherited. Wassom and Tranel (2005) reported that Palmer
amaranth and tall waterhemp do not necessarily hybridize with each other more than they would with other monoecious species. Understanding that resistance can be transferred from a monoecious species to a dioecious *Amaranthus* species, the question remains as to whether resistance traits express complete or incomplete dominance over susceptible traits and how frequently this transfer may occur in field situations.

Darmency (1994) reported that the majority of herbicide resistance traits are inherited in a dominant or semi-dominant manner. Does glyphosate resistance fall into one of these categories? When glyphosate-susceptible and -resistant Italian ryegrass [*Lolium rigidum* (Gaud.)] were crossed, the F$_1$ progeny exhibited incomplete dominance after glyphosate treatment (Lorraine-Colwill et al. 2001). Incomplete dominance of Italian ryegrass was evident when based on the LD$_{50}$ values because both the F$_1$ homozygous and heterozygous populations (857 and 937 g a ha$^{-1}$, respectively) were between the glyphosate susceptible and resistant populations (354 and 1833 g ha$^{-1}$, respectively), a characteristic of incomplete dominance (Lorraine-Colwill et al. 2001). Backcrossing the F$_1$ progeny with a susceptible parent produced phenotypic ratios typical of a single-gene trait; therefore, it is suspected that glyphosate resistance in Italian ryegrass is a pollen-mediated trait (Lorraine-Colwill et al. 2001).

Quantifying the frequency of resistance transfer in Palmer amaranth will depend largely on pollen viability and movement under field conditions. In situations where both the homozygous and heterozygous populations exhibit strong resistance, the rate of evolution will increase at a rate that is dependent on the ability of a species to transfer genes via pollen. The physical characteristics of Palmer amaranth pollen grains determine their ability to travel in field situations. While some research has been done on Palmer amaranth pollen grain size and density for modeling the settling velocity and subsequent ability to travel via wind (Sosnoskie et al. 2011).
2009), the transfer of resistant traits is less understood in Palmer amaranth. Much of the research in this area has focussed on *Brassicaceae* spp., a close relative to the herbicide-resistant canola (*Brassica napus* L.) (Warwick et al. 2008).

**Glyphosate Mode of Action**

Glyphosate [(N-phosphonomethyl)glycine], a non-selective herbicide, inhibits 5-enolpyruvalshikimate-3-phosphate synthase (EPSPS), which is encoded by the *aroA* gene involved in aromatic amino acid biosynthesis (Jin et al. 2007; Sikorski and Gruys 1997). The EPSPS is responsible for catalyzing phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) to form 5-enolpyruvalshikimate 3-phosphate (EPSP) and inorganic phosphate. EPSP directly precedes the branch-point intermediate chorismate, which is required for the synthesis of the essential aromatic amino acids phenylalanine, tyrosine, and tryptophan, as well as auxins, quinones, and other essential components of plants (Lorraine-Colwill et al. 1999; Sikorski and Gruys 1997). Glyphosate, an analog of PEP, is a competitive inhibitor of EPSPS and binds in a ternary complex of EPSPS-S3P-glyphosate, effectively inhibiting the activity of EPSPS. This reduces aromatic amino acid synthesis and growth, eventually leading to premature cellular death of the plant (Duke 1988). Because EPSPS is found only in plants and certain microorganisms, glyphosate is essentially nontoxic to birds, fish, insects, and kills a broad spectrum of plants. In addition, glyphosate is broken down rapidly in the soil by microbes, giving it no residual effects.

A naturally occurring EPSPS gene, whose protein product is glyphosate-resistant, was identified in an *Agrobacterium tumefaciens* sp. CP4 (Barry et al. 1992; Padgette et al. 1995). Cloning this gene, followed by genetic insertion into crop plants, gave rise to the first
glyphosate-resistant crops. With the evolution of glyphosate-resistant weeds, recent research has shifted to focus on determining the resistance mechanism, particularly in glyphosate-resistant weeds (Culpepper et al. 2006; Lorraine-Colwill 2001; Neve and Powles 2005; Wakelin and Preston 2006). Glyphosate resistance may occur through several mechanisms including, but not limited to, reduced absorption or translocation of glyphosate, increased levels of EPSPS, an insensitive target site on EPSPS, or glyphosate metabolism (Feng et al. 2004; Gaines et al. 2010; Powles and Preston 2006). Some soil microorganisms contain the enzyme glyphosate oxidoreductase (GOX), which cleaves the nitrogen-carbon bond in glyphosate to yield aminomethylphosphonic acid (AMPA). Another enzyme, glyphosate N-acetyl transferase, acetylates and deactivates glyphosate (Pline-Smic 2006).

Symptoms of glyphosate activity typically occur within 12 to 14 DAT; however, there are several changes that rapidly occur at the biochemical level (Maria et al. 2006). A foliar-applied herbicide such as glyphosate has to pass through several barriers, such as the epicuticular wax and the plasma membrane before reaching glyphosate’s target site, EPSPS. It is because of this that many different glyphosate formulations have been tested for uptake and weed control efficacy (Harring et al. 1998). Interest was directed to the changes that occur on the biochemical level because changes in metabolite concentrations occur soon after exposure and indicate glyphosate has reached the target site. Measuring shikimic acid accumulation, resulting from inhibition of EPSPS (blocking shikimate pathway), is one technique that can be used to quantify the effectiveness of glyphosate at the target site (Harring et al. 1998; Koger et al. 2005).

**Confirmation of Resistance**
To be successful in resistance management, one must be able to quickly and efficiently detect herbicide resistance. Efficient screening for herbicide resistance is a main component of resistance management (Beckie et al. 2000; Koger et al. 2005). Screening tests use discriminating doses of herbicide to differentiate between resistant and susceptible biotypes. Initial characterization of biotypes should be done using this method because many biotypes may vary in their discriminating doses (Beckie et al. 2000). However, this method of screening can be expensive and time consuming. Koger et al. (2005) suggest that when time is a factor, *in vivo* assays using seed, pollen, or plant tissue can be used to efficiently screen for resistance.

Seed collection for screening is easier today than in the past. With the increase of confirmed glyphosate-resistant species over the last decade (Heap 2011), many producers now report putative resistant species each growing season. Prior to the confirmation of glyphosate-resistant species, a survey was the only source of detecting suspected resistant species. While surveys are still a useful tool, agricultural extension agents are now faced with increasing reports of suspected resistance. Most resistant weeds will have a patchy distribution in agricultural fields (Beckie et al. 2000). A form of on-site herbicide screening allows extension agents to begin assessment before collecting seed. Species that are suspected of being resistant are sprayed with a discriminating herbicide rate prior to seed collection, to determine the likelihood that a patchy distribution is due to resistance, rather than to environmental or management practices.

Beckie et al. (2000) reported that seed should be collected from at least 40 putative plants that have at least 1,000 viable seeds each. However, the number of seed may vary depending on the species itself, the size of the resistant population, amount of seed needed to complete initial screening, mating pattern, and subsequent research. Enough seed should be collected to provide
sufficient numbers of plants to avoid a faulty conclusion (Beckie et al. 2000). Since field-collected samples will almost always be a mixture of susceptible and resistant biotypes, determining the discriminating doses is an essential step in separating resistant and susceptible biotypes.

The range of herbicide doses should be ample enough to quantify the level of glyphosate resistance. The glyphosate rate range should be low enough that both susceptible and resistant plants live, yet high enough to kill both the resistant and susceptible plants. In the case that there are survivors at the highest rate, additional screening may be done in order to quantify the level of resistance. There are several other methods that can be used to further explain the level of resistance in a particular species. One type of dose-response experiment determines the LD$_{50}$, or the dose of herbicide needed to kill 50% of a biotype (Neve and Powles 2005). The design of this experiment is such that the LD$_{50}$ of both the resistant and susceptible biotype can be compared to confirm presence of resistance.

**Shikimic Acid Accumulation**

Several researchers have used shikimic acid to differentiate between glyphosate-resistant and -susceptible biotypes (Shaner et al. 2005). Accumulation of shikimic acid indicates that glyphosate has reached its target site and is effectively inhibiting EPSPS activity. Shikimic acid analysis may also provide an indication of what type of resistance mechanism may be present in a given biotype. Within a biotype, a susceptible plant should have higher amounts of shikimate accumulation as glyphosate concentration increases. In any biotype, there may actually be more than one resistance mechanism responsible for resistance. In this case, a shikimate concentration vs. glyphosate concentration curve generated over time can actually separate the susceptible
plants from the resistant plants. If results from shikimate accumulation indicate multiple curves with different functional shapes, there could be multiple types of resistant mechanisms.

A plant with an altered herbicide absorption or translocation mechanism would be expected to have some shikimate accumulation, since some glyphosate is still likely to reach the target site. This curve would look similar to the susceptible biotype, with lower shikimate accumulation. Glyphosate metabolism would be dependent on the rate of glyphosate, but would still accumulate shikimate at high enough rates. However, it is not suspected that there are many plants that can metabolize glyphosate, making this a less viable possibility when looking for resistant mechanisms (Sammons et al. 2007). A plant with an altered target site (mutation) would have much less shikimate accumulation (Sammons et al. 2007; Wakelin and Preston 2006). In a situation where a plant has multiple resistance mechanisms, it would be expected that there would be even less shikimate accumulation than would be indicated by either the altered uptake or altered target site alone, even at the higher concentrations of glyphosate. Quantifying the level of shikimate accumulation after exposure to glyphosate is a critical step for initiating research on suspected glyphosate-resistant populations.

Many methods are used to quantify the level of shikimic acid accumulation, and most have produced significant differences between glyphosate-resistant and glyphosate-susceptible biotypes (Feng et al. 2004; Shaner et al. 2005). A whole-plant analysis, although destructive, can provide a large amount of information about potential resistance mechanisms. This type of analysis incorporates all above-ground portions of the plant, which is important because maturity levels of the plant dictate the development of the biochemical pathways and their constituents. Plants are harvested at the base and then ground to a fine powder in liquid nitrogen. Plant material is then digested with hydrochloric acid (HCL). Using a centrifuge for supernatant
separation, an HPLC can then be used to detect levels of shikimate in the harvested plants. There are other, non-destructive methods, available for use when screening plants for resistance. Koger et al. (2005) reported two rapid, non-destructive assays to differentiate glyphosate-resistant and glyphosate-susceptible horseweed \([Conyza canadensis \ (L.) \ Cronq.]\). One assay called for removal of a single plant leaf and dipping it into a known solution of glyphosate for 3 d. The other was an \textit{in vivo} evaluation using excised leaf disc tissue that were incubated in solutions of known glyphosate concentrations for 16 h. The leaf dip assay used only a visual rating of necrosis to differentiate between glyphosate-resistant and glyphosate-susceptible horseweed, while levels of shikimate in the leaf disc assay were measured using a spectrophotometer (Koger et al. 2005).

**Glyphosate Absorption and Translocation**

Glyphosate resistance in rigid ryegrass (Lorraine-Colwill et al. 2001) and horseweed (Feng et al. 2004) have been attributed to reduced translocation of glyphosate. The success of an exclusion mechanism such as reduced translocation can be characterized by the LD\(_{50}\) for that particular herbicide (Sammons et al. 2007). Because of this, the selection process for resistance of this type is highly dependent on the glyphosate rate applied. Low levels of resistance can be partially managed with higher rates of glyphosate, assuming that the rate was high enough to kill the plant or prevent seed development.

The primary mechanism of glyphosate resistance in horseweed is reduced translocation of glyphosate to the meristematic tissues (Koger and Reddy 2005). Yu et al. (2007) also reported that reduced translocation is partially responsible for the resistance mechanism in rigid ryegrass. This is important because glyphosate targets EPSPS, an enzyme found in high concentrations of
the young, growing portions of the plant (Weaver and Herman 1997). Without normal systemic movement of glyphosate to the actively growing apical meristem, herbicide failure becomes a major issue. Each herbicide application that does not effectively translocate to the growing point can result in glyphosate failure, increasing resistance evolution.

**Glyphosate Metabolism**

In the soil, microorganisms readily metabolize glyphosate to AMPA. It has been speculated that some plants may be able to metabolize glyphosate in this way (Healy-Fried et al. 2007). Reddy et al. (2004) reported that AMPA accumulated in glyphosate-resistant and glyphosate-susceptible soybean leaves, indicating that a plant GOX exist. Reddy et al. (2004) found that although AMPA is still phytotoxic to plant species, it has considerably less activity than glyphosate. Reddy et al. (2004) reported that external application of AMPA to glyphosate-resistant and glyphosate-susceptible canola caused no chlorotic injury, indicating that AMPA could have been further metabolized to nonphytotoxic, secondary metabolites before causing injury to glyphosate-resistant and glyphosate-susceptible canola. Other research suggests the presence of a C-P lyase, which metabolizes glyphosate to sarcosine (Reddy et al. 2008).

Some leguminous species are naturally more tolerant to glyphosate than other weed species. Could this be because of glyphosate metabolism? It is suspected that six of seven leguminous species tested in research by Reddy et al. (2008) have a plant GOX; however, AMPA data did not support the theory that metabolism of glyphosate was responsible for low levels of tolerance to glyphosate. Several other factors involved could be responsible for this, including interception and retention of spray, absorption, translocation, and sensitivity of EPSPS among the species.
Gene Flow

The development and use of GM crops has led to increasing concern of the environmental impact on agronomic ecosystems. One concern is that pollen-mediated gene flow (cross-fertilization) will spread GM genes to non-GM crops or to wild relatives of the crops. This has been particularly evident in recent struggles with the European Union over labeling GM adventitious presence thresholds for food and feed.

Recent research has focused on characterizing the relationship and frequency of outcrossing (hybrid formation) in field situations. Shivrain et al. (2007) reported that outcrossing was evident up to 6 m away, although at low levels, between herbicide-resistant Clearfield (CL) rice and red rice (*Oryza sativa* L.). Even at extremely low levels of outcrossing (0.003 to 0.008%), the results were approximately 170 resistant plants ha\(^{-1}\) (Shivrain et al. 2007).

Similar problems with pollen-mediated gene flow may play a major role in the spread of glyphosate resistance in Palmer amaranth. Dioecious species such as Palmer amaranth produce pollen and are considered obligate outcrossers. Trucco et al. (2005) reported that *Amaranthus* species can exhibit interspecific hybridization, and that this contributes to the evolution of herbicide resistance. Outcrossing levels were relatively high (33%), and a single tall waterhemp female plant was capable of producing more than 200,000 hybrids. Depending on the level of resistance, selection for resistance may increase or decrease according to the rate of herbicide used. This could play a critical role in how fast resistance spreads through pollen-mediated gene flow.

A limiting factor in pollen-mediated gene flow is how far viable pollen can travel in nature. This is an area not well understood or studied until recent problems with cross-pollinating, or
outcrossing. Most available models simulate gene flow well; however, these models are often restricted due to limited environmental variability in the datasets (Beckie and Hall 2008). Previous research from Franssen et al. (2001) has shown that ALS resistance in *Amaranthus* species can occur via interspecific hybridization. Specifically, ALS-resistant alleles from Palmer amaranth were transferred to non-ALS-resistant common waterhemp, a monoecious species. It is believed that glyphosate resistance alleles could be spread in the same way, and therefore more research is needed to fully understand the implications of pollen-mediated gene transfer in glyphosate-resistant Palmer amaranth.

**Seed Dispersal**

Understanding that you have resistance, the next step should be preventing the spread and distribution by intensive management strategies. Weed seeds can spread short or long distances by wind, water, animals, and humans (Thill and Mallory-Smith 1997). Because of this, it is extremely important to implement management strategies to prevent weed seed production and spread.

Long-distance weed seed spread is usually associated with the invasion and subsequent colonization by a foreign weed species (Thill and Mallory-Smith 1997). If, for instance, the foreign weed seeds happen to be from a glyphosate-resistant weed, problems only worsen. Short-distance weed seed spread is usually responsible for the local distributions and densities of natural weed populations in a cropping system. It is for this reason that it is important to fully understand the characteristics of weed seeds and the mechanisms by which they are dispersed.

Recently, glyphosate-resistant horseweed rapidly spread across the Midsouth, largely due to long-distance seed dispersal and subsequent colonization. Dauer et al. (2007) reported that
horseweed regularly disperses seed at least 500 m from source populations. As the population size increases, the chance of long-distance dispersal increases up to 1,500 m, which would easily affect surrounding farms. With selection pressure for glyphosate resistance at an all-time high, the spread of glyphosate-resistant horseweed will continue.

Although Palmer amaranth seed is not moved by wind as effectively as horseweed, there are several other mechanisms that may play a role in seed dispersal. In a review from Thill and Mallory-Smith (1997), weed seed that moved in manure did not increase the number of weed seeds in the seedbank (Mt. Pleasant and Schlather 1994); however, this mechanism could be responsible for spreading weeds long distances, including glyphosate-resistant Palmer amaranth. Gin trash would be one example of a medium that could transport Palmer amaranth seed. Mechanical movement by farm implements such as tractors, combine harvesters, spray units, and fertilizer spreaders are all possible mechanisms of seed movement (Thill and Mallory-Smith 1997).

Seed dispersal via animals or birds is also a possibility. Epizoochory and endozoochory, the terms for seed adherence to an animal’s fur and for the ingestion of seeds, respectively, are two ways in which animals or birds can spread seed (Benvenuti 2007). Research in this area is usually species specific, limiting the data for Palmer amaranth seed movement by animals or birds.

Hydrochory, or spatial movement of seed by floating in waterways and/or runoff processes, is another way in which Palmer amaranth seed may travel (Benvenuti 2007). The small size of Palmer amaranth seed make it a likely candidate for this type of dispersion. In a flood-prone area, such as the Delta in east Arkansas, it is likely that seed will travel long distances in this way.
Weed Species Shifts

The extensive use of glyphosate has resulted in unparamounted selection pressure for glyphosate resistance. Other than resistance, what ways have producers been affected by overuse of glyphosate? Weed populations often change in response to new or extensively used herbicides (Culpepper 2006). Extensively used herbicides can, and often will, shift the population of weed flora present in a given field. The adoption of glyphosate eliminated or limited the need for weed control practices such as tillage and herbicide rotations. In doing so, the continual use of glyphosate selects for weeds that are more tolerant, such as the *Ipomoea* species and the *Cyperus* species (Culpepper 2006).

Data for weed species shifts are extremely limited; however, we know that shifts from more susceptible to more tolerant species occurs by both chemical and non-chemical methods (Culpepper et al. 2004; Marshall et al. 2000). Previous research conducted by Reddy (2004) has shown that the shift in spectrum of weeds toward more tolerant species in bromoxynil-resistant (BR) cotton can be prevented by rotating BR cotton with GR cotton. While BR cotton is no longer an option for producers, glufosinate-resistant or Liberty Link® (LL) cotton is now available for commercial use as a viable option for rotating weed management systems in cotton.

Glufosinate inhibits glutamine synthase, the enzyme responsible for converting glutamic acid and ammonia into glutamine, leading to a rapid accumulation of ammonia and glyoxylate within the plant. This accumulation causes damage to the chloroplasts, essentially stopping photosynthetic activity and leading to necrosis of plant tissue (Everman et al. 2007). Because glufosinate is a nonselective, contact herbicide providing broad-spectrum grass and broadleaf weed control in Liberty Link cotton, it may potentially be integrated into many weed
management programs. With the evolution of glyphosate-resistant weed species, glufosinate has emerged as one of the best options for management of glyphosate-resistant weeds. In research from Mississippi, glufosinate applied alone provided at least 88% control of horseweed (Eubank et al. 2008), while other research suggests a tank mix of glufosinate plus dicamba or 2,4-D is needed for acceptable control under field conditions (Steckel et al. 2006).

