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Cotton Injury Due to Soil- or Foliar-Applied Herbicides: An Assessment Based on the Influences of Genetic, Agronomic, and Environmental Factors

Brandon William Schrage
University of Arkansas, Fayetteville

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Cotton Injury Due to Soil- or Foliar-applied Herbicides: An Assessment Based on the Influence
of Genetic, Agronomic, and Environmental Factors

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Sciences

by

Brandon William Schrage
Southern Illinois University
Bachelor of Science in Plant and Soil Science, 2012

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University of Arkansas

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Dr. Jason Norsworthy
Thesis Director

Dr. Fred Bourland
Committee Member

Dr. Tom Barber
Committee Member

Dr. Richard Norman
Committee Member

Abstract

Increasing populations of glyphosate-resistant weeds, such as Palmer amaranth, have prompted growers to pursue alternative means of weed control in cotton. In many cropping systems, this means the utilization of older chemistries and residual herbicides. The goal of this research was to evaluate and understand the agronomic and environmental factors that affect the inconsistent injury often associated with these herbicides as well as determine the impact of Palmer amaranth emergence date on seed production, biomass, and cotton yield. Experiments were conducted in three counties in Arkansas giving a distinct range of climate and soil texture. Injury, biomass, and number of plants per m of row, number of seed per female Palmer amaranth plant, and cotton yield were assessed in experiments under various conditions.

Seed vigor levels, seed size, stressed conditions, and planting depth constituted the majority of factors evaluated. Low seed vigor increased the risk of injury from diuron, fomesafen, and fluometuron. Increasing planting depth from 0.64 to 2.5 cm resulted in greater cotton injury from fomesafen but proved inconsequential when applying diuron or fluometuron preemergence. Cotton injury from glufosinate was observed on two Widestrike® cultivars and to a lesser extent on a Liberty Link® cultivar. Injury from glufosinate was significantly increased when cotton was shaded prior to application. Palmer amaranth emerging for the 10-week period after cotton emergence is capable of producing seed, which points to need for extended period of weed control in cotton.

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I. Literature Review

Cotton Production in Arkansas. In 2014, U.S. upland cotton covered 4,466,673 ha and was valued at over \$4.6 billion dollars (NASS 2015). An additional billion dollars was attributed to cottonseed oil production (NASS 2015). The delta region of the Mississippi River, extending from Southern Missouri to Louisiana encompasses a majority of the cotton production in the Midsouth. The state of Arkansas is no exception.

Like other cotton-growing states of the Midsouth, Arkansas growers have experienced difficulty regarding production as input costs have risen substantially and intensive weed control methods have become more critical. The susceptibility of cotton to yield loss from weed competition can be attributed to its relatively noncompetitive foliar canopy and slow inherent growth. In addition, cotton has very few available herbicides compared to corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] (Eaton 1955; Pankey et al. 2005). In 1975, Drs. Parker and Fry calculated that weeds eliminated 11.5% of the world's potential crop production and in 1965, estimated annual losses due to reduced crop yield and quality and costs of weed control in the United States were \$5.1 billion (Agric. Res. Serv. 1965; Parker and Fryer 1975). A 1992 survey concluded that losses to weeds and cost of control exceeded \$15 billion annually (Bridges 1992). The cost of weed competition is likely higher today and continues to climb, partly as a result of glyphosate-resistant weeds.

Weed Control in Cotton. Weed control in cotton has always been a crucial step in successful production. Cotton can require up to 8 wk of weed-free maintenance to maximize yields; a great deal longer than corn and soybean (Buchanan 1974). Therefore, successful production demands clean fields and weed-free maintenance throughout the growing season (Norsworthy et al. 2012a).

Prior to the 1970s, weeds were controlled mechanically (Appleby 2005), and the physical displacement of weed seeds was a strategic management practice in cotton production. Tillage systems that affect the depth, abundance, distribution, and composition of seeds in the soil seedbank (Cardina 2002) remained a vital part of weed control until conservation tillage gained acceptance mostly because of the wide-spread adoption of glyphosate-resistant crops.

The discovery and application of herbicides have provided sufficient control and have become important management tools to avoid reductions in both cotton yield and quality (Snipes and Mueller 1992). Early chemical weed control included residual herbicides such as trifluralin and fluometuron, which were applied to over 50 and 25%, respectively, of the cotton hectares from 1992 to 1999 (Young 2006). Early-season chemical-based control programs often involved the use of pre-plant incorporated (PPI) herbicides followed by a preemergence (PRE) application, while mid-season control was supplemented by directed applications of contact herbicides or cultivation.

Arkansas growers relied heavily on the effectiveness of burndown and PRE herbicides to provide early-season weed control. Glyphosate, a herbicide commonly applied for weed control a few weeks prior to planting (burndown), was first registered in 1974 (Nichols et al. 2009). Because of its relatively low selectivity, it was generally used for non-crop areas until 1997 when glyphosate-resistant cotton was introduced (Nichols et al. 2009)

Glyphosate-resistant (GR) crops have enabled growers to apply multiple over-the-top glyphosate applications, controlling weeds without disrupting crop growth. Essentially, GR crops allowed for easier and more economical weed control in the Midsouth. Glyphosate accomplishes control by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), hindering the synthesis of aromatic amino acids (Duke 1990). Tolerance in crops was achieved

by expressing the EPSPS gene derived from *Agrobacterium* spp. strain CP4 (Nida et al. 1996). Earlier GR cotton cultivars had high vegetative tolerance, but reproductive tolerance was lower because of a reduced expression of CP4 EPSPS in male tissues (Nida et al. 1996; Pline et al. 2002).

Growers widely accepted GR cotton technology as it resulted in cost savings, improved weed management, and simplicity of use (Duke and Powles 2009). In 2000, after the loss of patent rights to glyphosate, the price of glyphosate decreased by 40% in the United States (Duke and Powles 2009; USDA 2006). The low price of glyphosate, its relative high LD₅₀, and ability to control a broad spectrum of weed species has resulted in extensive use of glyphosate on annual weeds which have high rates of reproduction and therefore increased selection for weed populations having resistance (Nichols et al. 2009).

The evolution of target-site resistance in weeds is attributed to the frequent use of herbicides that share the same site of action and for their propensity to select for herbicide-resistant (HR) biotypes (Beckie 2006; Beckie et al. 2001; LeBron and McFarland 1990). Today there are 24 HR biotypes in Arkansas croplands, including Palmer amaranth (*Amaranthus palmeri* S. Wats.), which plagues Arkansas cotton fields at high densities (Heap 2015; Smith et al. 2000).

Palmer amaranth is a highly prolific, dioecious summer annual that is capable of producing over 600,000 seed per female plant (Keeley et al. 1987). Because of an extensive rooting system and high carbon and water efficiency, Palmer amaranth can reduce soybean yield 68% at densities of only 10 plants m⁻² (Klingaman and Oliver 1994). Its high vegetative and reproductive potential makes it arguably the most resistance-prone weed in the Midsouth, and today it has become resistant to five herbicide mechanisms of action in the United States (Heap 2015). Palmer amaranth has rapid erect growth and alleopathic potential that allow it to

successfully hinder the ability of cotton to obtain maximum levels of light, water, space, and nutrients (Morgan et al. 2001). Previous research concludes that with an increase of one Palmer amaranth per 10 m row⁻¹, yield was reduced 5.9 to 11.5% in Oklahoma cotton fields (Rowland et al. 1999). In a 1996 study, Palmer amaranth competition decreased cotton canopy volume 35 and 45% by 6 and 10 WAE, respectively (Morgan et al. 2001). Cotton lint yield is sensitive to Palmer amaranth competition and cotton yields decrease as Palmer amaranth densities increase (Rowland et al. 1999; Norsworthy et al. 2014). Currently, 87% of Arkansas cotton acreage is infested with GR Palmer amaranth biotypes (Norsworthy et al. 2012b).

Since 2005, GR Palmer amaranth populations have evolved throughout the southern U.S. (Webster 2005; Culpepper 2006). In ways similar to those constructed when rigid ryegrass (*Lolium rigidum* Gaudin) became glyphosate-resistant in Australia, models were created to simulate and estimate the future of glyphosate-resistant Palmer amaranth in Arkansas cotton. Glyphosate-use patterns and cropping practices were categorized and labeled as those that promote resistance selection and those that minimized selection (Diggle et al. 2003; Neve et al. 2003a,b). With five annual glyphosate applications in monoculture GR cotton, glyphosate resistance in Palmer amaranth was predicted to evolve in 32% of the fields after 4 years and an additional 19% after year 5 (Neve et al. 2010). This illustrates the likelihood of continued resistance issues and the need to explore additional management options. The widespread existence of GR Palmer amaranth in the Midsouth has prompted a return to the use of residual herbicides as well as integrated management approaches as suggested by recent modeling efforts (Neve et al. 2011).

Use of additional herbicides to control GR and troublesome weeds throughout the United States has increased weed management costs. It is imperative that cotton be maintained weed-

free until the crop can effectively compete with the weed. Knowing the impact of Palmer amaranth interference as a function of emergence date relative to cotton is needed to properly understand the contribution of non-controlled Palmer amaranth plants on the soil seedbank.

According to the Minnesota Department of Agriculture, integrated weed management (IWM) is “the combination of multiple management tools to reduce a weed population to an acceptable level while preserving the quality of existing habitat, water, and other natural resources” (MDA 2011). The presence of GR weeds and recent recommendations have forced many growers to explore the potential of additional mechanisms of action, resulting in more intensive (costly in time and finances) control measures. Resistance has made control in Arkansas cotton extremely difficult considering the resulting reduction of many research and development efforts due to the effectiveness of glyphosate prior to widespread resistance. Glufosinate, another broad-spectrum herbicide option, has gained popularity among producers combating glyphosate-resistant weeds since the release of PhytoGen® and LibertyLink® cultivars.

Glufosinate inhibits glutamine synthetase (Bellinder et al. 1987) and can be applied POST in glufosinate-resistant cotton (CDMS 2015). Although glufosinate-resistant crops have not been as successful as glyphosate and similarly provide no residual control, they can serve as an effective management tool as few weed biotypes are currently resistant to glufosinate (Duke and Powles 2009; Green 2009; Heap 2015). Many scientists believe the answer to acceptable control and prevention of resistance should involve the integration of soil-applied residual herbicides with a glyphosate/glufosinate rotation program (Norsworthy et al. 2012a). The use of both soil- and foliar-applied herbicides provides increased diversity and can be used as a tool to ensure use of multiple effective mechanisms of action within the crop. The use of multiple residual herbicides incorporated into a glyphosate-based weed management cotton program can

effectively reduce the number of needed applications, fuel, labor, and equipment costs and reduce selection for herbicide resistance (Wilcut et al. 2002; Norsworthy et al. 2012a). The University of Arkansas System Division of Agriculture (UADOA) endorses the practice of overlapping residual herbicides for pre-plant, PRE, POST, and at layby applications in cotton as well as encourages the rotation of chemistries. The use of residual herbicides such as diuron, fluometuron, and fomesafen in coordination with LibertyLink® systems to control high densities of GR Palmer amaranth has proven to be a successful tactic (York and Culpepper 2009, UADOA 2012).

Herbicide Injury to Cotton. One of the limitations of residual herbicides and the reason for the initial widespread adoption of glyphosate and GR crops is the frequent injury often associated with soil- and foliar-applied residual herbicides in cool, wet conditions (Askew et al. 2002; Hayes et al. 1981). Such conditions are a common occurrence during planting and early stages of cotton development when herbicides are being applied for Palmer amaranth control. In spite of this, insufficient research has been conducted relating the interaction of microenvironments, residual herbicides, and cotton injury in the Midsouth. There is existing evidence that seed vigor, seed size, and cultivar could also influence herbicide injury.

In peanut (*Arachis hypogaea* L.), root injury from residual herbicide increases with smaller seed sizes (Cargill and Santelmann 1971). Because cotton seed can be sensitive to mechanical handling like peanuts (Cargill and Santelmann 1971), it can be hypothesized that seed size will have an impact on whether or not a residual herbicide can cause injury. Seeds germinate successfully when conditions are favorable (Anderson 1962; Gibson and Mullen 1996; Holm and Miller 1972) and having low seed vigor can result in lower yield and unfavorable stands because the germinating seedling spends more time surrounded by the herbicide in solution (Edje and

Burris 1971; Fehr et al. 1973; Grabe 1966; Culpepper 2012). When emergence is slow, increased injury is likely because of reduced metabolism of soil-applied herbicides. Seed size, vigor, planting depth, and herbicide timing have a great impact on seedling success; the ultimate success of the plant depends on natural defense adaptations that promote survival during times of environmental stress.

A narrow margin of selectivity in cotton exists between achieving effective weed control and preventing injury from commonly used soil-applied residual herbicides (Kendig et al. 2007). There is not, however, a great deal of evidence linking cotton injury with microenvironments (Norsworthy et al. 2012b); although injury has been shown to increase in low-vigor seed and in shallow planting scenarios in Georgia (Culpepper 2012). Microenvironments pertain to the environmental conditions within close proximity to the seed or seedling. Microenvironments may differ in pH, soil moisture, light intensity, nutrient levels, and temperature and proper evaluations of such interactions between microenvironments and cotton seedling establishment should be conducted.

In response to growing concerns over glyphosate resistance, many growers have begun exploring glufosinate-resistant technology in cotton. Glufosinate can be used to effectively manage GR Palmer amaranth when applied at the appropriate timing and is a successful alternative to glyphosate where GR weeds are present (Culpepper et al. 2009). The recommendation of glufosinate-resistant systems requires a detailed analysis concerning injury to cotton from glufosinate applications. Developing an understanding between the interaction of microenvironments and injury in a Liberty Link/residual management system would provide Midsouth producers with the means to devise an efficient integrated weed management system for cotton production.

In an evaluation of two cotton cultivars, PHY 375 WRF and PHY 485 WRF, glufosinate injured cotton 18% more than that of *S*-metolachlor and glyphosate alone (Steckel et al. 2012). The injury observed in cotton from glufosinate in various scenarios must be evaluated for management strategies. Because glufosinate has no residual activity and losses in efficacy occur when applied to larger weeds (Corbett et al. 2004; Shaw and Arnold 2002), residual herbicides must be included to successfully manage resistant weeds like Palmer amaranth. Many documented cases involving herbicide induced injury explain the physiological and metabolic components; however, there exist gaps in the data. The interactions of seed vigor, microenvironments (soil moisture, temperature and light exposure), agronomic practices (planting depth and seed size), and herbicides could result in different levels of injury (Muzik 1976; Richardson 1977; Wanamarlta and Penner 1989).

Understanding the influence of genetic, agronomic, and environmental factors on cotton injury from soil- and foliar-applied herbicides is necessary to construct a successful weed management strategy for Midsouth producers. Environmental conditions can greatly alter morphological and physiological processes in plants resulting in altered herbicide absorption, translocation, or metabolism (Muzik 1976; Richardson 1977; Wanamarlta and Penner 1989). For example, high relative humidity (RH) increased efficacy of fluoxypyr (Lubbers et al. 2007). When grown at 90% RH, Palmer amaranth, redroot pigweed (*Amaranthus retroflexus* L.), and common waterhemp (*Amaranthus rudis* Sauer) control with glufosinate was greater than when these plants were grown at 35% RH. In several studies, green foxtail [*Setaria viridis* (L.) Beauv.] control from fenoxaprop, fluaxifop-P, haloxyfop, and sethoxydim improved with increased soil moisture. Johnsongrass [*Sorghum halepense* (L.) Pers] control from glyphosate, and kochia [*Kochia scoparia* (L.) Schrad.] control from imazethapyr was also improved in cases

of adequate soil moisture (Nalewaja et al. 1990, Boydston 1990, Nalewaja and Woznica 1985, McWhorter and Azlin 1978).

The cuticle of cotton is composed of long-chain aliphatic compounds derived from lengthy fatty acids and is the protective layer of aerial plant parts (Kolattukudy 1970; Kunst and Samuels 2003). The cuticle is weakest as a seedling, and reducing the length of time in which the seed is surrounded by the herbicide reduces cuticle stress and increases the success of germination and healthy cotton stands (Culpepper 2012). Epicuticular wax (ECW), which serves as the first barrier to herbicide absorption, is influenced by many environmental factors including temperature, light, relative humidity, and soil water content. These factors greatly impact ECW morphology and development and subsequently the efficacy of POST herbicides (Hatterman-Valenti et al. 2011). Hence, it is imperative that a proper evaluation be conducted exploring the association of environmental, agronomic, and genetic factors with residual herbicides, and glufosinate applications with cotton injury.

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II. Impact of Seed Vigor, Seed Size, Planting Depth, and Soil Conditions on Cotton Injury from Soil-Applied Herbicides

Abstract

Preemergence (PRE) herbicides, once a primary component of weed control in cotton, are reestablishing utility as glyphosate-resistant weeds become more prominent in the Midsouth. Although considerably effective, cotton injury is often associated with the use of soil-applied herbicides. The purpose of this study was to determine if seed vigor, seed size, planting depth, and soil conditions could potentially affect the tolerance of cotton to PRE-applied herbicides. Five seed sizes derived from a seed lot were planted and treated with three rates of the PRE-applied herbicide diuron. In addition, field studies were conducted evaluating low- and high-vigor cotton seed planted at a 0.6- and 2.5-cm depth treated with 1 and 2X rates of PRE herbicides: diuron, fluometuron, and fomesafen. A growth chamber experiment was conducted evaluating the interaction of low and high seed vigor applied with 1 and 2X rates of diuron and fomesafen at both stressed and non-stressed soil conditions. Smaller seed size often increased the risk of injury from diuron applied PRE and a trend for greater cotton biomass occurred with larger seed sizes, suggesting greater tolerance to PRE herbicides as seed size increases. In regards to vigor, planting depth, and soil conditions, there were varying results according to year and location; however, there was a general increase in injury when PRE herbicides were applied to low-vigor cotton under stressed conditions. This injury was often substantially increased with above-labeled herbicide rates.

Nomenclature: Diuron; fomesafen; fluometuron; cotton, *Gossypium hirsutum* L.

Key words: Pre-emergence (PRE); seed size; seed vigor; planting depth; soil conditions

Introduction

Growers widely accepted glyphosate-resistant (GR) cotton technology following its commercialization in 1997 because it provided cost savings, improved weed management, and simplicity of use (Duke and Powles 2009). In 2000, after the loss of patent rights to glyphosate, the price of glyphosate decreased by 40% in the United States (Duke and Powles 2009; Reddy 2001). The low price of glyphosate, its low mammalian toxicity, and ability to control a broad spectrum of weed species has resulted in extensive use of glyphosate on annual weeds, in turn increasing the risks of glyphosate resistance evolving.

The evolution of target-site resistance in weeds is attributed to the frequent use of herbicides that share the same mechanism of action and for their propensity to select for herbicide-resistant (HR) biotypes (Beckie 2006; Beckie et al. 2001; LeBaron and McFarland 1990). Today there are 24 HR biotypes in Arkansas crops, including Palmer amaranth (*Amaranthus palmeri* S. Wats.), which plagues Arkansas cotton fields with increased densities (Heap 2015; Smith et al. 2000).

Glyphosate resistance has created an ever-increasing need for diversified approaches to weed control. In reference to chemical control methods, integrated weed management (IWM) strategies require the use of multiple, effective, herbicide mechanisms of action to delay the onset and spread of resistant weeds (Norsworthy et al. 2012). Presently, control and IWM can be achieved by the incorporation of soil-applied herbicides into previously postemergence (POST)-herbicide dominated programs (Norsworthy et al. 2012; Neve et al. 2011).

Herbicide resistance has made weed control in cotton extremely difficult across much of the U.S. Cotton Belt because few effective herbicide options are available, especially for the control of glyphosate-resistant Palmer amaranth, and cotton inherently grows slower than other

row crops in this region. New herbicide chemistry is limited as industry research and development efforts slowed following the release of glyphosate-resistant crops. The subsequent lack of new innovative herbicide options have forced growers to return to the use of herbicides that were commonly used prior to commercialization of glyphosate-resistant cotton (Riar et al. 2013).

The difficulty with residual herbicides, and reason for their reduced use, aside from the effectiveness of glyphosate, is the inconsistent injury often associated with their application (Culpepper 2012; Main et al. 2012). Reducing the length of time in which the seed is surrounded by the herbicide increases the success of germination and healthy cotton stands (Culpepper et al. 2012; Kendig et al. 2007). Soil moisture has been found to affect the activity of soil-applied herbicides by altering the herbicide concentration and mobility in the soil (Zhang et al. 2001). Previous research suggests that early-season cotton injury with various soil-applied herbicides frequently occurs, especially under cool, moist conditions (Askew et al. 2002; Hayes et al. 1981). Excessive rainfall and cold temperatures have been reported to overexpose cotton seedlings to herbicides resulting in reduced metabolism and increased injury (Steckel et al. 2012). In contrast, some studies have reported no or slight cotton injury with residual herbicides in other environments (Faircloth et al. 2001; Riar et al. 2011). The variability in research could potentially attest to variations in environment or the agronomic factors involved in different production systems. Filling the gaps in research regarding the impact of soil environmental factors on emergence problems could potentially improve cotton establishment.

Seed size may influence the cotton tolerance to PRE-applied herbicides. It has been shown that small-seeded peanut (*Arachis hypogaea* L.) exhibited greater root injury from PRE herbicides compared to large-seeded peanut (Cargill and Santlemann 1971). Additionally, large-

seeded strains of soybean (*Glycine max*) were more tolerant of atrazine than small-seeded strains (Andersen 1970); possibly due to the roots of large-seeded plants expanding into nontreated soil more quickly. In reference to cotton, seed size within a seed lot is often characterized as simple variation in vigor (Bourland, Personal communication, 2013). It can be hypothesized that within a seed lot, seed sizes exhibiting injurious differences reflect the impact of vigor. For the soil types and production practices common to the Midsouth, little research has been conducted to determine the reasons for inconsistent cotton tolerance based on seed size within a germplasm.

