Evaluation of Rescue Applications on Glyphosate-Resistant Palmer Amaranth

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By

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Options for glyphosate-resistant (GR) Palmer amaranth [*Amaranthus palmeri* (S. Wats)] control are becoming limited. Research was conducted in 2014 and 2015 to evaluate the effectiveness of rescue herbicide applications on glyphosate-resistant Palmer amaranth. Research was established to evaluate efficacy provided by new and current herbicide programs on GR Palmer amaranth that was larger than recommended at the time of herbicide application. Studies included a postemergence application of different herbicides used singly and in combination at different initial application timings; sequential postemergence application timing evaluating herbicide tank mix combinations at five different time intervals between applications; and postemergence evaluation of herbicide tank mix combinations at multiple application timings.

DEDICATION

I would first like to dedicate this research to my parents, Boyette and Melissa Denton, and siblings, Lindsey, Natalie, and Blake Denton. The immense support and competiveness provided by my family has always allowed me to strive for more in life and achieve my goals. I would also like to dedicate this research to my grandparents, Dorris Mott, Rosemary Denton and in particular, my grandfathers, Huey Mott and the late Morris Denton. I am very thankful for the work ethic displayed by these two men and am privileged to have learned so much over the years about being a genuine individual. Lastly, I would like to dedicate this research to all of my friends and colleagues who have accompanied and supported me throughout this process.

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CHAPTER I

INTRODUCTION

Cotton is a perennial shrub native to a semi-desert habitat that requires warm temperatures and little water for optimum growth. Commercial cultivars are grown as a pseudo-annual shrub (Chaudhry and Guitchounts 2003). There are five different types of cotton species in the world with *Gossypium hirsutum* (L.) or Acala cotton, being the most popular. Upland cotton was originally selected from germplasm found in Mexico and is the primary species of cotton grown in Mississippi. Soil temperatures must be at least 16°C for seed germination and at least 50 heat units are necessary for successful seed germination and seedling emergence. Heat units or growing degree days, utilizing temperature rather than age, are used to describe growth and development of cotton. Cotton requires a minimum temperature of 60°F and DD_{60}'s are determined by adding the maximum and minimum daily temperatures (°F), dividing by 2, and subtracting the minimum threshold temperature (60°F) (Chaudhry and Guitchounts 2003).

$$DD_{60} = \frac{(°Fmax + °Fmin)}{2} - 60 \qquad (1.1)$$

Approximately five days pass from planting to seedling cotton emergence (Ritchie et al. 2008). Generally, 35 to 38 days are needed to reach pinhead square and an additional 21 days to reach first white bloom from pinhead square (Chaudhry and Guitchounts 2003; Ritchie et al. 2008). An additional 40 to 60 days generally pass from first white bloom to first open boll. Heat units needed to achieve corresponding growth stages are given in Table 1.1 (Ritchie et al. 2008).

Table 1.1 Growth stage of cotton with corresponding heat units.

Growth Stage	Heat Units
Planting to seedling establishment	50-60
Emergence to first square	425-475
First square to first white flower	300-350
Planting to first flower	775-850
First white flower to first open boll	850
Planting to harvest	2300

Cotton has an indeterminate growth habit which can result in very tall or rank growth under optimum growing conditions (Ritchie et al. 2008). Gowers manage excess vegetative cotton growth with plant growth regulators (PGR) such as mepiquat chloride (Chaudhry and Guitchounts 2003; Ritchie et al. 2008). Rank growth is commonly associated with excess foliage or vegetative growth due to excessive fertilizer (nitrogen) and/or fertile soils. Excessive vegetative growth can promote boll rot and fruit abscission and make cotton difficult to defoliate and harvest (Chaudhry and Guitchounts 2003; Siebert et al. 2006). Monopodial or vegetative branches generally do not bear fruit and are found on the first few nodes of the plant (Chaudhry and Guitchounts 2003). Plant spacing and variety characteristics can influence the number of vegetative branches with plants grown closer together having reduced number of vegetative braches. Sympodial or fruiting branches bear fruit and develop after vegetative branches. Sympodial branches generally start on node five or six (Ritchie et al. 2008).

The cotton floral bud goes through several stages. Typically, 35 to 38 days from planting are required for pinhead squares to appear (Ritchie et al. 2008). After pinhead square, match-head squares appear and prior to bloom the square forms a candle shape (Chaudhry and Guitchounts 2003). The first flower will appear approximately 21 to 28 days after the first square and flowering generally lasts four to six weeks depending on environmental conditions (Ritchie et al. 2008). The bloom process takes several days and can be identified by distinct characteristics. The day the flower opens it is white and pollination occurs within a few hours. The flower will turn pink on day two, red on day three, subsequently drying and falling off and exposing a developing boll. From this point forward, cotton development is referred to in terms of nodes above white flower (NAWF) and once blooming begins there are usually nine to ten NAWF.