Research is necessary to determine the effects of rotating Roundup Ready Flex and Liberty Link cotton systems on weed species shifts and cotton yields. Overreliance on Roundup Ready technology has led to the development of several resistant species (Heap 2011); therefore, it is important to understand the benefits of rotating Roundup Ready Flex and Liberty Link cotton systems. In addition, characterizing the shift in weed species during system rotations may allow more accurate predictions as to which species are more likely to develop resistance in the future.
LITERATURE CITED


CHAPTER I
GLYPHOSATE-RESISTANT PALMER AMARANTH IN ARKANSAS
ABSTRACT

The objective of this research was to confirm glyphosate resistance in two Palmer amaranth biotypes from Mississippi (MC-R) and Lincoln (LC-R) Counties, Arkansas. There were noticeable differences in response to field doses of glyphosate, suggesting varying levels of glyphosate resistance. A susceptible (S) biotype from Clarendon County, South Carolina, which had never been exposed to glyphosate, was used for comparison in each experiment. Shikimic acid concentration over time was significantly greater in the S biotype than both the MC-R and LC-R biotypes. The lethal dose required to kill 50% (LD₅₀) of the population was 2,255 and 3,223 g ae ha⁻¹ for the MC-R and LC-R biotypes, respectively, with no statistical difference between the LD₅₀ values of the two resistant biotypes. However, based on subtle differences in phenotypic response to glyphosate, mortality in response to glyphosate, and shikimic acid accumulation, it was hypothesized that the two Arkansas biotypes had a different resistance mechanism. Metabolism of glyphosate to its major metabolite, aminomethylphosphonic acid, was not responsible for resistance in either biotype. Reduced absorption in the LC-R and limited translocation from the treated leaf in the MC-R were at least partially responsible for the observed resistance to glyphosate; however, further investigation of other mechanisms such as EPSPS amplification would help clarify the observed resistance to these Arkansas Palmer amaranth populations.


Key words: Herbicide resistance, resistance mechanism.
INTRODUCTION

Glyphosate, a nonselective herbicide, was commercialized in 1974 for use in agriculture, primarily for broad spectrum, preplant burndown weed control, including perennial weeds (Franz et al. 1997). A shift towards conservation tillage and the introduction of glyphosate-resistant soybean [Glycine max (L.) Merr.], cotton (Gossypium hirsutum L.), and corn (Zea mays L.) in the mid-1990s has substantially increased the use of glyphosate over the last 15 years. The convenience of applying a single herbicide for broad-spectrum weed control either preplant burndown or postemergence (POST) over-the-top (OT) of resistant crops introduced flexibility and allowed producers to replace traditional herbicides and cultivation in weed management programs. Although glyphosate introduced a new herbicidal mechanism of action (MOA) for use in agronomic crops, it has reduced the use of traditional MOA’s with residual activity, such as the dinitroanalines and imidazolinones in soybean and the dinitroanalines and phenyl ureas in cotton (Corbett et al. 2004; Faircloth et al. 2001; Young 2006).

Glyphosate provided simple, cheap, flexible, and effective weed control, allowing producers to rely on a total-POST herbicide program consisting of sequential glyphosate applications (Franz et al. 1997; Grossbard and Atkinson 1985). Since glyphosate lacks residual activity, multiple applications may be required for season-long weed control (Grichar et al. 2004; Groves et al. 2001), a common practice across the southern states (Culpepper et al. 2006; Norsworthy et al. 2008; Steckel et al. 2008). Prior to the development of glyphosate-resistant crops, glyphosate was largely used for preplant burndown, which has been associated with low risks of resistance evolution (Neve 2008). Previous reports predicted weed resistance to glyphosate was unlikely due to its particular MOA (Baylis 2000) or other unique properties such as chemical structure, lack of metabolism, or absence of residual activity (Bradshaw et al. 1997). However, the
increased use of a single MOA applied POST over-the-top alone associated with herbicide-resistant cropping systems increases the selection pressure for resistance evolution and has led to the widespread occurrence of resistance to herbicides, including glyphosate-resistant weed species (Nandula 2010; VanGessel 2001).

Herbicide selection and application timing are two operational factors that influence resistance evolution in addition to genetic, biological, and ecological characteristics of weeds (Neve 2008). Currently, the U.S. has confirmed glyphosate-resistance in 13 weed species, including glyphosate-resistant Palmer amaranth [Amaranthus palmeri (S.) Watts], horseweed [Conyza canadensis (L.) Cronq.], common waterhemp [Amaranthus tuberculatus (Moq.) Sauer], Italian ryegrass [Lolium perenne spp. multiflorum (Lam.) Husnot], rigid ryegrass (Lolium rigidum Gaudin), giant ragweed (Ambrosia trifida L.), common ragweed (Ambrosia artemisiifolia L.), hairy fleabane [Conyza bonariensis (L.) Cronquist], goosegrass [Eleusine indica (L.) Gaertn], annual bluegrass (Poa annua L.), kochia [Kochia scoparia (L.) Schrad], junglerice [Echinochloa colona (L.) Link], and johnsongrass [Sorghum halepense (L.) Pers.] (Heap 2012).

The Amaranthus spp. are comprised of 60 to 70 species, with approximately 50 species native to the Americas (Kigel 1994; Sauer 1976). Palmer amaranth is an erect, branched, dioecious, summer annual broadleaf native to the Southern Great Plains (Bryson and DeFelice 2009). Palmer amaranth is highly competitive, reducing yields in cotton (Morgan et al. 2001; Rowland et al. 1999), corn (Massinga et al. 2001), soybean (Klingaman and Oliver 1994; Monks and Oliver 1988), grain sorghum [Sorghum bicolor (L.) Moench] (Moore et al. 2004), and peanut [Arachis hypogaea (L.) Perry] (Burke et al. 2007).
Several competitive characteristics of Palmer amaranth favor its resistance evolution. Palmer amaranth grows rapid and upright via a C4 photosynthetic pathway (Ehleringer 1983; Horak and Loughin 2000), and exhibits high fecundity and prolific seed production of up to 1.8 million seed plant\(^{-1}\) (Dr. Ken Smith, unpublished data). High growth rates under optimal conditions make herbicide application timings difficult to achieve for large- hectare farms. Palmer amaranth has high genetic diversity (Bond and Oliver 2006; Gray et al. 2007) and is an obligate outcrosser, causing concern about the heritability and spread of adventitious traits such as herbicide resistance (Davis et al. 2010). These characteristics place Palmer amaranth among the most resistance-prone dicots in the U.S., with resistance now confirmed to four different MOA (Heap 2012). Glyphosate-resistant Palmer amaranth has been confirmed in 15 U.S. states (Heap 2012) and is commonly considered one of the most troublesome species in southern U.S. agriculture (Webster 2009).

Glyphosate-resistant Palmer amaranth in Arkansas was first confirmed in Mississippi County (MC-R) in November 2006 (Norsworthy et al. 2008). After treatment with field doses of glyphosate, the MC-R biotype expresses varying levels of chlorosis, necrosis, and stunting, with regrowth occurring after the apical meristem was destroyed. Succeeding generations of the MC-R biotype continue to segregate with a scattered distribution of resistant and susceptible plants the following year. Glyphosate-resistant Palmer amaranth biotypes had already been reported in Georgia, Tennessee, and North Carolina (Cuppeper et al. 2006; Heap 2012; Steckel et al. 2008). Resistance in the Georgia biotype was high, with some plants surviving glyphosate at 10,000 g ae ha\(^{-1}\), while the Tennessee biotype was controlled with glyphosate at 3,360 g ha\(^{-1}\) (Culpepper et al. 2006; Steckel et al. 2008). The MC-R biotype was controlled 95% with glyphosate at 12,500 g ha\(^{-1}\), a similar response to the Georgia biotype (Norsworthy et al. 2008). Shikimic acid
accumulation in treated leaf tissue, an indicator that glyphosate has effectively reached its 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) target-site, occurred in the low-level resistant Tennessee biotype but not in the Georgia biotype (Culpepper et al. 2006; Steckel et al. 2008). Further investigation of possible resistant mechanisms in the Georgia biotype indicated no differences in $^{14}$C-glyphosate absorption or translocation or a ploidy change (Culpepper et al. 2006). Other known resistant mechanisms possible in weeds include: metabolism, altered or mutated target site, sequestration or compartmentalization, and gene amplification (Nandula 2010). The resistant mechanism in the Georgia biotype has since been confirmed as EPSPS gene amplification (Gaines et al. 2010), while the Tennessee biotype remains unknown.

A second biotype in Lincoln County, AR (LC-R) survived sequential field applications of glyphosate at 3,360 g ha$^{-1}$ in the summer of 2006 and was believed to have a higher level of resistance than the MC-R biotype. There were notable differences in the LC-R and MC-R biotypes field response to glyphosate (Smith et al. 2008). The MC-R biotype exhibited a lower level of resistance when treated with field rates of glyphosate, and tended to segregate. Chlorosis, necrosis, and stunting were noted after field applications, while the LC-R biotype did not segregate and showed a higher level of glyphosate resistance with little response to glyphosate applications (Smith et al. 2008). Preliminary greenhouse work indicated differences in response to glyphosate between the MC-R and LC-R biotypes; therefore, it was hypothesized that there may be different resistant mechanisms responsible for the observed resistance. The objectives of this research were: (1) to confirm resistance and determine the level of shikimic acid concentration after treatment with glyphosate in the MC-R and LC-R biotypes; (2) to quantify the level of resistance in the MC-R and LC-R biotypes compared to a known susceptible population never exposed to glyphosate; (3) to determine if glyphosate metabolism is
occurring and to investigate \(^{14}\)C-glyphosate absorption and translocation as possible resistance mechanisms.

**MATERIALS AND METHODS**

**Plant Materials.** The MC-R biotype seed used in these experiments were the F2 generation obtained from a controlled cross of F1 parents that had survived glyphosate\(^1\) at 8,960 g ha\(^{-1}\) (Norsworthy et al. 2008). Seed from the LC-R resistant biotype were sent to Fayetteville, AR where they were sown in 10 cm-diam pots containing commercial potting mix\(^2\) and placed in the greenhouse with a 30/20 C day/night temperature and a 16-h photoperiod. These plants were screened with glyphosate\(^1\) at 3,360 g ha\(^{-1}\) plus 0.25% v/v nonionic surfactant\(^3\) (NIS) at the four-to six-leaf growth stage with 100% survival. All spray applications were made in a stationary spray chamber equipped with two 8000\(^67\) nozzles\(^4\) calibrated to deliver 93.5 L ha\(^{-1}\) at 276 kPa. Male and female Palmer amaranth plants were selected at the 8- to 10-leaf growth stage and placed in a growth chamber with a 12-h diurnal cycle at 30/20 day/night temperature regime. A controlled cross was accomplished by shaking the pollinated male inflorescence with the female each day until pollen was no longer produced. Plants were given adequate water and nutrients\(^4\) through maturity and seeds were harvested and stored at 4 C. Because a Palmer amaranth population in Arkansas that had not been previously exposed to glyphosate was not readily available, Palmer amaranth seeds collected from Clarendon County, South Carolina (S) in 1986 that were believed never to have been exposed to glyphosate were used as susceptible standards. The aforementioned seeds from the MC-R, LC-R, and S biotypes were used for all greenhouse and laboratory experiments.
**Shikimate Accumulation.** These experiments were conducted from February to May of 2007. Palmer amaranth seeds from the MC-R, LC-R, and S biotypes were sown in separate trays containing potting mix and allowed to germinate in the greenhouse under 30/20 C day/night temperature with a 16-h photoperiod. Palmer amaranth seedlings were transplanted into 10 cm-diam pots containing potting mix at the cotyledon to one true-leaf stage. Plants were placed in the shade and allowed to root and acclimate for 2 d, then returned to normal conditions and allowed to grow.

The experiment was conducted in a completely randomized split-plot design with two factors and four replications. The whole plot was biotype (MC-R, LC-R, and S) and the sub-plot was harvest timings of 1, 3, 5, and 7 d after treatment (DAT). Methods were derived from Singh and Shaner (1998) and Mueller et al. (2003), with some modifications. Plants were treated with glyphosate\(^1\) at 210 g ha\(^{-1}\) at the five- to seven-leaf stage using a stationary spray chamber calibrated to deliver at 93.5 L ha\(^{-1}\). A nontreated control was also included, and the experiment was repeated.

One nontreated and four treated plants of each biotype were harvested at each time interval and the above-ground fresh weight of each was recorded. Subsamples were created from a single observational unit or replication for to determine repeatability. The nontreated plants from each biotype were used to correct for background levels of shikimic acid. Shoot tissue was finely ground in liquid nitrogen using a mortar and pestle. After grinding, the tissue was weighed in a 30-ml centrifuge tube, and 0.25 N HCl was added to the ground tissue at a ratio of 6 ml of HCl solution per 1 g of plant tissue. The samples were then placed on a rotary shaker at 250 rotations min\(^{-1}\) for 24 h. The extract was centrifuged at 10,000 rpm (9.6 x 10\(^3\) g) for 7 min and an aliquot of the supernatant was filtered through a 0.20-μm nylon filter and collected in a 2-
ml high performance liquid chromatography (HPLC) vial and stored at 4 C until analysis within 48 h.

An external standard calibration curve was established using 98% analytical grade shikimate. A 20-μl aliquot of the filtered sample was diluted with 980 μl of deionized water. The concentration of shikimate in Palmer amaranth was determined using a Hitachi HPLC system\(^5\) with an L-7100 pump, L-7455 diode array detector, L-7200 autosampler, D-7000 interface, ERC Inc. solvent degasser, and an Eppendorf column heater set to 30 C. The mobile phase was HCl (pH=2.1), at a flow rate of 0.250 ml min\(^{-1}\). Shikimate was quantified using peak height. Each 10-μl injection had a 10-min run time with shikimate eluted at 3.5 min. The level of shikimate in each treated sample is reported as μg shikimate g\(^{-1}\) plant tissue. Shikimate data were analyzed using PROC MIXED in SAS\(^6\) as a split-plot design with biotype as the main plot and harvest timing as the subplot. Experimental run was considered random. Shikimic acid was log transformed because variance was correlated with mean. Means were separated according to Fisher’s protected LSD with \(\alpha = 0.05\).

**Level of Glyphosate Resistance.** The MC-R, LC-R, and S biotypes were grown as previously described. The level of glyphosate resistance compared to a susceptible biotype that had never been exposed to glyphosate was quantified by determining the lethal glyphosate dose required to kill 50% of the population (LD\(_{50}\)). The experimental design was completely randomized with 8 replications of 11 glyphosate rates ranging from 13 to 13,440 g ha\(^{-1}\) of glyphosate, and the experiment was repeated. The lowest rate corresponds to 1/64 of a recommended glyphosate rate of 840 g ha\(^{-1}\). Seedlings were treated with glyphosate\(^6\) at the five- to seven-leaf stage (7 to 10 cm tall). A control treated with 0.25% v/v NIS alone was also included. The spray
applications were made in a stationary chamber with a boom containing two 800067 nozzles calibrated to deliver 93.5 L ha\(^{-1}\). After treatment, plants were returned to the greenhouse, with 30/20 C day/night temperatures and a 16-h photoperiod and were supplied adequate nutrients and water for an additional 28 d. Plant death (live or dead) was recorded at 28 DAT. The LD\(_{50}\) and LD\(_{95}\) were determined using PROC PROBIT in SAS. Confidence intervals (95%) were used to determine whether accessions responded differently to glyphosate.

**Glyphosate Metabolism.** MC-R, LC-R, and S biotypes were grown as previously described. Glyphosate metabolism was measured by quantifying the parent molecule and its metabolite, aminomethylphosphonic acid (AMPA), in treated tissue of resistant and susceptible plants using HPLC post-column derivatization. The experimental design was completely randomized with four replications and was repeated. The extraction procedure used was from Pickering Laboratories (2007) with some modifications.

Palmer amaranth biotypes were treated with glyphosate\(^1\) at 840 g ha\(^{-1}\) at the 12- to 14-leaf growth stage. For analysis, 15 g of treated leaves were harvested from each plant 48 h after treatment (HAT). Four separate 15 g samples were harvested and oven-dried at 60 C for 48 h to determine moisture content. To prepare each sample for solid phase extraction (SPE) cartridges (Pickering Laboratories 2007), the 15 g sample was homogenized with 60 ml H\(_2\)O in a high speed blender for 3 min and transferred to a 250 ml nalgene bottle and centrifuged for 10 min at 5,500 rpm. A 30 ml aliquot was transferred through glass wool into a centrifuge tube and centrifuged for 10 min at 3,500 rpm. A 20-ml aliquot was transferred to a 30-ml centrifuge tube and 6 ml methylene chloride was added before centrifuging at 3,500 rpm for 10 min. The addition and removal of methylene chloride was repeated two more times. A 9 ml aliquot was
transferred to another centrifuge tube and 1 ml of acidic modifier solution (16 g KH$_2$PO$_4$, 160 ml H$_2$O, 40 ml MeOH, 13.4 ml HCl) was added and centrifuged for 10 min. The SPE cartridge was conditioned with 10 ml deionized H$_2$O before placing 1 ml of extract in followed by 6 ml of elution solution (160 ml H$_2$O, 2.7 ml HCl, 40 ml MeOH). The elution solution was collected in a 10 ml volumetric vacuum (4 ml min$^{-1}$) and transferred to a 20 ml round-bottom flask. The samples were evaporated to dry under vacuum at 40 C for 15 min and then re-dissolved with 1.5 ml elution solution and filtered through a 0.20-µm nylon filter for HPLC analysis. Data were analyzed using PROC MIXED in SAS with run considered random.

$^{14}$C-glyphosate Absorption and Translocation. Palmer amaranth seeds from the MC-R, LC-R, and S biotypes were sown in separate trays containing potting mix and allowed to germinate in the greenhouse under 30/20 C day/night temperature with a 16-h photoperiod. Palmer amaranth seedlings were transplanted into 10 cm-diam pots containing potting mix at the cotyledon to one true-leaf stage. Plants were placed in the shade and allowed to root and acclimate for 2 d, then returned to a growth chamber with a 16-h diurnal cycle with 40% relative humidity and a 30/20 day/night temperature regime.

The experiment was designed as a split-plot in which the whole plot portion was a randomized complete block with four blocks and sampling time after herbicide application as the factor. Glyphosate-resistant Palmer amaranth biotype was the split-plot factor. The entire experiment was repeated twice. Runs of the experiment and blocks were treated as random effects and sampling time and biotype as fixed effects. Absorption and translocation of $^{14}$C-glyphosate in resistant and susceptible Palmer amaranth was determined as described by Norsworthy et al. (2001) and Brewer and Oliver (2009), with some modifications. Plants were
treated with glyphosate at 210 g ha\(^{-1}\) in a stationary spray chamber calibrated to deliver 93.5 L ha\(^{-1}\) at the four- to six-leaf stage. A small volume of spray solution was then spiked with \(^{14}\)C-phosphonomethyl-labeled glyphosate\(^8\) (\(^{14}\)C-glyphosate), (specific activity of 2.0 GBq mmol\(^{-1}\)) to make a stock solution with specific activity of 0.38 kBq \(\mu l^{-1}\). After the leaf surface had dried, the adaxial surface of the third youngest, fully opened leaf was spiked with four 1-\(\mu l\) droplets of \(^{14}\)C-glyphosate, containing a total of 92,000 dpm (23,000 dpm \(\mu l^{-1}\)).

Plants were separated into treated leaf, tissue above the treated leaf, aboveground tissue below the treated leaf, and the roots at 6, 12, 24, and 48 HAT. The treated leaf was rinsed with 5 ml deionized water in a 20-ml scintillation vial for 15 sec to remove nonabsorbed \(^{14}\)C-glyphosate. The aqueous rinsate was mixed with 10 ml scintillation cocktail\(^9\) and the radioactivity within each rinsate was quantified by liquid scintillation spectrometry\(^{10}\) to determine the amount of nonabsorbed \(^{14}\)C-glyphosate. Samples were stored at -18 C until they were combusted in a biological oxidizer\(^{11}\), and the evolved \(^{14}\)CO\(_2\) trapped in vials containing carbon dioxide absorbent and scintillation cocktail\(^{12}\). The trapped \(^{14}\)CO\(_2\) was quantified by liquid scintillation spectrometry. Data were expressed as proportions and analyzed as a generalized linear mixed model with a beta distribution for the response and a logit link function. Means for significant effects were separated on the logit scale and back transformed to the proportion scale for presentation.