Low seed vigor often results in lower yield and unfavorable stands because the germinating seedling spends more time surrounded by the herbicide in solution (Anderson 1962; Gibson and Mullen 1996; Holm and Miller 1972; Edje and Burri 1971; Fehr et al. 1973; Grabe 1966). Based on one-year experiment conducted in Georgia, it has been suggested that low-vigor seed and shallow planting may increase injury from PRE-applied herbicides (Culpepper et al. 2012). Improved seed vigor has been documented to improve germination in controlled stressful conditions such as deep planting and may be further affected by herbicide applications (Burriss 1976; Johnson and Wax 1978; TeKrony et al. 1987; Hamman et al. 2002).

Cotton has exhibited tolerance to fomesafen applied PRE, a diphenylether herbicide that inhibits protoporphyrinogen oxidase (PPO) (Baumann et al. 1998; Gardner et al. 2006; Troxler et al. 2002). There is, however, limited research evaluating PPO- and photosystem II (PSII)-inhibiting herbicides in varying soil types and moisture regimes (Main et al. 2012). Sulfentrazone, another PPO herbicide, was reported to cause more injury as soil pH increased (Reiling et al. 2006), and Taylor-Lovell et al. (2001) suggested that PPO induced injury on cotton increased under wet, low-organic matter soil conditions. Based on the need to better understand the factors contributing to cotton injury from soil-applied herbicides, the objective of

this research was to determine the impact of seed vigor, seed size, planting depth, and soil conditions on cotton tolerance to PRE herbicides.

Materials and Methods

Seed Vigor and Seed Size—Within A Genotype. A field experiment was conducted at the Arkansas Agricultural Research and Extension Center (AAREC) in Fayetteville, AR in 2012 and 2013 as a 5 x 3 factorial in a randomized complete block. An unreleased red-leaf germplasm cotton seed originally developed at Texas A&M University named “Texas Maroon” was acquired from Dr. Fred Bourland at the University of Arkansas Northeast Research and Extension Center in Keiser, AR. Five seed size categories (9.3, 10.4, 11.6, 12.1, 13.1 g 100 seed⁻¹) were established from the seed lot of Texas Maroon using a fabricated forced-air separator in 2012 and were used both years. Each seed size was planted at a rate of 15 seed m⁻¹ of row, 2 cm deep after the soil was prepared by use of conventional tillage on May 24, 2012 and May 15, 2013. Four 92-cm wide row plots were planted in mid-May using a 7100 four-row John Deere planter pulled by a John Deere 6403 medium-frame tractor (Deere & Company, Moline, IL) resulting in a 3.7 by 7.6 m plot dimension. The planter was retrofitted with an electric, cone-type seeder (ALMACO, Nevada, IA) to enable accurate planting of the various seed sizes.

Herbicide treatments included a nontreated control and a 1X (1.12 kg ai ha⁻¹) and 2X rate of diuron (Direx®4L, MANA Inc., Raleigh, NC). Applications were made using a CO₂-pressurized backpack sprayer with TTI 110015 nozzles (Teejet Technologies, Springfield, IL) spaced 51 cm apart and calibrated to deliver 187 L ha⁻¹ at a pressure of 276 kPa and a speed of 4.8 kph immediately after planting. Plots were sprinkler irrigated as needed and a one-time application of pyriithiobac sodium (Staple LX, DuPont Crop Protection, Wilmington, DE) at 56 g

ai ha⁻¹ was made at 3 weeks after planting (WAP), in conjunction with hand-weeding to help ensure weed-free plots.

Assessments of necrosis, chlorosis, and stunting (rated visually on a scale of 0 to 100% compared to the nontreated control) were recorded at 2 and 4 weeks after treatment (WAT). At 4 WAT, cotton density was recorded as plants per 2 m of row and biomass (all above-ground cotton tissue) was harvested in 2 m of row and reported as g plant⁻¹. Biomass was oven dried for 7 d at 60 C before weighing. After initial analysis, it was determined that the five seed indices would be divided into two groups in order to achieve normality. Groups were composed of those weighing less and those weighing more than 11 g 100 seed⁻¹. Using Fit Model programming under an effect screening personality, data were analyzed in JMP Pro 11 (SAS Institute Inc., Cary, NC), and means were separated using Tukey's HSD at $\alpha = 0.05$.

Seed Vigor and Seed Size—Across Genotypes. To distinguish potential variability associated with the characterization of seed vigor and seed size an additional experiment was conducted at the AAREC in Fayetteville and the Lon Mann Cotton Research Center (LMCRC) near Marianna, AR in 2013. Planting dates were May 13, 2013 at the AAREC and May 20, 2013 at the LMCRC. The soil series at LMCRC was a Convent silt loam (Coarse-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) with 9% sand, 80% silt, 11% clay, 1.8% organic matter, soil pH of 6.6, and an estimated cation exchange capacity (ECEC) of 11 cmolc kg⁻¹. At AAREC, the soil series was a Leaf silt loam (Fine, mixed, active, thermic Typic Albaquults) with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.9 (USDA-NRCS 2015). Both locations exhibited a plot dimension of 1.78 by 20.32 m. A randomized complete block with a factorial experimental setup and four replications utilized 36 commercial cotton cultivars provided by Dr. Fred Bourland, each given a specific index based on the average weight of 100 seed (Bourland et

al. 2013). Each seed index was subjected to a 0, 1, and 2X application of diuron applied PRE where the 1X rate was 1,120 g ai ha⁻¹. Plot construction, planting, injury rating, and biomass harvesting procedures were conducted similarly to the previous experiment. A multivariate statistical analysis was performed using JMP PRO 11(SAS Institute Inc. 2014).

Seed Vigor and Planting Depth. In 2012 and 2013, field experiments were conducted at the AAREC in Fayetteville and the Rohwer Research Station (RRS) near Rohwer, AR on Leaf and Herbert silt loams, respectively. Planting dates in Fayetteville were April 13, 2012 and May 8, 2013. Planting dates in Rohwer were April 22, 2012 and April 26, 2013. The Hebert silt loam soil (Fine-silty, mixed, active, thermic Aeric Epiaqualfs) consisted of 16% sand, 67% silt, 17% clay, 2.2 % organic matter and a pH of 7.1, whereas the Leaf silt loam (Fine, mixed, active, thermic Typic Albaqualts) was comprised of 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.9 (USDA-NRCS 2015). A randomized complete block with a split-strip plot design with four replications was employed where the sub-sub factor consisted of seed vigor (high and low), the subfactor was herbicide treatment [PRE application of diuron (1.12 and 2.24 kg ha⁻¹), fluometuron (1.12 and 2.24 kg ai ha⁻¹), fomesafen (0.28 and 0.56 kg ai ha⁻¹), and a non-treated control], and the main plot factor was planting depth (0.6 and 2.5 cm). Fluometuron (Cotoran®4L, MANA Inc., Raleigh, NC, USA) and fomesafen (Reflex®, Syngenta Crop Protection, Greensboro, NC, USA) are registered for PRE and preplant applications, respectively, in Midsouth cotton. FM 1944 GLB2 (Bayer Crop Science, Research Triangle Park, NC, USA) was artificially aged to create a low-vigor seed as described below.

The reason for using low-vigor seed is to account for the potential reduction in vigor associated with damage from harvesting, storage, and weathering that may ultimately increase herbicide injury to cotton. Using principles established by Bourland et al. (1988) cotton seed

were subjected to a 40 C bath for 20 min. This artificial aging is exacerbated with increases in humidity and temperature as illustrated by Basra et al. (2003). The subsequent low-vigor seed were dried, and subsequently treated with a fungicide and insecticide. A John Deere 6403 medium-frame tractor and John Deere 7100 four-row planter (Deere & Company, Moline, IL) retrofitted with an electric cone-type seeder (ALMACO, Nevada, IA) served as planting equipment. To promote stress, cotton was planted on April 13, 2012 and May 8, 2013 and supplemented with overhead irrigation (2.54 cm) immediately after planting and PRE application. Plots consisted of two, 92-cm wide rows 7.6 m long at AAREC and 97-cm wide rows 7.6 m long at RRS.

Applications were made using a CO₂-pressurized backpack sprayer with TTI 110015 nozzles that were calibrated to deliver 187 L ha⁻¹ at a pressure of 276 kPa and walking speed of 4.8 kph. Plots were sprinkler irrigated 2.5 cm, 24 hours after application (HAA). Injury was assessed at 1, 2, 3, and 4 WAT as a visual percentage (0 to 100%) as described in the previous experiments. Plants per 2 m of row were counted, and the associated biomass (all above-ground tissue) was collected, oven-dried for 7 d at 60 C and weighed. Data were subjected to a mixed model analysis in JMP Pro 11. Utilizing a full factorial arrangement of fixed effects, ANOVA was generated and means were separated using Tukey's HSD (SAS Institute Inc. 2014).

Seed Vigor and Environmental Conditions. In 2013, an automated growth chamber experiment was conducted twice as a randomized complete block with a two (seed vigor: low and high) by three (herbicide treatments: nontreated and PRE application of diuron and fomesafen) by two (planting and herbicide application conditions: stressed vs non-stressed) factorial design with four replications. FM 1944 GLB2 cotton seed were planted 0.6 cm deep into 15-cm-diameter plastic pots (Hummert International™, Earth City, MO) filled to 95% capacity with Leaf silt

loam soil obtained from the AAREC (Fine, mixed, active, thermic Typic Albaquults) with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.9 (USDA-NRCS 2015). Low-vigor seed was established using the same method as field studies previously mentioned. To promote stress pots were held at field capacity (Askew et al. 2002; Zhang et al. 2001). Using established estimations for bulk density and organic matter content as determined by the 24-h hydrometer method (Gee and Bauder 1986), Leaf silt loam soil textural values were entered in Soil-Plant-Atmosphere-Water (SPAW) program (v6.02.75, USDA- ARS, Washington D.C.; Saxton and Rawls 2006) to establish daily watering requirements necessary to maintain field capacity. Herbicide applications (diuron at 1.12 kg ha⁻¹ and fomesafen at 0.28 kg ha⁻¹) were made immediately after planting using an automated spray chamber with a boom consisting of two flat fan 800067 nozzles (Teejet Technologies, Springfield, IL) calibrated to deliver 187 L ha⁻¹. After planting, the high moisture, stressed condition was achieved by setting the PGW36 Conviron growth chamber (Controlled Environment Limited, Edmonton, AL, CAN) at 13/24 C within a 14-h photoperiod for seven days followed by then raising temperatures to normal spring-like conditions (18/25 C). The nonstressed condition was never exposed to the seven day stress period. Visual estimates of chlorosis, necrosis, and stunting were recorded at 2, 3, and 4 WAT. At 4 WAT, all above-ground cotton biomass was collected, dried for 7 d at 60 C and weighed. Data were subjected to an effect screening personality to provide ANOVA in JMP Pro 11. Significant means and separation were achieved using Tukey's HSD at $\alpha = 0.05$ (SAS Institute Inc. 2014).

Results and Discussion

Seed Size and Seed Vigor—Within a Genotype. Visual estimates of cotton injury and plant biomass varied by year. With over twice as much precipitation and nearly 4.5 C lower average daily temperatures in 2013, experiments conducted in 2012 had less measureable differences (Table 1). In light of initial model parameters and data structure, it was determined that analysis would best be achieved by grouping the cotton seed indices into two groups rather than five. Small-sized seed (<11 g 100 seed⁻¹) and large-sized seed (>11 g 100 seed⁻¹) formed the two classification indices. At 2 WAT in 2012, injury assessments were significantly higher when the 2X rate of diuron was applied to plots planted to small-sized seed (<11 g 100 seed⁻¹) (Table 2). The increased injury from the higher rate of diuron was not surprising since similar results have been noted elsewhere (Kendig et al. 2007). At 2 WAT in 2012, small-seeded cotton seedlot exhibited greater injury than large-seeded cotton seedlot at the 1 and 2X rates. In 2013 at 2 WAT, a similar injury response to seed size occurred at the 2X rate of diuron (Table 2).

The interaction of application rate and seed size was not significant at 4 WAT in 2012 and 2013 (Table 1). However, the main effect of seed size was significant for injury estimates and biomass at 4 WAT both years (Table 3). Additionally, diuron rate influenced the degree of injury observed on cotton and biomass production in 2012, but not 2013. At 4 WAT in 2012, small-sized seed (averaged over diuron rates) and the highest diuron rate (averaged over seed sizes) resulted in greater injury to cotton and less cotton biomass (Table 3). Application rate failed to influence the injury observed on cotton at 4 WAT in 2013 as well as the production of biomass. The small-sized seed had greater injury and less biomass than plants from the larger seed size (Table 3).

According to Wanjura et al. (1969), the primary indicator of the potential for a seed to survive early-season stresses is the time required for germination and emergence. Seed quickly emerging from the soil (or the activated herbicide zone) have the most favorable chance of survival. Seed weight, which should influence vigor, did impact the ability of cotton to tolerate a herbicide that routinely causes early-season injury (Table 1). Even if large seed fail to possess a germination advantage, the larger cotyledons, endosperm, and seed coat could enable larger seed to withstand adverse environmental conditions in the soil. Pettigrew and Meridith (2009) concluded that seed heavier than $11 \text{ g } 100 \text{ seed}^{-1}$, similar to the “large-sized seed” in this experiment, produced greater plant height, leaf area index, leaf weight, and total dry weight than seed lighter than $11 \text{ g } 100 \text{ seed}^{-1}$. One can hypothesize that the larger seed index, expressing higher vigor within a seedlot, would possess greater potential tolerance to herbicide residues in the soil as Pettigrew and Meridith (2009) suggested.

Seed Size and Seed Vigor—Across Genotypes. The supplemental experiment conducted at AAREC and LMCRC in 2013 provided an additional assessment of seed index on cotton tolerance to various rates of diuron applied PRE over vastly different cultivars. The geographical separation of study locations promoted a difference in both climatic and soil texture conditions (Figure 1). Unique interactions between diuron applications and the selected cultivars are could potentially be related to differences in climatic and soil conditions. Similar to observations made by Nieuwenhuizen and Nel (1980) in South Africa, greater phytotoxicity was observed on the finer-textured (lighter) soil at AAREC (Table 4). The Convent silt loam soil at LMCRC generally contains up to 16 percentage points lower clay composition than the silt loam at the AAREC location (USDA NRCS 2014). Increasing diuron application rates resulted in increased injury, irrespective of location. At the AAREC location, seed size failed to influence

injury levels yet did impact plant biomass production (Figure 2). Larger-seeded cotton significantly resulted in greater biomass production, suggesting a possible increase in yield potential. At the LMCRC location, seed size proved to significantly influence biomass as well as visual injury assessments. At both the 1 and 2X rates of diuron, there was over 5 g more biomass per 2 m of row with every gram increase of seed weight (Figure 2). Locations saw significantly different result, influencing the impact that seed size had on cotton tolerance to diuron applied PRE at above-labeled rates, yet larger seed often resulted in larger biomass, irrespective of location. Seed index and vigor have been suggested to be related when within the same seed lot. Growers should consider seed size when selecting cultivars, especially if cotton tolerance to PRE-applied herbicides has been less than acceptable in past years.

Seed Vigor and Planting Depth. Variability of cotton tolerance to soil-applied herbicides remains under-documented regarding the soil types and agronomic practices common to the Midsouth.

Convent Silt Loam Soil. In 2012 and 2013 at the AAREC on a silt loam soil, planting depth significantly affected cotton tolerance to PRE-applied herbicides (Table 5). At 2 WAT, tolerance to the herbicides on shallow planted cotton was greater than the deeper depth (Table 6). The increase in injury to cotton planted 2.5 cm deep persisted through 4 WAT but failed to impact cotton biomass.

The main effect of herbicide likewise influenced injury to cotton at 2 and 4 WAT in 2012 and 2013 at AAREC (Table 6). Increasing application rates to 2X that of the label recommendation of diuron significantly increased the injury to cotton in 2012 and diuron was generally more injurious to cotton than fluometuron in both years. As a result of the injury risk associated with PRE-applied fomesafen applications on silt loam soils in the Midsouth, the

herbicide is currently labeled preplant rather than PRE in cotton (Anonymous 2015). Even though herbicide injury differences were noted among herbicides, these injury symptoms did not result in differences in cotton biomass at 4 WAT in 2012 or 2013.

Additionally, the main effect of seed vigor was significant for cotton injury on the silt loam soil at AAREC in 2013 at 2 and 4 WAT, but not in 2012 for similar assessment timings (Table 5). Plants emerging from low vigor seed had greater injury at 2 and 4 WAT and less biomass at 4 WAT than plants from high vigor seed (Table 6).

Herbert Silt Loam Soil. At RRS in 2012, the main effect of vigor and the interaction of seeding depth and choice of herbicide impacted injury to cotton (Table 7). Even though the increased clay content of the Herbert silt-loam soil likely resulted in greater herbicide adsorption, plants in plots having low-vigor seed had greater injury than those in plots having high vigor seed at 2 and 4 WAT as well as lower biomass production (Table 8).

The two-way interaction of planting depth x herbicide was significant for cotton injury and biomass production in 2012 (Table 7). More than 50% injury to cotton at 2 WAT resulted from the highest rate of diuron, regardless of planting depth (Table 9). Cotton treated with labeled rates of fluometuron, diuron, and fomesafen generally resulted in greater biomass production than the nontreated control, regardless of planting depth. The lower biomass of the nontreated control is likely a result of early-season weed competition prior to initiating hand removal and an over-the-top application of glufosinate.

In 2013 at RRS, there was a three-way interaction among planting depth, herbicide, and vigor at 2 WAT (Table 7). The same interaction was non-significant at 4 WAT ($p = 0.0512$). Diuron and fomesafen were often most injurious to cotton in shallow planted, low-vigor plots whereas fluometuron caused more injury when the low-vigor cotton was seeded at the 2.5-cm

depth. Even though the interaction of factors makes it difficult to understand each factor individually, the need for high vigor seed for reducing injury to cotton is obvious.

Only the main effects of herbicide and planting depth significantly influenced cotton biomass production at 4 WAT at RRS in 2013 (Table 7). Planting cotton at a 2.5-cm depth rather than a 0.6-cm depth resulted in 27% less biomass (data not shown). Deeper planting translated to an extended period of the hypocotyl within the activated herbicide zone. In regards to the herbicides evaluated, the PPO-inhibiting herbicide fomesafen resulted in the least amount of cotton biomass among the three herbicides, which partially confirms the reason for the current fomesafen label requiring that the product be applied preplant rather than preemergence on silt loam soils (Anonymous 2014). The 1X rate of fomesafen resulted in an average of 0.72 g plant⁻¹ biomass compared to 0.98 g plant⁻¹ in the nontreated control. Due to the high density of weeds in this location and its general distance from Fayetteville, weed-free maintenance efforts suffered the first two weeks after planting. It is likely that competition with weeds resulted in some reduction in biomass in the nontreated plots, which likely diminished the differences between herbicide-treated and non-treated treatments.

Seed Vigor and Environmental Conditions. The variability encountered with field trials is often attributed to the inability of researchers to control climactic or environmental conditions, especially those at or near the time of a herbicide application. Under controlled conditions of a growth chamber, the main effect of herbicide choice as well as the interaction of vigor by environment were significant for cotton injury at 2 and 4 WAT (Table 11). Applications of fomesafen resulted in greater injury than diuron at 2 and 4 WAT. The cooler conditions associated with the stressed environment resulted in greater injury to cotton, which was further

accentuated by the low vigor seed (Figure 3). By 4 WAT, only when a herbicide application was followed by stressed conditions did seed vigor play a role in increased injury to cotton.

In regards to cotton biomass present at 4 WAT, the interaction of herbicide by environment was significant (Table 11). The cause of the interaction is likely a result of reduced biomass of the nontreated control relative to the herbicide-treated cotton in the non-stressed environment. The reason for the reduced biomass is not known. Averaged over low- and high-vigor seed, cotton that began in a stressed environment had less biomass than cotton beginning in a nonstressed environment (Figure 4). Additionally, no differences were found in biomass production among herbicide treatments in the stressed environment; albeit, the nontreated control was numerically greater than the herbicide-treated cotton. It is known that soil temperature greatly influences cotton seedling emergence and in turn the uptake of water and herbicides (Wanjura et al. 1969). In times of stress during emergence, such as those evaluated in this experiment, seed quality is of utmost importance (Ferguson and Turner 1971). Since large-scale producers have a very narrow planting window and no control of the potential for cool, wet conditions that may arise after planting, utilizing high-vigor seed could reduce the risk of injury to cotton from soil-applied residual herbicides.

Summary

These experiments were established to provide Midsouth cotton producers with accurate information pertaining to the integration of soil-applied residual herbicides into current cotton production systems while minimizing the risk for cotton injury. Variability was observed between years and locations, and interactions existed with many of the factors evaluated. Although planting depth did affect cotton injury from preemergence herbicides, the fact that

cotton responded differently to planting depths across herbicides and locations makes it difficult to fully recommend one planting depth over another. During periods of stress, seed planted at greater depths are likely to experience a greater period of herbicide uptake by the hypocotyl; hence, a shallower planting depth may be preferred if it is imperative that cotton be planted and cool, wet conditions are forecast. Overall, fluometuron was often less injurious to cotton than was diuron or fomesafen and the excessive injury from fomesafen further substantiates the lack of a preemergence recommendation for this product in Arkansas (Scott et al. 2014). As seed size increased, there was a tendency for cotton to better tolerate soil-applied herbicides; albeit, some smaller seeded cultivars did appear to have high vigor and increased tolerance to diuron. In regards to seed vigor, which may be partially linked to seed size, low-vigor seed had a higher risk for elevated injury over high-vigor seed. The use of high-vigor seed will improve cotton tolerance and often biomass production following the application of PRE herbicides. Proper harvesting, processing, and storage can help ensure that seed has high vigor at the time of planting; notwithstanding there is likely a genetic component involved with seed vigor. In regards to locations, the lower clay content of the Convent silt loam soil likely contributed to greater injury over the Hebert silt loam. Growers need to be cognizant of the soil texture and use past experiences to slightly lower rates if excessive injury has been observed in the past. During cool, wet conditions common to early-season cotton planting, large, high vigor seed can improve crop tolerance and ensure rapid germination.