Boll development begins immediately after pollination and approximately 50 days pass from pollination to open boll (Ritchie et al. 2008). Cut-out occurs when cotton growth shifts from vegetative to reproductive and the rate of dry matter accumulation equals the growth rate of the crop (Chaudhry and Guitchounts 2003). As this happens, all photosynthates are channeled to existing bolls and new fruit typically sheds. Boll development occurs in phases which include: enlargement, filling, and maturation (Ritchie et al. 2008). The enlargement phase occurs when fibers are being produced on the seed after which time they elongate and fill the area within the boll. During the filling phase, elongation stops and cellulose is deposited inside the elongated fiber. The fiber in both the enlargement and filling phase is very sensitive to adverse environmental conditions. Maturation occurs when the boll has achieved maximum size and weight capacity (Main 2012). At this time, seed and fiber have reached maturity and the capsule walls of the boll dry and shrink resulting in boll opening. One of the final management practices performed prior to harvest is defoliation. Defoliants, or harvest aids, are used to defoliate the cotton plant and enhance boll opening (Ritchie et al. 2008). These products give the producer more control over harvest timeliness and efficiency.

Palmer amaranth

Palmer amaranth [*Amaranthus palmeri* (S. Wats)], is a fast growing broadleaf weed that is very problematic in agriculture throughout the Mid-South and Southeastern U.S. The genus *Amaranthus* is a member of the Amaranthaceae family which contains approximately 75 species worldwide (Ward et al. 2013). Within the genus *Amaranthus*, Palmer amaranth is one of 10 dioecious species that are native only to Native America (Steckel 2007). Being native to the Sonoran desert (Ehleringer 1983), Palmer amaranth is very adaptive to the heat of southern United States. Although Palmer amaranth has invasive tendencies and a history of expansion, its presence as a major agronomic weed pest is somewhat recent (Ward et al. 2013). However, by 2009, Palmer amaranth was ranked as the most troublesome weed in cotton in the southern United States (Webster and Nichols 2012). Palmer amaranth is the most common and troublesome weed in cotton for the state of Mississippi (Webster 2013).

Palmer amaranth normally has one central reddish-green stem that may grow up to 2 m in height with many lateral branches (Bryson and DeFelice 2009; Sauer 1955). Leaves are hairless in an alternate arrangement with long petioles that usually exceed the length of the leaf. Palmer amaranth, along with spiny amaranth (*Amaranthus spinosus*

L.), will often have a distinct, darker V-shaped chevron on the upper surface of the ovate leaf (Franssen et al 2001; Steckel 2007). Being a dioecious plant, Palmer amaranth has pistillate and staminate flowers on separate plants (Bryson and DeFelice 2009; Ward et al. 2013). The difference between the female and male can be identified by touch with the male spikes (inflorescence) being softer and thinner, while the female spikes are prickly due to the stiff, thick bracts.

Male Palmer amaranth plants produce large amounts of pollen which can transfer glyphosate-resistance to susceptible female plants up to 300 m away (Sosnoskie et al. 2012; Ward et al. 2013). Female Palmer amaranth plants are excellent seed producers (Jha et al. 2008; Ward et al. 2013). Palmer amaranth plants with seed heads emerging as late as October may produce up to 80,000 seeds per plant (Keeley et al. 1987). Palmer amaranth seeds are primarily gravity dispersed due to their small, smooth, round shape (Sauer 1955; Costea et al. 2004, 2005: Norsworthy et al. 2009). Palmer amaranth seed can also be dispersed geographically by water movement such as irrigation, animal movement by birds and mammals, and various agricultural management practices such as plowing, mowing, harvesting and spreading manure or gin trash (Costea et al. 2004, 2005; Norsworthy et al. 2009).

Glyphosate

Glyphosate is a nonselective, systemic herbicide that has been used extensively throughout the world for the last four decades (Nandula et al. 2012). Glyphosate was commercialized in 1974 and has been used in both crop and noncrop areas; however, due to its nonselective nature, glyphosate use was initially limited to preplant, postdirected, preharvest, and postharvest applications (Nandula et al. 2012). Glyphosate-resistant crops were introduced in 1996 and glyphosate has been widely used in-crop for weed control since that time (Owen and Zelaya 2005; Nandula et al. 2012). Glyphosate-resistant crops are grown in several countries with the most prevalent use in the United States, Canada, Argentina, and Brazil (Nandula et al. 2012; James 2014).

Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, which in turn inhibits biosynthesis of aromatic amino acids (Nandula et al. 2012). Through this inhibition, many metabolic disturbances occur including inhibition of protein and secondary product biosynthesis and deregulation of the shikimate pathway, leading to general metabolic disruption (Franz et al. 1997). Inhibition of EPSPS leads to reduced feedback inhibition of the pathway, resulting in substantial carbon flow to shikimate-3-phosphate, which is converted into high levels of shikimate (Duke and Powles 2008). Once glyphosate enters the phloem, phytotoxic levels of glyphosate translocate to meristems, young roots, leaves and other actively growing tissue or organs.

Due to the widespread adoption of glyphosate-resistant crops and subsequent overuse of glyphosate, glyphosate-resistant weed populations have become problematic (Nandula et al. 2012; Webster and Sosnoskie 2010). Worldwide, 35 weed species are glyphosate-resistant (Heap 2016). The first confirmed case of glyphosate-resistant Palmer amaranth occurred in Georgia in 2005 (Culpepper et al. 2006; Heap 2016). The Georgia population produced an abundant amount of EPSPS which was able to act as a molecular sponge to absorb glyphosate, making it possible for uninhibited EPSPS to continue functioning after glyphosate treatment (Powles 2010; Gaines et al. 2011). Since 2005, 25 states have confirmed the presence of glyphosate-resistant Palmer amaranth with several states having biotypes resistant to multiple site of actions (SOA).