**RESULTS AND DISCUSSION**

**Shikimate Accumulation.** There was a significant interaction between biotypes and harvest interval (p = <.0001). Shikimate accumulated in all biotypes following glyphosate application, indicating that EPSPS activity was affected; however, there were significant differences between
resistant and susceptible biotypes within each timing (Table 1.1). At 7 DAT, shikimate accumulation in the S biotype continued to increase (11,691 μg g tissue⁻¹), while shikimate accumulation in the MC-R and LC-R biotypes peaked at 3 and 5 DAT, respectively, and began to recover by 7 DAT. Both resistant biotypes accumulated significantly less shikimate than the S biotype at all harvest timings. At 1 DAT, the MC-R and LC-R biotypes had accumulated 26 and 24%, respectively, of the total shikimate quantified in the S biotype (2,817 μg g tissue⁻¹), indicating there were differences in the inhibition of EPSPS.

Differences in shikimate accumulation between resistant and susceptible plants have been reported in glyphosate-resistant horseweed, Italian ryegrass, rigid ryegrass, and Palmer amaranth, among others (Culpepper et al. 2006; Meuller et al. 2003; Nandula et al. 2008; Wakelin and Preston 2006). Several studies have suggested the pattern of shikimate accumulation, as well as the subsequent recovery of resistant plants can indicate that different, or even multiple mechanisms may be responsible for the observed resistance to glyphosate (Culpepper et al. 2006; Mueller et al. 2003; Wakelin and Preston 2006). It has also been suggested that an altered target-site is associated with no shikimate accumulation and that differences in absorption or translocation exhibit transient differences in shikimate accumulation over time (Culpepper et al. 2006; Mueller et al. 2003; Nandula et al. 2008); however, Gaines et al. (2010) have since confirmed EPSPS gene amplification in glyphosate-resistant Palmer amaranth from Georgia associated with no shikimate accumulation. Furthermore, altered target-site mechanisms have also been associated with low levels of shikimate accumulation (Wakelin and Preston 2006).
Table 1.1. Shikimate accumulation at 1, 3, 5, and 7 d after treatment (DAT) in the MC-R, LC-R, and S Palmer amaranth.\textsuperscript{a,b,c}

<table>
<thead>
<tr>
<th>Biotype</th>
<th>1 DAT</th>
<th>3 DAT</th>
<th>5 DAT</th>
<th>7 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-R</td>
<td>732</td>
<td>1,830</td>
<td>1,152</td>
<td>122</td>
</tr>
<tr>
<td>LC-R</td>
<td>675</td>
<td>679</td>
<td>829</td>
<td>340</td>
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<tr>
<td>S</td>
<td>2,817</td>
<td>6,461</td>
<td>9,472</td>
<td>11,691</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: MC-R, glyphosate-resistant biotype from Mississippi County, AR; LC-R, glyphosate-resistant biotype from Lincoln County, AR; S, susceptible biotype from Clarendon County, SC.

\textsuperscript{b} Biotypes were treated with glyphosate at 210 g ae ha\textsuperscript{-1}. All data were corrected for the shikimate concentration in the untreated biotype.

\textsuperscript{c} Fisher’s protected LSD ($\alpha = 0.05$) to compare means within a biotype = 1,119 $\mu$g g tissue\textsuperscript{-1}.

\textsuperscript{c} Fisher’s protected LSD ($\alpha = 0.05$) to compare different biotypes in the same or different time frame = 1,455 $\mu$g g tissue\textsuperscript{-1}. 


Differences in shikimate accumulation have also been attributed to different tissue types sampled, extraction procedures, quantification methods, as well as diversity within resistance mechanisms (Pline et al. 2002; Powles and Preston 2006; Simarmata et al. 2003). Although there were no differences in shikimate accumulation between the MC-R and LC-R biotypes at 5 DAT, the different trend in shikimate accumulation peak and recovery may suggest that different, or possibly multiple, resistant mechanisms may be responsible for the observed glyphosate resistance in these Arkansas biotypes.

**Level of Glyphosate Resistance.** The level of resistance was similar in the MC-R and LC-R biotypes, based on 95% confidence intervals. These biotypes were determined to be 19- and 27-fold more resistant, respectively, to glyphosate than the S biotype (Figure 1.1). The LD50 values of the MC-R, LC-R, and S biotypes were 2,254 g ha⁻¹, 3,222 g ha⁻¹, and 120 g ha⁻¹ of glyphosate, respectively. The level of resistance in the MC-R and LC-R biotypes is 3- to 4-fold greater than the labeled use rate of 840 g ha⁻¹ glyphosate, and is higher than others previously reported in Tennessee and Georgia (Culpepper et al. 2006; Steckel et al. 2008). Previous research from Norsworthy et al. (2008) has indicated that comparison of resistance across populations can be difficult due to different environmental conditions, different susceptible standards, and different growth stages.

Glyphosate is no longer a viable option for controlling the MC-R and LC-R biotypes, as evident by LD95 values of 13,538 and 18,611 g ha⁻¹ of glyphosate, respectively. Although 95% confidence intervals indicate similar response to glyphosate, a subtle difference in the level of resistance in the MC-R and LC-R biotype is apparent, both through visual observations in response to glyphosate, as well as trends in shikimate accumulation peak and recovery (Table
Figure 1.1. Probit analysis with 95% confidence intervals (thin lines) to predict the glyphosate dose needed to kill the MC-R, LC-R, and S Palmer amaranth biotypes when treated at the five- to seven-leaf growth stage. The MC-R, LC-R, and S biotypes were from Mississippi County, AR, Lincoln County, AR, and Clarendon County, SC.
Possible explanations for these observations include diversity of resistance mechanisms, different mechanisms entirely, or the presence of multiple mechanisms.

**Glyphosate Metabolism.** The metabolism of glyphosate to AMPA is not common in weed species but has been reported in some genetically modified crops such as soybean (Nandula et al. 2007; Nandula et al. 2008). There were no differences in AMPA accumulation in MC-R, LC-R, and S biotypes after treatment with glyphosate (Figure 1.2). The concentration of AMPA was extremely low, ranging from 0.76 to 0.84 μg AMPA g⁻¹ tissue. These results indicate glyphosate metabolism does not contribute to the glyphosate resistance observed in the MC-R and LC-R biotypes from Arkansas.

**¹⁴C-glyphosate Absorption and Translocation.** The main effect of time, or harvest interval, was significant for percent recovery and translocation (Table 1.2). At 6 HAT, >90% of the applied ¹⁴C- glyphosate was recovered (Table 1.2). Percent recovery of ¹⁴C-glyphosate declined over time, with 68% recovered at 48 HAT. The percent of ¹⁴C-glyphosate remaining in the treated leaf was highest at 6 HAT (Table 1.2), and declined over time as glyphosate translocation occurred. At 24 HAT, only 62 % of the absorbed ¹⁴C-glyphosate remained in the treated leaf. This coincided with an increase of ¹⁴C-glyphosate translocation to the aboveground plant portion below the treated leaf at 24 HAT (18%) and to 10% to the roots. While there were no significant differences in ¹⁴C-glyphosate translocation above the treated leaf, glyphosate movement below the treated leaf and to the roots increased over time, and was highest in each of these parts at 48 HAT (Table 1.2).
Figure 1.2. Glyphosate metabolism to aminomethylphosphonic acid (AMPA) in the MC-R, LC-R, and S biotypes 48 hours after treatment with 840 g ha$^{-1}$ of glyphosate at the 12- to 14-leaf Palmer amaranth growth stage. The MC-R, LC-R, and S biotypes were from Mississippi County, AR, Lincoln County, AR, and Clarendon County, SC.
Table 1.2. Percent recovery and translocation of $^{14}$C-glyphosate averaged over all three biotypes at 6, 12, 24, and 48 h after treatment.$^{a-b}$

<table>
<thead>
<tr>
<th>HAT</th>
<th>Recovery</th>
<th>$^{14}$C-glyphosate translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>6</td>
<td>92.1 (1.1) a</td>
<td>80.5 (2.3) a</td>
</tr>
<tr>
<td>12</td>
<td>88.3 (1.4) ab</td>
<td>77.5 (2.4) a</td>
</tr>
<tr>
<td>24</td>
<td>82.2 (1.7) b</td>
<td>61.9 (2.9) b</td>
</tr>
<tr>
<td>48</td>
<td>68.0 (2.1) c</td>
<td>47.5 (3.0) c</td>
</tr>
</tbody>
</table>

$^{a}$ Percent recovery based off of total $^{14}$C-glyphosate applied, percent translocation based off of the percent of $^{14}$C-glyphosate applied that was absorbed.

Translocation is the percent of $^{14}$C-glyphosate remaining in the treated leaf, the aboveground portion below the treated leaf, and the roots.

$^{b}$ Standard error of the mean in parentheses for comparing within a column; letters of significance for mean separation within a column.
There were significant differences in recovery, absorption, and translocation of $^{14}$C-glyphosate as a result of biotype (Table 1.3). Absorption of $^{14}$C-glyphosate was slightly less than reported in some literature, and may be related to study design and environmental conditions. The 40% relative humidity held in these experiments is lower than others previously reported, and is one possibility for lower absorption by Palmer amaranth plants. Brewer and Oliver (2009) reported increased $^{14}$C-glyphosate absorption from 38 to 80% by increasing relative humidity from 40 to 70%, respectively. Culpepper et al. (2006) reported 36% absorption by 48 HAT when plants were grown in 50% relative humidity. Another factor that may have limited absorption is the maximum 48 HAT harvest interval used in these experiments; some literature indicates increased absorption at 72 to 168 HAT (Brewer and Oliver 2009; Burke et al. 2007; Everman et al. 2009).

Glyphosate absorption occurred in all biotypes; however, there were significant differences among Palmer amaranth biotypes, with the LC-R biotype absorbing less $^{14}$C-glyphosate (11%) than the S (19%) or MC-R (21%) biotype (Table 1.3). Visual observations in the greenhouse and growth chambers show different phenotypes for the MC-R, LC-R, and S biotypes, as previously mentioned. The MC-R biotype exhibited varying levels of chlorosis, necrosis, and stunting after treatment with glyphosate with less vigor than the LC-R biotype. The LC-R biotype exhibited less chlorosis, necrosis, and stunting with a darker green leaf color and more vigor.

Movement out of the treated leaf occurred in all biotypes, but was significantly less in the MC-R biotype, with 76% of the $^{14}$C-glyphosate remaining in the treated leaf (Table 1.3). Less $^{14}$C-glyphosate translocated below the treated leaf (10%) in the MC-R biotype than in the S (18%) or the LC-R (16%). This may partially explain why the apical meristem was killed, with re-growth occurring on lower portions of the MC-R biotype. Subtle differences in translocation
Table 1.3. Percent recovery, absorption, and translocation of $^{14}$C-glyphosate for S, MC-R, and LC-R Palmer amaranth, averaged over time and experiment.$^{a,c}$

<table>
<thead>
<tr>
<th>Biotype</th>
<th>Recovery</th>
<th>Absorbed</th>
<th>$^{14}$C-glyphosate translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>S</td>
<td>82.3 (1.5) b</td>
<td>19.2 (1.5) a</td>
<td>61.2 (2.6) b</td>
</tr>
<tr>
<td>MC-R</td>
<td>87.9 (1.3) a</td>
<td>20.5 (1.5) a</td>
<td>76.3 (2.2) a</td>
</tr>
<tr>
<td>LC-R</td>
<td>82.3 (1.5) b</td>
<td>11.2 (1.2) b</td>
<td>65.8 (2.5) b</td>
</tr>
</tbody>
</table>

$^{a}$ Abbreviations: MC-R, glyphosate-resistant biotype from Mississippi County, AR; LC-R, glyphosate-resistant biotype from Lincoln County, AR; S, susceptible biotype from Clarendon County, SC; NS, not significant.

$^{b}$ Percent recovery based off of total $^{14}$C-glyphosate applied, percent translocation based off of the percent of $^{14}$C-glyphosate applied that was absorbed. Translocation is the percent of $^{14}$C-glyphosate remaining in the treated leaf, above the treated leaf, the aboveground portion below the treated leaf, and the roots.

$^{c}$ Standard error of the mean in parentheses for comparisons; letters of significance for mean separation within a column; the portion above the treated leaf and the roots were not significantly different (Above p-value = 0.0575; Roots p-value = 0.1810)
may be attributed to plant growth stages, herbicide absorption, and source-sink relationships (Dewey and Appleby 1983; Sandberg et al. 1980). Palmer amaranth also exhibits extreme genetic variability, and this could also account differences in absorption and translocation. The S biotype used in this experiment was collected in 1986 and was believed to have never been exposed to glyphosate. Overall, $^{14}$C-glyphosate translocation out of the treated leaf was significantly less in the MC-R biotype and $^{14}$C-glyphosate absorption was less in the LC-R biotype. These differences are at least partially responsible for the observed resistance to glyphosate, although they may not be the major mechanism of glyphosate resistance in these biotypes of Palmer amaranth.

**SUMMARY AND CONCLUSIONS**

Results from shikimic acid analysis and dose-response experiments confirmed that both the MC-R and LC-R Palmer amaranth were resistant to glyphosate. Although 95% confidence intervals indicated a similar response to glyphosate, a subtle difference in the level of resistance in the MC-R and LC-R biotype is apparent through visual observations in response to glyphosate. This observation was further supported by noticeable differences in shikimic acid accumulation peak and recovery for the glyphosate-resistant biotypes. These differences in shikimic acid accumulation could be due to inherent variability among Palmer amaranth biotypes, or an indication that multiple resistant mechanisms are present. Since metabolism experiments indicated this mechanism was not responsible for the resistance to glyphosate, and other research has shown that neither the MC-R nor the LC-R biotype exhibits a mutation altering the EPSPS target site (Burgos et al. 2008), the observed differences in resistance levels could stem from a combination of factors. Translocation of $^{14}$C-glyphosate out of the treated leaf
was less in the MC-R biotype, and although minimal, it is suspected this mechanism plays a partial role in the resistance to glyphosate. The LC-R biotype absorbed significantly less $^{14}$C-glyphosate than the S and MC-R biotypes, and this could potentially explain part of the resistance in this biotype; however, it is suspected that both the MC-R and LC-R biotypes may potentially have increased EPSPS gene copy number and expression similar to that of the previously reported resistant biotype from Georgia (Gaines et al. 2010).

Furthermore, other research has shown that multiple glyphosate resistant mechanisms exist in Arkansas Palmer amaranth populations, including increased EPSPS copy number (Doug Sammons, personal communication). At least one resistant biotype was identified that did not exhibit increased EPSPS copy number, supporting the results from this research that other resistant mechanisms are present. Differences within and among biotypes across these experiments could partially be due to the variability of increased EPSPS gene copy number and expression; however, there is still concern that multiple mechanisms of glyphosate resistance may be present in some of these populations. This creates a major concern for managing these resistant biotypes, particularly in situations where pollen-mediated gene flow may be responsible for creating biotypes with a variety of glyphosate resistance mechanisms. More research is needed to better characterize the resistant mechanisms present in glyphosate-resistant Palmer amaranth populations in Arkansas.
SOURCES OF MATERIALS

1. RoundupPowerMax®, Monsanto Company, St. Louis, MO 63167.

2. Professional growing mix, LC1 Mix, Sungro Horticulture Distribution Inc., 15831 N.E. 8th Street, Suite 100, Bellevue, WS 98008.

3. Miracle-Gro All Purpose Plant Food, Scotts Miracle-Gro Company, 14111 Scotts Lawn Road, Marysville, OH 43041.

4. MON 78623, K-salt of glyphosate, Monsanto Company, St. Louis, MO 63167.


7. OX-500 Biological Oxidizer, R.J. Harvey Instrument Corp., 11 Jane Street, Tappan, NY 10983.

8. Glyphosate-(phosphonomethyl-14C), American Radiolabeled Chemicals, Inc., 101 Arc Drive, St. Louis, MO 63178.

9. Ultima Gold Liquid scintillation cocktail, Perkin Elmer Life and Analytical Sciences, 940 Winter St., Waltham, MA 02451.

10. Tricarb 2900 TR, Perkin Elmer Life and Analytical Sciences, 940 Winter St., Waltham, MA 02451.

11. OX-161 14C-cocktail, R.J. Harvey Instrument Corp., 11 Jane Street, Tappan, NY 10983.
LITERATURE CITED


CHAPTER II
SPATIAL MOVEMENT OF GLYPHOSATE-RESISTANT PALMER AMARANTH IN
ARKANSAS COTTON
ABSTRACT

This research was aimed at understanding how far and how fast glyphosate-resistant (GR) Palmer amaranth will spread in cotton and the consequences associated with allowing a single plant to escape control. Specifically, research was conducted to determine the collective impact of dispersing agents on the rate of expansion of GR Palmer amaranth, and any resulting yield reductions in a Roundup Ready Flex® cotton system where the crop was managed similar to that by producers, except glyphosate was the only means of weed control. The introduction of 20,000 GR seed from Lincoln County (LC-R), AR, in 2008 was used to represent survival through maturity of a single GR female Palmer amaranth in four cotton fields ranging from 0.53 to 0.77 ha in size. In 2008, over 28 cm of rain fell in the month of March, and it is believed this rainfall resulted in longitudinal seed movement as far as 114 m downslope, resulting in a GR female Palmer amaranth setting seed and creating a separate GR Palmer amaranth patch in 2009. Movement was greater in 2009 than in 2008, likely a result of increased seed production from 2008 survivors. In less than two years from introduction, the LC-R biotype had expanded to the borders of all fields, infesting over 20% of the total field area. Spatial regression estimates indicate there was no significant yield penalty as a result of 2008 Palmer amaranth density, which is not surprising since only 0.56% of the field area was infested with GR Palmer amaranth. Yield penalty was evident in 2009, with a single Palmer amaranth reducing lint yield up to 17 kg ha⁻¹ for some areas of the fields. In 2010, all fields were a complete failure, resulting from Palmer amaranth infesting 95 to 100% of the area in all fields. Results from these data indicate resistance management options such as the ‘zero tolerance theory’ should be used in managing and/or mitigating the spread of GR Palmer amaranth. This research demonstrates the need for proactive resistance management and that the consequence of allowing a few plants to escape control occurs before yield loss is detectable.

**Key words:** Spatial analysis, zero threshold, crop yield loss.
INTRODUCTION

The U.S. ranked first globally with 8 genetically-modified crops for commercial production and 62.5 million hectares planted in 2008 (James 2008). More recently, data suggest that a total of 93, 78, and 70% of the U.S. soybean \(\text{Glycine max (L.) Merr}\), cotton \(\text{Gossypium hirsutum L.}\), and corn \(\text{Zea mays L.}\) hectares were planted in genetically-modified crops in 2010 (USDA 2011). The majority of these hectares were planted with glyphosate-resistant varieties, which were introduced in the mid-1990s. The use of glyphosate for in-season weed control increased dramatically as a result, and has been associated with the selection of several glyphosate-resistant weed species around the world. In 2011, the U.S. had confirmed glyphosate-resistance in 12 weed species, 52% of the total glyphosate-resistant species in the world (Heap 2011).