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Table 1. Analysis of variance effect tests for injury to cotton at 2 and 4 weeks after treatment (WAT) and cotton biomass at 4 WAT as influenced by herbicide rate and seed index in 2012 and 2013 at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b}

Source	Df ^a	2 WAT	4 WAT	Biomass ^b
		Prob > F ^c		
2012				
Rep	3	0.6659	0.3040	0.9095
Seed index	1	<0.0001	<0.0001	0.0011
Rate	1	<0.0001	<0.0002	0.0005
Seed index*Rate	1	0.0007	0.0867	0.9470
2013				
Rep	3	0.7507	0.0247	0.8728
Seed index	1	0.0602	0.0131	0.0057
Rate	1	<0.0001	0.0803	0.9009
Seed index*Rate	1	0.0797	0.4375	0.8394

^a Abbreviation: Df, degrees of freedom.

^b Biomass includes all above ground plant tissue harvested at 4 WAT.

^c Source values less than the alpha level of 0.05 are significant.

Table 2. Cotton injury as influenced by the interaction of seed indice and diuron rate at 2 weeks after treatment in 2012 and 2013 at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b}

Diuron rate kg ai ha ⁻¹	Injury			
	2012		2013	
	Large ^a	Small	Large	Small
	%			
1.12 (1X)	4 B	15 A	6	7
2.24 (2X)	14 B	38 A	11 B	27A

^a Cotton seed weighed >11 g 100 seed⁻¹ and <11 g 100 seed⁻¹ for the large and small seed indices, respectively.

^b Letters indicates significant difference between seed indices within a year and diuron rate based on Tukey's HSD.

Table 3. Cotton injury and biomass as influenced by the interaction of seed indice and diuron rate at 4 weeks after treatment in 2012 and 2013 at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b,c}

Main effect	Injury		Biomass	
	2012	2013	2012	2013
	%		g m ⁻¹ of row	
Seed indice ^a				
Large	8 B	8 B	16 A	18 A
Small	28 A	28 A	10 B	14 B
Diuron rate ^b				
0X	—	—	17 A	17 A
1X	10 B	20 A	13 B	19 A
2X	27 A	22 A	7 C	16 A

^a Cotton seed weighed >11 g 100 seed⁻¹ and <11 g 100 seed⁻¹ for the large and small seed indices, respectively.

^b The 1X and 2X rates of diuron were 1.12 and 2.24 kg ai ha⁻¹, respectively.

^c Letters indicates significantly higher values between seed indices or diuron rates within a year based on Tukey's HSD.

Table 4. Correlation of injury on 34 commercial cotton cultivars at 2 and 4 weeks after treatment (WAT) with seed index, and biomass with seed index at 4 WAT.^{a,b,c}

Location	Rating	Rate ^a	Variable	by Variable	Correlation	Confidence interval		Prob > F ^b	
						Lower 95%	Upper 95%		
AAREC ^c	2 WAT	1X	Injury	Seed index	0.0269	-0.1415	0.1937	0.7553	
		2X	Injury	Seed index	0.1276	-0.0398	0.2880	0.1345	
	4 WAT	1X	Injury	Seed index	0.0095	-0.1585	0.1769	0.9124	
		2X	Injury	Seed index	0.1052	-0.0636	0.2682	0.2210	
		0X	Biomass	Seed index	0.3319	-0.1780	0.4699	<.0001	
		1X	Biomass	Seed index	0.2767	0.1173	0.4222	0.0009	
		2X	Biomass	Seed index	0.1806	0.0146	0.3370	0.0333	
	LMCRC ^c	2 WAT	1X	Injury	Seed index	-0.3272	-0.4641	-0.1751	<.0001
			2X	Injury	Seed index	-0.3490	-0.4831	-0.1988	<.0001
4 WAT		1X	Injury	Seed index	-0.0845	-0.2426	0.0779	0.3071	
		2X	Injury	Seed index	-0.3082	-0.4473	-0.1546	0.0001	
		0X	Biomass	Seed index	0.4344	0.2927	0.5575	<.0001	
		1X	Biomass	Seed index	0.3698	0.2172	0.5048	<.0001	
		2X	Biomass	Seed index	0.3444	0.1887	0.4832	<.0001	

^a 1X and 2X represent the 1.12 and 2.24 kg ai ha⁻¹ application rates of diuron, respectively.

^b Values lower than the alpha level of 0.05 are statistically significant.

^c Arkansas Agricultural Research and Extension Center (AAREC) in Fayetteville, AR; Lon Mann Cotton Research Center (LMCRC) near Marianna, AR.

Table 5. Analysis of variance for low- and high-vigor cotton, planted at various depths and treated with various rates of different herbicides on a silt loam soil at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b,c}

Source	2012			2013		
	2 WAT ^a	4 WAT	Biomass ^b	2 WAT	4 WAT	Biomass
	Prob > F ^c					
Depth	0.0221	0.0005	0.0950	0.0181	0.0001	0.8832
Herbicide	0.0015	0.0006	0.0685	0.0417	0.0001	0.2626
Vigor	0.3082	0.2294	0.0802	0.0001	0.0006	0.0216
Depth*Herbicide	0.4374	0.2894	0.3116	0.3762	0.3656	0.1257
Depth*Vigor	0.6582	0.5311	0.7031	0.1058	0.3476	0.6811
Herbicide*Vigor	0.6033	0.5001	0.4616	0.7946	0.9706	0.4957
Depth*Herbicide*Vigor	0.9468	0.9006	0.4309	0.3296	0.9177	0.8721

^a 2 and 4 WAT refer to early-season cotton injury observed 2 and 4 weeks after treatment.

^b Biomass includes all above-ground plant tissue harvested, dried and weighed 4 WAT.

^c Source values lower than the alpha level of 0.05 are statistically significant.

Table 6. Cotton injury and biomass affected by planting depth, herbicide, and seed vigor 2 and 4 weeks after treatment on a silt loam soil at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b,c,d,e}

Source	Rate	2012			2013		
		Injury		Biomass ^b g plant ⁻¹	Injury		Biomass g plant ⁻¹
		2 WAT ^a %	4 WAT		2 WAT	4 WAT	
Planting depth ^c							
Shallow		57 B ^e	52 B	1.14	15 B	14 B	0.66
Deep		74 A	67 A	0.90	35 A	36 A	0.66
Herbicide ^d							
Nontreated	0X	—	—	1.29 A	—	—	0.66
Diuron	1X	51 B	46 B	1.01 AB	21 AB	16 B	0.60
	2X	84 A	76 A	0.73 B	36 A	35 A	0.66
Fluometuron	1X	58 B	51 B	1.19 AB	12 B	10 B	0.64
	2X	63 B	58 AB	0.93 AB	19 AB	25 AB	0.67
Fomesafen	1X	57 B	51 B	1.03 AB	35 A	35 A	0.76
	2X	80 A	73 A	0.97 AB	28 A	31 A	0.64
Vigor							
Low		68	61	0.95	31 A	31 A	0.63 B
High		63	57	1.09	20 B	19 B	0.70 A

^a 2 and 4 WAT refer to early-season cotton injury observed 2 and 4 weeks after treatment.

^b Biomass includes all above-ground plant tissue harvested, dried, and weighed 4 WAT.

^c Planting depth refers to the mechanical placement of seed 0.6 and 2.5 cm within the soil for both shallow and deep scenarios, respectively.

^d Herbicides included treatments of diuron, fluometuron, and fomesafen at 0, 1, and 2X labeled rates (diuron, 1.12 kg ai ha⁻¹; fluometuron, 1.12 kg ai ha⁻¹; fomesafen, 0.28 kg ai ha⁻¹).

^e Letters represent significant differences between means within each main source effect, year, and WAT according to Tukey's HSD.

Table 7. Analysis of variance effects tests for low- and high-vigor cotton planted at various depths and treated with various rates of different herbicides on a silt loam soil at the Rohwer Research Station near Rohwer, AR.^{a,b,c}

Source	2012			2013		
	2 WAT ^a	4 WAT	Biomass ^b	2 WAT	4 WAT	Biomass
Prob > F ^c						
Depth	0.9927	0.6716	0.8145	0.0014	0.0022	0.0248
Herbicide	0.0001	0.0001	0.0001	0.0001	0.0001	0.0004
Vigor	0.0001	0.0001	0.0230	0.0001	0.0001	0.6734
Depth*Herbicide	0.0492	0.0192	0.0060	0.0005	0.0017	0.0614
Depth*Vigor	0.5295	0.8875	0.4605	0.0003	0.0012	0.0863
Herbicide*Vigor	0.0973	0.7445	0.4093	0.0001	0.0001	0.2487
Depth*Herbicide*Vigor	0.3977	0.8279	0.1785	0.0357	0.0512	0.4808

^a 2 and 4 WAT refer to early-season cotton injury observed 2 and 4 weeks after treatment.

^b Biomass includes all above ground plant tissue harvested, dried, and weighed 4 WAT.

^c Source values lower than the alpha level of 0.05 are statistically significant.

Table 8. Cotton injury at 2 and 4 weeks after treatment (WAT) and biomass production at 4 WAT as influenced by cotton seed vigor averaged over herbicides and planting depths on a silt loam soil at Rohwer Research Station near Rohwer, AR in 2012.^{a,b,c}

Vigor	Injury		Biomass ^b g plant ⁻¹
	2 WAT ^a	4 WAT	
	%		
Low	38 A	31 A	0.60 B
High	24 B	20 B	0.67 A

^a 2 and 4 WAT refer to early-season cotton injury observed 2 and 4 weeks after treatment.

^b Biomass includes all above ground plant tissue harvested, dried, and weighed 4 WAT.

^c Letters represent significant differences within respective ratings made weeks after treatment (WAT) according to Tukey's HSD.

Table 9. Cotton injury at 2 and 4 weeks after treatment (WAT) and biomass production at 4 WAT as affected by planting depth and herbicide, averaged over seed vigor on a silt loam soil at the Rohwer Research Station in Rohwer, AR in 2012.^{a,b,c,d}

Herbicide	Rate kg ai ha ⁻¹	Injury				Biomass ^b	
		2 WAT ^a		4 WAT		Shallow	Deep
		Shallow ^c	Deep	Shallow	Deep	g plant ⁻¹	
Nontreated	—	—	—	—	—	0.49 B	0.47 B
Diuron	1.12	19 B	13 B	12 B	4 B	0.69 AB	0.85 A
	2.24	67 A	51 A	54 A	41 A	0.44 B	0.55 AB
Fluometuron	1.12	17 B	8 B	11 B	8 B	0.87 A	0.79 A
	2.24	22 B	26 B	18 B	23 B	0.59 AB	0.75 A
Fomesafen	0.28	31 B	39 A	34 A	33 A	0.77 A	0.63 AB
	0.56	31 B	49 A	29 B	45 A	0.69 AB	0.40 B

^a 2 and 4 WAT refer to early-season cotton injury observed 2 and 4 weeks after treatment.

^b Biomass includes all above ground plant tissue harvested, dried, and weighed 4 WAT.

^c Shallow and deep planting depths consisted of 0.6 and 2.5 cm, respectively.

^d Letters represent significant differences according to Tukey's HSD.

Table 10. Influence of the interactions of herbicide, planting depth, and seed vigor on cotton injury at 2 and 4 WAT on a silt loam soil at the Rohwer Research Station near Rohwer, AR in 2013.^{a,b,c}

Herbicide	Rate kg ai ha ⁻¹	Injury							
		2 WAT ^a				4 WAT			
		Shallow ^b		Deep		Shallow		Deep	
		Low	High	Low	High	Low	High	Low	High
		%							
Diuron	1.12	5 B	10 B	10 B	2 B	8 B	6 B	4 B	3 B
	2.24	38 A	13 B	47 A	13 B	29 A	13 B	40 A	10 B
Fluometuron	1.12	6 B	2 B	9 B	4 B	9 B	4 B	8 B	1 B
	2.24	9 B	5 B	38 A	11 B	6 B	13 B	34 A	5 B
Fomesafen	0.28	6 B	4 B	10 B	13 B	11 B	5 B	11 B	11 B
	0.56	23A	4 B	44 A	12 B	14 B	5 B	36 A	13 B

^a 2 and 4 WAT refer to early-season cotton injury observed 2 and 4 weeks after treatment.

^b Shallow and deep plots were mechanically planted 0.6 and 2.5 cm deep, respectively.

^c Letters represent significant differences within a WAT according to Tukey's HSD.

Table 11. Analysis of variance effect tests for low- and high-vigor cotton treated with preemergence herbicides in stressed and non-stressed environments.^{a,b,c}

Source	2 WAT ^a	4 WAT	Biomass ^b
	Prob > F ^c		
Vigor	0.0482	0.0009	0.8676
Herbicide	0.0323	0.0045	0.3880
Environment	<0.0001	0.0131	<0.0001
Vigor*Herbicide	0.0774	0.0927	0.8400
Vigor*Environment	0.0041	0.0351	0.7440
Herbicide*Environment	0.4704	0.3710	0.0112
Vigor*Herbicide*Environment	0.1927	0.5699	0.5196

^a 2 WAT refers to early-season cotton injury observed 2 weeks after treatment.

^b Biomass includes all above-ground plant tissue harvested, dried and weighed 4 WAT.

^c Source values lower than the alpha level of 0.05 are statistically significant.

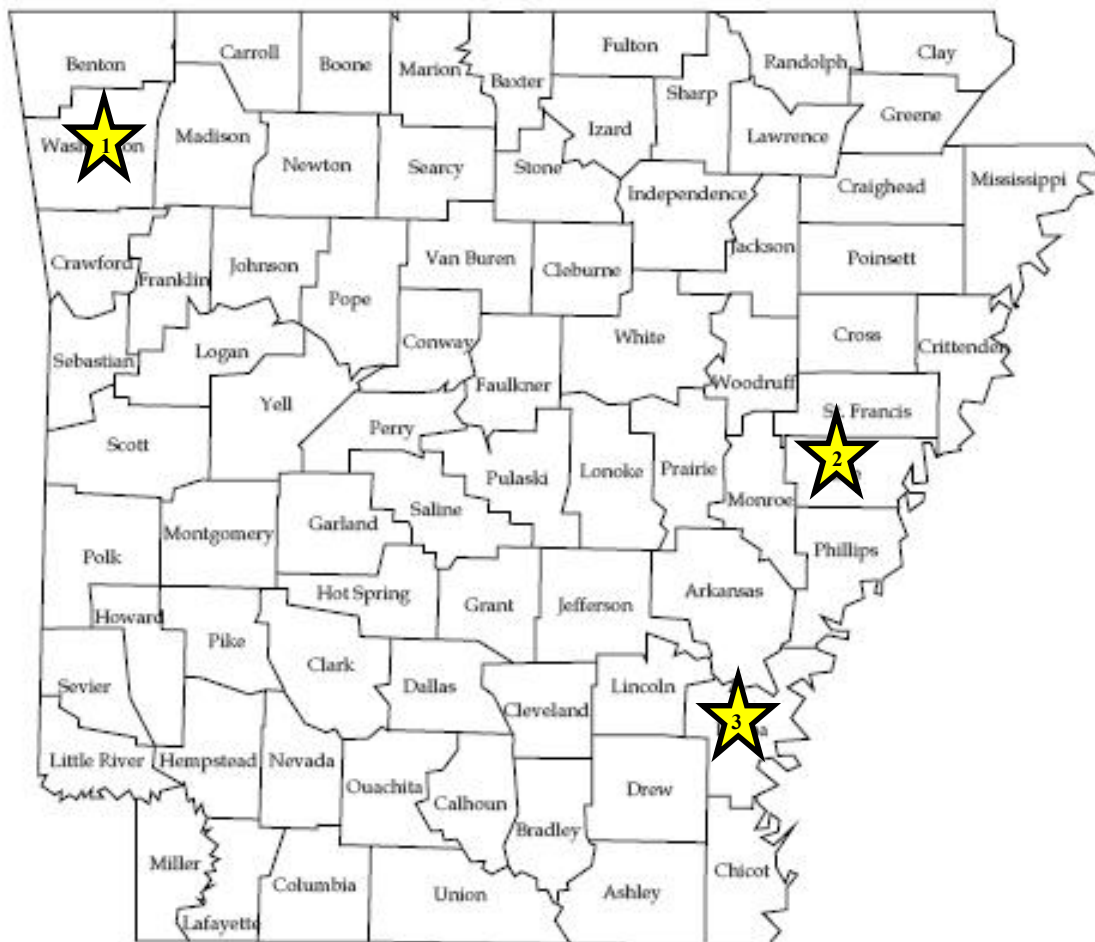


Figure 1. Location of Arkansas counties [1 – Washington (Fayetteville); 2 – Lee (Marianna); 3 – Desha (Rohwer)] where experiments were conducted in 2012 and 2013.

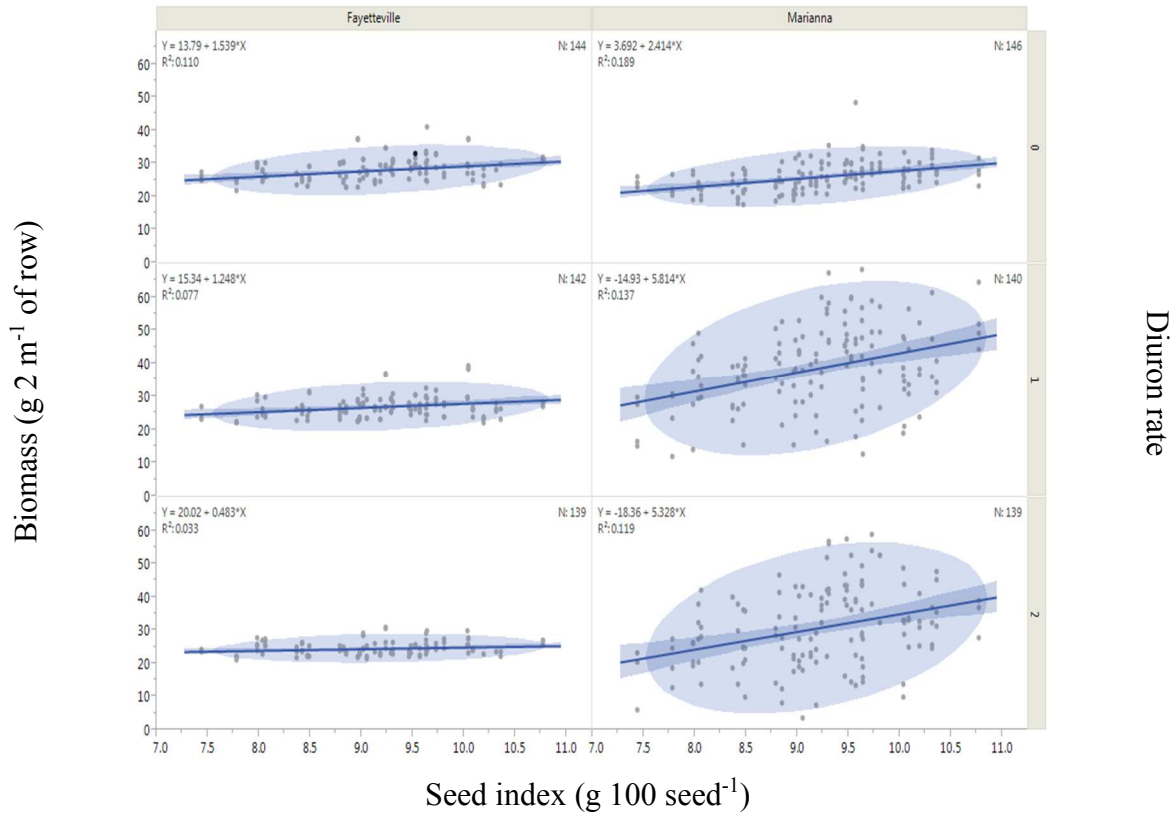


Figure 2. Cotton biomass production at the Arkansas Agricultural Research and Extension Center in Fayetteville and the Lon Mann Cotton Research Center near Marianna, AR in response to various seed indices and diuron application rates (0, 1, and 2X the labeled field rate of 1.12 kg ai ha⁻¹). Dark shading represents 95% of the data set.

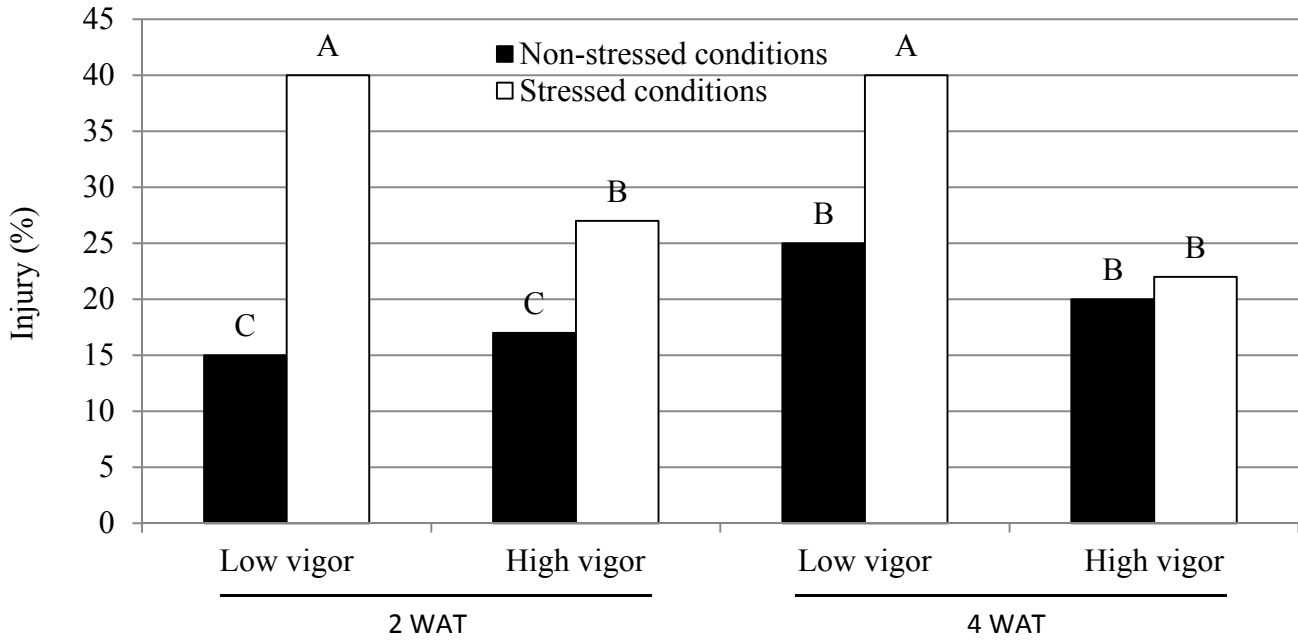


Figure 3. Injury observed on low- and high-vigor cotton 2 and 4 weeks after treatment (WAT) following stressed and non-stressed environmental conditions. Within a WAT, letters represent significant difference at the alpha level 0.05 using Tukey's HSD.