Mississippi has Palmer amaranth populations resistant to glyphosate and ALS-inhibiting herbicides (Heap 2016).

Glufosinate

Glufosinate is a non-selective, contact herbicide that inhibits the glutamine synthetase enzyme which converts glutamic acid and ammonia into glutamine (Everman et al. 2007). Accumulation of ammonia destroys cells and inhibits photosystem I and II (Senseman 2007). Little to no glufosinate is absorbed through the roots due to rapid microbial breakdown as well as minimal movement in the xylem or phloem (Senseman 2007). Glufosinate is labeled for broadcast applications on canola (*Brassica napus* L.), corn (*Zea mays* L.), cotton and soybean [*Glycine max* (L.) Merr.] designated as LibertyLink® to control a broad spectrum of emerged annual and perennial grass and broadleaf weeds (Anonymous 2013). Glufosinate-resistant cotton was introduced in 2004 as LibertyLink®, and was created by the insertion of the bialaphos resistance (*bar*) gene isolated from *Streptomycyes viridochromogenes* which encodes for phosphinothricin-acetyl-transferase (PAT) (Culpepper et al. 2009; Gardner et al. 2006). In cotton, the *bar* gene expresses the PAT enzyme which makes the plant resistant to glufosinate (Culpepper et al. 2009; Gardner et al. 2006). Application rates in cotton range from 0.4 to 0.6 kg ai ha^{-1} from emergence to early bloom in up to three applications with a season maximum of 1.8 kg ai ha^{-1}. If environmental conditions prevent timely herbicide application, a single application of up to 0.9 kg ai ha^{-1} may be made; however, when a single application exceeds 0.6 kg ai ha^{-1}, the seasonal maximum is reduced to 1.2 kg ai ha^{-1} (Anonymous 2013).

Dicamba

Dicamba is a benzoic acid herbicide and is a part of the synthetic auxin class of herbicides (Senseman 2007). Dicamba penetrates plant leaves, roots and stems, and is transported by both the phloem and xylem and accumulates at the growing points. Dicamba acidifies the cell wall and the change in pH causes cell elongation that, in turn, leads to cell wall loosening and vascular tissue destruction. Symptoms associated with dicamba application includes twisting and curling of stems and petioles along with stem swelling and elongation, and leaf cupping. These symtoms are followed by chlorosis, wilting, and necrosis.

Dicamba was traditionally used for control of annual, biennial and perennial broadleaf weeds and was applied preplant in cotton. Dicamba can be applied at up to 0.3 kg ae ha^{-1} to control emerged broadleaf weeds prior to planting (Anonymous 2014). Following application, a minimum accumulation of 2.5 cm of rainfall or overhead irrigation is needed followed by a 21 day waiting interval per 0.3 kg ae ha^{-1} or less depending on formulation. Dicamba is mobile in soil but degrades rapidly. Dicamba may persist longer under low soil moisture conditions (Senseman 2007).

With glyphosate-resistant weeds becoming more problematic, crops resistant to dicamba along with glyphosate and glufosinate have been developed and will be offered as a triple stack weed control package. Dicamba tank mixed with glyphosate has been shown to provide 30 to 65% greater control of Palmer amaranth than glyphosate alone (Johnson et al. 2010).

2,4-Dichlorophenoxyacetic acid

2,4-D is a chlorophenoxy herbicide (Senseman 2007). 2,4-D is formulated in many different chemical forms including salts, esters, and acids with some of these chemical forms being more volatile than others. Plant roots absorb salt forms of 2,4-D more readily than esters whereas the ester form more rapidly penetrate foliage. 2,4-D kills plants by increasing the plasticity of cell walls, increasing the amount of proteins being made, and increasing the amount of ethylene being produced (Cox 2005). These changes in the plant cause cells to divide uncontrollably. Symptomology following 2,4-D application is similar to that of dicamba and is characterized by epinasty with bending and twisting of the stems and petioles along with leaf cupping and curling (Senseman 2007).

2,4-D has been used for decades to control broadleaf weeds. Since monocots are naturally tolerant to 2,4-D, it has used for weed control in cereals, *Saccharum officinarum* (L.), turf grass and used to manage forest understory (Bayley et al. 1992). As the number of glyphosate-resistant weeds continues to increase, crops resistant to 2,4-D along with glyphosate and glufosinate have been developed and will be offered in a triple stack weed control package.

Project Justification

Glyphosate-resistant Palmer amaranth is one of the most troublesome weeds in cotton production systems throughout the Cotton Belt. Palmer amaranth has an aggressive growth habit and prolific seed production. New auxin herbicide technologies have been shown to provide good Palmer amaranth control under ideal conditions;

however, producers need reliable data for Palmer amaranth control when it has exceeded optimum height requirements prior to herbicide application.