Horseweed \(\text{Conyza canadensis (L.) Cronq}\) from Delaware was the first glyphosate-resistant weed species in the U.S., confirmed in 2001 (VanGessel 2001). Glyphosate-resistant horseweed rapidly spread across the U.S., confirmed in 20 states over the last decade (Heap 2012). There were several reasons horseweed became one of the major weeds in U.S. agriculture (Norsworthy et al. 2007; Webster 2009). Prior to the adoption of conservation tillage and glyphosate-resistant crops, horseweed was easily controlled with conventional tillage practices (Brown and Whitwell 1988). Some horseweed in the U.S. evolved resistance to multiple herbicide families, reducing the available management options even further (Heap 2011). Also, horseweed emerges throughout the year and is capable of producing 50,000 to 200,000 wind-dispersed seeds plant\(^{-1}\), replenishing the soil seedbank and spreading large distances of up to 483 km when left uncontrolled (Holm et al. 1997; Shields et al. 2006). The dispersal of horseweed seed is a major concern for weed management, particularly in situations threatening the spread of multiple resistance traits.
With the confirmation of glyphosate-resistant Palmer amaranth in 2006, many Arkansas producers were already familiar with the impact of glyphosate-resistant horseweed in glyphosate-resistant cropping systems. Although management strategies for controlling the spread of glyphosate-resistant horseweed were largely in place (Norsworthy et al. 2009; VanGessel et al. 2009), these strategies were not necessarily effective for managing Palmer amaranth, a weed that exhibits a different emergence pattern. Glyphosate resistance in these two species can be further characterized by understanding biological and ecological characteristics associated with each species.

Both species exhibit the potential for outcrossing, increasing the risks of spreading resistance traits as well as homogenizing local populations over short distances (Davis et al. 2010; Jasieniuk et al. 1996; Stallings et al. 1995). The major mechanism for survival and dispersal of these annual weeds is via seed production, with horseweed capable of producing over 200,000 seed plant\(^{-1}\), and Palmer amaranth producing from 200,000 to 1,800,000 seed plant\(^{-1}\) (Holm et al. 1997; Keeley et al. 1987; Smith et al. 2012; Sosnoskie et al. 2011). One factor involved with managing resistant weed species is how persistent their seeds are in the soil seedbank. A percentage of horseweed and Palmer amaranth seed persist in the soil at least three years, making seed production a major concern for managing and eliminating resistance (Comes et al. 1978; Sosnoskie et al. 2011).

Glyphosate-resistant horseweed and glyphosate-resistant Palmer amaranth are both examples of weeds that have evolved resistance, separating their resistant-biotypes from the rest of the susceptible weed community because they are particularly problematic to control (Swanton and Booth 2004). In this situation, previous economic-based thresholds of acceptable levels of weed control are no longer viable options for sustaining glyphosate-resistant cropping
systems (Norsworthy et al. 2012). This statement can further be understood by discussing the origin and definition of an economic threshold.

The economic threshold was first developed as a decision-making tool in entomology and was based on the biological life cycle of arthropods (Stern et al. 1959). Several differences in the population ecology of weeds and arthropods exist, indicating that economic thresholds can lead to different outcomes in weed management strategies. Weed populations are long-lived through the seedbank and usually have only one generation each year, whereas arthropods are relatively short-lived and can have multiple generations each year (Norris 1999). Also, arthropod populations usually rapidly decrease at some point in the year, whereas weed populations survive over winter through seed production, indicating management practices should target seed production and seed dispersal, with a ‘zero tolerance’ theory serving as the basis for weed control (Jones and Medd 2000; Norris 1999; Swanton et al. 1999; Swanton and Booth 2004). Rejmanek and Pitcairn (2002) reported success rates of eradicating exotic weeds was greatest with early detection, prior to infestations greater than 1 ha in size; therefore it is extremely important to understand the mechanisms of seed dispersal and how fast these resistant biotypes can colonize fields.

There are several mechanisms of weed seed dispersal involved in the spread of resistant species. Glyphosate-resistant horseweed spread short and long distances by wind, known as anemochory (Van der Pijl 1972), a dispersal mechanism not highly involved in long-distance Palmer amaranth seed movement. However, several characteristics of Palmer amaranth seed make other dispersal mechanisms viable pathways for seed movement.
Palmer amaranth seed is very small, having a diameter of only 1 to 1.3 mm (Bryson and DeFelice 2009). Hydrochory, or seed movement by water, is a mechanism that could potentially disperse Palmer amaranth seed. Li and Qiang (2009) reported that over 74 species, belonging to 20 different families, were found to float and travel via water and that movement and species composition was related to irrigation frequencies. Redroot pigweed (*Amaranthus retroflexus* L.), a closely related *Amaranthus* species, was one of the most common weed seed found in irrigation canals in early research by Kelley and Bruns (1975). Wind and water are examples of abiotic dispersal mechanisms, but there are still several biotic mechanisms, such as movement via animals by adhesion (epizoochory) or ingestion (endozoochory), and even movement resulting from human activities (anthropochory) (Van der Pijl 1972).

Tillage and harvest equipment have also been reported to disperse seed. Furthermore, the combination of harvest equipment followed by cultivation prior to weed seed shed increased the distance seed was dispersed to over 100 m in a corn cropping system (Heijting et al. 2008). Other dispersal mechanisms to consider are through animal manure and gin trash. Norsworthy et al. (2009) reported that Palmer amaranth seed was viable at a depth of 25 cm after 2 years of gin trash composting. Since gin trash is sometimes used as cattle feed, and both gin trash and manure are commonly spread over agricultural fields, this could represent short- and long-distance dispersal mechanisms for Palmer amaranth.

A survey conducted with Arkansas cotton consultants by Norsworthy et al. (2007) indicated that 79% of the scouted hectares accounted for in the survey were already infested with resistant weeds and that the major educational and research efforts should continue to focus on herbicide resistance issues. University of Arkansas weed science and agricultural extension groups began to focus on documenting the spread of resistance at both the state and local levels; however,
research was limited in this area and needed to be addressed. Monitoring patch expansion and the soil seedbank using site-specific technology such as global positioning systems (GPS) is considered a useful practice for resistance management (Beckie 2006) and has been documented in wild oat (Avena fatua) (Beckie et al. 2005), purple nutsedge (Cyperus rotundus L.), yellow nutsedge (Cyperus esculentus L.) (Webster 2005), and hemp dogbane (Apocynum cannabinum L.) (Webster et al. 2000), among others. Furthermore, precision agricultural continues to evolve as an economical best management practice and has been incorporated in variable-rate fertilizer applications in cotton (Velandia et al. 2008), creating yield-based management maps for multiple crop rotations (Blackmore 2000), and generating management maps for variable rate herbicide applications (Mohammadzamani and Rashidi 2009; Wiles 2008).

Incorporation of new technologies such as GPS, yield monitors, and other sensors can provide thousands of observations and provide visual support for furthering producer knowledge. The objectives of this research were to develop a geo-spatial data set to characterize the patch expansion of glyphosate-resistant Palmer amaranth through seed production over 3 yrs in a glyphosate-resistant cotton production system in which glyphosate was the only herbicide used for weed control, and to determine the effect of glyphosate-resistant Palmer amaranth density on cotton yields.

MATERIALS AND METHODS

Research was initiated to evaluate the patch expansion of glyphosate-resistant Palmer amaranth. In an attempt to further our ability to incorporate GPS and precision agriculture techniques in Arkansas weed management research, field studies were conducted from 2007 to 2009 in Fayetteville, AR, at the Main Experiment Station of University of Arkansas. This was a
large-scale experiment, using four fields ranging from 0.53 to 0.77 ha in size. The soil types in these fields included a mix of Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults), a Pickwick silt loam (fine-silty, mixed, semiactive, thermic Typic Paleudults), and a Leaf silt loam (fine, mixed, active, thermic Typic Albaqults) (SSURGO 2012).

These fields, known as field G2, G4, G5, and G6 (Figure 2.1), were monitored in 2007 for the presence of Palmer amaranth and none were found to exist. Each year, Stoneville 4554 B2/RRF cotton was planted and managed according to Arkansas recommendations and was furrow irrigated as needed. Each of these fields had 20,000 glyphosate-resistant Palmer amaranth seeds from Lincoln County, AR, (LC-R) sown into a circular 1-m² area on the high-end of the field (South) in February 2008, centered approximately 15 m from the field edge. The center and edge of these 1-m² patches were georeferenced (±4 cm) using a Trimble AgGPS 332 Ultimate Choice GPS¹ receiver with OmniSTAR HP² correction. This initial introduction was intended to represent a conservative estimate of seed production from a single glyphosate-resistant plant that survived to maturity in 2007. Since Palmer amaranth seeds are capable of floating in water, rainfall events and irrigation totals in 2007, 2008, and 2009 are shown in Table 2.1.

Each year, glyphosate was applied as needed (four applications) to control all other weed species in the field. In 2007, 2008, and 2009 the final density of Palmer amaranth was taken using a 1.0-m² grid, collecting densities in a Cartesian coordinate system using a continuous scale of 0, 1, 2, 3, 4, 5, and 6 (>5) Palmer amaranth m⁻¹ of row. Spatial cotton yield data were collected using a cotton yield monitor kit³ equipped with Insight display and the previously described GPS. Yield data was collected every second from the two border rows of each grid.
Figure 2.1. Aerial image of fields G2, G4, G5, and G6 at the Main Experiment Station of the University of Arkansas in Fayetteville, AR. (Google Earth 2011)
cell, with an approximate harvest speed of 3 kph. After cotton harvest, cotton stalks were shredded prior to working and re-bedding the ground.

Since yield data were geo-spatially referenced, spatial variability must be accounted for, rendering standard ANOVA and least squares regression methods unreliable for statistical analysis. The original yield data was imported into ArcGIS along with latitude, longitude, elevation, speed, time, and lint mass as the major attributes for each data point. Additional information, such as field name, was added to the attribute table and it was converted to an .shp file. A soils map was obtained through the soil survey geographic database, SSURGO, and imported to ArcGIS. The soils map was added as a separate layer and a polygon was drawn around each soil type, creating a soil polygon for selecting yield data points within each soil type. Soil types were added to the attribute table for use as covariates and again saved as an .shp file.

In a separate ArcGIS layer, a 1 m² grid was created and aligned for fields G2, G4, G5, and G6 and Palmer amaranth density data were added for each year. The layer containing Palmer amaranth density data was then spatially joined or snapped to the original yield data layer, with each Palmer amaranth density cell taking the average of the nearest yield data points to represent cotton lint yield. This data set was then saved as a single .shp file for future analysis. To help assess spatial variability, cotton yields and Palmer amaranth density data were subjected to exploratory spatial data analysis (ESDA) using GeoDa 0.9.5-I. Row-standardized spatial weights matrices were created based on either queen (8 directions) or rook (4 directions) contiguity, since the data set contained aerial units. These spatial weight matrices were used in Moran’s I (Anselin 1999) test for global spatial autocorrelation, as well as in a local indicator of spatial association (LISA) (Anselin 2003) to determine if significant local clustering occurred.
Table 2.1. Precipitation and furrow irrigation totals in 2007, 2008, and 2009 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.a

<table>
<thead>
<tr>
<th>Month</th>
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<th>2009</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>October</td>
<td>9.1</td>
<td>6.0</td>
<td>26.0</td>
</tr>
<tr>
<td>November</td>
<td>0.9</td>
<td>2.2</td>
<td>4.0</td>
</tr>
<tr>
<td>December</td>
<td>6.3</td>
<td>6.0</td>
<td>7.3</td>
</tr>
</tbody>
</table>

*a A single irrigation accounts for 5.1 cm of rainfall; multiple irrigations are totaled together based off this value.
The results from ESDA suggest further statistical analysis using spatial regression to help account for the spatial structure of the data.

Moran’s $I$ test for regression residuals is assumed to be normally distributed under the null hypothesis of no spatial dependence, given by:

$$ I = \frac{n}{S_o} \frac{x'Wx}{x'x}, $$

where $x$ is a $n \times 1$ vector of observations as deviations from the mean, $W$ is an $n \times n$ spatial weights matrix, and $S_o$ is the sum of elements of $W$. This test statistic has previously been interpreted as a correlation coefficient (Anselin 1988), with a large positive Moran’s $I$ value indicating high (low) values having neighbors of high (low) values, and a negative Moran’s $I$ indicating that high and low value observations occur as neighbors. Palmer amaranth density and cotton yields were used as the variable of interest in Moran’s $I$ to determine if spatial autocorrelation existed in each field.

It was suspected that several field variables were correlated with site-specific cotton yield, including Palmer amaranth density, soil type, and elevation. Since elevation and slope were likely responsible for some Palmer amaranth seed dispersal and yield variability, a relative elevation variable was created for each data point to help account for spatial structure. Topographic modeling techniques have been incorporated into statistical models in the form of digital elevation models and hydrologic models, and have been used to account for the noise component associated with spatial datasets (Griffin et al. 2006); however, in situations where agricultural fields have been precision leveled such as rice ($Oryza sativa$ L.) and cotton (Griffin et al. 2005), elevation may not be considered an important covariate for yield.

The fields in this study were furrow irrigated with slopes at some locations greater than 5%, and seed dispersal was expected to be correlated to elevation and water flow. Although spatial
regression techniques have been implemented in other areas of research (Anselin 2001; Goodchild et al. 2000), the application of spatial models in agriculture has been less extensive, with fewer models for addressing large-scale yield monitor data sets (Anselin et al. 2004). Previous attempts to address spatial variability have included: the use of state-space analysis to remove spatial autocorrelation (Wendroth et al. 1999), comparison between regression model- and ANOVA-generated estimates for spatial autoregressive response models, and, more recently, Anselin et al. (2004) reported exploiting the spatial structure of the data to give more precise site-specific estimates of the parameters involved in yield response. Exploratory spatial data analysis has indicated spatial structure exist; therefore, it is necessary to investigate spatial regression modeling techniques.

To determine if spatial regression was more appropriate, a spatial specification search was carried out for 2007, 2008, and 2009 lint yields using standard OLS and general moments (GM) estimation for a linear aspatial model and a spatial autoregressive error model (SERROR). The GM estimation can be conducted for very large datasets of several thousand observations. The maximum likelihood (ML) estimator has computational limitations in large datasets, and also requires a known distribution, while GM does not.

The SERROR model is given as $y = X\beta + \varepsilon$, where $\varepsilon = \lambda W\varepsilon + \mu$ or in reduced form as $y = X\beta + (I - \lambda W)^{-1} \mu$ where $y$ is an $n \times 1$ vector of dependent variables, $X$ a $n \times k$ matrix of explanatory variables, $\beta$ a $k \times 1$ vector of regression coefficients, $\varepsilon$ an $n \times 1$ vector of residuals, $\lambda$, a spatial autoregressive parameter, $W$ is an $n \times n$ spatial weights matrix, and $\mu$ a well behaved, non-heteroskedastic uncorrelated error term (Anselin, 1988). The spatial autoregressive lag model (SLAG) was not chosen due to conceptual reasons, as well as diagnostic results from using the Lagrange Multiplier (LM) test on the OLS residuals. The models were estimated in R 2.13.17.
using the *rgdal* and *spdep* packages. The Akaike information criterion (AIC) was used to
determine which statistical model was more appropriate (Anselin 2001). The field explanatory
variables of interest are found in Table 2.3.

The model

\[ Y_i = \text{intercept} + PA_{count} + PA_{count}^2 + W_iPA_{count} + RE + RE \times PA_{count} + LE + CAB + LE \times \]

\[ PA_{count} + CAB \times PA_{count} + LE \times W_iPA_{count} + CAB \times W_iPA_{count} \]

where \( Y_i \) is cotton lint yield in kg ha\(^{-1}\) at location \( i \), \( PA_{count} \) is the density of Palmer amaranth,
\( W_iPA_{count} \) is the \( i \)th weighted matrices density average, \( RE \) is the relative elevation, \( RE \times PA_{count} \) is
the interaction term of relative elevation and Palmer amaranth density, \( LE \) was an indicator
variable for the presence of a Leaf silt loam soil, \( CAB \) was an indicator variable for the presence
of a Captina silt loam soil, \( LE \times PA_{count} \) was the interaction term for Leaf silt loam and Palmer
amaranth density, and \( CAB \times PA_{count} \) was the interaction term for Captina silt loam and Palmer
amaranth density, \( LE \times W_iPA_{count} \) was the interaction of the Leaf silt loam soil and Palmer
amaranth density, and \( CAB \times W_iPA_{count} \) was the interaction of the Captina silt loam soil and
Palmer amaranth density, was used to determine the effects on cotton lint yields. This model
was used to generate three different equations (one for each soil type). Two soil types are
present in the model, while the third is accounted for in the intercept.

**RESULTS AND DISCUSSION**

**Count Data.** Palmer amaranth counts for 2008 and 2009 in fields G2, G4, G5, and G6 are
presented in Figures 2.2 to 2.9. In 2008, after only one year from introduction, Palmer amaranth
had moved linearly (down slope) as far as 118 m in field G6 (Figure 2.8). Of those Palmer
amaranth that moved 118 m in field G6, both male and female Palmer amaranth were present,
Table 2.3. Description of explanatory variables for cotton lint yields in fields G2, G4, G5, and G6 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>Intercept</td>
</tr>
<tr>
<td>PA\text{count}</td>
<td>Palmer amaranth density</td>
</tr>
<tr>
<td>PA\text{count}^2</td>
<td>Square of Palmer amaranth density</td>
</tr>
<tr>
<td>W_1 \cdot PA\text{count}</td>
<td>Weighted average of Palmer amaranth density</td>
</tr>
<tr>
<td>RE</td>
<td>Relative elevation</td>
</tr>
<tr>
<td>RE \times PA\text{count}</td>
<td>Interaction term of RE and PA\text{count}</td>
</tr>
<tr>
<td>LE</td>
<td>Leaf silt loam soil type covariate</td>
</tr>
<tr>
<td>Cab</td>
<td>Captina silt loam soil type covariate</td>
</tr>
<tr>
<td>LE \times PA\text{count}</td>
<td>Interaction term of LE and PA\text{count}</td>
</tr>
<tr>
<td>CAB \times PA\text{count}</td>
<td>Interaction term of CAB and PA\text{count}</td>
</tr>
</tbody>
</table>
Figure 2.2. Glyphosate-resistant Palmer amaranth density maps from 2008 for field G2 (0.53 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.3. Glyphosate-resistant Palmer amaranth density maps from 2009 for field G2 (0.53 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.4. Glyphosate-resistant Palmer amaranth density maps from 2008 for field G4 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.5. Glyphosate-resistant Palmer amaranth density maps from 2009 for field G4 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.6. Glyphosate-resistant Palmer amaranth density maps from 2008 for field G5 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.7. Glyphosate-resistant Palmer amaranth density maps from 2009 for field G5 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.8. Glyphosate-resistant Palmer amaranth density maps from 2008 for field G6 (0.77 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.9. Glyphosate-resistant Palmer amaranth density maps from 2009 for field G6 (0.77 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
leading to the formation of a second Palmer amaranth patch in 2009 (Figure 2.9). Since
glyphosate-resistant Palmer amaranth seeds were not introduced until February 2008, fall tillage
and re-bedding were not responsible for seed movement. Movement of this distance is likely a
result of significant rainfall events in the spring (Table 2.1). Although Palmer amaranth
movement in fields G2, G4, and G5 was less than 16 m in 2008 (Figures 2.2, 2.4, and 2.6), patch
expansion reached the borders of all fields in 2009, infesting 14, 31, 24, and 12% of fields G2,
G4, G5, and G6, respectively (Table 2.4).