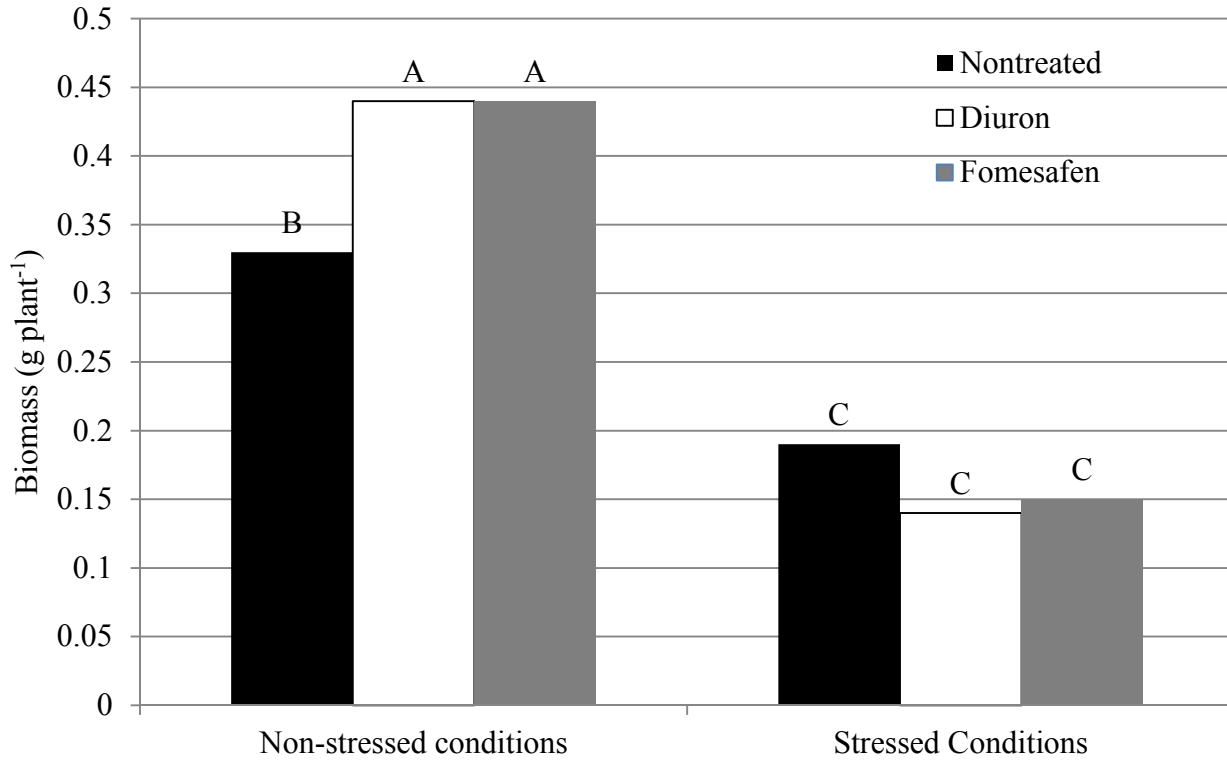


Figure 4. Cotton biomass (all above-ground plant tissue) collected, dried, and weighed at 4 WAT. Letters represent significant differences at the alpha level of 0.05 using Tukey's HSD.

III. Effect of Shading, Cultivar, and Application Timing on Cotton Tolerance to Glufosinate

Abstract

With the increasing presence of glyphosate-resistant (GR) weeds in the Midsouth, cotton producers are having to implement new control strategies and technologies to achieve maximum cotton yields. Early-season residual herbicides are again common; however, inconsistent crop injury and dependence on moisture for activation causes the need for effective postemergence (POST) options. Glufosinate-resistant technology in cotton can be effective in controlling GR weeds like Palmer amaranth when applied at appropriate times and rates. The objective of this study was to determine if differences exist among PhytoGen[®] and Liberty Link[®] cultivars to recover from injury with glufosinate applied at different growth stages and in the presence of low-light conditions. In 2012 and 2013, field studies were conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR where three cultivars were treated with various rates of glufosinate at three growth stages in the presence and absence of a preceding low-light condition. At 2 weeks after emergence (WAE), cotton tolerance to glufosinate differed by cultivar, although some injury was observed on Liberty Link cotton. Injury was often greatest when applied at the 1-leaf stage to PhytoGen[®] cultivars, but by 4 to 5 weeks after treatment, all cultivars showed similar potential to recover. In general, cotton plants that were shaded 3 d prior to applying glufosinate were injured to a greater extent than nonshaded plants. Yields were reduced 72 g m⁻¹ of row when cotton was shaded 3 d prior to applying 2X rates of glufosinate in 2012 and similarly, shaded plots produced 76 g m⁻¹ of row less seedcotton in 2013. There was little difference among cultivar yields; however, shade did significantly reduce yield when present 3 d prior to application, illustrating the importance of avoiding glufosinate application during prolonged periods of cloudy conditions.

Nomenclature: Glufosinate; PhytoGen[®]; Liberty Link[®]; cotton, *Gossypium hirsutum* L.

Key words: Postemergence (POST); shading; application timing, crop tolerance

Introduction

Since the 1950s, synthetic herbicides have become an increasingly critical tool in the improvement of cotton yields through the control of problematic weed species that compete for light, nutrients, and moisture (Duke and Powles 2008; McWhorter and Bryson 1992). Cotton yield and quality throughout the southern United States has increased while reducing labor costs and time requirements associated with weed control. Arguably, the most influential achievement in weed control over the past 50 years was the increased availability of postemergence (POST) herbicides.

Glyphosate, a non-selective, systemic herbicide, was first registered in 1974 for burndown purposes and the control of perennial weeds in non-crop areas. The absence of analogs or alternative chemical classes that inhibit EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) made this single compound an ideal herbicide for non-selective weed control. Increased utility of glyphosate was recognized in 1996 with the release of glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.], which was subsequently followed by the release of GR cotton, corn (*Zea mays* L.), and canola (*Brassica napus* L.). The discovery of the CP4 gene of *Agrobacterium* sp. provided a method of encoding a GR form of EPSPS (Padgett et al. 1996). Accompanied by a promoter, high levels of glyphosate-resistance were expressed when the gene was placed into certain crop genomes. The earliest GR cotton cultivars had high vegetative tolerance, but reproductive tolerance was lower because of reduced expression of CP4 EPSPS in male tissues (Nida et al. 1996; Pline et al. 2002).

Midsouth cotton producers widely accepted GR technology as it resulted in cost-savings, improved weed management, and simplicity of use (Duke and Powles 2009). In 2000, after the loss of patent rights to glyphosate, the price of glyphosate decreased by 40% in the United States

(Duke and Powles 2009; USDA 2006). The low price of glyphosate, its low toxicity, and ability to control a broad spectrum of weed species resulted in extensive use of glyphosate on annual weeds. The reduction in diversity among modes of action increased the selection on weed populations for potential resistance. The eventual occurrence of GR weeds was contradictory to the earlier predictions by Bradshaw et al. (1997). The main risk factors associated with the evolution of HR weeds are a simple cropping system that favors recurrent application of highly efficacious herbicides with the same site of action to genetically diverse, annual weed species with high fecundity that occur at high densities over vast geographies and have efficient gene (seed or pollen) dissemination (Beckie 2006). Many of the cropping scenarios and problematic weed species involved with Midsouth cotton production attest to this statement as 23 herbicide-resistant (HR) weed biotypes infest Arkansas croplands (Heap 2015).

The adoption and justification for continued use of HR crops is due to the adopted simplistic approach to improved weed control and higher returns embraced by many growers (Burnside 1992; Devine and Buth 2001). The future of cost-effective weed control depends heavily on the protection of HR technology from the evolution of HR weeds; therefore, reducing selection for resistance evolution while maintaining sufficient weed control is essential (Beckie 2006). The presence of GR weeds has forced many growers to adopt additional herbicide mechanisms of action, resulting in more intensive (costly in time and finances) control measures. Resistance has made weed control in cotton increasingly difficult as crop seedlings are slow growing and there are fewer herbicide options relative to other crops like corn and soybean (Eaton 1955; Pankey et al. 2005). New herbicide chemistry is limited as industry research and development efforts were greatly slowed following the release of glyphosate-resistant crops (Reddy 2001).

The presence of GR weeds has encouraged many to evaluate the efficacy of the glutamine synthetase inhibitor glufosinate (Bellinder et al. 1987). Glufosinate can be applied POST over the top to glufosinate-resistant cotton cultivars, namely Liberty Link[®] (Anonymous 2014). Although glufosinate provides no residual control, it can serve as an effective management tool as no weed biotypes in cotton are currently resistant to glufosinate (Duke and Powles 2009; Green 2009). Many scientists believe the answer to acceptable control and prevention of resistance should involve the integration of soil-applied residual herbicides with a glyphosate/glufosinate rotation program (Norsworthy et al. 2012). Exploring the efficacy of glufosinate in various environmental and cultural conditions could potentially reduce the number of applications, fuel, labor, and equipment costs and reduce selection for herbicide resistance (Wilcut et al. 2002; Norsworthy et al. 2012; UADOA 2012).

In croplands with high populations of GR weeds like Palmer amaranth, glufosinate can provide adequate control when applied at appropriate times and rates (Culpepper et al 2009; Steckel et al. 1997; Everman et al. 2007). Because glufosinate is a contact herbicide, efficacy is greatly dependent on several factors including coverage, relative humidity (RH), and weed size (Coetzer et al. 2001; Hoss et al. 2003; Riar et al. 2011). Properly evaluating the environmental and agronomic factors that influence the efficacy of glufosinate could potentially improve the utility of this herbicide as a tool for the management of GR weeds.

Seeds germinate, develop, and reproduce most effectively under favorable conditions (Anderson 1962; Gibson and Mullen 1996; Holm and Miller 1972) because plant physiological processes benefit from adequate sunlight, water, and nutrients. Similarly, the efficacy of POST herbicides is influenced by environmental conditions before, during, or after the time of application as plant foliar and root uptake is impacted (Cole 1983).

Plant growth stage, rate of growth, leaf wax surface development, and degree of turgor can influence the susceptibility of plants to herbicides (Hammerton 1967); therefore, herbicide efficacy is dependent upon a lethal amount of herbicide penetrating the cuticle and reaching the active site. Many POST herbicides are considered effective by associated retention and penetration of the leaf surface which has been documented to be affected by light quantity. The protective layer on aerial plant parts is known as the cuticle and it serves as the primary barrier to POST herbicide penetration (Kolattukudy 1970). The outer layer of the cuticle, known as the epicuticular wax (ECW) layer, is composed of long-chain aliphatic compounds derived from very long chain fatty acids and can be highly altered, physiologically, from environmental factors (Kunst and Samuels 2003). Factors such as temperature, sunlight, RH, and soil water content have been documented to affect the foliar absorption of POST herbicides such as glufosinate (Garcia et al. 2002; Stevens and Baker 1987). Field experiments emphasized that plants subjected to 80% shading were injured more by carfentrazone-ethyl, a selective POST herbicide registered for use in corn, than nonshaded corn plants. Similarly, shading 5 d prior to application resulted in a 4 to 8% increase in injury on wheat (*Triticum aestivum* L.) from carfentrazone-ethyl. Soybean exhibited the greatest response to shading as plants were injured 24 to 41% more by carfentrazone-ethyl than those grown in complete sunlight (Thompson and Nissen 2002).

Little research has been conducted evaluating the impact of glufosinate on vegetative cotton injury under low-light conditions. Based on soybean, crop injury from herbicides as a result of shading could be associated with reduced herbicide metabolism, herbicide sequestration, chlorophyll biosynthesis, protogen degradation, or free radical detoxification (Dayan and Duke 1997). The existence of lowered BAR gene activity in WideStrike (Phytogen)

cultivars translates into incomplete glufosinate tolerance compared to LibertyLink® (Steckel et al. 2012). This reduced tolerance has been documented to result in 11 to 25% crop injury with single applications of glufosinate (Culpepper et al. 2009; Whitaker et al. 2011; Sweeney and Jones 2014). There has been, however, weak evidence suggesting this injury could result in reduced yield and lint quality (Culpepper et al. 2009; Dodds et al. 2011) and in many cases injury symptoms were not observed (Wallace et al. 2011). Plants with low soil moisture status achieved by fully exposing them to sunlight could experience dehydrated cuticles and less foliar absorption of herbicides (Peregoy et al. 1990) and this reaction could vary among different biotypes or cultivars as well as growth stage during application. Additionally, injury to LibertyLink® cotton in the form of necrosis of the upper most leaves can result at times following a labeled application of glufosinate; albeit, the extent of injury is often quite variable and transient (Norsworthy, personal communication). Therefore, the objective of this research was to assess the response of PhytoGen® and LibertyLink® cotton to glufosinate applied at different growth stages when low-light conditions precede the application.

Materials and Methods

In 2012 and 2013, a split-split-strip plot field experiment with four replications was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR on a Leaf silt loam soil (Fine, mixed, active, thermic Typic Albaquults; with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.9) (USDA-NRCS 2015). The main plot consisted of three cotton cultivars (PHY 375 WRF, PHY 499 WRF, and ST 4145LLB2). PhytoGen® seed was obtained from Dow AgroSciences in Indianapolis, IN, USA, and Stoneville® seed was acquired from Bayer Crop Science in Research Triangle Park, NC, USA. A John Deere 6403

medium-frame tractor and John Deere 7100 four-row planter (Deere & Company, Moline, IL, USA) retrofitted with an electric cone-type seeder (ALMACO, Nevada, IA, USA) were utilized to plant 125,000 seed ha⁻¹ at a 2-cm depth in mid-May. The strip-plot dimensions included 2 bedded rows spaced 91 cm apart, 3.8 m long. The subplot consisted of three cotton growth stages (1-, 4-, and 6-leaf stage) at application and the subsubplot consisted of three herbicide treatments (glufosinate at 0.88 and 1.76 kg ai ha⁻¹ and a non-treated control). Glufosinate was applied as Liberty 280 SL. The strip of this experiment consisted of light intensity (shade and non-shaded) organized horizontally across replications. The shaded plots (front 3 m of subsubplot) were covered with shade cloth allowing for 50% light penetration without spectrum limitation 3 d prior to herbicide treatment. Shade cloth covers (Gempler's, Madison, WI, USA) were supported by a polyvinyl chloride pipe frame 40 cm above the plant canopy. Weed control was supplemented by a preemergence (PRE) application of fluometuron (Cotoran®4L, MANA Inc., Raleigh, NC, USA) and *S*-metolachlor (Dual Magnum, Syngenta, Greensboro, NC, USA) at 1.12 and 1.07 kg ai ha⁻¹, respectively, along with hand weeding.

Applications were made using a CO₂-pressurized backpack sprayer calibrated to deliver 187 L ha⁻¹ at 276 kPa and 4.8 kph. Cotton injury was assessed at 2 and 4 to 5 WAT on a 0 to 100 scale, with 0 being no injury and 100 being complete death. The test site was routinely scouted for insects and diseases. In early-July of 2012 and 2013, a one-time application of 0.27 kg ha⁻¹ of dimethoate (Dimethoate 4E, Cheminova, Research Triangle Park, NC, USA) was applied to control tarnished plant bug [*Lygus lineolaris* (Palisot de Beauvois)].

In 2013, cotton leaves were sampled to determine cuticle quantity on the day of herbicide application by means of bagging three of the youngest mature leaves from both the shade portion and exposed portion of each plot. Three 3-cm² sections from each sampled leaf were submersed

in chloroform and shaken for 10 min. After removing the cuticle, the chloroform was permitted to evaporate and the vials were weighed to determine the amount of epicuticular wax (ECW). In both years, aboveground cotton biomass was harvested from 2 m of row and the number of harvested plants counted at 12 WAE. The harvested biomass was oven-dried for 7 d at 60 C and weighed. Upon maturity, seedcotton was hand harvested from 2 m of row from all plots and weighed.

Data were subjected to a fixed effects test in JMP Pro 11 (SAS Institute Inc., Cary, NC, USA) with the randomization of replication. The variability of irrigation and precipitation events led to years being analyzed separately as a by-variable using GLM Mixed under an effect leverage personality. Under this mixed model and residual maximum likelihood (REML), p-values were generated and means associated with significant interactions were separated using Tukey's HSD at the alpha level of 0.05 (SAS Institute Inc. 2014)

Results and Discussion

In 2012, the malfunction of irrigation equipment accompanied by extended periods of hot, dry weather contributed to an overall reduction in cotton growth compared to 2013. The irrigation and environmental differences between years in Fayetteville, AR prompted the separation of trial years as a by-variable during statistical analyses (Figure 1).

2012. At 2 WAT, statistical significance was observed on the main effects of leaf stage at application, herbicide rate, and shading (Table 1); however by 4 to 5 WAT, these main effects were no longer significant, likely as a result of the ability of cotton to recover from the initial stresses from the herbicide and shading. Late-season assessment of cotton aboveground biomass was impacted by the main effects of shading and herbicide, but not cultivar.

Initial Injury (2 WAT). Cotton that had been shaded prior to treatment at the 1-leaf stage exhibited more damage at 2 WAT when compared to other growth stages at application, averaged over cultivar and glufosinate rate (Figure 2). No difference was observed between cultivars or shading when cotton was treated with glufosinate at $0.88 \text{ kg ai ha}^{-1}$ although trends for injury to increase were present when all cultivars were subjected to simulated cloud cover 3 d prior to application (Figure 3).

Recovery from Injury (4 to 5 WAT). At 4 to 5 WAT, all cotton cultivars had shown measurable potential to recover (Table 1). Tukey's HSD as well as a Student's t-test failed to produce statistical separation of means, prompting a more detailed contrast for two-way components within the two-way interaction of shading and cultivar (personal communication, K. Thompson). Data were then run through SAS 9.3 in which the interaction of cultivar and shade on cotton injury 4 to 5 WAT in 2012 were shown to be nonsignificant (data not shown). Having shown greater susceptibility to injury at high rates, LibertyLink® cultivars display potentially weaker tolerance to glufosinate when application follows periods of reduced photosynthetic activity. According to Zhao and Oosterhuis (1998a), shading negatively impacts photosynthetically active radiation (PAR) absorbed by the plant and decreases the net photosynthetic rate. Cotton possesses a mechanism to compensate by increasing chlorophyll biosynthesis which could hinder the plant's ability to combat leaf damage by increasing the partitioning of N into leaves and subsequently increasing absorption of glufosinate.

Late-Season Biomass (12 WAP). The biomass collected 12 weeks after planting (WAP) proved to display a shade by growth stage and shade by herbicide interaction following analysis in 2012 (Table 1). Cotton treated at the 4-leaf stage in absence of shade was found to produce significantly more biomass. When cotton was treated with an above-labeled rate of glufosinate

(1.76 kg ha⁻¹), shaded plants produced less biomass in 2012, averaged over cultivar (Figure 5). In addition, nonshaded cotton not treated with glufosinate produced 67 g m⁻¹ of row more biomass compared to nontreated plants.

Seedcotton Yield. Significant influences of leaf stage, herbicide, shade presence, and a herbicide by shade interaction were observed regarding seedcotton yield (Table 2). Though statistical separation is lacking, numeric trends suggest reduced yield when cotton was shaded 3 d prior to application of 1 and 2X rates of glufosinate (Figure 6). With proper fertilization, weed-free maintenance, and irrigation, leaf stages 1 and 4, averaged over shading and herbicide applications, exhibited greater yields than 6-leaf cotton (Figure 7). It seems unlikely that the 18% injury to cotton that occurred following the 6-leaf application resulted in yield loss compared to the 1-leaf application which was injured 28 to 36% by glufosinate (Figures 2 and 7).

2013. *Initial Injury (2 WAT).* In 2013, there was a shade by cultivar by leaf stage interaction for injury at 2 WAT (Figure 8). Numerically more injury was observed to cotton that had been shaded prior to glufosinate application than plants that were not shaded, and differences for 1-leaf cotton treated with glufosinate were significant between shaded and nonshaded treatments. The low leaf photosynthesis of shaded cotton may be associated with decreased electron transport capacity (Zhao and Oosterhuis 1998b) severely hindering the detoxification of ammonia. Glufosinate could then potentially lead to the uncoupling of photophosphorylation resulting in both membrane disruption and lipid peroxidation. Just as Culpepper et al. (2009) and Steckel et al. (2012) concluded, the lowered tolerance presented by PhytoGen® cultivars often resulted in greater injury. This injury was often proliferated during periods of simulated cloud cover at both the 1- and 4-leaf stage.

Recovery from Injury (4 to 5 WAT). The variability among cultivars in initial injury from glufosinate and ability to recover from injury resulted in an interaction of cultivar, leaf stage, and glufosinate rate for assessments taken at 4 to 5 WAT in 2013 (Table 1). This experiment supplied variations in injury symptoms, biomass, and seedcotton yield often due to cultivar differences. There exists an inability for some cultivars to respond similarly to all cloud events (Goodman 1955). ANOVA indicated the interaction to be significant though the conservative nature of Tukey's HSD failed to produce significant separation of treatments. Greater injury was observed when glufosinate was applied at 1.76 kg ai ha⁻¹ to PhytoGen® cultivars compared to the Liberty Link® variety. Additionally, injury was escalated when applications took place during the 1-leaf stage (Figure 9).