Many factors can influence timely herbicide applications. Weather is one of the more prevalent factors associated with timely application. Not only can a weather event such as rain keep a sprayer out of the field, extended periods of heavy rainfall can reduce the effectiveness of residual preemergence herbicides making weed escapes more common. Wind speed can also be a limiting factor when making timely applications. Not only can herbicide drift damage other crops, ornamentals, etc., reduced herbicide effectiveness can result from drift as well.

Due to the aggressive growth of Palmer amaranth, as well as the possibility of weed escapes, data are needed regarding rescue herbicide applications on glyphosate-resistant Palmer amaranth. Therefore, research was initiated to evaluate potential herbicide programs for Xtend® and Enlist™ cotton on Palmer amaranth 20- to 25- and 40- to 50-cm tall at the time of herbicide application.

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CHAPTER II

EVALUATION OF ONE-PASS HERBICIDE PROGRAMS FOR CONTROL OF GLYPHOSATE-RESISTANT PALMER AMARANTH

Abstract

Since 2005, glyphosate-resistant (GR) Palmer amaranth has spread throughout the Mid-South and Southeastern U.S. Growers have dramatically altered weed control practices in areas where this weed is problematic. Cotton cultivars resistant to dicamba and 2,4-D were available in 2015 and 2016, respectively. However, in-crop application of these herbicides is prohibited as of 2016. While timely application is critical with any herbicide, timely herbicide applications are not always feasible due to unforeseen circumstances such as weather. Therefore, data are needed regarding control of GR Palmer amaranth that is larger than recommended at the time of herbicide application. Substantial research regarding postemergence applications of glufosinate on GR Palmer amaranth is available; however, little published data are available regarding GR Palmer amaranth control with dicamba and 2,4-D. Research was conducted at Hood Farms in Dundee, MS in 2014 and 2015 as well as at the Mississippi State University Delta Research and Extension Center in Stoneville, MS in 2015 to evaluate one-pass herbicide programs on GR Palmer amaranth in a rescue scenario. The rescue scenario was simulated by allowing Palmer amaranth to grow to a desired height for this study with no prior forms of control. Two initial application timings used in this study included 20- to

25-cm and 40- to 50-cm tall Palmer amaranth plants. A total of eight herbicide treatments were evaluated consisting of glyphosate, glufosinate, dicamba, and 2,4-D applied alone as well as tank mixes including glyphosate plus dicamba, glyphosate plus 2,4-D, glufosinate plus dicamba, and glufosinate plus 2,4-D. Palmer amaranth control was increased one week after application when applications were made to 20- to 25-cm Palmer amaranth (64%) compared to applications being made to 40- to 50-cm Palmer amaranth (49%). Applications of herbicide tank mixes provided greater Palmer amaranth control than treatments containing glyphosate or glufosinate alone three weeks after application.

Introduction

Palmer amaranth [*Amaranthus palmeri* (S. Wats.)], is a fast growing broadleaf weed that is very problematic in agriculture throughout the Mid-South and Southeastern U.S. (Ward et al. 2013; Webster 2013). The genus *Amaranthus* is a member of the Amaranthaceae family which contains approximately 75 species worldwide (Ward et al. 2013). Being native to the Sonoran desert, Palmer amaranth is very adaptive to the heat of the southern United States (Ehleringer 1983). Although Palmer amaranth has invasive tendencies and a history of expansion, its presence as a major agronomic weed pest is somewhat recent (Ward et al. 2013). In 2009, Palmer amaranth was ranked as the most troublesome weed in cotton in the southern United States (Webster and Nichols 2012). Palmer amaranth is also the most common and troublesome weed in cotton for the state of Mississippi (Webster 2013).

Glyphosate-resistant (GR) crops were introduced in 1996 and glyphosate has been widely used for in-crop weed control since that time (Owen and Zelaya 2005; Nandula et al. 2012). Due to widespread adoption of glyphosate-resistant crops and subsequent overuse of glyphosate, GR weed populations have become problematic throughout the U.S. (Nandula et al. 2012; Webster and Sosnoskie 2010). Worldwide, 35 weed species are glyphosate-resistant (Heap 2016). Glyphosate-resistant Palmer amaranth was first reported in 2005 in Georgia and has been confirmed in 25 states since that time (Culpepper et al. 2006; Heap 2016). Nearly 680,000 hectares across the Southern United States were infested with GR Palmer amaranth by 2009 (Nichols et al. 2009). The entire Mid-South and Southeastern U.S. has confirmed cases of GR Palmer amaranth with many of these states having Palmer amaranth biotypes resistant to multiple sites of action (SOA) such as acetolactate synthase (ALS) inhibitors and photosystem II (PSII) inhibitors (Heap 2016).

Herbicide application timing is critical to optimize weed control and reduce yield loss (Knezevic et al. 2002). Preeemergence (PRE) herbicide application is recommended when developing a season-long weed control strategy in multiple crops (Irby et al. 2010; Loux et al. 2011; Sosnoskie et al. 2010). While PRE herbicides are recommended, they can slow crop development as well as injure the crop potentially leading to yield reductions (Kendig et al. 2007). Postemergence (POST) weed control systems may provide the only option for weed control when weather events or time constraints prevent PRE applications. In these situations, weeds may germinate with the crop and compete for similar resources and ultimately reduce yield (Loux et al. 2011). Timely POST applications will increase herbicidal efficacy and crop yield (Gower et al. 2002; Sosnoskie et al. 2010).