These figures indicate that the majority of Palmer amaranth movement occurred linearly
(North to Northwest direction). Lateral movement occurred to a lesser extent in all fields in
2008, with Palmer amaranth moving up to 6 m from the source in field G6 (Figure 2.8). A
decrease in lateral seed movement was somewhat expected due to the presence of beds for
furrow irrigation and also because the general direction of equipment was linear. Palmer
amaranth patch expansion had increased to $\geq 95\%$ of all fields in 2010, causing total crop failure
(Figure 2.10). The fact that crop failure occurred after only three years from the introduction of
only 20,000 seed in a square meter, simulating a single glyphosate-resistant female Palmer
amaranth, is a major concern for producers. It is extremely important to monitor fields for
suspected glyphosate-resistant Palmer amaranth to ensure methods of control can be
implemented in a timely fashion, most notably removal of all escapes (zero tolerance theory).
The critical period for removing plants is relatively short after pollination has occurred, as
determined for the closely related waterhemp \textit{[Amaranthus tuberculatus} (Moq) Sauer.] species,
where over 75% of seeds germinated only 12 days after pollination (Bell and Tranel 2010).
Table 2.4. Total area (%) infested by glyphosate-resistant Palmer amaranth in fields G2, G4, G5, and G6 in 2008 and 2009 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Field</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>0.58</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>G4</td>
<td>0.56</td>
<td>31</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>G5</td>
<td>0.60</td>
<td>24</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>G6</td>
<td>0.51</td>
<td>12</td>
<td>&gt; 95</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Percent infestation calculated by dividing the number of grid cells containing Palmer amaranth counts by the total number of cells for that field.

\textsuperscript{b} Infestation in 2007 = 0\%.
Figure 2.10. Field G4 in at the time of normal cotton harvest in October, 2010. Palmer amaranth infested more than 95% of fields G2, G4, G5, and G6, causing total crop failure (no harvest) only three years after glyphosate-resistant Palmer amaranth was introduced.
Table 2.5. Descriptive statistics for cotton lint yield in fields G2, G4, G5, and G6, in 2007, 2008, and 2009 at Main Experiment Station of the University of Arkansas, Fayetteville, AR.

<table>
<thead>
<tr>
<th>Field</th>
<th>Year</th>
<th>Mean</th>
<th>Std Error Mean</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>2007</td>
<td>673</td>
<td>285</td>
<td>6</td>
<td>1,712</td>
</tr>
<tr>
<td>G2</td>
<td>2008</td>
<td>735</td>
<td>346</td>
<td>0</td>
<td>1,848</td>
</tr>
<tr>
<td>G2</td>
<td>2009</td>
<td>1,073</td>
<td>370</td>
<td>0</td>
<td>1,846</td>
</tr>
<tr>
<td>G4</td>
<td>2007</td>
<td>706</td>
<td>235</td>
<td>0</td>
<td>1,837</td>
</tr>
<tr>
<td>G4</td>
<td>2008</td>
<td>872</td>
<td>236</td>
<td>0</td>
<td>1,844</td>
</tr>
<tr>
<td>G4</td>
<td>2009</td>
<td>970</td>
<td>349</td>
<td>0</td>
<td>1,847</td>
</tr>
<tr>
<td>G5</td>
<td>2007</td>
<td>1,057</td>
<td>325</td>
<td>74</td>
<td>1,841</td>
</tr>
<tr>
<td>G5</td>
<td>2008</td>
<td>972</td>
<td>295</td>
<td>0</td>
<td>1,839</td>
</tr>
<tr>
<td>G5</td>
<td>2009</td>
<td>1,231</td>
<td>437</td>
<td>0</td>
<td>1,847</td>
</tr>
<tr>
<td>G6</td>
<td>2007</td>
<td>578</td>
<td>213</td>
<td>0</td>
<td>1,827</td>
</tr>
<tr>
<td>G6</td>
<td>2008</td>
<td>813</td>
<td>254</td>
<td>0</td>
<td>1,792</td>
</tr>
<tr>
<td>G6</td>
<td>2009</td>
<td>1,053</td>
<td>347</td>
<td>0</td>
<td>1,847</td>
</tr>
</tbody>
</table>
Table 2.6. Moran’s I statistic using queen contiguity and minimum distances of 1.42 and 3 m for Palmer amaranth density and cotton lint yield in 2007, 2008, and 2009 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.\textsuperscript{a-c}

<table>
<thead>
<tr>
<th>Year</th>
<th>Density\textsuperscript{W1}</th>
<th>Lint yield\textsuperscript{W1}</th>
<th>Density\textsuperscript{W2}</th>
<th>Yield\textsuperscript{W2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>0.08</td>
<td>0.83</td>
<td>0.01</td>
<td>0.76</td>
</tr>
<tr>
<td>2008</td>
<td>0.48</td>
<td>0.76</td>
<td>0.31</td>
<td>0.66</td>
</tr>
<tr>
<td>2009</td>
<td>0.72</td>
<td>0.82</td>
<td>0.62</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\textsuperscript{a} W\textsubscript{1} represents a minimum distance of 1.42 m; W\textsubscript{2} represents a minimum distance of 3m.

\textsuperscript{b} All values were significant, with p-value <0.0001.

\textsuperscript{c} All values were significant, with p-value <0.0001.
Cotton Lint Yield Maps. Cotton lint yields varied each year as a result of environmental conditions as well as increasing Palmer amaranth densities (Figures 2.11 to 2.22). General descriptive statistics for lint yields are found in Table 2.5. In general, lint yields were lowest in 2007, likely because of increased reliance on irrigation due to the limited rainfall during the growing season (Table 2.1). It was suspected that the standard deviation increased each year as a result of increasing Palmer amaranth density and competition in 2008 and 2009 (Tables 2.4 and 2.5). Lint yield maps created in ArcGis to help visualize the localized effect of increasing Palmer amaranth densities from 2007 to 2009 (Figures 2.11 to 2.22). The minimum and maximum yields were similar for all years, largely due to the natural effects of environment within these fields (Table 2.5). A visual comparison of the 2009 G6 Palmer amaranth density map (Figure 2.9) and the lint yield map (Figure 2.22) indicate yield reduction patterns similar in structure, represented in Figure 2.23. A more precise statistical analysis on Palmer amaranth density and cotton lint yield was accomplished using spatial regression techniques and will be further discussed.

Application of ESDA. Palmer amaranth count data and continuous cotton lint yield were used as the variable of interest in Moran’s I to characterize the spatial autocorrelation across all fields. Spatially weighted matrices were created using queen contiguity with minimum distances of 1.42 and 3 m, the previous being the minimum distance to ensure each observation has at least one neighbor. Significant spatial autocorrelation existed for cotton lint yields in all years and for Palmer amaranth density in 2008 and 2009 (Table 2.6). Traditional regression techniques using OLS estimation fail to address any spatial autocorrelation, rendering estimates that are unreliable and introducing bias test statistics (Anselin 1988). Results from LISA also indicate significant
Figure 2.11. Cotton lint yield map from 2007 for field G2 (0.53 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.12. Cotton lint yield map from 2008 for field G2 (0.53 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.13. Cotton lint yield map from 2009 for field G2 (0.53 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.14. Cotton lint yield map from 2007 for field G4 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.15. Cotton lint yield map from 2008 for field G4 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.16. Cotton lint yield map from 2009 for field G4 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.17. Cotton lint yield map from 2007 for field G5 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.18. Cotton lint yield map from 2008 for field G5 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.19. Cotton lint yield map from 2009 for field G5 (0.57 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.20. Cotton lint yield map from 2007 for field G6 (0.77 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.21. Cotton lint yield map from 2008 for field G6 (0.77 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.22. Cotton lint yield map from 2009 for field G6 (0.77 ha) at the Main Experiment Station of the University of Arkansas, Fayetteville, AR. (ArcGis)
Figure 2.23. Comparison of cotton lint yield map and glyphosate-resistant Palmer amaranth count map in field G6 in 2009. (ArcGis)
local clustering occurred, suggesting that spatial modeling techniques should be used to account for spatial variability (data not shown).

**Empirical Analysis.** Estimates for cotton lint yields in 2007, 2008, and 2009 were generated as a function of Palmer amaranth counts, the square of Palmer amaranth counts, spatially weighted averages of Palmer amaranth counts, soil types, relative elevation, and the interactions using a cumulative data set from fields G2, G4, G5, and G6. The square of Palmer amaranth count was included because the relationship was expected to be quadratic. As Palmer amaranth density increases, cotton yield is expected to decrease. The gaps between these fields were taken into account when building the cumulative data set by using xy coordinates. The soil data was expected to account for the field to field differences. Our focus is on the model comparison between standard aspatial models estimated by OLS and the SERROR model estimated using GM.

Results from 2007 estimates are shown in Table 2.7. Since no Palmer amaranth was present in 2007, a reduced version of the model including relative elevation and soil type was used to demonstrate the inherent variability in yield associated with those parameters. The spatial autoregressive parameter \( \lambda \) was 0.93, indicating that spatial dependence inherently exists and that a spatial model is a better alternative than a traditional model. This is also supported by the AIC values, which show the SERROR model as the best fit model for this data (Table 2.7). The CAB soil type was significant for increasing lint yields in 2007, as was the relative elevation. Higher elevations yielded higher in 2007, likely a result of the direct proximity to the source of furrow irrigation.
Table 2.7. Coefficient estimates and diagnostic statistics for cotton lint yield in 2007 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.\(^a\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>OLS</th>
<th>SERROR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>539.1</td>
<td>4.7</td>
</tr>
<tr>
<td>RE</td>
<td>-6.4</td>
<td>2.1</td>
</tr>
<tr>
<td>LE</td>
<td>98.1</td>
<td>8.0</td>
</tr>
<tr>
<td>CAB</td>
<td>268.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Lambda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIC</td>
<td>319,889</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Abbreviations: OLS; aspatial model with ordinary least squares estimation; SERROR, spatial autoregressive error model; SE, the standard error of the estimate; RE, relative elevation; LE, Leaf silt loam soil; CAB, Captina silt loam soil; AIC, akaike information criterion.
Table 2.8. Coefficient estimates and diagnostic statistics for cotton lint yield in 2008 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.a

<table>
<thead>
<tr>
<th>Variables</th>
<th>OLS Estimate</th>
<th>SE</th>
<th>SERROR Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>750.8</td>
<td>4.0</td>
<td>791.0</td>
<td>11.2</td>
</tr>
<tr>
<td>PA\text{count}</td>
<td>-29.9</td>
<td>33.2</td>
<td>32.1</td>
<td>18.5</td>
</tr>
<tr>
<td>PA\text{count}^2</td>
<td>2.0</td>
<td>6.0</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>W_1 PA\text{count}</td>
<td>-80.8</td>
<td>36.5</td>
<td>-42.5</td>
<td>35.7</td>
</tr>
<tr>
<td>RE</td>
<td>5.1</td>
<td>2.0</td>
<td>5.2</td>
<td>6.3</td>
</tr>
<tr>
<td>RE × PA\text{count}</td>
<td>-77.3</td>
<td>88.7</td>
<td>-36.7</td>
<td>47.6</td>
</tr>
<tr>
<td>LE</td>
<td>76.5</td>
<td>4.1</td>
<td>71.4</td>
<td>13.1</td>
</tr>
<tr>
<td>CAB</td>
<td>106.3</td>
<td>4.6</td>
<td>53.3</td>
<td>10.4</td>
</tr>
<tr>
<td>LE × PA\text{count}</td>
<td>-49.1</td>
<td>99.6</td>
<td>-38.0</td>
<td>52.8</td>
</tr>
<tr>
<td>CAB × PA\text{count}</td>
<td>46.1</td>
<td>100.2</td>
<td>-15.3</td>
<td>54.0</td>
</tr>
<tr>
<td>LE × W_1 PA\text{count}</td>
<td>-4.8</td>
<td>39.5</td>
<td>2.7</td>
<td>38.4</td>
</tr>
<tr>
<td>CAB × W_1 PA\text{count}</td>
<td>3.0</td>
<td>7.4</td>
<td>5.7</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Lambda | 0.90 |
AIC | 317,620 | 287,506 |

a Abbreviations: OLS; aspatial model with ordinary least squares estimation; SERROR, spatial autoregressive error model; SE, the standard error of the estimate; PA\text{count}, Palmer amaranth count; PA\text{count}^2, Palmer amaranth count squared; W_1 PA\text{count}, spatially weighted average of Palmer amaranth counts; RE, relative elevation; RE × PA\text{count}, the interaction of PA\text{count} and RE; LE, Leaf silt loam soil; CAB, Captina silt loam soil; LE × PA\text{count}, interaction of LE and PA\text{count}; CAB × PA\text{count}, interaction of CAB and PA\text{count}; LE × W_1 PA\text{count}, interaction of LE and W_1 PA\text{count}; CAB × W_1 PA\text{count}, interaction of CAB and W_1 PA\text{count}; AIC, akaike information criterion.
Overall, mean cotton lint yields were higher in 2008 than in 2007 (Table 2.5), regardless of the introduction of glyphosate-resistant Palmer amaranth. This is not surprising, since only 0.56% of these fields were infested with Palmer amaranth in 2008 (Table 2.4). The same scenario often occurs in a producer’s field very early in resistance evolution, when small densities of resistant weeds show no yield penalty over large field areas. The coefficients for predicting the 2008 yield estimations are shown in Table 2.8. The spatial model was a better fit for estimation, as indicated by the lower AIC value. The positive \( \lambda \) value indicates that inherent spatial variability exists in this data. The SERROR model had an AIC value of (287,506) and was the best fit model for estimating lint yield. Soil type was significant for yield gain in 2008, with CAB and LE increasing overall yield. The majority of Palmer amaranth remained in the ‘high’ end of the field in 2008 (Figures 2.2, 2.4, 2.6, and 2.8), with spatial movement limited to 16 m or less in fields G2, G4, and G5.

In 2009, after less than two years from introduction, the Palmer amaranth population had expanded to the borders of all fields, infesting over 20% of the total area (Table 2.4). Glyphosate-resistant Palmer amaranth was more widespread in 2009, as can be seen from the Palmer amaranth count maps (Figures 2.3, 2.5, 2.7, and 2.9). In some cases, this is the first indication to producers that they have issues with resistant weeds. The estimates for 2009 cotton lint yield are shown in Table 2.9. Yields were significantly impacted by several parameters in 2009, including Palmer amaranth count, weighted counts, relative elevation, soil type, and the interaction of weighted Palmer amaranth counts and the Captina silt loam soil. The SERROR was a better fit for estimation, as indicated by the lower AIC value (287,506). The positive \( \lambda \) indicates that inherent spatial variability existed in these data. Relative elevation was significant, with the higher elevations yielding less than lower elevations. This effect is visually
Table 2.9. Coefficient estimates and diagnostic statistics for cotton lint yield in 2009 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.⁶

<table>
<thead>
<tr>
<th>Variables</th>
<th>OLS Estimate</th>
<th>OLS SE</th>
<th>SERROR Estimate</th>
<th>SERROR SE</th>
</tr>
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<tr>
<td>(Intercept)</td>
<td>1005.8</td>
<td>4.8</td>
<td>997.6</td>
<td>13.2</td>
</tr>
<tr>
<td>PA\textsubscript{count}</td>
<td>-105.8</td>
<td>8.0</td>
<td>-16.6</td>
<td>5.3</td>
</tr>
<tr>
<td>PA\textsubscript{count}\textsuperscript{2}</td>
<td>2.3</td>
<td>1.3</td>
<td>1.8</td>
<td>0.7</td>
</tr>
<tr>
<td>W\textsubscript{1} PA\textsubscript{count}</td>
<td>-94.3</td>
<td>7.8</td>
<td>-60.8</td>
<td>7.6</td>
</tr>
<tr>
<td>RE</td>
<td>-101.8</td>
<td>2.4</td>
<td>-88.0</td>
<td>7.5</td>
</tr>
<tr>
<td>RE × PA\textsubscript{count}</td>
<td>12.4</td>
<td>5.5</td>
<td>-2.1</td>
<td>3.9</td>
</tr>
<tr>
<td>LE</td>
<td>186.7</td>
<td>5.1</td>
<td>160.6</td>
<td>15.8</td>
</tr>
<tr>
<td>CAB</td>
<td>116.2</td>
<td>5.6</td>
<td>93.5</td>
<td>12.0</td>
</tr>
<tr>
<td>LE × PA\textsubscript{count}</td>
<td>56.6</td>
<td>7.6</td>
<td>-1.1</td>
<td>4.3</td>
</tr>
<tr>
<td>CAB × PA\textsubscript{count}</td>
<td>5.0</td>
<td>6.2</td>
<td>-2.6</td>
<td>4.8</td>
</tr>
<tr>
<td>LE × W\textsubscript{1} PA\textsubscript{count}</td>
<td>-74.8</td>
<td>7.8</td>
<td>-12.3</td>
<td>9.5</td>
</tr>
<tr>
<td>CAB × W\textsubscript{1} PA\textsubscript{count}</td>
<td>7.5</td>
<td>1.5</td>
<td>-3.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

| Lambda | 0.92         |
| AIC    | 323,382      |

⁶Abbreviations: OLS; aspatial model with ordinary least squares estimation; SERROR, spatial autoregressive error model; SE, the standard error of the estimate; PA\textsubscript{count}, Palmer amaranth count; PA\textsubscript{count}\textsuperscript{2}, Palmer amaranth count squared; W\textsubscript{1} PA\textsubscript{count}, spatially weighted average of Palmer amaranth counts; RE, relative elevation; RE × PA\textsubscript{count}, the interaction of PA\textsubscript{count} and RE; LE, Leaf silt loam soil; CAB, Captina silt loam soil; LE × PA\textsubscript{count}, interaction of LE and PA\textsubscript{count}; CAB × PA\textsubscript{count}, interaction of CAB and PA\textsubscript{count}; LE × W\textsubscript{1} PA\textsubscript{count}, interaction of LE and W\textsubscript{1} PA\textsubscript{count}; CAB × W\textsubscript{1} PA\textsubscript{count}, interaction of CAB and W\textsubscript{1} PA\textsubscript{count}; AIC, akaike information criterion.
represented by Figures 2.12, 2.15, 2.18, and 2.21, where the lowest yielding areas of each field are on the South end (higher elevation) of the field. Yields were reduced 17 kg ha\(^{-1}\) for each Palmer amaranth. As expected, the weighted average of Palmer amaranth counts for a given location was also significant in reducing cotton lint yields. This parameter might be more important in understanding the relationship between Palmer amaranth and yield, because it takes into account the surrounding Palmer amaranth for a given location in the field.

The expectation that each Palmer amaranth has the same effect on yield and that the yield penalty is directly proportional to Palmer amaranth count is not necessarily true. In some instances, this may be the case; however, several factors play a role in determining yield loss per Palmer amaranth plant. Some of these factors were not accounted for in this study. Examples include the time of Palmer amaranth emergence and duration of weed/crop competition. The intraspecific competition of Palmer amaranth will also have an effect on the competitive ability of Palmer amaranth to reduce crop yields; i.e. a single Palmer amaranth plant in the right environment can be as competitive as or more dominant than a small group of Palmer amaranth in the same given area.

**Palmer Amaranth and Yield.** A reduced input model was created in order to demonstrate the effect that Palmer amaranth density has on cotton lint yields for a given soil type in this study. The model:

\[
Y_i = \text{intercept} + PA_{count} + PA_{count}^2 + W_iPA_{count} + LE + CAB
\]

where \(Y_i\) is cotton lint yield in kg ha\(^{-1}\) at location \(i\), \(PA_{count}\) is the density of Palmer amaranth, \(W_iPA_{count}\) is the \(i^{th}\) weighted matrices density average, \(LE\) was a Leaf silt loam soil, and \(CAB\) was a Captina silt loam soil, was used to determine the effect Palmer amaranth density has on...
cotton lint yields in a given soil. The SERROR model was chosen based on AIC and GM estimation was used for determining the yield penalty/gains. The estimates were used to build Figure 2.22, which represents the relationship of all Palmer amaranth present in a given area of the field, including the spatially weighted average of neighboring Palmer amaranth. This model represents a quadratic relationship for increasing Palmer amaranth densities and decreasing cotton lint yields. Cotton in the Leaf silt loam soil yielded the highest in the absence of any Palmer amaranth, followed by the Captina silt loam soil. Cotton in the Pickwick silt loam soil, which was far less abundant and located in the lower end of the fields, yielded the lowest when no Palmer amaranth were present. Regardless of soil type, increasing Palmer amaranth densities significantly lower lint yields.
Figure 2.22. Cotton lint yields for a Leaf silt loam (LE), Captina silt loam (CAB), and a Pickwick silt loam (PKC), as affected by cumulative Palmer amaranth densities across fields G2, G4, G5, and G6 in 2009 at the Main Experiment Station of the University of Arkansas, Fayetteville, AR.
SUMMARY AND CONCLUSIONS

Results from this research highlight the importance of awareness in managing glyphosate-resistant Palmer amaranth. In only two years from introduction, glyphosate-resistant Palmer amaranth had colonized each field, spreading from border to border. Although yields were not affected as a direct result of Palmer amaranth in 2008, the implications of resistance evolution going ‘unnoticed’ in the first year can have devastating effects in the second or third year. The amount of seed produced by glyphosate-resistant Palmer amaranth allows it to rapidly spread throughout a field and/or entire farm. In 2010, a complete crop failure was noted in this study. The competition from high densities of Palmer amaranth resulted in little to no cotton at harvest. Moreover, the high densities in 2010 made harvest virtually impossible due to potential equipment failure and there appeared to be little or no cotton lint present in these fields that fall.