Late-Season Biomass (12 WAP). Shading cotton 3 d prior to application at the 4-leaf stage resulted in 27% less biomass than nonshaded plots (Figure 10). Numeric trends show that simulated cloud cover consistently reduced biomass which is consistent with the findings of Dusserre et al. (2002).

Epicuticular Wax Quantity. As the leaf cuticle plays a crucial role in defending leaves from chemical penetration, thicker cuticles can be expected to reduce the penetration of foliar-applied herbicides (Oosterhuis et al. 1991). Cotton leaves sampled just prior to the 1-leaf application stage contained 0.6 to 0.15 g greater ECW per 3.14 cm² sample than 4- and 6-leaf samples (Figure 11). It is possible that cotton possesses physiological properties that include equipping younger leaves with thicker cuticles in effort to protect itself during initial root establishment and vegetative development. In a study conducted in Fayetteville, AR in 1991, water-stressed cotton increased ECW by 44% (Oosterhuis et al. 1991). According to Fick's second law, the flux of a herbicide should increase as cuticle thickness decreases, which likely means that there other

factors at play regarding differences in tolerances across growth stages, some of which may involve wax composition or may be physiological in nature (Nobel 1970, Price 1982).

Seedcotton Yield. Similar to 2012, the main effect of shade in 2013 impacted seedcotton yield (Table 2). By mid-August, all treatments had visually recovered from injury sustained by the application of glufosinate (data not shown). These visual assessments of phytotoxicity did not, however, illustrate the potential metabolic disruptions that took place resulting in shaded plots producing 820 kg ha⁻¹ less seedcotton than nonshaded plots (data not shown). Dunlap (1943) reported that interruptions for two or three days in high sunlight intensities often causes shedding of fruiting forms and documented a significant yield reduction. Cotton is extremely sensitive to low photosynthetic photon flux density (PPFD) stress and numerous studies have documented yield reductions reaching 67% (Eaton and Ergle 1954; Knight 1935; Zhao and Oosterhuis 2000). Most research, including the before mentioned, explored simulated cloud cover during the fruiting period rather than early vegetative growth. This research would suggest that shading cotton at younger growth stages (prior to 7-leaf) can have lasting effects, and in certain years like 2013, can negatively impact yield. Granted, it is unusual that young cotton would be detrimentally affected by a mere three days of simulated cloud cover, but cotton is very sensitive to early season interference or stress of any kind and any evidence suggesting a potential lag in vegetative and ultimately reproductive growth cannot be overlooked (Wells, personal communication). Zhao and Oosterhuis (2000) hypothesized that “the effects of low PPFD at different developmental stages on cotton growth and yield may be quite different because cotton is perennial with an indeterminate growth habit, and it is very responsive to changes in environments, especially PPFD.” This research compliments that hypothesis.

Summary

The impact of cloud cover, which was simulated by means of shade cloth in this research, has been documented as a challenge to global cotton production regarding variability in injury, biomass, ECW, and yield by various cultivars treated with glufosinate. The decrease in photosynthetic irradiance by shading can increase otherwise irrelevant injury and yield losses. It has become evident that the application of glufosinate, namely to control GR weed species such as Palmer amaranth, should be reserved for times of high photosynthetic activity by cotton (Zhao and Oosterhuis 1998). It is suggested that cotton producers refrain from applying high rates of glufosinate on cotton that has been subject to cloudy conditions for 3 d. Although no significant yield differences were observed, special caution is advised when the use of glufosinate is employed to control GR weeds in typically less tolerant PhytoGen® cotton systems compared to Liberty Link®.

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Table 1. Fixed effects test for three cotton cultivars treated with various rates of glufosinate at three leaf stages in the presence and absence of preceding shade at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b,c}

Source	2012			2013		
	2 WAT	4 to 5 WAT	Biomass ^b	2 WAE	4 to 5 WAT	Biomass
	Prob > F ^c					
Cultivar	0.0700	0.2373	0.5673	0.0008	0.0893	0.9597
Stage	0.0001	0.7544	0.0188	0.0001	0.0613	0.6139
Herbicide	0.0008	0.1198	0.0005	0.0001	0.7346	0.6588
Shade	0.0247	0.2702	0.0533	0.0031	0.0582	0.1063
Cultivar*Stage	0.0190	0.6366	0.3044	0.0006	0.7346	0.5490
Cultivar*Herbicide	0.8487	0.3525	0.6473	0.0780	0.0531	0.6368
Cultivar*Shade	0.2349	0.0240	0.9181	0.0131	0.0966	0.2143
Stage*Herbicide	0.0688	0.1052	0.4099	0.0068	0.7094	0.6366
Stage*Shade	0.0146	0.1863	0.0385	0.0066	0.0832	0.7982
Herbicide*Shade	0.3045	0.9696	0.0068	0.2861	0.7160	0.7014
Cultivar*Stage*Herbicide	0.3504	0.3372	0.0689	0.1517	0.0393	0.2845
Cultivar*Stage*Shade	0.6772	0.7815	0.3884	0.0360	0.0817	0.7748
Cultivar*Herbicide*Shade	0.0413	0.1666	0.7116	0.3747	0.5445	0.8540

Stage*Herbicide*Shade	0.1399	0.5397	0.2997	0.5985	0.7439	0.1847
Cultivar*Stage*Herbicide*Shade	0.2175	0.1103	0.0957	0.6164	0.5310	0.5045

^a 2 WAT refers to early-season cotton injury observed 2 weeks after treatment with glufosinate.

^b Biomass includes all above ground plant tissue harvested, dried, and weighed 12 WAP.

^c Source values lower than the alpha level of 0.05 are statistically significant.

Table 2. Fixed effects tests for the seedcotton yield of three cotton cultivars applied with various rates of glufosinate at three leaf stages in the presence and absence of shade at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR.^{a,b,c,d,e,f}

Source	Seedcotton yield ^a	
	2012	2013
	Prob > F ^b	
Cultivar ^c	0.1772	0.9559
Stage ^d	0.0081	0.4295
Herbicide ^e	0.0001	0.5711
Shade ^f	0.0003	0.0001
Cultivar*Stage	0.6314	0.2324
Cultivar*Herbicide	0.9830	0.5214
Cultivar*Shade	0.6795	0.3314
Stage*Herbicide	0.7648	0.5399
Stage*Shade	0.1117	0.8849
Herbicide*Shade	0.0123	0.9065
Cultivar*Stage*Herbicide	0.0762	0.1603
Cultivar*Stage*Shade	0.5412	0.8949
Cultivar*Herbicide*Shade	0.9756	0.9507
Stage*Herbicide*Shade	0.6964	0.5177
Cultivar*Stage*Herbicide*Shade	0.4503	0.8974

^a Seedcotton was collected upon reproductive maturity in the form of g m row⁻¹.

^b Source values lower than the alpha level of 0.05 are statistically significant.

^c Cotton cultivars tested include PHY 375 WRF, PHY 499 WRF, and 4145 LLB2.

^d Glufosinate was applied at the 1-, 4-, and 6-leaf stage.

^e Herbicide treatments included glufosinate at 0.88 and 1.76 kg ai ha⁻¹ and a nontreated control.

^f Treatments included the presence and absence of simulated cloud cover (shade cloth) 3 days prior to treatment with glufosinate.

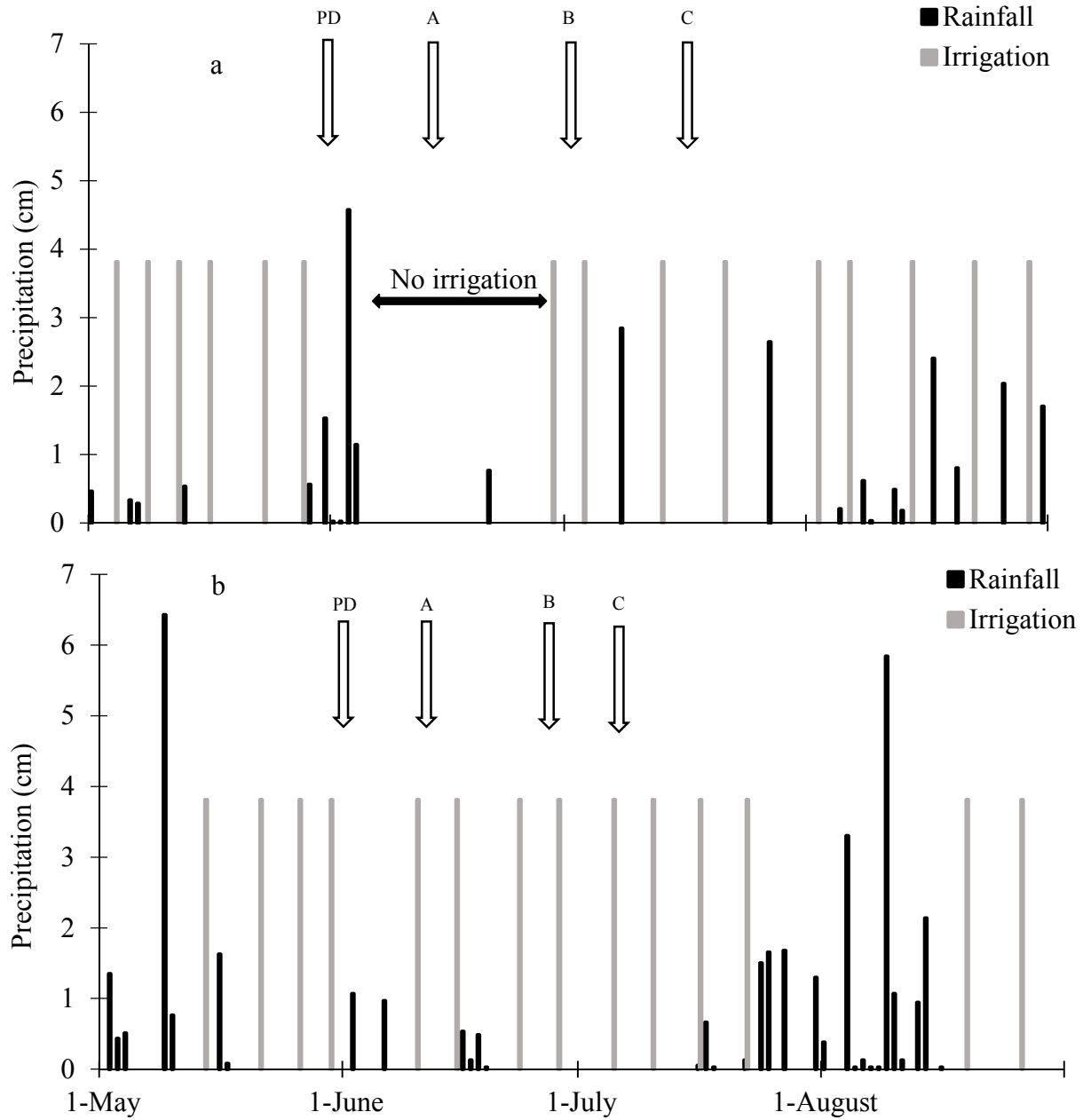


Figure 1. Rainfall and irrigation distribution at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR in 2012 (a) and 2013 (b) displaying planting dates (PD) and application timings (A,B,C).

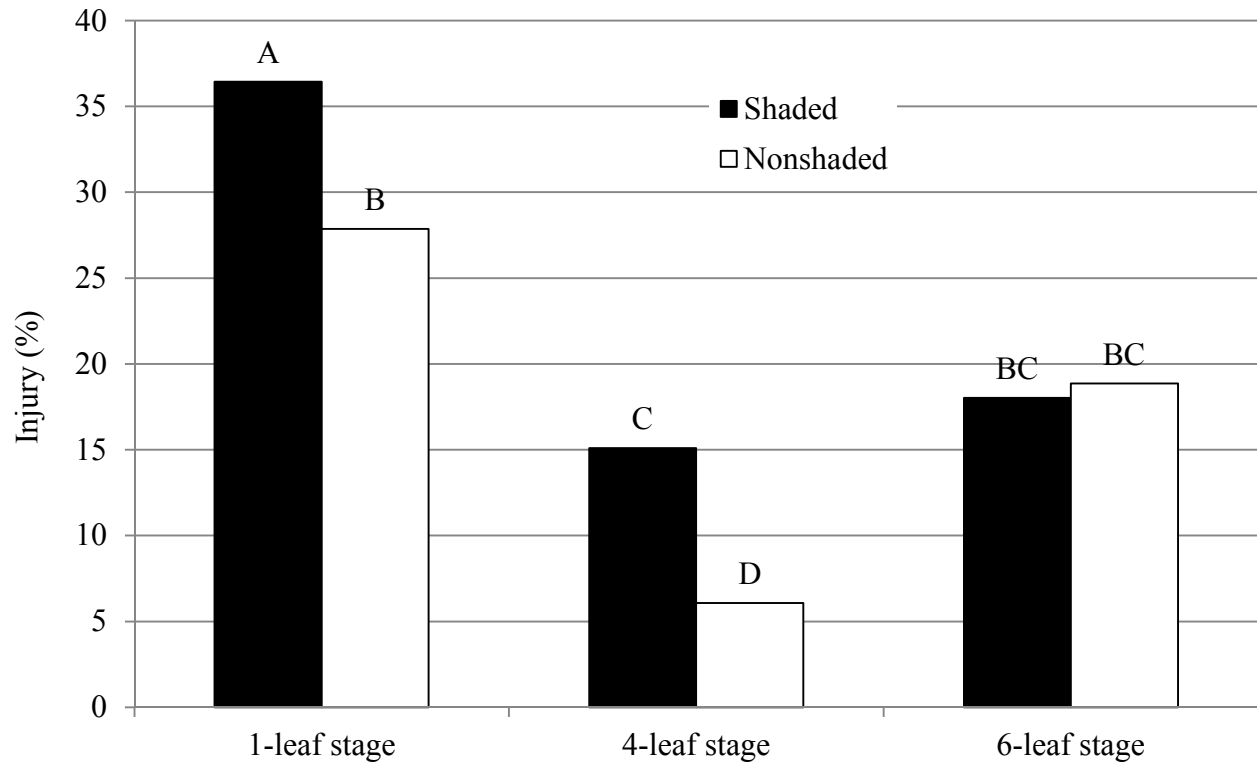


Figure 2. Cotton injury observed 2 weeks after treatment in 2012 on cotton of three growth stages applied with glufosinate in the presence and absence of shade 3 days prior to application. Letters represent significant differences according to Tukey's HSD.

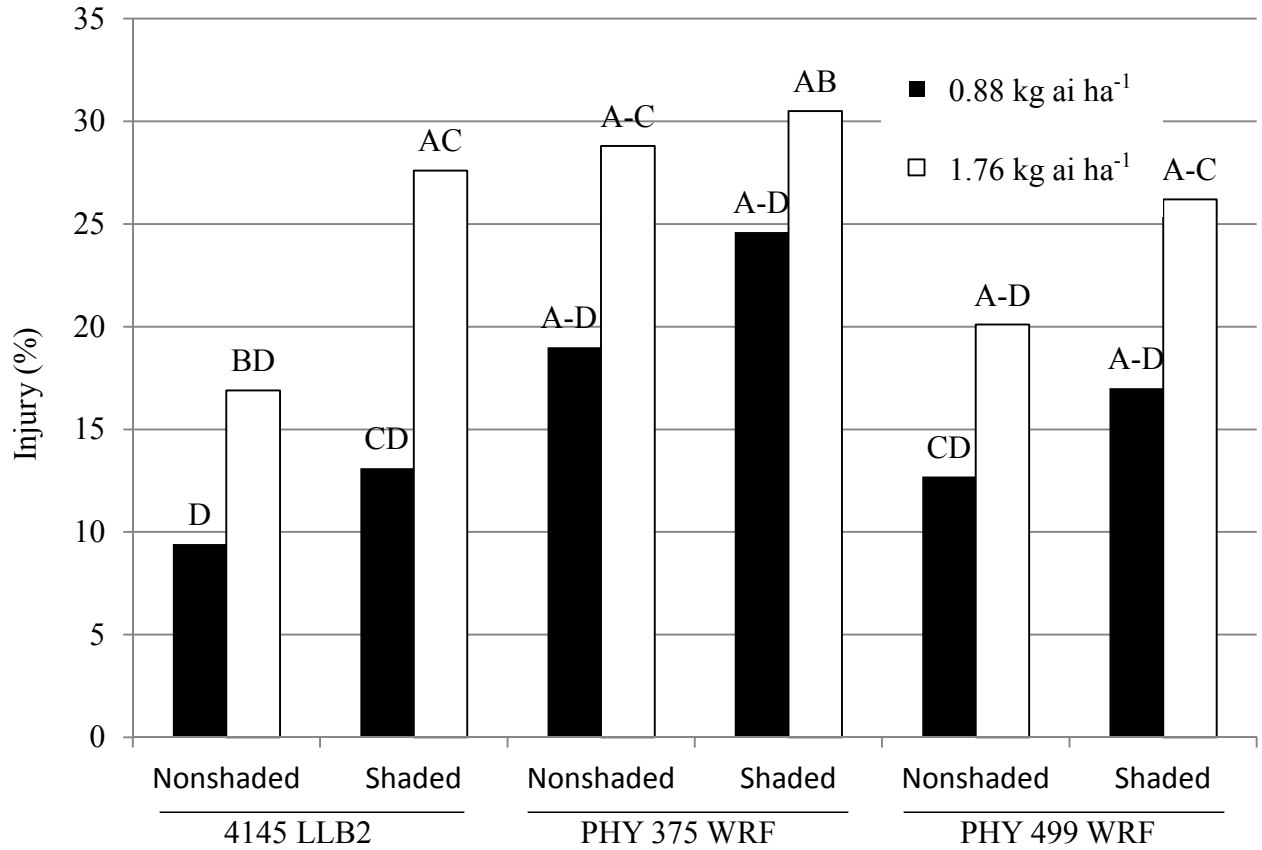


Figure 3. Cotton injury at 2 weeks after treatment in 2012 on three cotton cultivars following applications of glufosinate that had been shaded or not shaded for 3 days prior to application. Means were averaged over growth stage at application. Letters represent significant differences according to Tukey's HSD.

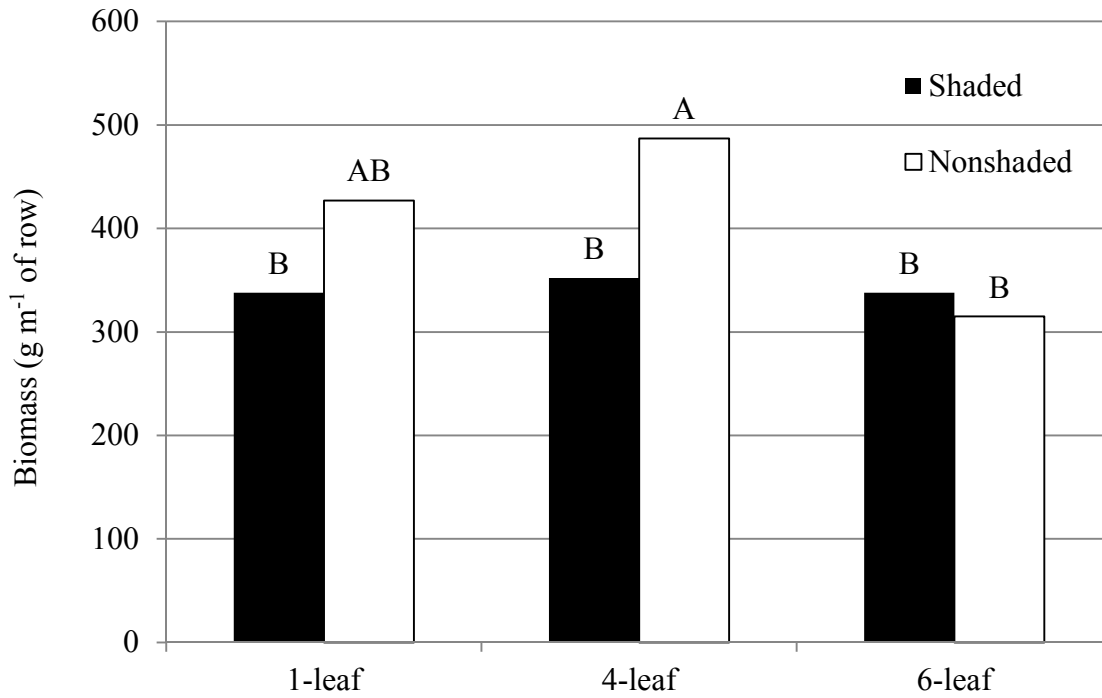


Figure 4. Cotton biomass at 12 weeks after planting from plots of various growth stages treated with various rates of glufosinate in the presence and absence of shade 3 days prior to the glufosinate application in 2012. Means are averaged over application rate and cultivar. Letters represent significant differences according to Tukey's HSD test.