Glufosinate is a non-selective, contact herbicide that inhibits the glutamine synhtetase enzyme which converts glutamic acid and ammonia into glutamine (Everman et al. 2007). Accumulation of ammonia destroys plant cells and inhibits photosystem I and II (Senseman 2007). Little to no glufosinate is absorbed through the roots due to rapid microbial breakdown as well as minimal movement in the xylem or phloem (Senseman 2007). LibertyLink® cotton was introduced in 2004 and was created by the insertion of the bialaphos resistance (bar) gene isolated from *Streptomycyes viridochromogenes*, a soil fungus, which encodes for phosphinothricin-acetyl-transferase (PAT). In cotton, the bar gene expresses the *pat* enzyme which results in tolerance to glufosinate (Culpepper et al. 2009; Gardner et al. 2006). WideStrike® cotton from Dow AgroSciences was released in 2005 offering two proteins of *Bacillus thuringiensis* for genetically modified insect protection (Wright et al. 2014). Both Cry1Ac and Cry1F proteins in WideStrike® cotton were inserted with the *pat* gene to be used as a selectable marker to detect the presence of the *Bt* proteins (CERA 2015; Dodds et al. 2015). However, the levels of the *pat* gene in WideStrike® cotton are less resulting in lower tolerance than exhibited by LibertyLink® cultivars (Dodds et al. 2015; Steckel et al. 2012). In 2015, four of the top five cotton cultivars planted in the U.S. were glufosinate-resistant (USDA-AMS 2015).

Given the increased incidence of GR weeds, crops resistant to dicamba, glyphosate, and/or glufosinate tolerance have been developed and deregulated (USDA-APHIS 2015a). Cotton resistant to glyphosate, glufosinate, and dicamba was available for 2015; however, an in-crop application of dicamba is still prohibited as of 2016. Dicamba is a benzoic acid herbicide and is a part of the synthetic auxin class of herbicides (Senseman 2007) and has been traditionally used for pre-plant control of annual, biennial and perennial broadleaf weeds in cotton. Dicamba can be applied at up to 0.3 kg ae ha^{-1} to control emerged broadleaf weeds prior to planting (Anonymous 2014). Following application, a minimum accumulation of 2.5 cm of rainfall or overhead irrigation is required followed by a 21 day waiting interval per 0.3 kg ae ha^{-1} or less (Anonymous 2014). Dicamba is mobile in soil but degrades rapidly. Dicamba persists longer in the soil when low soil moisture is present (Senseman 2007). Dicamba has been shown to effectively control several weed species when tank mixed with glyphosate or glufosinate. Dicamba provided 30 to 65% greater control of GR Palmer amaranth when mixed with glyphosate as opposed to sequentially applied glyphosate alone (Johnson et al. 2010). In addition, dicamba tank mixed with glufosinate provided 15% greater Palmer amaranth control one week after application compared to glufosinate alone (Chafin et al. 2010).

Cotton resistant to 2,4-dichlorophenoxyacetic acid (2,4-D) has also been developed and will be available for commercial use for the 2016 planting season (USDA-APHIS 2015b). However, no 2,4-D formulation is labeled for POST application in cotton as of 2016. 2,4-D-resistant technology will allow for POST application of glyphosate, glufosinate, and/or 2,4-D. 2,4-D kills plants by increasing the plasticity of cell walls, increasing the amount of proteins being made, and increasing the amount of ethylene being produced (Cox 2005). Since monocots are naturally tolerant to 2,4-D, it has used for weed control in cereals, *Saccharum officinarum* (L.), turf grass and used to manage forest understory (Bayley et al. 1992).

Weed escapes due to failed herbicide applications are becoming more common due to weed resistance (Heap 2016). The rapid growth and prolific seed production of Palmer amaranth has made control very challenging when herbicide applications are not timed properly. Therefore, this research was conducted to evaluate one-pass herbicide programs for control of GR Palmer amaranth at two application timings that were larger than a recommended height of 10-cm (L. Steckel, personal communication) at the time of herbicide application.

Materials and Methods

Studies were conducted at Hood Farms in Dundee, MS, in 2014 and 2015 as well as at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, in 2015 to evaluate control of GR Palmer amaranth. Treatments were arranged in a factorial arrangement of treatments within a randomized complete block design with four replications. Factor A consisted of herbicide program and included glyphosate (Roundup PowerMAX- Monsanto Company, St. Louis, MO) at 0.8 kg ae ha^{-1}, dicamba (Clarity- BASF Corporation, Research Triangle Park, NC) at 0.6 kg ae ha^{-1}, glufosinate (Liberty 280 SL- Bayer CropScience, Research Triangle Park, NC) at 0.6 kg ai ha^{-1}, 2,4-D amine (Opti-Amine- Helena Chemical Company, Collierville, TN) at 1.1 kg ae ha^{-1}, glyphosate plus dicamba; glufosinate plus dicamba; glyphosate plus 2, 4-D; and glufosinate plus 2, 4-D. An untreated check was included for comparison. Factor B consisted of application timing and included applications to 20- to 25-cm and 40- to 50-cm Palmer amaranth. Studies were initiated in fields containing no crop and heavy natural infestations of GR Palmer amaranth. S-metolachlor (Dual Magnum- Syngenta Crop Protection, Greensboro, NC) was applied to all plots in a separate application at the time of initial application at

1.4 kg ai ha^{-1} to prevent subsequent Palmer amaranth germination. Herbicide applications were made with a CO_2 – powered backpack sprayer at 317 kPa of pressure and an application volume of 140 L ha^{-1}. All herbicides were applied using Turbo Teejet Induction 110015 tips.