Glyphosate-resistant Palmer amaranth potentially spread long distances in this research via furrow irrigation, tillage and harvest equipment, as well as significant rainfall events after seed maturity. Seed movement perpendicular to the bedded rows may have also resulted from wind, insects, rodents, or other animals. Seed spread was not limited to the confines of field borders in this study, as glyphosate-resistant Palmer amaranth plants were noted outside the test area in 2010. In a production situation, seed dispersal becomes more critical, because there is potential for spreading resistance over thousands of hectares for any given farm.

Another critical issue to point out lies in the selection pressure associated with continued postemergence over-the-top applications of glyphosate. This type of cultural practice rapidly selects for individuals with high levels of glyphosate resistance. The fact that yields were not significantly affected by Palmer amaranth densities in 2008 might actually be a function of increased levels of glyphosate resistance in 2009, leading to a more competitive population of
Palmer amaranth. These results indicate that the economic threshold of Palmer amaranth is in reality a ‘zero tolerance’ threshold when managing a resistant population or trying to prevent resistance from evolving. No Palmer amaranth should be allowed to reach reproductive maturity, meaning that multiple means of control will be needed over an extended growing season due to the season-long emergence pattern of Palmer amaranth (Jha and Norsworthy 2009; Norsworthy et al. 2012). In this research, it took only 20,000 seed initially introduced into a square meter to effectively colonize a field in less than two years, which is far fewer than the number of seed produced by most Palmer amaranth females.
SOURCES OF MATERIALS

1 Trimble AgGPS 332 Ultimate Choice GPS, Laserplane Arkansas Inc., 882 East Park St, Carlisle, AR, 72024.

2 OmniSTAR HP correction, FURGO; OmniSTAR, Inc., 8200 Westglen, Houston, TX, 77063.

3 Cotton yield monitor with Insight display, Case-IH 1822 kit; Ag Leader Technology, 2202 S. Riverside Dr., Ames, IA, 50010.

4 ArcGIS, ESRI software; http://www.esri.com/software/arcgis/.

5 SSURGO Database, Soil Survey Staff, NRCS-USDA, for Fayetteville, AR. http://soildatamart.nrcs.usda.gov/.)

6 GeoDa 0.9.5-I, Arizona State University; http://geodacenter.asu.edu/.

LITERATURE CITED


Webster, T. M.  2005.  Patch expansion of purple nutsedge (Cyperus rotundus) and yellow nutsedge (Cyperus esculentus) with and without polyethylene mulch.  Weed Sci. 53:839-845.


CHAPTER III
EFFECT OF HERBICIDE PROGRAMS IN GLUFOSINATE- AND GLYPHOSATE-
RESISTANT COTTON ON WEED SEED RAIN, SOIL SEEDBANK, AND SPECIES
SHIFTS OVER A THREE-YEAR ROTATION
ABSTRACT

Weed control programs need to alleviate the intense selection pressures associated with using a single mode of action and provide effective control of all weed species. Technologies such as the Liberty Link® (LL) cotton trait are being used along with additional herbicide modes of action to help manage glyphosate-resistant Palmer amaranth and enhance agricultural sustainability. Integrating a residual herbicide in a cotton weed control program applied either preemergence (PRE), postemergence (POST), or post-directed (PD) at layby (LAYBY) may broaden the weed spectrum and provide extended weed control. The objectives of this research were to (1) determine how cotton system rotations and herbicide programs affect annual weed seed rain and changes in the weed seedbank over a 3-yr period, and (2) to evaluate the economic returns associated with herbicide programs in LL and Roundup Ready Flex® (RRF) cotton rotations over a 3-yr period. This research demonstrates the criticalness of herbicide application timing on the effectiveness of glufosinate in a LL cotton system. Use of glufosinate in LL cotton was not effective in reducing Palmer amaranth in the seedbank over a 3-yr period. Averaged over cotton trait rotation, the Palmer amaranth seedbank increased regardless of the herbicide program used. When averaged over herbicide programs within a herbicide resistance trait, seedcotton yields in 2009 for the LL trait were at least 400 kg ha\(^{-1}\) lower than for the RRF trait. The economics associated with these treatments indicate that cotton lint prices would need to be as high as $1.75 kg\(^{-1}\) lint in order to break even over a 3-yr period when rotating the LL-RRF-LL traits and applying a residual herbicide PRE and PD at LAYBY. The market price of cotton lint each year may well determine which resistant management techniques producers opt to employ.


Key words: Species shift, weed control, weed spectrum.
INTRODUCTION

The management of pests in crop production is a major component of successful farming. Weed management, in particular, has been one component that has evolved over the years as new technologies and cultural practices were introduced. Weed management has been characterized as “the skillful combination of prevention, eradication, and control to manage weeds in a crop, or environment” (Zimdahl 1993a). Since weed management is continuously evolving, there are several key components in understanding the systematic approach to controlling weeds, including the history of all management practices in a given field.

One aspect that is particularly important is that there is usually a time frame late in the season in which weed competition is no longer detrimental to yield, so weed control measures cease, allowing weeds to produce seed. The ability of weeds to survive and produce seed each year incorporates the concept of the soil seedbank, or weed seedbank. The weed seedbank, which includes all viable seeds in the soil or associated litter (Simpson et al 1989) represents a major survival mechanism for most weeds, particularly summer annuals (Zimdahl 1993c), and is a key factor driving the overall dynamics of weed populations. The species composition of the weed seedbank is influenced by the evolution of individual species by selection and adaptation, with species exhibiting high genetic diversity more likely to survive and thrive over time (Dekker 1999). The size and diversity of weed seedbanks are influenced by withdrawals and deposits (see Dekker 1999), factors often linked to crop/weed management practices.

Weed management practices have evolved over the years as new agricultural technologies are introduced. Prior to the widespread use of herbicides for weed management, producers typically relied on non-chemical practices such as crop selection and rotation, mechanical tillage, mowing, flooding, burning, and hand weeding for selective weed control (Parish 1990; Zimdahl
Herbicides have gained importance since the 1950’s with the synthesis of chemical molecules for weed control (Young 1987; Zimdahl 1993b). As new herbicide chemistries were discovered and used in weed management, several advantages became evident. Herbicides provided more efficient weed control in the crop rows than cultivation alone and reduced the number of times heavy tillage equipment or hand labor was required (Bridges 1994; Zimdahl 1993b). The adoption of chemical weed control provided distinct advantages, including economic feasibility as well as improving soil structure and reducing tillage-based soil erosion. Chemical weed management has witnessed a new era over the past few decades with the introduction of herbicide-resistant crop cultivars.

The commercial introduction of glyphosate-resistant soybean [Glycine max (L.) Merr.], cotton (Gossypium hirsutum L.), and corn (Zea mays L.) in the mid-1990’s provided successful examples of rapid adoption by producers. This technology increased the economic benefits resulting from excellent in-crop postemergence weed control with glyphosate. With the rapid adoption and increasing land area planted to this type of technology, herbicide use patterns changed dramatically (Young 2006). The adoption of one or two pass herbicide programs that relied solely on glyphosate became common practice (Clay et al. 2005), increasing the selection pressure for difficult-to-control species (Reddy and Norsworthy 2010), such as the Ipomoea spp. Furthermore, increased selection pressure from glyphosate has led to the selection/evolution of glyphosate-resistant weed species such as glyphosate-resistant Palmer amaranth [Amaranthus palmeri (S.) Wats.] and horseweed [Conyza canadensis (L.) Cronq.], among others (Heap 2011). The change in relative frequency of weeds within a species (resistant weeds), or among species (diversity), are examples of weed species shifts resulting from overuse of a single management
practice (Reddy and Norsworthy 2010). In this situation, the weed seedbank becomes a major factor in determining weed management strategies.

Recent examples of increasing weed densities and decreased weed diversity, as well as weed species shifts, have resulted from increased use of glyphosate-resistant crops (Duke 2005; Reddy and Koger 2006). Specific examples are evident in Georgia cotton production, where Benghal dayflower \( \textit{Commelina benghalensis} \) became one of the most troublesome weeds after only three years of glyphosate-resistant cotton use (Webster 2001), and was deemed the most troublesome weed in Georgia cotton production after seven years (Webster 2005). This shift is commonly attributed to inadequate control by glyphosate (Culpepper et al. 2004), the single herbicide applied to most glyphosate-resistant cotton. Furthermore, glyphosate-resistant Palmer amaranth was confirmed in Georgia cotton in 2005 (Culpepper et al. 2006), and by 2009 it became the most troublesome weed throughout the Midsouth (Webster 2009), likely due to inadequate weed management diversity as a result of almost sole use of glyphosate for weed control and inadequate control of late-season escapes (Reddy and Norsworthy 2010).

Since herbicide resistance has become a widespread issue, the focus has turned towards integrated tactics for mitigating/controlling the resistant species. One of the key considerations in this regard is to prevent weed seed return and reduce the seedbank size as much as possible. Several options exist for managing weed seed return and seedbank size, which include, but are not limited to, implementing crop rotation, using residual herbicides, timing of herbicide applications, and incorporating different herbicide modes of action (MOA) (Givens et al. 2011; Reddy and Norsworthy 2010).
Cotton crop rotation is governed by several factors such as the availability of suitable equipment (i.e. row spacing), economic-based decisions such as market futures and production costs, rotation restrictions of herbicides used in the previous or rotating crop, and also pest management decisions such as managing nematodes or resistant weeds. Technologies such as the glufosinate-resistant cotton system are being used along with different herbicide rotations to help manage glyphosate-resistant species. Rotating glyphosate- and glufosinate-resistant cotton systems allow producers to eliminate hard-to-control weeds and reduce seedbank persistence.

Herbicide diversity and application timing are two components of a weed management program that can directly affect crop yields. Mismanaged application timings, or failure to incorporate a residual herbicide can lead to increased weed competition and seed production from early-season escapes (Clay et al. 2005; Loux et al. 2011). To help manage glyphosate-resistant species, many researchers and producers have begun to investigate the use of glufosinate-resistant cotton, such as the Liberty Link® (LL) cotton system, for diversifying herbicide MOA’s and enhancing agricultural sustainability. Incorporating a residual herbicide in a cotton weed control program applied either preemergence (PRE), postemergence (POST), or post-directed (PD), may broaden the weed spectrum and provide extended weed control. To better understand how these practices may alleviate selection pressure and how effective they are in managing glyphosate-resistant species, research is needed to determine the effects on the soil seedbank and population dynamics as they relate to herbicide exposure and selection pressure.

Effective weed control programs need to alleviate the intense selection pressures associated with using a single MOA and also provide acceptable control of glyphosate-resistant species. The adoption rate of these management strategies prior to an “instance” of local resistance evolution has been inconsistent (Givens et al. 2011). It was hypothesized that the convenience
and economic feasibility of incorporating such strategies influence producer adoption; therefore, the objectives of this research were many-fold. Despite the potential importance of rotating cotton traits and choosing appropriate herbicide options for managing the weed seedbank, research is limited in this regard. The objectives of this study were to (1) determine how cotton system rotations and herbicide programs effect annual weed seed rain and changes in the weed seedbank over a 3-yr period, and (2) to evaluate the economic returns associated with herbicide programs in glyphosate- and glufosinate-resistant cotton rotations over a 3-yr period.

MATERIALS AND METHODS

Experimental Set-up. Research was conducted in a 6-hectare cotton field at the Northeast Research and Extension Center at Keiser, AR, in 2007, 2008, and 2009. This field was planted with corn the 4 yrs prior to the initiation of the study. Weed control programs during this period consisted of combinations of paraquat, S-metolachlor, and atrazine along with early-season cultivation. The experimental design was a split-split-plot with crop trait sequences as the main plot factor (four levels) and herbicide program as the sub-plot factor (three levels), and year as the sub-sub plot, consisting of three replications. Cotton trait sequence consisted of the following combinations of LL and Roundup Ready Flex® (RR) cotton in a 3-yr period: (1) LL-LL-LL, (2) LL-RR-LL, (3) RR-RR-RR, and (4) RR-LL-RR. The three herbicide programs were: (1) a total POST over-the-top (OTT) with no residual herbicides (P-P-P) consisting of either glufosinate at 0.59 kg ai ha⁻¹ (for main plot treatments with LL cotton, 1X field rate) or glyphosate at 0.87 kg ae ha⁻¹ (for main plot treatments with RR cotton, 1X field rate) applied to 1- to 3-node cotton, followed by (fb) 5- to 6-node cotton, fb ≥10-node cotton at LAYBY (i.e., three non-residual POST OTT applications, P-P-P), (2) S-metolachlor at 1.4 kg ai ha⁻¹ +
fluometuron at 2.24 kg ai ha\(^{-1}\) applied PRE, fb either glufosinate or glyphosate at the 1X rate at 5- to 6-node cotton, fb 1X rate of glufosinate or glyphosate to ≥10-node cotton as an OTT application at LAYBY (i.e., a residual PRE fb two OTT POST applications, R-P-P), (3) S-metolachlor + fluometuron applied PRE, fb either glufosinate or glyphosate at the 1X rate at 5- to 6-node cotton, fb a PD residual of flumioxazin at 0.071 kg ai ha\(^{-1}\) + MSMA at 2.24 kg ai ha\(^{-1}\) at ≥10-node cotton at LAYBY (i.e., residual PRE fb a non-residual POST fb a PD residual at LAYBY, R-P-RP).

The experiment was comprised of 12 (4 x 3) experimental treatments in total, with each plot measuring 7.72 m (8 rows of cotton) by 180 m in length. Each year, Stoneville 4554 B2/RRF (resistant to glyphosate) and Fibermax 955 B2/LL (resistant to glufosinate) cotton cultivars were planted in respective plots. In all years, cotton was managed according to the Arkansas Cooperative Extension Service (UA 2011a) recommendations for cotton production and the crop was furrow irrigated as needed during the growing season. All systems were re-bedded in the fall and received a spring herbicide application for burndown at least 30 d before re-bedding and planting in the next year.

**Data Collection.** Changes in seedbank size for each naturally occurring weed species under each treatment over the course of the study (3-yr period) was estimated by comparing the initial and final viable seedbank size in each plot. To estimate the viable soil seedbank size prior to initiating the experiment, soil cores were taken in April 2007 to a depth of 10 cm using a 10 cm-diam cup cutter\(^1\). In each plot, five soil cores were taken from between rows 3 and 4, and five soil cores from between rows 5 and 6, at distances of 30, 60, 90, and 120 m (40 cores plot\(^{-1}\)). Soil cores were immediately returned to the Northwest Arkansas Research and Extension Center.
in Fayetteville, AR, and placed in 40- by 52- by 7-cm flats. The trays were left in an open environment and were watered as required to facilitate germination. All emerged species were identified, counted, and removed within 2 wk after emergence. The soil was then stirred to induce germination of any remaining viable seeds. The same procedure was followed to estimate the final seedbank level from each plot at the end of the experiment in April 2010.

In addition, weed seed rain was estimated from each plot in early August each year to determine the effect of different treatments on weed seed production. Seed traps were made using 15- by 23- by 7-cm flats containing 580 μm mesh screen small enough to catch all weed seeds present in the plots, including that of Palmer amaranth, yet porous enough for water penetration. The traps were placed in the exact locations of original soil cores collected prior to the initiation of the experiment and were left in the field until cotton harvest. Seed traps were then collected for weed seed identification and enumeration.

**Data Analysis.** Seed trap counts were used to determine the treatment effects in 2007, 2008, and 2009. Data from soil cores and seed traps were discrete and lacked normality. In some cases, counts were zeros, giving no variability among reps; therefore, descriptive statistics were generated for various species. Observations of the studentized residuals indicated the data set had a Poisson distribution. A generalized linear mixed model procedure, PROC GLIMMIX, was performed using SAS to allow for more accurate sample-specific inference. The PROC GLIMMIX procedure performed maximum likelihood estimates based on Laplace approximation of the marginal log likelihood. Data are presented as the least square means of significant interactions, with the standard error of the means used to separate differences in
treatment combinations. Means and standard errors presented were converted to a per m² basis for presentation.

Viable seed counts from the initial soil cores taken in 2007 and the final soil cores taken in 2010 were used to determine actual change in seedbank density under each treatment for the major species detected over the 3-yr period. These data were analyzed using PROC GLM in SAS and mean separation was carried out using Fisher’s protected LSD (α = 0.05). Treatment means and LSD’s were presented on a per m² basis.

Seedcotton yields (kg ha⁻¹) were taken by harvest the inside 2 rows of 8-row plots. The seedcotton yields were analyzed using PROC MIXED (with year considered random) in SAS and mean separation was carried out using Fisher’s protected LSD (α = 0.05). To determine the economics associated with the 3-yr systems, mean lint yields (3-yr average) were used in an economic analysis. Each system was managed to maximize profit, consistent with practices recommended by the University of Arkansas Cooperative Extension Service (UA 2011a). Fertilization, irrigation, and plant growth regulator and harvest-aid applications were held constant across all systems.

Enterprise budgets were created using Mississippi State Budget Generator⁴ v6.0. Input prices used to generate 2009 Crop Production Budgets for Farm Planning by the University of Arkansas’ Division of Agriculture were selected to reflect current input prices in Arkansas. Random cotton samples from each variety were taken and ginned in order to determine the percent lint turnout in each year. Expected 3-yr lint yields from each of the 12 systems (4 x 3 combinations of treatments) were converted to gross revenue and adjusted for input and fixed costs to calculate net returns.
RESULTS AND DISCUSSION

Initial Soil Seedbank. There were 12 weed species detected during the initiation of the experiment (Table 3.1), and four more species, velvetleaf (*Abutilon theophrasti* Medik.), hophornbeam copperleaf (*Acalypha ostryifolia* Riddell), common ragweed (*Ambrosia artemisiifolia* L.), and bermudagrass (*Cynodon dactylon* (L.) Pers.), were later found during the course of the experiment. Of those 16 weed species, spotted spurge (*Chamaesyce maculate* (L.) Small) initially had the highest viable seed density (7,738 seed m$^{-2}$), followed by prostrate spurge (*Chamaesyce humistrata* (Engelm. ex Gray Small)) (4,844 seed m$^{-2}$). These species are common in cultivated areas of the Mississippi Delta region and may have been more prevalent as a result of late season escapes in the previous corn rotation (Bryson and DeFelice 2009; Reddy and Norsworthy 2010). The densities of Palmer amaranth, carpetweed (*Mollugo verticillata* L.), and prickly sida (*Sida spinosa* L.) were comparatively less than other broadleaf weeds found (Table 3.1). Large crabgrass (*Digitaria sanguinalis* (L.) Scop.], barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.], broadleaf signalgrass (*Urochloa platyphylla* (Nash) R.D. Webster], and red sprangletop (*Leptochloa panicea* (Retz.) Ohwi] were the grass species present, although at lower densities than the broadleaf weed species detected.