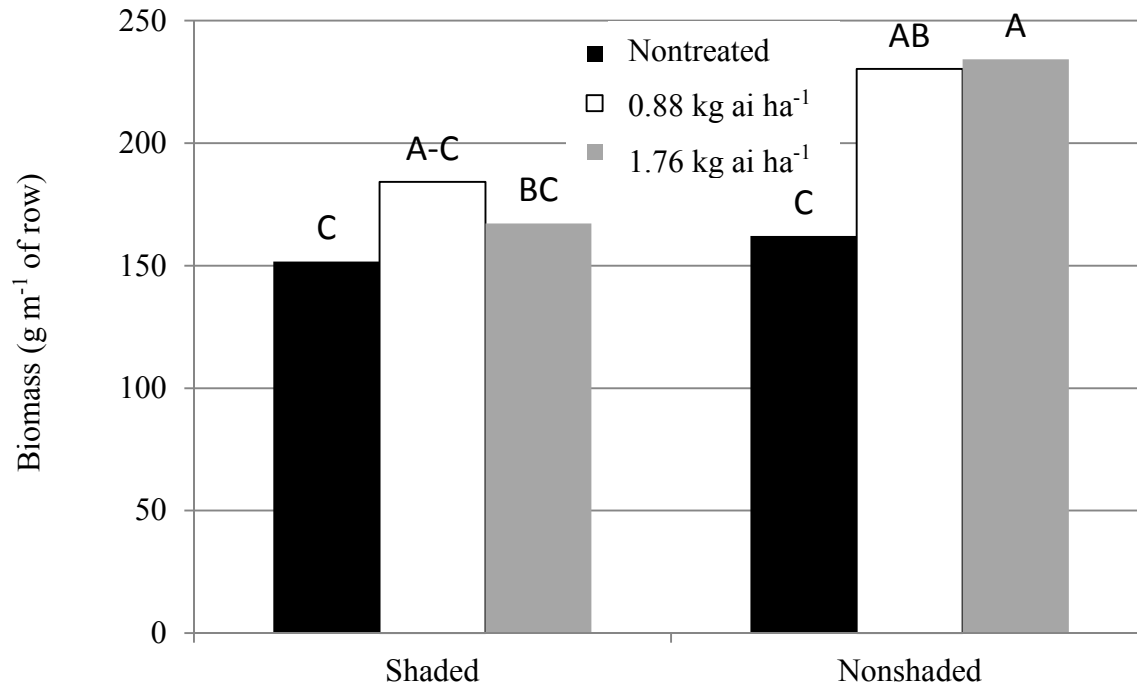


Figure 5. Cotton biomass at 12 weeks after planting from plots treated with various rates of glufosinate in the presence and absence of shade 3 days prior to the glufosinate application in 2012. Means are averaged over cotton stage at application and cultivar. Letters represent significant differences according to Tukey's HSD test.

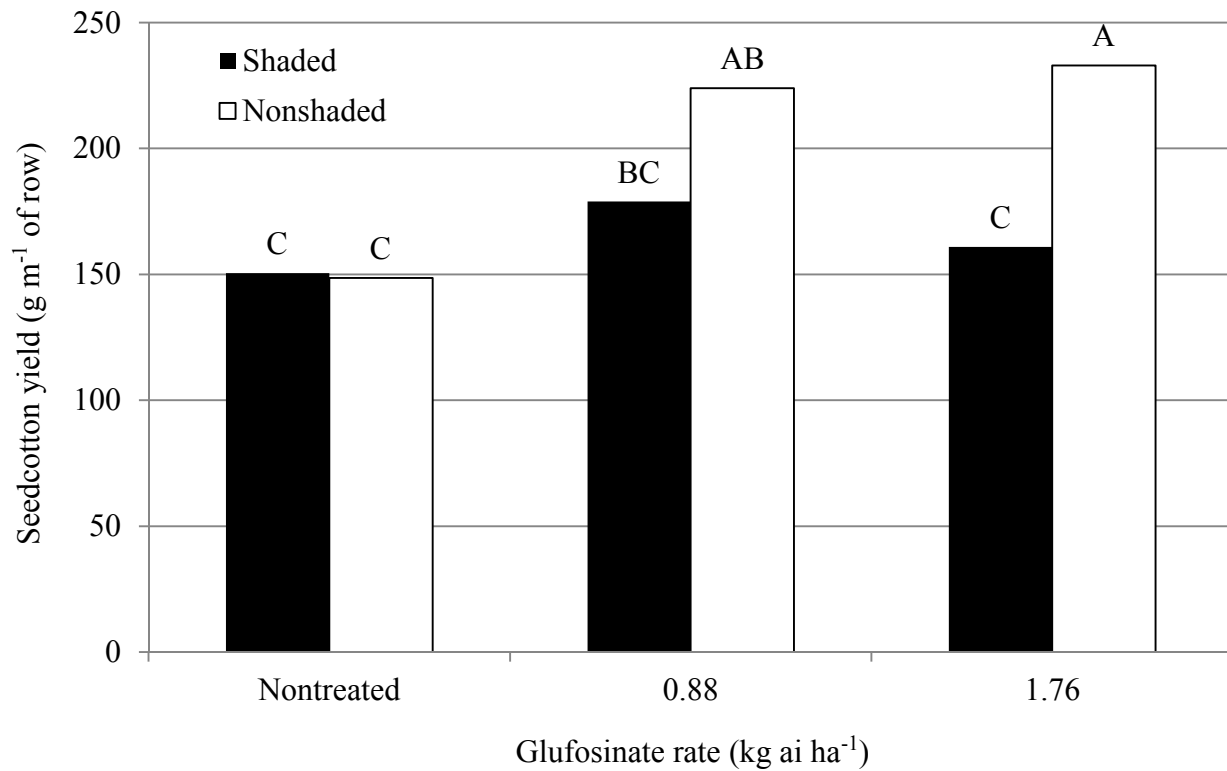


Figure 6. Seedcotton yield after early-season glufosinate applications made in the presence and absence of shade 3 days prior to application in 2012, averaged over cotton stage at application and cotton cultivar. Letters represent significant differences according to Tukey's HSD test.

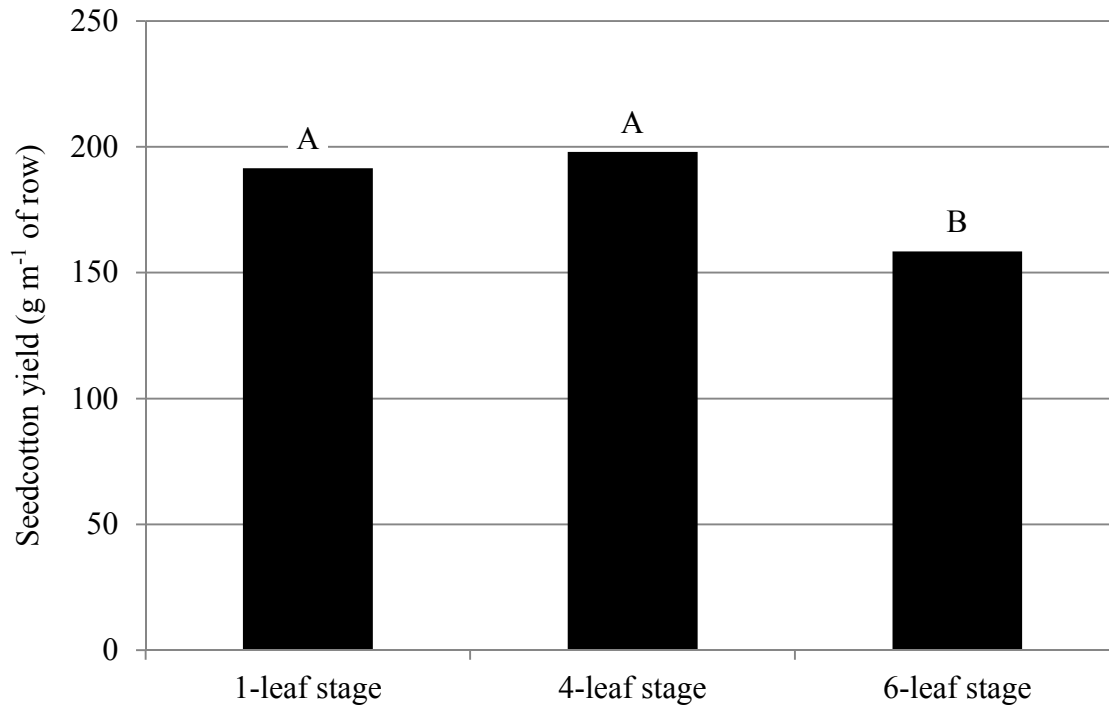


Figure 7. Seedcotton yield as influenced by cotton growth stage when treated with glufosinate in 2012, averaged over glufosinate rate, cotton cultivar, and presence or absence of shade prior to treatment with glufosinate. Letters represent significant differences according to Tukey's HSD.

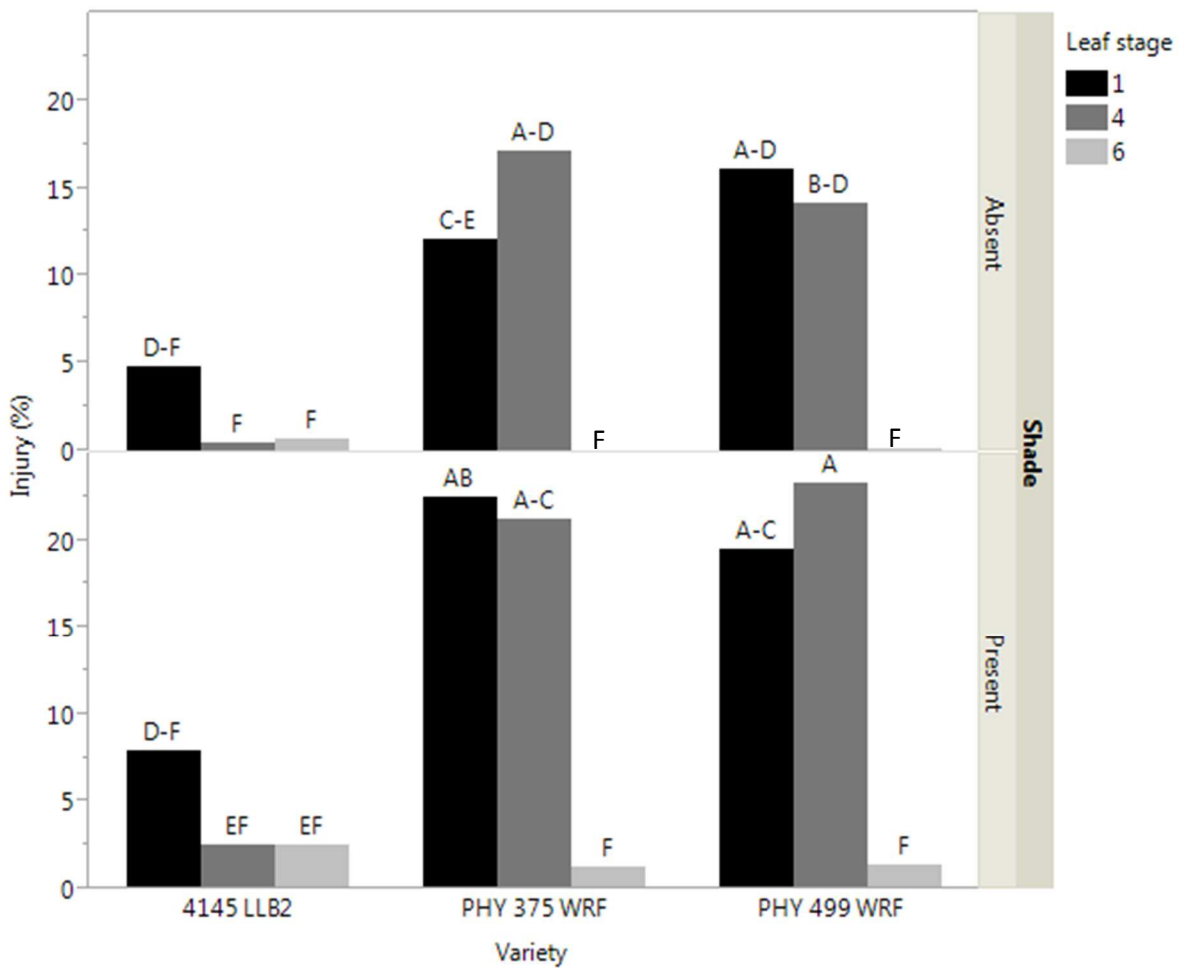


Figure 8. Cotton injury at 2 weeks after treatment in 2013 on three cotton cultivars following glufosinate applications at three different growth stages in the presence and absence of shade at 3 days prior to the glufosinate application. Means are averaged over glufosinate rates. Letters represent significant differences according Tukey's HSD test.

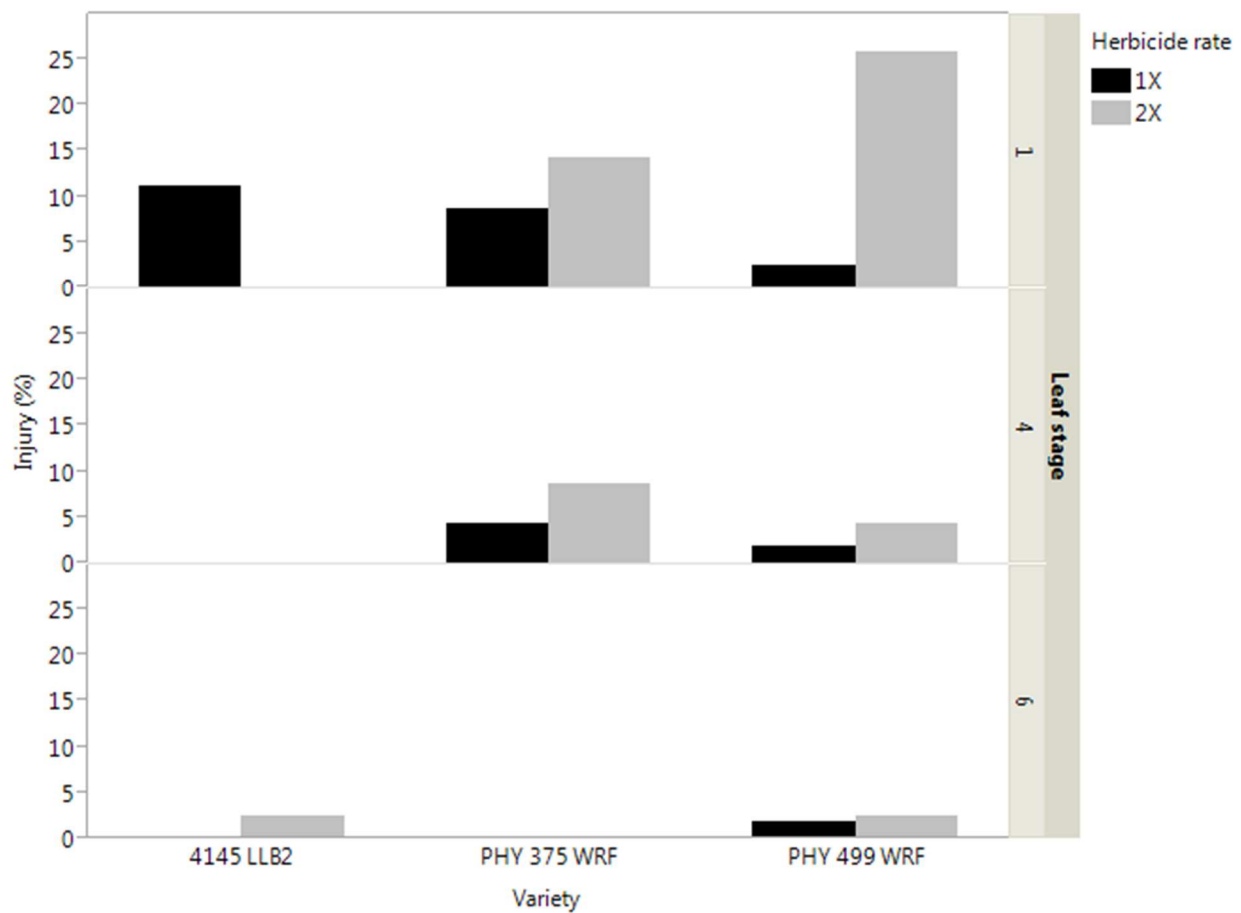


Figure 9. Cotton injury at 4 to 5 weeks after treatment of three cotton cultivars treated with glufosinate at $0.88 \text{ kg ai ha}^{-1}$ (1X) and $1.76 \text{ kg ai ha}^{-1}$ (2X) at three growth stages in 2013, averaged over the presence and absence of shade. Analysis of variance indicated the interaction to be significant; however, the conservative nature of Tukey's HSD failed to provide mean separation.

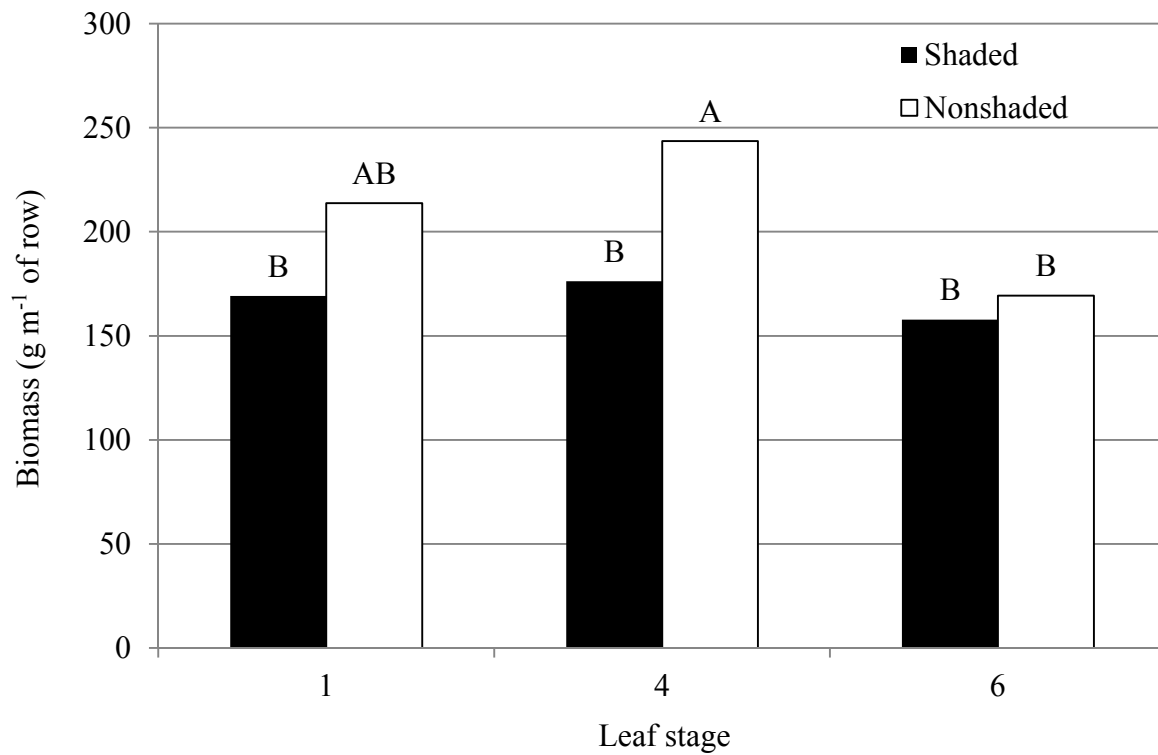


Figure 10. Cotton biomass 12 weeks after planting in 2013 from cotton treated at different leaf stages in the presence and absence of cotton. Letters represent significant differences according to Tukey's HSD test.

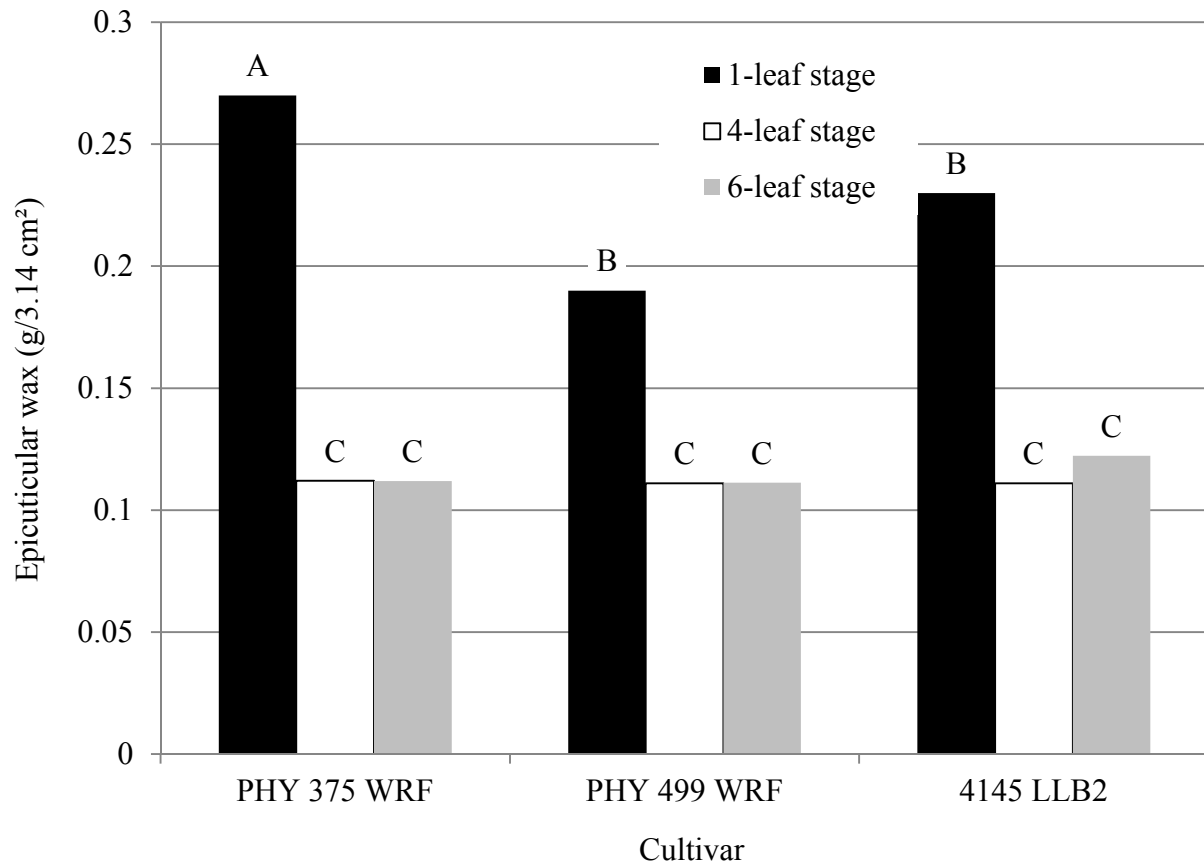


Figure 11. Epicuticular wax extracted from leaf samples collected from three cotton cultivars at time of the glufosinate application at three cotton growth stages. Letters represent significant differences according to Tukey's HSD test.