Plots consisted of three 97-cm rows that were 12.2 m in length in Dundee and four 76-cm rows that were 12.2 m in length in Stoneville. Untreated rows were utilized between each plot for comparison purposes at each location.

Visual estimates of weed control were collected one, two, three and four weeks after application (WAA) using a scale from 0 to 100 with 0 being no control and 100 being complete plant death (Frans et al. 1986). Palmer amaranth heights were collected at the initiation of the experiment and at one, two, three and four WAA by measuring the tallest point of five plants within a one m^2 quadrat. Palmer amaranth densities were collected at the same rating periods by counting the total number of live Palmer amaranth plants in the same one m^2 quadrat in each plot. The one m^2 area from which heights and counts were collected was established prior to herbicide application by marking four corners of one square meter and this one m^2 area was maintained for the duration of the experiment. Palmer amaranth height and density reductions were determined by comparing initial height and density to height and density within each plot at each rating period. Visual estimates of weed control, plant height, plant height reduction, density per square meter, and density reduction data were analyzed using the PROC MIXED procedure in SAS v9.4 with site year and replication (nested within site year) as random effect parameters (Blouin et al. 2011). All data were subjected to analysis of variance and means were separated using Fisher's Protected LSD the 0.05 level of significance.

Results and Discussion

Weed control of Palmer amaranth

Herbicide program (p=0.0007) and application timing (p=0.0215) significantly affected Palmer amaranth control at one WAA (Tables 2.1 and 2.2). Applications made to 20- to 25-cm Palmer amaranth provided 64% control whereas applications made to 40- to 50-cm Palmer amaranth provided 49% control, when pooled over herbicide program (Table 2.2). Dicamba or 2,4-D in combination with glyphosate provided 49 and 66% control, respectively, compared to 26% control following application of glyphosate alone at one WAA (Table 2.1). However, Palmer amaranth control was similar when dicamba or 2,4-D were applied alone (Table 2.1). Similar results were found by Johnson et al. (2010) where glyphosate plus dicamba provided 40% greater Palmer amaranth control compared to glyphosate alone. Glufosinate alone, glufosinate plus dicamba, and glufosinate plus 2,4-D provided(67%, 76%, and 80% control, respectively, which was greater than the 26% with glyphosate alone. Applications of glufosinate plus dicamba resulted in greater visual Palmer amaranth control (76%) compared to dicamba alone (42%) at one WAA (Table 2.1).

Herbicide program had a significant effect on Palmer amaranth visual control two WAA (p=0.0012) (Tables 2.1). The addition of 2,4-D increased Palmer amaranth control when tank mixed with glyphosate compared to glyphosate alone (Table 2.1). In addition, Palmer amaranth control from application of glufosinate plus 2,4-D was greater than control from glufosinate alone. Application of glyphosate plus dicamba resulted in similar Palmer amaranth control (53%) as applications of glufosinate (45%), dicamba (50%), or 2,4-D (53%) alone. Chafin et al. (2010) observed similar results with respect to dicamba or 2,4-D tank mixed with glufosinate providing increased Palmer amaranth control compared to glufosinate alone. Glyphosate (20%) provided less control than dicamba (50%) and 2,4-D (53%) two WAA. Application of glufosinate resulted in 45% control two WAA; however, control from dicamba and 2,4-D did not differ.

Palmer amaranth control three WAA was affected by herbicide program only (p=0.0013) (Table 2.1). Application of 2,4-D as well as tank mixes of glyphosate or glufosinate with 2,4-D or dicamba resulted in greater control than that provided by glufosinate (37%) or glyphosate (24%) alone three WAA (Table 2.1). Application of dicamba resulted in similar control to tank mix combinations including glyphosate or glufosinate plus 2,4-D or dicamba. The same trend in control observed two WAA from tank mixing 2,4-D or dicamba with either glyphosate or glufosinate with respect to increased Palmer amaranth control compared to control from glyphosate or glufosinate alone was also present three WAA (Table 2.1). Tank mix applications of glyphosate or glufosinate plus 2,4-D or dicamba provided greater control than glyphosate alone and glufosinate alone three WAA. These findings are similar to that of Chafin et al. (2010) who found that dicamba plus glufosinate provided improved Palmer amaranth control compared to glufosinate alone on 18- to 22-cm GR Palmer amaranth.