Annual Seed Rain. Weed seed rain was composed of several broadleaf weed and grass weed species; however, due to discrete counts, seed rain will only be reported for Palmer amaranth and for cumulative broadleaf weed seed totals (Table 3.2), as well as barnyardgrass and total grass weed seeds (Table 3.3). There was a significant three way interaction for these species. For annual broadleaf weed species such as Palmer amaranth, seed rain is extremely important for persistence. Under ideal growing conditions, a single Palmer
Table 3.1. Viable weed seed density in the top 10 cm of soil profile prior to initiation of the experiment in 2007 at the Northeast Research and Extension Center in Keiser, AR.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Species</th>
<th>Scientific name</th>
<th>Bayer code</th>
<th>Seed m\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>spotted spurge</td>
<td>\textit{Chamaesyce maculate} (L.) Small</td>
<td>EPHMA</td>
<td>7,738 \textsuperscript{a}</td>
</tr>
<tr>
<td>prostrate spurge</td>
<td>\textit{Chamaesyce humistrata} (Engelm. ex Gray)</td>
<td>EPHHT</td>
<td>4,844 \textsuperscript{b}</td>
</tr>
<tr>
<td>Palmer amaranth</td>
<td>\textit{Amaranthus paler i} S. Watts</td>
<td>AMAPA</td>
<td>2,839 \textsuperscript{c}</td>
</tr>
<tr>
<td>Carpetweed</td>
<td>\textit{Mollugo verticillata} L.</td>
<td>MOLVE</td>
<td>2,745 \textsuperscript{c}</td>
</tr>
<tr>
<td>prickly sida</td>
<td>\textit{Sida spinosa} L.</td>
<td>SIDSP</td>
<td>1,968 \textsuperscript{cd}</td>
</tr>
<tr>
<td>large crabgrass</td>
<td>\textit{Digitaria sanguinalis} (L.) Scop.</td>
<td>DIGSA</td>
<td>1,283 \textsuperscript{de}</td>
</tr>
<tr>
<td>Barnyardgrass</td>
<td>\textit{Echinochloa crus-galli} (L.) Beav.</td>
<td>ECHCG</td>
<td>420 \textsuperscript{ef}</td>
</tr>
<tr>
<td>common purslane</td>
<td>\textit{Portulaca oleracea} L.</td>
<td>POROL</td>
<td>277 \textsuperscript{ef}</td>
</tr>
<tr>
<td>pitted morningglory</td>
<td>\textit{Ipomoea lacunosa} L.</td>
<td>IPOLA</td>
<td>201 \textsuperscript{ef}</td>
</tr>
<tr>
<td>broadleaf signalgrass</td>
<td>\textit{Urochloa platyphylla} (Nash) R.D. Webster</td>
<td>BRAPP</td>
<td>147 \textsuperscript{ef}</td>
</tr>
<tr>
<td>ivyleaf morningglory</td>
<td>\textit{Ipomoea hederacea} Jacq.</td>
<td>IPOHE</td>
<td>38 \textsuperscript{f}</td>
</tr>
<tr>
<td>red sprangletop</td>
<td>\textit{Leptochloa panicea} (Retz.) Ohwi</td>
<td>LEFFI</td>
<td>32 \textsuperscript{f}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$ according to Fisher’s protected LSD.
amaranth plant is capable of producing over 1,500,000 seed plant\(^1\) (Smith et al. 2012) and consequently, it can quickly become a dominant species in the soil. Data from the present experiment indicate that, in the absence of glyphosate-resistant Palmer amaranth, a glyphosate-based total POST weed control program is more effective than a glufosinate-based total POST weed control program for reducing the number of Palmer amaranth seed returning to the soil seedbank over a 3-yr period (Table 3.2). This was also the case regardless of whether a residual herbicide was added PRE, PD, or both, except for 2009, where no difference was observed between continuous 3-yr LL and RR systems that incorporated a residual herbicide both PRE and PD. In general, the addition of a residual herbicide PRE or PD reduced the number of Palmer amaranth seed rain, except in 2007, where the addition of a residual herbicide either PRE or PD in the LL system did not reduce Palmer amaranth seed rain below 212 seed m\(^{-2}\) (Table 3.2). The inclusion of RR cotton in a 3-yr LL cotton sequence (i.e. LL-RR-LL) significantly decreased the number of seeds produced by Palmer amaranth in the second year compared to a LL only system (i.e. LL-LL-LL) with the herbicide programs P-P-P and R-P-P.

Conversely, the incorporation of LL cotton in a 3-yr RR cotton sequence significantly increased the number of Palmer amaranth seed m\(^{-2}\) in year two (Table 3.2), regardless of herbicide program. The fact that these herbicide applications were based off the growth stage of cotton rather than weed size at the time of application is one of several reasons that might possibly explain increased seed rain in the LL cotton systems (Table 3.4). Because glufosinate does not translocate as efficiently as glyphosate (Everman et al. 2007), control efficacy was lower in the LL system, particularly with larger weeds. In addition, it is well known that
Table 3.2. Palmer amaranth and total broadleaf weed seed rain as influenced by cotton trait sequence and herbicide program over three years (2007-2009) at the Northeast Research and Extension Center at Keiser, AR.a,b

<table>
<thead>
<tr>
<th>Trait Sequence</th>
<th>Herbicide program</th>
<th>AMAPA</th>
<th>All broadleaf weeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2007</td>
<td>2008</td>
</tr>
<tr>
<td>LL-LL-LL</td>
<td>P-P-P</td>
<td>999 (675)</td>
<td>820 (554)</td>
</tr>
<tr>
<td></td>
<td>R-P-P</td>
<td>585 (396)</td>
<td>862 (582)</td>
</tr>
<tr>
<td></td>
<td>R-P-RP</td>
<td>212 (143)</td>
<td>950 (642)</td>
</tr>
<tr>
<td>LL-RR-LL</td>
<td>P-P-P</td>
<td>475 (322)</td>
<td>16 (11)</td>
</tr>
<tr>
<td></td>
<td>R-P-P</td>
<td>1044 (706)</td>
<td>68 (46)</td>
</tr>
<tr>
<td></td>
<td>R-P-RP</td>
<td>725 (490)</td>
<td>1,557 (1,051)</td>
</tr>
<tr>
<td>RR-LL-RR</td>
<td>P-P-P</td>
<td>14 (10)</td>
<td>1,550 (1,046)</td>
</tr>
<tr>
<td></td>
<td>R-P-P</td>
<td>5 (4)</td>
<td>293 (199)</td>
</tr>
<tr>
<td></td>
<td>R-P-RP</td>
<td>14 (10)</td>
<td>294 (200)</td>
</tr>
<tr>
<td>RR-RR-RR</td>
<td>P-P-P</td>
<td>47 (33)</td>
<td>32 (21)</td>
</tr>
<tr>
<td></td>
<td>R-P-P</td>
<td>4 (3)</td>
<td>7 (6)</td>
</tr>
<tr>
<td></td>
<td>R-P-RP</td>
<td>6 (5)</td>
<td>168 (114)</td>
</tr>
</tbody>
</table>
Abbreviations:  AMAPA, Palmer amaranth; LL, Liberty Link® (glufosinate-resistant) cotton; RR, Roundup Ready Flex® (glyphosate-resistant) cotton; R, residual PRE herbicide application; P, non-residual POST OTT application; RP, residual PD herbicide application.

Values in parentheses represent standard errors of the means for comparing 2007, 2008, and 2009 means within a species or group. Trait sequence X herbicide program X year interaction p = <.0001 (both AMAPA and all broadleaves)
Table 3.3. Barnyardgrass and total grass weed seed production as influenced by cotton trait sequence and herbicide program over three years (2007-2009) at the Northeast Research and Extension Center at Keiser, AR.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Trait sequence</th>
<th>Herbicide program</th>
<th>ECHCG</th>
<th>All grass weeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007</td>
<td>2008</td>
<td>2009</td>
</tr>
<tr>
<td>LL-LL-LL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-P-P</td>
<td>111(107)</td>
<td>850 (826)</td>
<td>1,231 (1,042)</td>
</tr>
<tr>
<td>R-P-P</td>
<td>50 (40)</td>
<td>140 (124)</td>
<td>140 (121)</td>
</tr>
<tr>
<td>R-P-RP</td>
<td>79 (70)</td>
<td>319 (308)</td>
<td>36 (36)</td>
</tr>
<tr>
<td>LL-RR-LL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-P-P</td>
<td>17 (10)</td>
<td>4 (4)</td>
<td>212 (178)</td>
</tr>
<tr>
<td>R-P-P</td>
<td>36 (25)</td>
<td>4 (4)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>R-P-RP</td>
<td>69 (60)</td>
<td>161 (152)</td>
<td>79 (76)</td>
</tr>
<tr>
<td>RR-LL-RR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-P-P</td>
<td>18 (16)</td>
<td>660 (648)</td>
<td>1,037 (1,007)</td>
</tr>
<tr>
<td>R-P-P</td>
<td>4 (4)</td>
<td>32 (32)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>R-P-RP</td>
<td>0 (0)</td>
<td>32 (32)</td>
<td>39 (37)</td>
</tr>
<tr>
<td>RR-RR-RR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-P-P</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>14 (11)</td>
</tr>
<tr>
<td>R-P-P</td>
<td>4 (4)</td>
<td>4 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>R-P-RP</td>
<td>0 (0)</td>
<td>11 (11)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Abbreviations: ECHCG, barnyardgrass; LL, Liberty Link® (glufosinate-resistant) cotton; RR, Roundup Ready Flex® (glyphosate-resistant) cotton; R, residual PRE herbicide application; P, non-residual POST OTT application; RP, residual PD herbicide application.

Values in parentheses represent standard errors of the means for comparing 2007, 2008, and 2009 means within a species or group. Trait sequence X herbicide program X year interaction p = <.0001 (both barnyardgrass and all grasses)
Table 3.4. Herbicide timing, cotton growth stage, weed height, and precipitation for the three years (2007-2009) at the Northeast Research and Extension Center at Keiser, AR.

<table>
<thead>
<tr>
<th>Year</th>
<th>Application (cotton stage)</th>
<th>Date</th>
<th>Maximum weed height$^a$</th>
<th>Precipitation (14DAT)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cm</td>
<td>cm</td>
</tr>
<tr>
<td>2007</td>
<td>PRE</td>
<td>May 9</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POST 1- to 3-node</td>
<td>May 15</td>
<td>13</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>POST 5- to 6-node</td>
<td>June 8</td>
<td>36</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>LAYBY ≥ 10-node</td>
<td>July 16</td>
<td>152</td>
<td>0.3</td>
</tr>
<tr>
<td>2008</td>
<td>PRE</td>
<td>May 20</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POST 1- to 3-node</td>
<td>June 3</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>POST 5- to 6-node</td>
<td>June 18</td>
<td>36</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>LAYBY ≥ 10-node</td>
<td>July 16</td>
<td>152</td>
<td>2.0</td>
</tr>
<tr>
<td>2009</td>
<td>PRE</td>
<td>May 19</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POST 1- to 3-node</td>
<td>June 2</td>
<td>20</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>POST 5- to 6-node</td>
<td>June 18</td>
<td>46</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>LAYBY ≥ 10-node</td>
<td>July 15</td>
<td>152</td>
<td>25.1</td>
</tr>
</tbody>
</table>

$^a$ Palmer amaranth was the largest weed at each application.

$^b$ Cumulative rainfall total within 14 DAT.
glyphosate is highly effective on susceptible Palmer amaranth (Whitaker et al. 2010). For example, when the last POST glyphosate application in a RR system was replaced with a PD residual application of flumioxazin + MSMA at LAYBY, there was an increase in Palmer amaranth seed rain in the latter (Table 3.2). However, after only three years of continuous RR cotton (RR-RR-RR) using a total POST (P-P-P) herbicide program, Palmer amaranth seed rain substantially increased in the third year (Table 3.2), which is probably due to the evolution of resistance to glyphosate and our observations in the following year (2010, data not shown) also supported this conclusion.

Weed seed rain for total broadleaf weeds followed a similar pattern (Table 3.2), possibly because Palmer amaranth was the dominant broadleaf species. Overall, the LL cotton system accounted for greater seed rain in the first year compared to the RR systems, likely due to the aforementioned issues with weed size and herbicide efficacy (Tables 3.2 and 3.4).

Among the grass weeds, barnyardgrass was the dominant species in the experimental site, therefore it will be presented along with cumulative weed seed rain from all grass species (Table 3.3). In the second year of the sequence, plots with the LL system in the first year resulted in more barnyardgrass seed rain than any sequence containing the RR trait, except for the LL-RR-LL sequence with a total POST herbicide program (P-P-P), which was similar (18 seed m\(^{-2}\)). The RR-LL-RR sequence with a total POST herbicide program was the only RR cotton system with grass seed rain considered to be greater than zero in the first year. These results also indicate that in a 3-yr LL cotton sequence, regardless of whether or not RR cotton is incorporated in the second year, the addition of a residual herbicide PRE is necessary to reduce barnyardgrass seed rain (Table 3.3).
Total seed rain for all grasses in the third year had a similar trend to barnyardgrass, with the total POST herbicide program in the 3-yr LL cotton sequence resulting in greater seed rain than all other treatment combinations. Replacing LL cotton with RR cotton in the second year reduced both barnyardgrass and cumulative grass seed rain compared to continuous LL cotton. This is likely due to greater grass control with glyphosate compared to glufosinate (Culpepper et al. 2000; Price et al. 2008).

Another important difference regarding the herbicide programs was also evident when evaluating total grass and broadleaf weed seed rain. The PD application of flumioxazin + MSMA at LAYBY was more effective in reducing seed production of the grasses, but not in the broadleaf weed species (Tables 3.2 and 3.3). This is due, in part, to the efficacy of flumioxazin and MSMA on emerged grasses compared to emerged broadleaf weeds (Price et al. 2008). Flumioxazin provides better weed control when applied PRE than POST for most species, while MSMA is more effective on grasses than on larger broadleaves (Everman et al. 2007). Some grasses are more capable of achieving seed production following late-season emergence, and the addition of residual herbicide at LAYBY may be more effective in reducing seed rain. At the time of PD applications, the grass weeds were shorter than the broadleaf weeds such as Palmer amaranth; therefore, coverage and efficacy of the PD application could have differed for various species. Moreover, barnyardgrass plants can typically produce up to 25,000 seed plant⁻¹, somewhat less than can be expected from a Palmer amaranth plant (Keeley et al. 1987; Keeley and Thullen 1991).

**Seedcotton Yields.** The trait sequence by herbicide by year interaction was not significant; therefore, only the trait sequence by year and herbicide by year interactions were presented.
(Tables 3.5 and 3.6). It was expected that environmental conditions in 2007, 2008, and 2009 would affect seedcotton yields; therefore minimum and maximum temperatures and rainfall totals are shown in Figures 3.1 to 3.3. In general, seedcotton yields were lower in LL systems compared to RR systems in the first year (Table 3.5). In previous research, LL varieties yielded up to 448 kg ha\(^{-1}\) less than available RR varieties (UA 2011b; UG 2011). However, incorporating these varieties into a resistance management program may increase producer’s ability to mitigate/manage resistant weeds (Culpepper et al. 2009).

Seedcotton yields of RR and LL cotton rotations were similar in year one, except for the LL-RR-LL trait sequence (Table 3.5). Although the 3-yr LL cotton rotation yielded 3,005 kg ha\(^{-1}\) in year one, seedcotton yield in this system was significantly lower in year two (1,355 kg ha\(^{-1}\)), likely a result from increased weed pressure from Palmer amaranth and barnyardgrass (Tables 3.2 and 3.3). In the second year, seedcotton yields were the highest (3,282 kg ha\(^{-1}\)) in the continuous RR system and the lowest (1,355 kg ha\(^{-1}\)) in the continuous LL system (Table 3.5). Including RR cotton in LL cotton sequence either in the first year (RR-LL) or second year (LL-RR) increased seedcotton yields in year two, yet continuous RR sequence (RR-RR) provided the greatest yields in the year (Table 3.5).

In the third year, seedcotton yields were lower compared to the first and second years irrespective of the cotton trait sequence. In 2009, the experimental site witnessed more rainfall events with considerably greater rainfall compared to 2007 and 2008 (Figures 3.1 to 3.3), allowing multiple flushes of weeds throughout the growing season, leading to greater levels of weed pressure and subsequent yield reduction. A contrast analysis has indicated that planting LL cotton rather than RR cotton in the first year resulted in more yield loss in the third year (P<0.0091).
Table 3.5. Seedcotton yields as influenced by cotton trait sequence over three years (2007-2009) at the Northeast Research and Extension Center at Keiser, AR.\textsuperscript{a,b,c,d,e}

<table>
<thead>
<tr>
<th>Rotation</th>
<th>Seedcotton yields kg ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL-LL-LL</td>
<td>3,005 1,355 1,340</td>
</tr>
<tr>
<td>LL-RR-LL</td>
<td>2,662 2,609 1,510</td>
</tr>
<tr>
<td>RR-LL-RR</td>
<td>3,388 2,372 1,966</td>
</tr>
<tr>
<td>RR-RR-RR</td>
<td>3,342 3,282 1,937</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: LL, Liberty Link\textsuperscript{®} (glufosinate-resistant) cotton; RR, Roundup Ready Flex\textsuperscript{®} (glyphosate-resistant) cotton.

\textsuperscript{b} Fisher’s protected LSD ($\alpha = 0.05$) to compare means within a rotation = 486 kg ha\(^{-1}\).

\textsuperscript{c} Fisher’s protected LSD ($\alpha = 0.05$) to compare means in different rotations in the same or different year = 502 kg ha\(^{-1}\).

\textsuperscript{d} Contrast on the effect of LL vs. RR cotton in the first year of a 3-yr rotation on seedcotton yields in the second year (p < 0.0001).

\textsuperscript{e} Contrast on the effect of LL vs. RR cotton in the first year of a 3-yr rotation on seedcotton yields in the third year (p < 0.0001).
Table 3.6. Seedcotton yields for the interaction of herbicide program and year at the Northeast Research and Extension Center at Keiser, AR.\textsuperscript{a,b,c,d}

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-P-P</td>
<td>3,187</td>
<td>2,058</td>
<td>1,719</td>
</tr>
<tr>
<td>R-P-P</td>
<td>3,033</td>
<td>2,716</td>
<td>1,870</td>
</tr>
<tr>
<td>R-P-R</td>
<td>3,078</td>
<td>2,439</td>
<td>1,476</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: R, residual PRE herbicide application; P, non-residual POST OTT application; RP, residual PD herbicide application.

\textsuperscript{b} Fisher’s protected LSD (\(\alpha = 0.05\)) to compare within a herbicide program = 243 kg ha\(^{-1}\).

\textsuperscript{c} Fisher’s protected LSD (\(\alpha = 0.05\)) to compare different herbicide program means in the same or different years = 246 kg ha\(^{-1}\).

\textsuperscript{d} Contrast for the effect of including a residual either PRE or at LAYBY in the first year of a 3-yr rotation on seedcotton yields in the second year (\(p < 0.0001\)).
Figure 3.1. Minimum and maximum temperatures, precipitation, and furrow irrigation from April to October in 2007 at the Northeast Research and Extension Center in Keiser, AR.
Figure 3.2. Minimum and maximum temperatures, precipitation, and furrow irrigation from April to October in 2008 at the Northeast Research and Extension Center in Keiser, AR.
Figure 3.3. Minimum and maximum temperatures, precipitation, and furrow irrigation from April to October in 2009 at the Northeast Research and Extension Center in Keiser, AR
However, continuous RR cotton (RR-RR-RR) did not perform any better than RR-LL-RR in the third year, perhaps due to the increased weed pressure in the third year, resulting from possible glyphosate resistance evolution in Palmer amaranth. As such, incorporation of a trait requiring an alternative herbicide mode of action (LL in this case) has a tremendous value in preserving available herbicides, even if yield levels are to be compromised to some extent.