IV. Palmer Amaranth Seed Production and Height as Influenced by Emergence Date in Cotton

Abstract

With the confirmation of glyphosate-resistant Palmer amaranth in Arkansas, cotton growers have reached a new level of difficulty regarding the effective control of this weed. Rapid carbon sequestration and efficient water use make Palmer amaranth a strong competitor for nutrients in crops as it decreases water and space availability in turn reducing growth of cotton. Research was conducted in 2012 and 2013 at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas to evaluate the impact of Palmer amaranth emergence date on its biomass, height, and seed production as well as the corresponding influence on cotton biomass and yield. For each emergence date, Palmer amaranth was evaluated in the presence and absence of cotton. As Palmer amaranth emergence was delayed in cotton, the resulting seed production per female plant was reduced to a greater extent than delayed emergence in the absence of cotton. Palmer amaranth plants emerging as late as 10 weeks after cotton emergence were able to produce on average 880 seed per female plant, an amount sufficient to replenish a soil seedbank. The late emerging plants competing with cotton were smaller in size than earlier emerging plants and Palmer amaranth biomass production was correlated ($r^2 = 0.63$) with seed production in the presence of cotton. The later emerging cohorts responded to the presence of cotton by producing less biomass more so than a reduction in plant height with delayed emergence. This research shows that Palmer amaranth cohorts emerging as late as 10 weeks after cotton emergence must be removed if the goal of a weed management program is to prevent weed seed production.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats; cotton, *Gossypium hirsutum* L.

Key words: Cotton production; weed seed production; crop yield loss; weed interference

Introduction

The susceptibility of cotton to yield loss from weeds can be attributed to its relatively noncompetitive foliar canopy and slow inherent growth. Furthermore, cotton has few available herbicides compared to corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] (Pankey 2005). It has been estimated that weeds reduced the yield potential of cotton worldwide by 11.5% in 1975, whereas cotton yield and quality losses and cost of weed control in the United States in 1965 was estimated to be \$5.1 billion (Agric. Res. Serv. 1965; Parker and Fryer 1975). More recently, it was concluded in a survey published in 1992 that losses from weeds and cost of control in U.S. cotton exceeded \$15 billion annually (Bridges 1992). The cost of weed interference in U.S. cotton is likely higher today and continues to increase as a result of herbicide resistance, particularly with the ineffectiveness of glyphosate and acetolactate synthase (ALS)-inhibiting herbicides on Palmer amaranth.

Weed control has always been a crucial step in successful cotton production as problematic weed species, if not controlled, can effectively out-compete cotton for light, nutrients, space, and water. Cotton can require up to 8 weeks of weed-free maintenance to maximize yields; a great deal longer than corn and soybean (Buchanan and Burns 1970). The release of glyphosate-resistant (GR) cotton in 1997 enabled growers to make multiple over-the-top glyphosate applications, controlling a broad spectrum of weeds without disrupting the growth of the crop (Funke et al. 2006). Ultimately, the availability of the GR cotton prompted growers to widely adopt the technology because of cost savings, improved weed management, and simplicity of the system (Duke and Powles 2009). In 2000, after the loss of patent rights to glyphosate, the price of glyphosate decreased by 40% in the United States (Duke and Powles 2009; USDA 2006). The low price of glyphosate and ability to control a broad spectrum of weed

species with over-the-top applications resulted in extensive use of the herbicide. Annual weeds having high rates of reproduction were the main target for control, and sole use of the herbicide, especially early in the cropping season resulted in immense selection for herbicide resistance (Nichols et al. 2009; Neve et al. 2011). Today, there are 32 glyphosate-resistant weed biotypes worldwide and seven of these occur in Arkansas, of which Palmer amaranth is most problematic in cotton (Heap 2015; Riar et al. 2013).

Palmer amaranth is a dioecious, summer annual capable of producing over 600,000 seed per female plant (Keeley et al. 1987). It is highly competitive with crops, having been found to reduce soybean yield 68% at densities of 10 plants m⁻² (Klingaman and Oliver 1994). In cotton, for every one Palmer amaranth per 10 m of row, yield was reduced 5.9 to 11.5% at two sites in Oklahoma (Rowland et al. 1999). Additionally, its rapid erect growth and alleopathic potential directly hinder the yield potential of cotton (Morgan et al. 2001). Palmer amaranth densities of 1 to 10 plants/9.1 m of row in cotton decreased crop canopy volume 35 and 45% by 6 and 10 weeks after cotton emergence (WAE), respectively (Morgan et al. 2001). The high level of Palmer amaranth interference with cotton results in the need for effective control, even to the point of complete elimination of escape plants in cotton (Norsworthy et al. 2014).

Light is considered to have the greatest impact on cotton canopy volume, biomass, and yield when soil moisture and nutrients are not limiting (Donald 1958; Morgan et al. 2001). The rapid erect growth of Palmer amaranth can result in individuals reaching over 2 m in height, leaving little doubt that cotton in close proximity could experience decreased cotton yield via shading (Rowland et al. 1999; Keeley et al. 1987).

New herbicide chemistry is limited as industry research and development efforts slowed following the release of GR crops. While great attention has been focused on redeveloping

existing technologies, the use of integrated weed management (IWM) strategies has also gained momentum. In 2012, best management practices (BMPs) were put forth for addressing the ever increasing occurrence of herbicide-resistant weeds (Norsworthy et al. 2012). Diversity in weed management strategies was highlighted as a means to reduce the risk of herbicide resistance. Understanding the biology of the targeted weed is of utmost importance in best designing resistance management strategies and essential for modeling the evolution of herbicide resistance. The best management strategies to mitigate herbicide resistance encourages attention to weed biology and ecology; namely, seed production, growth potential, and overall competitiveness in a given crop (Bagavathiannan et al. 2012). Weed seed production and biomass are highly dependent upon time of emergence with a crop and weed and crop density or configuration of the spacing among crop plants (i.e., impacted by seeding rate and rows spacing) (Murphy et al. 1996; Knezevic and Horak 1998; Clay et al. 2005). Previous research shows that as emergence date becomes later in the growing season, plant fecundity decreases (Knezevic and Horak 1998; Clay et al. 2005). Continued exploration of weed biology and ecology benefits cotton producers striving to quantify the competitive interactions of cotton and Palmer amaranth with varying environments and agronomic scenarios (Clay et al. 2005; Van Acker 2009; Gressel 2011; Uscanga-Mortera et al. 2007). Hence, the objective of this research was to determine to what extent emergence date of Palmer amaranth in cotton affects its height, biomass, seed production and its resulting effect on cotton biomass and seedcotton yield.

Materials and Methods

In 2012 and 2013, a field experiment was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas as a randomized complete block with a

factorial treatment structure. Cotton cultivar PHY 375 WRF (Dow AgroSciences, Indianapolis, IN, USA) was planted at a 2-cm depth into a Leaf silt loam soil (Fine, mixed, active, thermic Typic Albaquults with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.9) (USDA-NRCS 2015) at 125,000 seed ha⁻¹ and supplemented with over-head sprinkler irrigation to maintain optimal growing conditions. Planting occurred on June 1, 2012 and May 15, 2013 using a John Deere 6403 medium-frame tractor and John Deere 7100 four-row planter (Deere & Company, Moline, IL, USA). Twelve treatments included various planting dates of glyphosate-resistant Palmer amaranth seed both in and out of competition with cotton on a 92 cm row spacing. Approximately 20 Palmer amaranth seeds were hand planted in close proximity to the inner two rows (<13 cm from row center) of four row plots approximately 4 d after seeding cotton in order for Palmer amaranth emergence to coincide with cotton emergence. Cotton emerged on June 5, 2012 and May 23, 2013 and was shortly thereafter removed in one treatment of each of the six Palmer amaranth emergence dates (0, 2, 4, 6, 8, and 10 weeks after cotton emergence). Removal of cotton in one-half of the plots allowed for the effect of cotton on Palmer amaranth to be assessed, accounting for the delayed emergence of cohorts after typical planting of cotton. Palmer amaranth seedlings were manually thinned to 1 plant per m⁻¹ of row within 2 weeks after emergence, resulting in a final density of 1.1 plants m⁻² competing with the two innermost rows of cotton in each plot.

A known glyphosate- and trifloxysulfuron-resistant Palmer amaranth biotype was used in the plots, which allowed for use of glyphosate (Roundup PowerMax[®], Monsanto, St. Louis, MO, USA) and trifloxysulfuron for control of unwanted weeds. Additionally, clethodim was used later in the growing season to remove grasses and some unwanted Palmer amaranth plants were hand removed throughout the season to promote as close of a weed-free environment as possible.

Only slight injury to Palmer amaranth was observed following any of the herbicide applications and the plants had often fully recovered by 2 to 3 weeks after treatment. All applications were made using a CO₂-pressurized backpack sprayer equipped with four TTI 110015 nozzles (Teejet Technologies, Springfield, IL, USA) calibrated to deliver 187 L ha⁻¹ at a pressure of 276 kPa and a walking speed of 4.8 kph.

Prior to cotton defoliation each fall, the height of three Palmer amaranth and three cotton plants in each plot were measured and aboveground biomass of all existing Palmer amaranth plants were harvested for biomass determination. Palmer amaranth biomass was placed in individual bags and oven dried at 66 C and then weighed. The inflorescence of the female plants was then removed and threshed to determine seed production per plant. Seed production was determined by counting the number of seed in four 100 g samples of threshed seed and then extrapolating for the mass of the entire sample. Following cotton defoliation, seedcotton was harvested from 4 m row⁻¹ of the two center rows of each treatment and weighed.

All data were subjected to ANOVA using JMP PRO 11 (SAS Institute Inc., Cary, NC, USA) (Table 1). Data were square-root transformed to meet normality assumptions for Palmer amaranth height, seed production, and biomass, and in all cases, replications were nested within years and ran as a random effect. Nonlinear models were established based on the best pseudo-R² value ($\text{Pseudo-R}^2 = 1 - \text{SS}(\text{Residual}) / \text{SS}(\text{Total}_{\text{Corrected}})$) (Chism et al. 1992). Based on this process, a two parameter exponential decay model was utilized ($Y = a * \exp(-b * x)$) to describe end-of-season Palmer amaranth biomass over the evaluated cohorts. The interacting effect of cotton competition and year proved to significantly impact Palmer amaranth height and a mixed model with an effect leverage personality under a residual maximum likelihood (REML) was utilized. This analysis is comparable to ProcMixed GLM in SAS (Statistician, Dr. Weisz, NC

State University, personal communication). Means were separated using Tukey's HSD at the alpha level of 0.05. Transformation of data achieved homoscedasticity for linear regression methods and fitted equations with associated pseudo- R^2 values were presented. Years were not pooled in regards to the interaction of Palmer amaranth biomass and seed production. For Palmer amaranth biomass and seed production, a bivariate fit was constructed blocking for replication and applying year as a by-variable for the associated linear equation, $Y = ax + b$, and pseudo- R^2 values. All figures were constructed in SigmaPlot (Systat Software, Inc., San Jose, CA, USA).

Results and Discussion

The establishment of successful IWM requires an understanding of the biology of major weed species. In current Midsouth cotton production, this directly relates to Palmer amaranth as a major competitor for light, space, water, and nutrients. Averaged over 2012 and 2013, Palmer amaranth end-of-season height and biomass production were significantly impacted by the interaction of Palmer amaranth emergence date in the presence and absence of cotton (Table 1). Of the three Palmer amaranth parameters measured, only end-of-season height was influenced by the effect of year.

Cotton Height and Seedcotton Yield. Cotton height was significantly greater in 2013 than in 2012 (Table 1). In 2012, there was a 4- to 5-week period when the overhead irrigation system was not functioning (Figure 1), which likely contributed to reducing cotton heights in addition to the interference imposed by Palmer amaranth. The greater rate of seedcotton yield loss as a function of Palmer amaranth emergence date in 2012 than in 2013 may partially be a result of the drier conditions in 2012. However, Palmer amaranth emergence date did not interact with year

nor did the main effect of Palmer emergence date relative to cotton impact cotton height.

Conversely, the interaction of Palmer amaranth emergence date and year did interact in regards to seedcotton yield.

For both years, seedcotton yield declined as Palmer amaranth emergence date occurred earlier in the year relative to that of cotton (Figure 2), illustrating the impact of early-season emergence on Palmer amaranth competitiveness with cotton and in turn reduction in seedcotton yield. This relationship of competition is well documented (Rowland et al. 1999; Dowler 1995; Ehleringer 1983; Jha et al. 2008; Menges 1987, 1988; Morgan et al. 2001). At a density of 1.1 Palmer amaranth plants m^{-2} , seedcotton yields increased by 487 kg ha^{-1} for every week delay in Palmer amaranth emergence through 10 weeks after cotton emergence in 2012. At the same density in 2013, seedcotton yields were less impacted by Palmer amaranth emerging in cotton; hence, seedcotton yields were improved only 278 kg ha^{-1} for each week delay in weed emergence relative to the crop. Webster and Grey (2015) conducted a closely related experiment in Georgia on Coastal Plain soils in 2011 and 2012. They concluded that there was a log-logistic relationship between seedcotton yield loss and relative timing of Palmer amaranth establishment—beginning with a 67% seedcotton reduction when Palmer amaranth was established at cotton planting at a density of 0.42 plants m^{-2} . It was evident in both studies that delayed emergence resulted in higher seedcotton yields.

Palmer Amaranth Height and Biomass. The presence of cotton had a greater impact on Palmer amaranth end-of-season height averaged over emergence dates in 2012 than in 2013 (Figure 3). Palmer amaranth heights averaged over emergence dates were similar in 2012 and 2013 in the absence of cotton ranging from 119 to 123 cm. The lack of irrigation for a short period in 2012 may have enhanced the level of interference between cotton and Palmer amaranth, but in the

absence of cotton, the drier conditions did not influence Palmer amaranth height. Furthermore, the spring of 2012 was uncharacteristically warmer than normal, which may have added early-season growth of cotton, resulting in greater suppression of Palmer amaranth. The fact that these plots were oversprayed with glyphosate and trifloxysulfuron during the growing season and transient injury was sometimes observed may have contributed to the heights being lower than that reported in other research. For instance in Kansas, Palmer amaranth heights ranged from 174 to 231 cm when grown without crop competition at a density of one plant per 0.76 m² of row (Horak and Loughlin 2000). Furthermore, the cool growing conditions at Fayetteville, AR are likely to have a greater impact on growth of Palmer amaranth, a C₄ plant that normally thrives under hot, dry conditions. Trends for Palmer amaranth height in Fayetteville experiments complimented those conducted by others. Hartzler et al. (2004) determined that a linear decline in plant height existed as common waterhemp (*Amaranthus rudis* Sauer) emergence date became later in soybean.

Biomass of Palmer amaranth was significantly reduced when grown in the presence of competition with cotton (Table 1). Just as competition for light could have been the deciding factor in regards to significant differences in Palmer amaranth height so too was the case of biomass. Year did not significantly impact Palmer amaranth growth and competition became more intense as emergence date of Palmer amaranth became later in the year (Figure 4). Those plots not burdened with cotton presence maintained similar heights throughout the year. Similar to the findings of Uscanga-Mortera et al. (2007), there was a significant exponential decay associated with weed biomass as emergence date became later in the year (Figure 5). In Georgia, it was found that early emerging Palmer amaranth (comparable to 2 to 4 WAE) growing in competition with cotton produced 29 and 40% less biomass compared to the absence of cotton

(Webster and Grey 2015). Palmer amaranth plants did not display appreciable phototropism as heights decreased linearly as weed emergence was delayed relative to the crop, and biomass per plant was likewise reduced with delayed emergence, even in the absence of cotton competition.

Palmer Amaranth Seed Production. Palmer amaranth emerging as late as 10 WAE was still able to produce 880 seed female plant⁻¹ (Figure 6). This displays the weed species' ability to reach reproductive maturity and disperse viable seed rather quickly even under the reduced light quantity imposed by the existing cotton crop. The decreasing day lengths of late-emerging weeds could result in the hastening of flowering similar to the findings of Bagavathiannan et al. (2015) and Keeley et al. (1987). Palmer amaranth seed production was found to be highly correlated with plant biomass (Figure 7). Biomass and associated seed production were significantly reduced when in competition with cotton in both 2012 and 2013 field studies complimenting existing research (Keeley et al. 1987; Webster and Grey 2015). The correlation between biomass and seed production, irrespective of cotton presence, allows late-emerging Palmer amaranth to produce viable seed. This is similar to the findings of Uscanga-Mortera et al. (2007) regarding common waterhemp (*Amaranthus tuberculatus*) fecundity in corn. Seed production per Palmer amaranth female averaged considerably less in all treatments compared to Keeley et al. (1997), Webster and Grey (2015), and MacRae et al. (2013). This is most likely attributed to the cooler, finer textured soils in Northwest Arkansas compared to other cotton producing regions of the United States. Additionally, the glyphosate and trifloxysulfuron applications and the transient injury following these applications may have contributed to the lower seed production in this research. The similarities in response to delayed Palmer amaranth emergence found between this research and those of Webster and Grey (2015) suggest that

herbicide-resistance in Palmer amaranth has initiated a need for IWM tactics (Norsworthy et al. 2012).

Practical Implications and Conclusions. Palmer amaranth seed production decreases as emergence occurs later relative to cotton. Late-emerging Palmer amaranth, though lesser in biomass and less prolific, can still produce viable offspring that can result in failure to maintain a static seedbank. A 50% annual recruitment (Keeley et al. 1987) supplemented by an estimated 33 to 55% female birth rate (Smith and Norsworthy, unpublished observation; Keeley et al. 1987), suggests that even minimal escapes can become detrimental to fields where cotton is grown. The delayed emergence of Palmer amaranth can also simulate the premature loss of herbicide efficacy as concluded by Culpepper et al. (2013) and even in the absence of significant seedcotton yield reduction; it is the recommendation of current BMPs and findings from this research that producers make every effort to control or remove Palmer amaranth throughout the season in Midsouth cotton production.

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Table 1. Effects tests for the impact of Year, Palmer amaranth emergence date relative to cotton (WAE), and presence and absence of cotton (Cotton) on Palmer amaranth end-of-season height, aboveground biomass, seed production per female plant, and cotton end-of-season height and seedcotton yield.

Source	Palmer amaranth			Cotton	
	Height ^a	Biomass ^b	Seed production ^c	Height ^d	Seedcotton yield
	Prob > F ^e				
Year	0.0351	0.2092	0.5872	0.0007	0.0022
WAE ^g	<.0001	<.0001	<.0001	0.1936	<.0001
Year*WAE	0.7906	0.4794	0.8649	0.0702	0.0022
Cotton	<.0001	0.9560	0.0022	-	-
Year*Cotton	0.0203	0.3444	0.4356	-	-
WAE*Cotton	<.0001	0.0038	0.3839	-	-
Year*WAE*Cotton	0.2634	0.6119	0.5678	-	-

^a Palmer amaranth height was measured at 17 weeks after cotton emergence

^b Aboveground Palmer amaranth biomass collected 17 weeks after cotton emergence, oven-dried, and weighed

^c Seed production per female Palmer amaranth plant collected immediately prior to defoliating cotton

^d Height of cotton at 17 weeks after emergence

^e Source values less than 0.05 are statistically significant

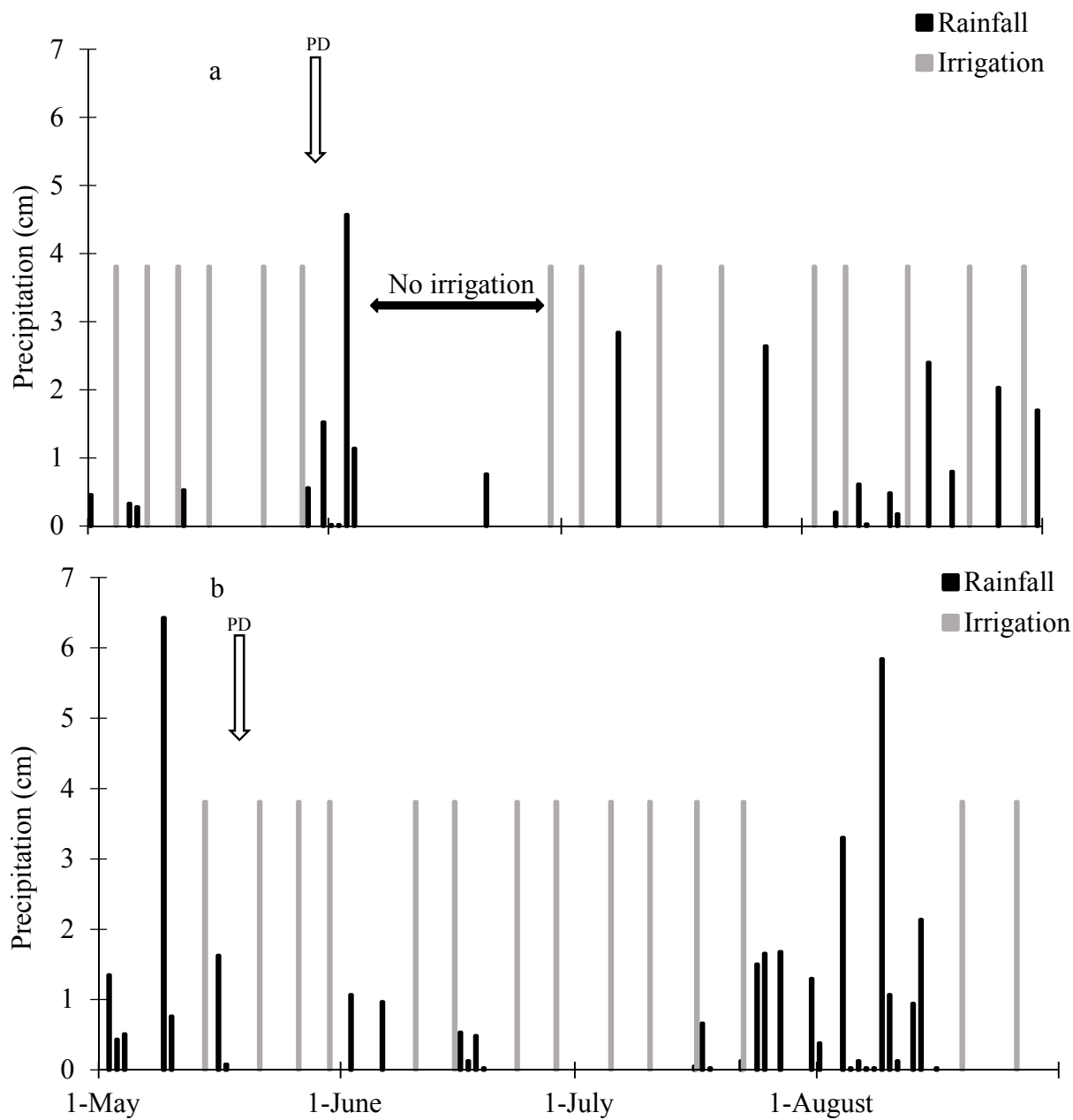


Figure 1. Rainfall and irrigation distribution at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in 2012 (a) and 2013 (b) with respective planting dates (PD).