Herbicide program (p=0.0199) had a significant effect on Palmer amaranth control at four WAA (Table 2.1). Application of glyphosate resulted in 15% Palmer amaranth control at four WAA (Table 2.1). Application of glufosinate (18%) and glufosinate plus 2,4-D (33%) provided the same level control as glyphosate. The addition of 2,4-D to glyphosate resulted in greater control (59%) compared to glyphosate (15%) at four WAA. However, the addition of 2,4-D to glufosinate did not result in a significant difference in Palmer amaranth control compared to that from glufosinate four WAA. Dicamba and 2,4-D resulted in the same level of Palmer amaranth control four WAA (Table 2.1). The presence of dicamba or 2,4-D alone or in combination with glyphosate resulted in 50 to 59% Palmer amaranth control which was greater than the 15% following glyphosate alone four WAA (Table 2.1) Fifteen percent control four WAA following application of glyphosate is attributed to glyphosate-susceptible Palmer amaranth biotypes present within the environments.

Palmer amaranth Height and Density

No significant difference in Palmer amaranth density was observed at the initiation of the study as well as at any other rating period due to herbicide program or application timing (Tables 2.3 and 2.4). Palmer amaranth density ranged from one to seven Palmer amaranth plants m^{-2} throughout the study (Table 2.3).

Application timing had a significant effect on Palmer amaranth height one WAA (p=0.0475) (Tables 2.3 and 2.4). Average Palmer amaranth height one WAA was 14-cm when herbicide treatments were applied to 20- to 25-cm Palmer amaranth whereas the average Palmer amaranth height one WAA was 30-cm when applications were made to 40- to 50-cm Palmer amaranth (Table 2.4). Average Palmer amaranth heights were affected by herbicide program (p=0.0186) two WAA (Table 2.3). Application of glufosinate alone resulted in taller Palmer amaranth plants (46 cm) compared to Palmer amaranth height following 2,4-D (16-cm), dicamba (22-cm), glyphosate plus dicamba (20-cm), glyphosate plus 2,4-D (15-cm), and glufosinate plus dicamba (26-cm) applications two WAA (Table 2.3). Application of glufosinate plus 2,4-D resulted in no difference in Palmer amaranth height compared to height following application of glufosinate alone at two WAA. Application of glyphosate plus dicamba (20-cm) or glyphosate plus 2,4-D (15-cm) resulted in shorter Palmer amaranth compared to the average height following application of glyphosate (36-cm) two WAA (Table 2.3). Average Palmer amaranth height three WAA was affected by application timing (p=0.0234) (Tables 2.3 and 2.4). When pooled across herbicide treatments, average Palmer amaranth height following the 20- to 25-cm application was 19-cm whereas average Palmer amaranth height following the 40- to 50-cm application timing was 29-cm (Table 2.4). Neither herbicide program (p=0.1662) nor application timing (p=0.3448) affected average Palmer amaranth height four WAA (Tables 2.3 and 2.4). Average Palmer amaranth height four WAA ranged from 16- to 45-cm (Table 2.3).

In conclusion, one-pass control options for escaped Palmer amaranth are limited. Herbicide programs in which one application is utilized to control large, escaped Palmer amaranth places immense pressure on herbicides. Although combinations of some herbicides performed better than glyphosate alone, escaped Palmer amaranth plants should be tended to as quickly as possible. Addressing the problem as quickly as time permits will reduce the impact of competition as well as facilitate a more efficient harvest at the end of the season.

Table 2.1 Control of Palmer amaranth one, two, three, and four weeks after application (WAA) based on herbicide treatment in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Herbicide Treatment	Rate	Palmer amaranth control			
		1 WAA[a]	2 WAA[a]	3 WAA[a]	4 WAA[a]
	kg ae ha^{-1}	%			
Glyphosate	0.8	26 d	20 e	24 c	15 c
Glufosinate[b]	0.6	67 ab	45 d	37 bc	18 c
Dicamba	0.6	42 cd	50 cd	53 ab	46 ab
2,4-D	1.1	49 bc	53 bcd	68 a	46 ab
Glyphosate + Dicamba	0.8 0.6	49 bc	53 bcd	61 a	50 ab
Glyphosate + 2,4-D	0.8 1.1	66 ab	69 abc	71 a	59 a
Glufosinate[b] + Dicamba	0.6 0.6	76 a	79 a	69 a	49 ab
Glufosinate[b] + 2,4-D	0.6 1.1	80 a	73 ab	62 a	33 bc

Data were pooled over application timing at three locations in two years.
[a]Means within a column following by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Rates of this herbicide are expressed in kg ai ha^{-1}.

Table 2.2 Control of Palmer amaranth one, two, three, and four weeks after application (WAA) based on application timing in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Application Timing	Palmer amaranth control			
	1 WAA[a]	2 WAA[a]	3 WAA[a]	4 WAA[a]
	%			
20- to 25-cm	64 a	47 a	59 a	40 a
40- to 50-cm	49 b	64 a	52 a	39 a

Data were pooled over herbicide treatment at three locations in two years.
[a]Means within a column following by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.