Averaged over cotton trait sequence, there were no differences in seedcotton yields among the herbicide programs in the first year (3,033 to 3,187 kg ha\(^{-1}\)) (Table 3.6). However, failure to incorporate a residual PRE herbicide in the first year lowered seedcotton yields in the second year. Among the herbicide program containing residuals, the program with a residual PRE alone yielded 11% greater than the program containing residuals both PRE and PD at LAYBY in the second year. Replacing the LAYBY application of glyphosate and glufosinate (applied OTT) with flumioxazin and MSMA (applied PD) reduced seedcotton yields due to the comparatively lower efficacy of the latter herbicides on larger weeds, especially broadleaves that survived earlier herbicide treatments (see Table 3.4 for weed size at the time of application) and to the inadequate coverage on larger weeds resulting from PD application.

In the third year, seedcotton yields were less than the first and second years, irrespective of the herbicide program. The experimental site received comparatively greater rainfall with high intensity of rainfall events in the third year compared to the first or second year (Figures 3.1 to 3.3), leading to greater weed emergence and competition. Moreover, rainfall was often excessive at or near the time of some herbicide applications (Table 3.4), which was detrimental to herbicide activity, particularly the loss of residual activity. The loss of residual activity early in the season allowed weeds to reach sizes that were too large (off label) for control with glyphosate- and, more importantly, glufosinate-based herbicide systems.
Regardless of whether or not a residual herbicide was used PRE, seedcotton yields were higher in the third year when the LAYBY application consisted of glyphosate or glufosinate rather than flumioxazin and MSMA, likely because of the comparatively greater efficacy of glyphosate or glufosinate on late-season escapes (Table 3.6).

**Final Soil Seedbank.** The actual change in the soil seedbank sizes of Palmer amaranth, prickly sida, red sprangletop, barnyardgrass, and large crabgrass was determined using the initial and final soil core estimates (Tables 3.7 and 3.8). Averaged over cotton trait sequence, the effect of herbicide program on change in seedbank level was significant for Palmer amaranth, prickly sida, and red sprangletop. For Palmer amaranth, the greatest increase in the seedbank was noted with the R-P-RP herbicide program (1,637 seed m\(^{-2}\)), this is attributed to the low efficacy of flumioxazin and MSMA on this weed, especially when applied as a PD spray. These observations corroborate the seed rain data obtained for this herbicide program.

For prickly sida, the total POST program (P-P-P) did not change the seedbank level compared to other herbicide programs, although substantially more seeds were extracted from these plots compared to the total POST program (Table 3.7). One possible explanation for the slight increase in seedbank level with the total POST program is the timing of these applications with the emergence periodicity of seedbank prickly sida. For example, it is possible that prickly sida had already emerged prior to planting, escaping the residual PRE application. Furthermore, prickly sida doesn’t exhibit the prolific seed production or the extended emergence pattern that are often observed in Palmer amaranth, leading to differences in seedbank levels between these two species.
Table 3.7. Change in estimates in the top 10 cm of soil profile over a three-year period (2007-2008) as affected by herbicide program at the Northeast Research and Extension Center in Keiser, AR.

<table>
<thead>
<tr>
<th>Herbicide program</th>
<th>AMAPA</th>
<th>SIDSP</th>
<th>LEFFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-P-P</td>
<td>+970</td>
<td>+2</td>
<td>+287</td>
</tr>
<tr>
<td>R-P-P</td>
<td>+774</td>
<td>+49</td>
<td>+42</td>
</tr>
<tr>
<td>R-P-R</td>
<td>+1,637</td>
<td>+76</td>
<td>+36</td>
</tr>
<tr>
<td>LSD</td>
<td>494</td>
<td>61</td>
<td>98</td>
</tr>
</tbody>
</table>

Abbreviations: AMAPA, Palmer amaranth; SIDSP, prickly sida; LEFFI, red sprangletop; R, residual PRE application; P, non-residual POST OTT application; RP, residual PD LAYBY application.

LSD was calculated using Fisher’s protected LSD at $\alpha = 0.05$. 

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Table 3.8. Change in estimates in the top 10 cm of soil profile over a three-year period (2007-2009) as affected by trait sequence at the Northeast Research and Extension Center in Keiser, AR.\textsuperscript{a,b,c,d}

<table>
<thead>
<tr>
<th>Rotation</th>
<th>ECHCG</th>
<th>DIGSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-P-P</td>
<td>R-P-P</td>
</tr>
<tr>
<td>LL-LL-LL</td>
<td>+1,726</td>
<td>+237</td>
</tr>
<tr>
<td>LL-RR-LL</td>
<td>+968</td>
<td>+70</td>
</tr>
<tr>
<td>RR-LL-RR</td>
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<td>+62</td>
</tr>
<tr>
<td>RR-RR-RR</td>
<td>+211</td>
<td>+50</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: ECHCG, barnyardgrass; DIGSA, large crabgrass; LL, Liberty Link\textsuperscript{®} (glufosinate-resistant) cotton; RR, Roundup Ready Flex\textsuperscript{®} (glyphosate-resistant) cotton; R, residual PRE application; P, non-residual POST OTT application; RP, residual PD LAYBY application.

\textsuperscript{b} Fisher’s protected LSD (\(\alpha = 0.05\)) to compare two ECHCG rotational means in the same or different herbicide programs = 344 seed m\(^{-2}\).

\textsuperscript{c} Fisher’s protected LSD (\(\alpha = 0.05\)) to compare two ECHCG herbicide program means in the same rotation = 433 seed m\(^{-2}\).

\textsuperscript{d} Fisher’s protected LSD (\(\alpha = 0.05\)) to compare two means within the DIGSA column = 153 seed m\(^{-2}\).
Red sprangletop interacted differently with the herbicide programs. Increase in seed bank was greatest with the total POST program (287 seed m$^{-2}$), possibly due to the lack of PRE herbicides (Table 3.7). The application and timely activation of S-metolachlor PRE is highly effective on grass species such as red sprangletop, resulting in efficient early-season control. Yet, the substantial seed production in the treatments containing a residual PRE application is indicative of late-season grass emergence and seed production, but there was no additional benefit of tank-mixing a residual herbicide such as flumioxazin with MSMA at LAYBY (Table 3.7).

There was a significant interaction between cotton trait sequence and herbicide program averaged over years for changes in barnyardgrass seedbank level (Table 3.8). The total POST program in a continuous LL system (LL-LL-LL) allowed the greatest increase in the barnyardgrass seedbank 1,726 seed m$^{-2}$). Regardless of the cotton trait sequence, incorporating a residual herbicide either PRE, or PRE and PD at LAYBY decreased barnyardgrass seed production, except for the continuous RR cotton system (Table 3.8). The seedbank densities of spotted spurge, prostrate spurge, and carpetweed, the dominant species prior to initiating the study, declined over the course of the study (data not shown). The main effect of cotton trait sequence was significant in changes in seedbank levels for large crabgrass (Table 3.8). The continuous LL cotton (LL-LL-LL) increased large crabgrass seedbank level by 378 seed m$^{-2}$. Replacing one or more LL crops with the RR system in the three-year sequence significantly reduced the large crabgrass seedbank level compared to the continuous LL system (Table 3.8).
**Economic Evaluation.** Resistance management requires a renewed focus on producer’s weed management programs, with several cultural practices playing a critical role in how successful the outcome. In order to help determine the economic feasibility of cotton trait rotations and herbicide programs associated with resistance management strategies in this study, enterprise budgets were created using Mississippi State Budget Generator\(^4\) v6.0. Additional inputs that are necessary and common practice in cotton production were included in this economic evaluation in order to obtain a more realistic data set.

The direct expenses were associated with input variables such as harvest aids, fertilizers, insecticides, custom hires, ginning, labor, irrigation, diesel fuel, and repair and maintenance. The fixed expenses were associated with the implements, tractors, a self-propelled sprayer, and furrow irrigation used over the 3-yr period. Table 3.9 shows the 3-yr summary of each treatment combination for fixed and direct costs, lint yield totals and seed income, as well as the net returns for each system. The seed costs, technology fees, and herbicide programs associated with resistance management options are shown separately for each treatment combination. The cost of direct inputs differed slightly each year depending on equipment used for herbicide application, the number of irrigations required during the growing season, and the subsequent amount of diesel fuel use associated with each. Differences in lint yield indicate that the continuous RR trait sequence performed better than the continuous LL trait sequence, regardless of the herbicide program used (Table 3.9). Only when a residual herbicide was used either PRE or PD at LAYBY did the incorporation of the LL trait in the second year yield comparably to a continuous RR system. Because glyphosate resistant Palmer amaranth did not exist in this field
Table 3.9. Economics of a 3-yr summation across all cotton trait and herbicide program treatment combinations at the Northeast Research and Extension Center in Keiser, AR

<table>
<thead>
<tr>
<th>Trait Sequence</th>
<th>Herbicide program</th>
<th>Lint b</th>
<th>Seed income c</th>
<th>Direct expenses</th>
<th>Fixed expenses</th>
<th>Herbicide program</th>
<th>Seed technology fees</th>
<th>Net return (low price) d</th>
<th>Net return (high price) e</th>
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<tr>
<td>LL-LL-LL</td>
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<td></td>
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<tr>
<td>P-P-P</td>
<td>2048</td>
<td>674.91</td>
<td>2385.28</td>
<td>926.40</td>
<td>263.01</td>
<td>220.61</td>
<td>263.31</td>
<td>-454.38</td>
<td>1089.20</td>
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<tr>
<td>R-P-P</td>
<td>2363</td>
<td>778.40</td>
<td>2401.94</td>
<td>926.40</td>
<td>446.34</td>
<td>220.61</td>
<td>263.31</td>
<td>-100.82</td>
<td>1679.92</td>
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<tr>
<td>R-P-RP</td>
<td>2036</td>
<td>677.15</td>
<td>2405.39</td>
<td>933.22</td>
<td>462.72</td>
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<td>-696.39</td>
<td>837.59</td>
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<tr>
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<td>R-P-RP</td>
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<td>470.58</td>
<td>206.38</td>
<td>416.91</td>
<td>1259.23</td>
<td>3722.91</td>
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</table>
a LL, Liberty Link® (glufosinate-resistant) cotton; RR, Roundup Ready Flex® (glyphosate-resistant) cotton; R, residual PRE herbicide application; P, non-residual POST OTT application; RP, residual PD herbicide application.

b Yield means followed by the same letter are not significantly different at \( \alpha = 0.05 \) according to Fisher’s protected LSD.

c Calculated by multiplying the kilograms of seed produced/ha by the Arkansas average price received for cottonseed in 2009.

d The cotton lint price was $1.43 kg\(^{-1}\), an arbitrary representation of a low lint price.

e The cotton lint price was $2.20 kg\(^{-1}\), an arbitrary representation of a high lint price.
prior to initiating this study, the benefit of incorporating another MOA for controlling this species might not be realized, particularly when herbicide applications were based off of the size of the cotton, rather than the size of Palmer amaranth. The purpose of this study was not to demonstrate the benefits of resistance management techniques in the presence of resistant species, but to evaluate the effect of these treatments on the soil seedbank and to determine if any are economically feasible for the producer adoption.

Two arbitrary cotton prices of $1.43 and $2.20 kg\(^{-1}\) were used to represent a low and high price, respectively, for cotton lint. In years where lint prices are lower, a continuous LL trait sequence managed with application timings based on the stage of cotton may not cover the cost of farming, regardless of which herbicide program is chosen (Table 3.9). The glufosinate label indicates applications should be made to \(\leq 10\)-cm Palmer amaranth for best efficacy. The first glufosinate application in this study was made on Palmer amaranth larger than 10 cm (Table 3.4), and efficacy suffered as a result. This type of application is also common in large-scale farming, where it may take several days to cover more farm hectares, allowing Palmer amaranth to exceed the recommended size. Glyphosate; however, has a labeled maximum size of 46 cm for control of susceptible Palmer amaranth, providing some flexibility in application timing.

Even when the RR trait is substituted in the second year of a three year LL system, net returns for a low lint price are not favorable for adoption. A net return of $469.49 over a three year period is only $156.50 ha\(^{-1}\) yr\(^{-1}\), and probably would not break even, particularly in situations where rent or land payments are involved. Even at the lower lint price, the continuous RR trait sequence provided suitable returns, regardless of herbicide program (Table 3.9). Incorporating the LL trait in the second year of a three year RR trait sequence is one resistance
management technique that returns similarly to the continuous RR sequence, but only when a residual herbicide is used either PRE or PD at LAYBY.

Cotton prices have fluctuated over the last decade, affecting the number of hectares planted each year. In a year where cotton lint prices are projected high, the number of hectares planted will generally increase over years with a low lint price. At the high lint price of $2.20 kg⁻¹ lint, the returns were much more favorable across all treatments in this study. In this scenario a producer would be much more likely to adopt management strategies incorporating other trait sequences and MOA’s. For instance, the returns for a 3-yr LL trait sequence with a R-P-P herbicide program ($1679.92) were similar to the 3-yr RR trait sequence in a year when lint prices were low (Table 3.9).

**SUMMARY AND CONCLUSIONS**

A single herbicide program or rotation of herbicide resistance traits is not a completely effective resistance management strategy. The LL trait sequence introduces an additional MOA which has been used to successfully manage many glyphosate-resistant broadleaves, particularly Palmer amaranth; however, there are long-term implications associated with relying on a single MOA for weed control, including the use of glufosinate. Moreover, this research shows that a herbicide-only weed management program is not effective in reducing the soil seedbank.

This research demonstrates the criticalness of timely herbicide applications for effectively managing the soil seedbank. Furthermore, use of the LL trait in rotation with the RRF trait was not effective in reducing the Palmer amaranth seedbank over a 3-yr period probably because of the use of only three in-crop herbicide applications and the marginal control of Palmer amaranth with glufosinate because plants were too large at application. For the aforementioned reason of
weed size, Palmer amaranth seed rain was highest in the total POST glufosinate-only program. Adding an additional herbicide timing (four in-crop applications rather than three), which would shorten the interval between applications, would likely have had a profound impact on the results obtained in this experiment. Currently, as many as six residual herbicide applications are applied in cotton to ensure season-long residual weed control (Norsworthy, personal communication).

Even the best herbicide programs for protecting against herbicide-resistant weeds have flaws, rendering them unsuccessful at times. First, it can be challenging for producers to timely apply herbicides across large acreages, particularly when weed size or environmental conditions play such a critical role in efficacy. The simple fact that a weed control program has worked in the past or has been demonstrated as successful does not mean it will be effective in every situation or following repeated use. For example, integrating residual herbicides into weed control programs to reduce selection pressure on postemergence-applied herbicides is of little value in the absence of an activating rainfall. Weeds must be removed prior to planting and fields kept weed-free throughout much of the growing season using an array of tactics to minimize species shifts or evolution of resistant weeds (Norsworthy et al. 2012). This goal will only be achieved through weed control programs much more intense than those evaluated here.

Herbicide programs incorporating a residual PD herbicide at LAYBY provided no benefit in reducing the Palmer amaranth seedbank, in part because the Palmer amaranth plants that escaped earlier POST applications were too large at LAYBY. Again, more timely applications and activation of residual herbicides would guarantee extended weed control and overall success of a weed management program.

Another important concept taken from this research lies in the evolution of glyphosate-resistant Palmer amaranth as a result of a continuous RR trait sequence with a total POST (P-P-
P) herbicide weed management program. Although glyphosate effectively controlled Palmer amaranth in the first two years of this research, there were plants in the third year that were non-responsive to POST OT glyphosate applications; albeit, these plants did not impact yields in the final year of this 3-yr experiment. This is the same situation that many producers face today with the evolution of glyphosate-resistant weeds. The presence of glyphosate-resistant Palmer amaranth plants may not lower yield or have short-term economic consequences when the infested area is still small or limited to a few plants; however, lower cotton yields and reduced harvest efficiency will most definitely become a weed management issue and will have a profound economic impact in subsequent years if action is not taken prior to resistance becoming widespread.

The goal of this research was to evaluate trait (sequence) rotations and subsequent herbicide programs on the soil seedbank and economics of the production systems. Although the soil seedbank generally increased for the weed species in this research following multiple years of effective weed control in corn, several treatment options, particularly use of the LL trait and glufosinate would provide increased control of the initial glyphosate-resistant Palmer amaranth that was observed in the final year of this 3-year experiment. Use of the LL trait in rotation with RRF cotton does not guarantee against the evolution of glyphosate-resistant Palmer amaranth in cotton; however, the risks of glyphosate-resistant Palmer amaranth evolving are most notably lower when rotating the LL and RRF traits (Neve et al. 2011).

With the current severity of glyphosate-resistant Palmer amaranth infestations throughout the southern U.S., producers may be forced to adopt LL cotton and additional MOAs; however, preemptive use of LL cotton, especially considering the lower yields observed here, and use of additional herbicide MOAs come at an expense. Ultimately, short-term economics as seen in
this research over a 3-yr period drive the weed management programs being used by many growers today, including their lack of adoption of proactive resistance management strategies as long as a simple, easy to use, effective weed control option is available. Additionally, many producers are still limited in their management options; whether it is due to lack of equipment, cost of herbicide programs, or fees associated with technologies, further complicating the adoption of preemptive resistance management. In this research, it was evident that cotton price received by growers plays a critical role in net returns, which will most likely influence the decision to rotate to other crops or a seed-trait technology. There is potential for increased producer adoption of resistant management strategies such as the ‘zero tolerance’ threshold, unlike the weed control programs evaluated in this research, but only when crop prices are high enough to offset these added expenses. Economics are certainly involved and will continue to change, but managing the seedbank is more appropriate, particularly with the evolution of resistant weeds.
SOURCES OF MATERIALS

1 Golf coarse cup cutter, Doyle Golf, Cedar Falls, IA 50613.

2 Teflon mesh screen, Sefar AG, Depew, NY 14043.

3 SAS 9.2 for Windows, SAS Institute, Inc., 100 SAS Campus Dr., Cary, NC 27513.

4 Mississippi State Budget Generator Version 6.0
(http://www.agecon.msstate.edu/what/farm/generator/)
LITERATURE CITED


OVERALL CONCLUSION

This research confirms glyphosate resistance in two Arkansas Palmer amaranth populations. Although the LD50’s indicate a similar response to glyphosate, differences in shikimic acid accumulation and in \(^{14}\)C-glyphosate absorption and translocation suggest multiple resistant mechanisms are present in these populations. Furthermore, other research has shown that multiple glyphosate resistant mechanisms do exist in Arkansas Palmer amaranth populations, including increased EPSPS copy number. Pollen-mediated gene flow could lead to biotypes with a variety of glyphosate-resistant mechanisms, complicating potential management strategies.

Managing the spread of glyphosate-resistant Palmer amaranth is critical for alleviating potential yield loss in agronomic production systems. This research highlights the impact that Palmer amaranth seed production has in colonizing a field and/or entire farm. Although yields may not be affected during the early stages of resistance evolution, the effects can be devastating to yields and potential management options in the succeeding years. Several mechanisms of seed dispersal were noted in this study, including rainfall, irrigation, and mechanical spread by equipment such as stalk shredders and harvesters. This reinforces the concept of a ‘zero tolerance’ theory when managing a resistant population or trying to prevent resistance evolution. In this research, it took only 20,000 seed representing a single glyphosate-resistant female Palmer amaranth to effectively colonize a field in less than two years. Multiple methods of control should be utilized each year to alleviate the selection pressure associated with multiple glyphosate applications. After the evolution of glyphosate-resistant Palmer amaranth, continued
selection from glyphosate applications will only increase the resistant/susceptible ratio for a given population.

Although other herbicide management options do exist for controlling glyphosate-resistant Palmer amaranth, these management strategies are not flawless and require increased attention and input from producers to be successful. This research demonstrated several current management options ineffective when used without adapting to the environment and/or Palmer amaranth populations. The current severity of glyphosate-resistant Palmer amaranth infestations throughout the southern U.S. have warranted the use of crops with different traits such as the LL system; however, the preemptive use of these technologies for managing resistance evolution may ultimately be driven by short-term economics, limiting the adoption of proactive management strategies.