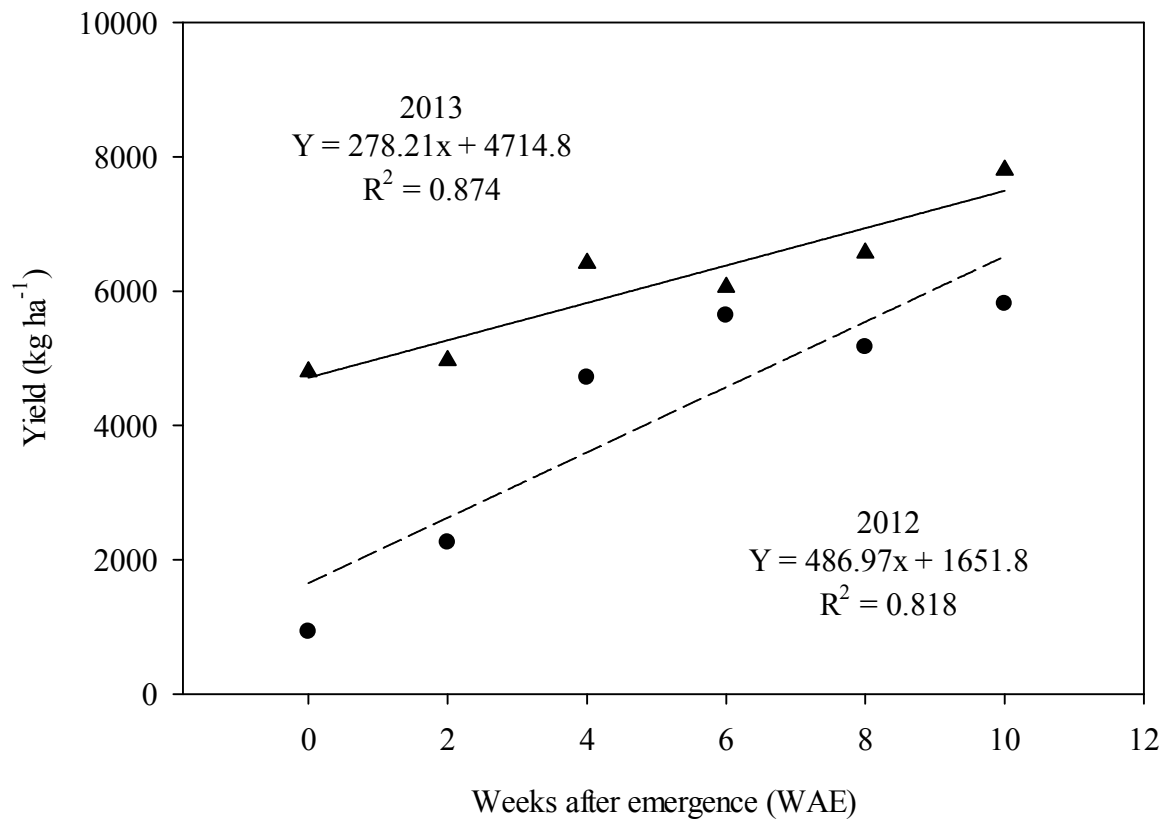


Figure 2. Seedcotton yield in 2012 and 2013 at the Arkansas Agricultural Research and Extension Center. Significance interaction of WAE and year was achieved using a mixed model in JMP Pro 11. Under a standard least squares personality, the REML method conceived linear regression emphasizing effect leverage.

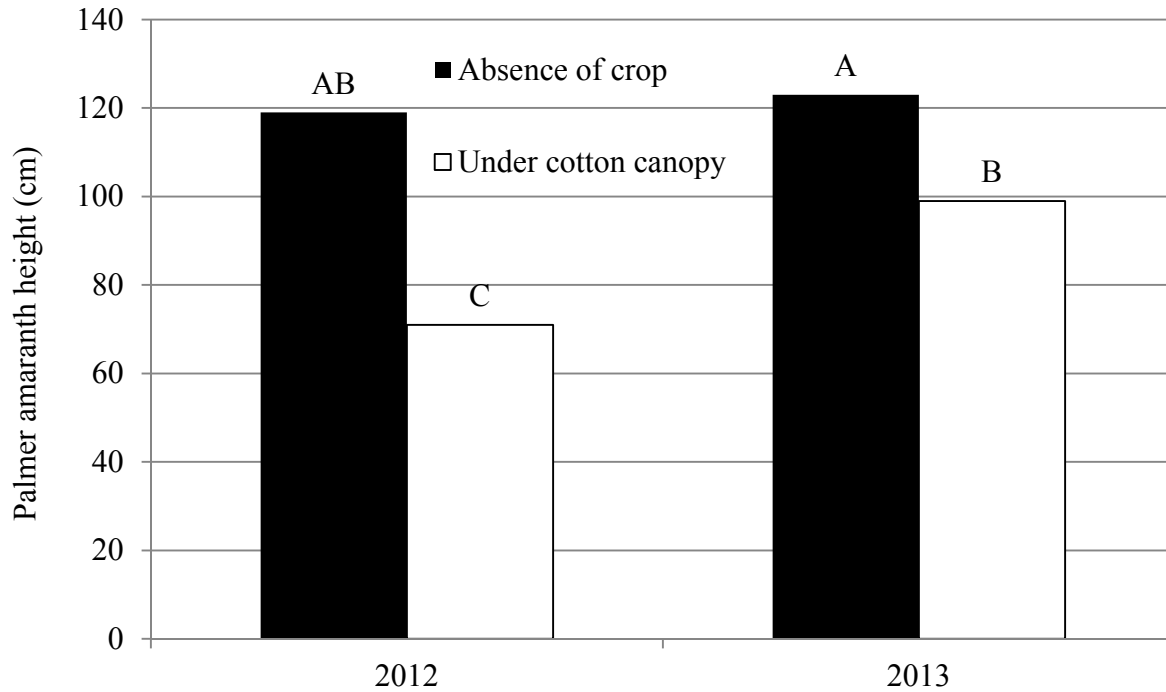


Figure 3. Palmer amaranth heights at 17 weeks after cotton emergence at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in 2012 and 2013 in the presence and absence of cotton, averaged over emergence cohorts (weeks after cotton emergence). A mixed model emphasizing effect leverage, REML method, and standard least squares personality presented significance with means separated by Tukey's HSD at the alpha level of 0.05.

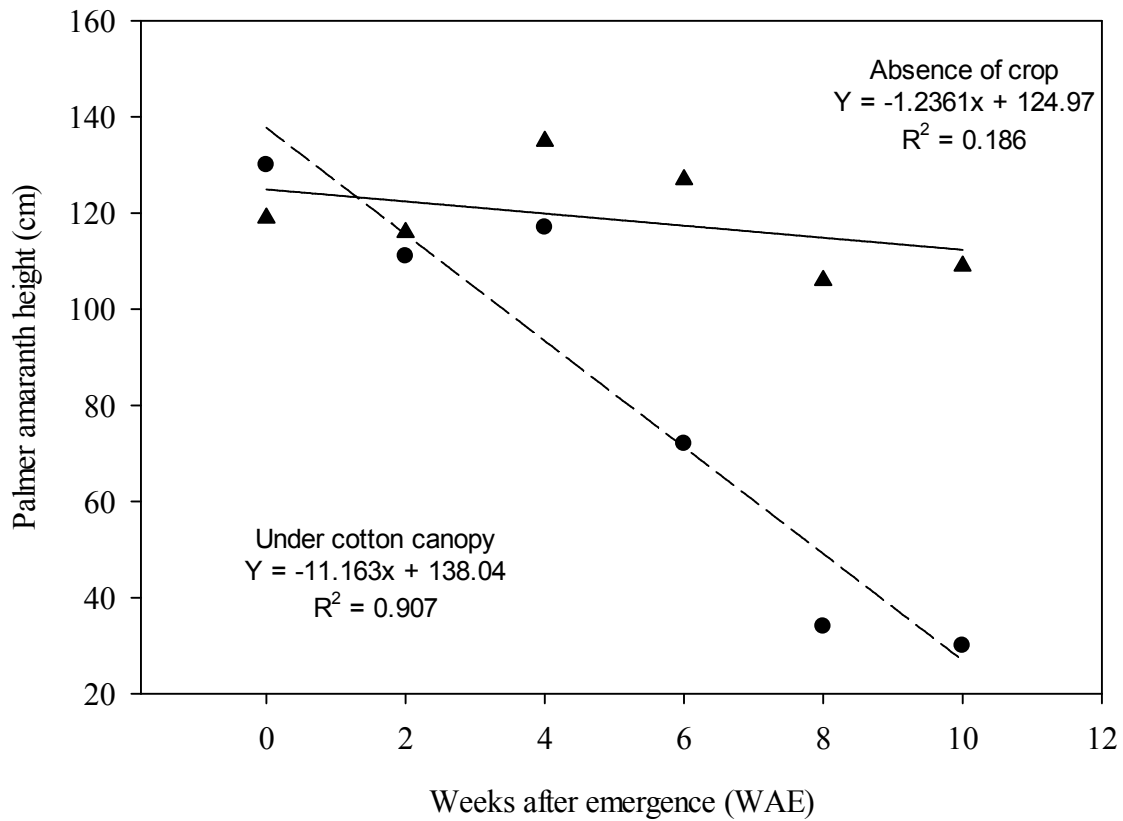


Figure 4. Palmer amaranth heights at 17 weeks after cotton emergence at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in the presence and absence of cotton competition as a function of Palmer amaranth emergence date relative to cotton (x-axis; WAE). Data was pooled over years and analysis conducted as a mixed model in JMP Pro 11. Effect leverage emphasis under the REML method provided linear regression.

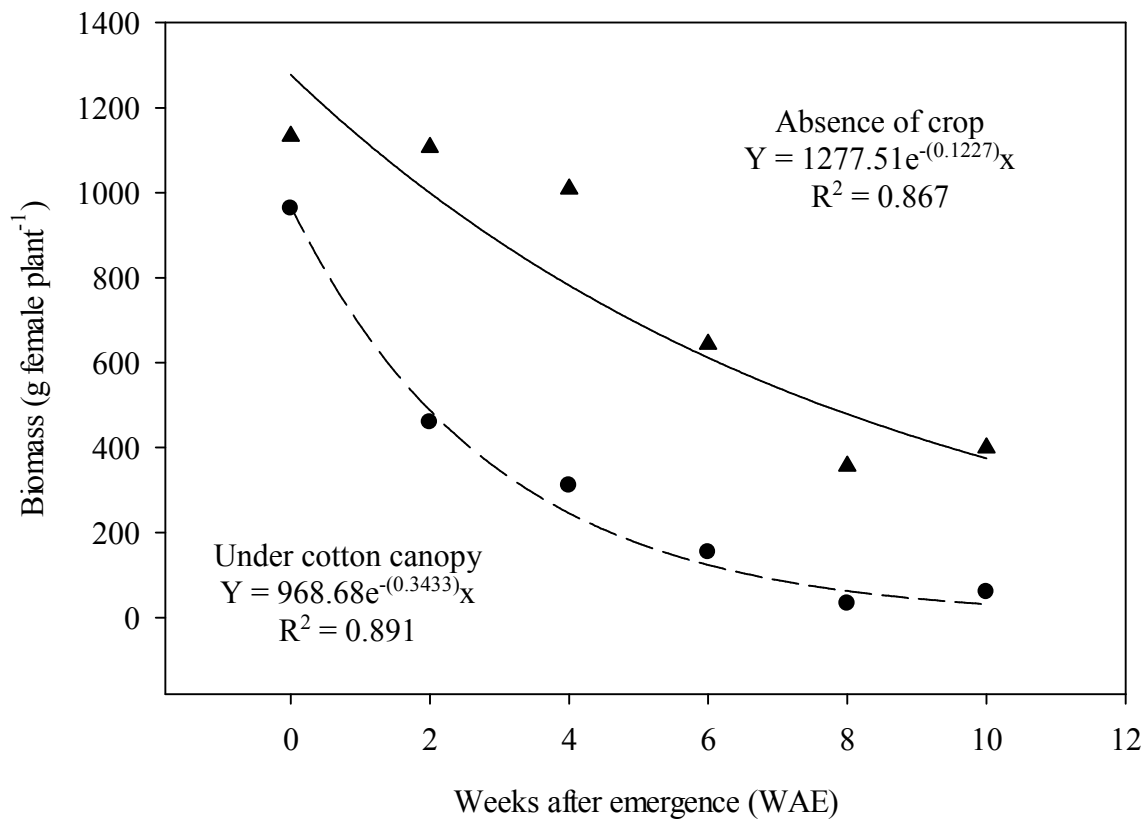


Figure 5. Amount of biomass collected from female Palmer amaranth plants within cotton and noncrop plots that were planted at different timings following the emergence date of cotton at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas. Mixed model analysis in JMP Pro 11 fitted for an exponential decay function; data were pooled over 2012 and 2013.

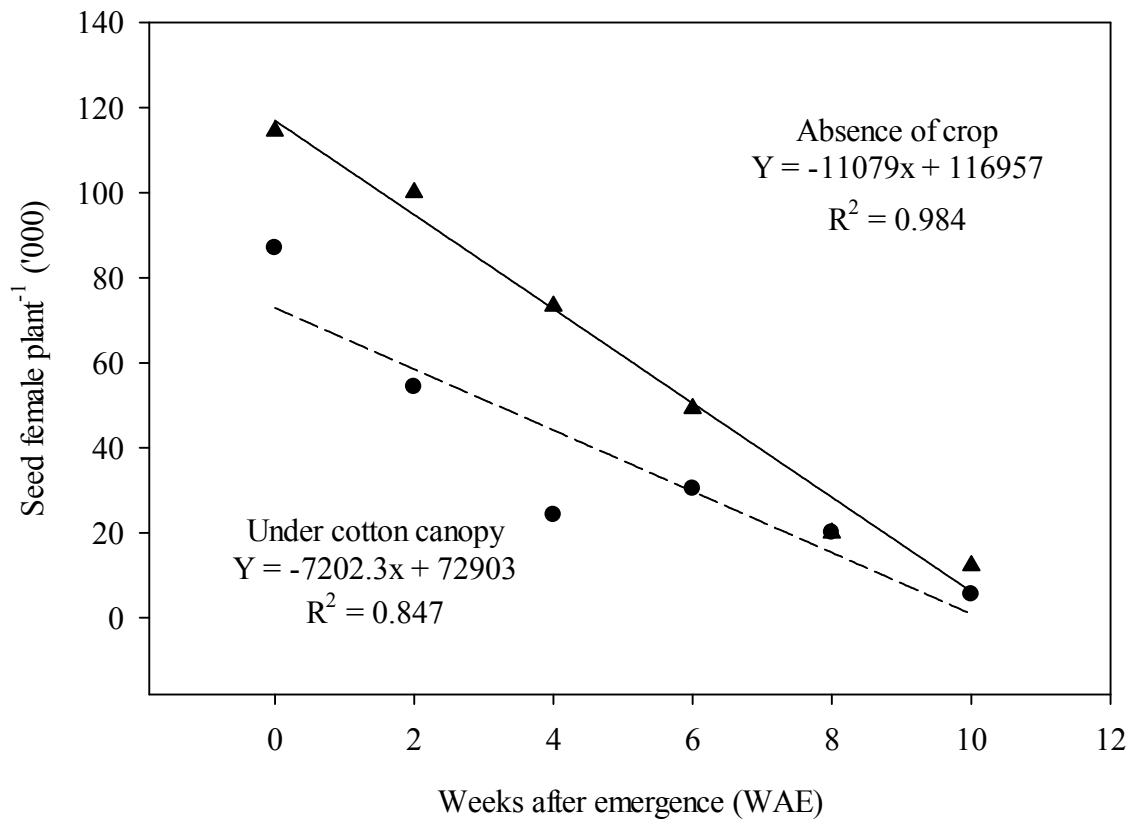


Figure 6. Number of Palmer amaranth seeds produced per female plant as a function of Palmer amaranth emergence dates at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in 2012 and 2013. A mixed model was utilized in JMP Pro 11. Under a standard least square personality the REML method conceived linear regression emphasizing effect leverage.

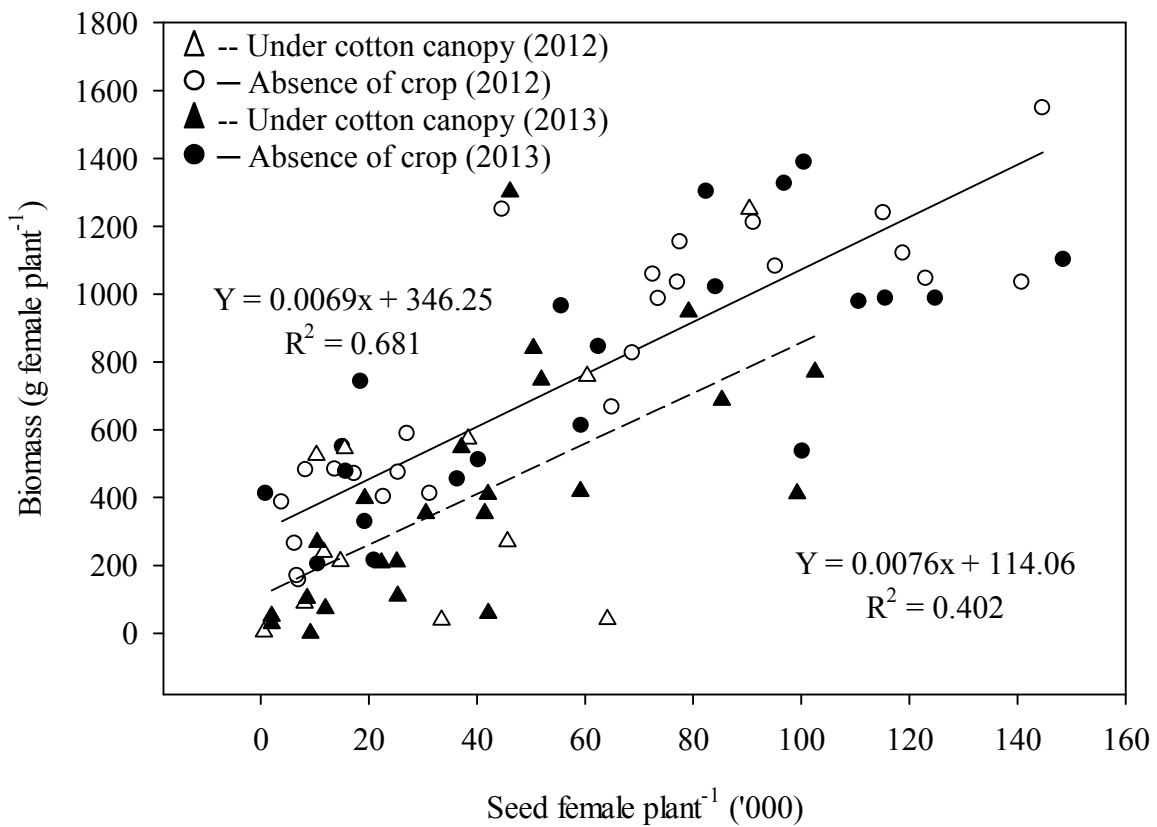


Figure 7. Relationship between Palmer amaranth biomass and seed production at the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in 2012 and 2013. Analysis achieved with a bivariate fit conceived in JMP Pro 11; blocking for replication and utilizing a by-variable (year).

V. Conclusions

Successful production of cotton in the Midsouth now involves utilizing integrated weed management (IWM) to control increasing populations of glyphosate-resistant (GR) Palmer amaranth. Resistant biotypes can hinder production by competing for light, water, nutrients, and space. The ineffectiveness of certain postemergence (POST) herbicide options prompted the exploration of soil-applied herbicides and research evaluating how they interact as a function of environmental, agronomic, and genetic factors.

There was little conclusive evidence involving planting depth as variability existed between years and locations albeit planting at shallow depths might limit negative effects presented by cool, wet conditions. There was a tendency for cotton to better tolerate soil-applied herbicides when seed size increased (>11 g/100 seed). Increased seed size and associated seed vigor was shown to improve cotton tolerance and cotton biomass production following the application of preemergence (PRE) herbicides.

Additionally, glufosinate has been shown to successfully control GR weeds as a POST option when applied at appropriate times and rates. Glufosinate applied during times of high photosynthetic activity, such as absence of cloudy conditions, can reduce potential injury issues and optimize yield. Having secondary POST options can assist cotton growers with controlling escapes that would otherwise compete with cotton and produce viable seed.

This research finds that in the era of weed resistance to glyphosate and other mechanisms of action, season-long control is essential. Even minimal escapes, which might not hinder cotton production, can produce viable offspring leading to failures in maintaining a static seedbank. The prolific nature of Palmer amaranth and its ability to produce seed under full cotton canopy late in the growing season can mean increased difficulty in future years of production.

It is the recommendation of best management practices and findings from this research that IWM be implemented in Midsouth cropping systems. Understanding how various soil-applied herbicides interact with specific climatic conditions and soil textures can add control options to herbicide programs. Using high-vigor seed can likewise prevent potential injury from soil-applied herbicides. Glufosinate, when applied appropriately, can assist in the control of GR Palmer amaranth and fields should remain free of this weed throughout the growing season.