Table 2.3 Height and density of Palmer amaranth one, two, three, and four weeks after application (WAA) based on herbicide treatment in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Herbicide Treatment	Rate	Palmer amaranth height				Palmer amaranth density			
		1 WAA[a]	2 WAA[a]	3 WAA[a]	4 WAA[a]	1 WAA[a]	2 WAA[a]	3 WAA[a]	4 WAA[a]
	kg ae ha^{-1}	cm				m^{-2}			
Glyphosate	0.8	28 a	36 ab	40 a	45 a	3 a	7 a	7 a	6 a
Glufosinate[b]	0.6	22 a	46 a	27 a	28 a	2 a	2 a	2 a	3 a
Dicamba	0.6	21 a	22 bcd	22 a	24 a	5 a	3 a	3 a	3 a
2,4-D	1.1	18 a	16 d	22 a	22 a	6 a	5 a	3 a	2 a
Glyphosate + Dicamba	0.8 0.6	20 a	20 cd	22 a	21 a	6 a	4 a	5 a	6 a
Glyphosate + 2,4-D	0.8 1.1	18 a	15 d	14 a	16 a	4 a	4 a	3 a	4 a
Glufosinate[b] + Dicamba	0.6 0.6	25 a	26 bcd	24 a	29 a	5 a	4 a	3 a	1 a
Glufosinate[b] + 2,4-D	0.6 1.1	19 a	35 abc	22 a	23 a	4 a	1 a	2 a	1 a

Data were pooled over application timing at three locations in two years.
[a]Means within a column following by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Rates of this herbicide are expressed in kg ai ha^{-1}.

Table 2.4 Height and density of Palmer amaranth at initial application and one, two, three, and four weeks after application (WAA) based on application timing in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Application Timing	Palmer amaranth height					Palmer amaranth density				
	Initial[a]	1 WAA[a]	2 WAA[a]	3 WAA[a]	4 WAA[a]	Initial[a]	1 WAA[a]	2 WAA[a]	3 WAA[a]	4 WAA[a]
	cm					m^{-2}				
20- to 25-cm	21 b	14 b	16 a	19 b	21 a	6 a	5 a	5 a	4 a	4 a
40- to 50-cm	43a	30 a	38 a	29 a	31 a	3 a	3 a	2 a	2 a	2 a

Data were pooled over herbicide treatment at three locations in two years.
[a]Means within a column following by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.

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CHAPTER III

EVALUATION OF SEQUENTIAL HERBICIDE TIMING FOR CONTROL OF GLYPHOSATE –RESISTANT PALMER AMARANTH

Abstract

Gowers in the Mid-South and Southeastern U.S. have been forced to alter weed control practices in areas where glyphosate-resistant (GR) Palmer amaranth is problematic. Cotton cultivars that are resistant to glyphosate, glufosinate, and dicamba were available for purchase in the 2015 planting season; however, in-crop application of dicamba is prohibited. Timely herbicide applications are critical with any herbicide; however, timely herbicide applications are not always feasible due to unforeseen circumstances. Therefore, data are needed regarding control of GR Palmer amaranth that is larger than recommended at the time of herbicide application. This research was conducted in 2014 and 2015 at Hood Farms in Dundee, MS and at the Delta Research and Extension Center in Stoneville, MS in 2015 to determine the effect of herbicide combinations and timing between sequential applications on GR Palmer amaranth control. Experiments were initiated in fields with heavy natural infestations of GR Palmer amaranth. Herbicide applications were initiated when Palmer amaranth plants were 20- to 25-cm in height and 40- to 50-cm in height. Sequential applications were made at one of five different timings which included one, two, three, four and five weeks after initial treatment for each growth stage. Treatments utilized in this experiment

included: glyphosate plus dicamba at 0.8 kg ae ha^{-1} and 0.6 kg ae ha^{-1} and glufosinate plus dicamba at 0.6 kg ai ha^{-1} and 0.6 kg ae ha^{-1}. Application of glyphosate plus dicamba decreased Palmer amaranth height (≥49%) compared to applications of glufosinate plus dicamba at the time of a sequential application as well as 2 weeks after the sequential application when initial applications were made to 20- to 25-cm Palmer amaranth. No differences in Palmer amaranth visual control due to herbicide program were observed when initial applications were made to 20- to 25-cm Palmer amaranth at any rating period. Palmer amaranth treated at 40- to 50-cm was controlled more effectively with glufosinate plus dicamba than glyphosate plus dicamba at all rating periods. Sequential herbicide applications at either growth stage provided effective rescue control of Palmer amaranth which may help facilitate crop harvest and minimize Palmer amaranth seed production.

Introduction

Palmer amaranth [*Amaranthus palmeri* (S. Wats.)], is a fast growing broadleaf weed that is very problematic in agriculture throughout the Mid-South and Southeastern U.S. (Ward et al. 2013; Webster 2013). Being native to the Sonoran desert, Palmer amaranth is adapted to the heat of the southern United States (Ehleringer 1983). The presence of Palmer amaranth as a major agronomic weed pest is somewhat recent (Ward et al. 2013). In 2009, Palmer amaranth was ranked as the most troublesome weed in *Gossypium hirsutum* (L.) in the southern United States (Webster and Nichols 2012). Palmer amaranth is the most common and troublesome weed in cotton for the state of Mississippi (Webster 2013).

Glyphosate-resistant (GR) crops were introduced in 1996 and glyphosate has been widely used for in-crop weed control since that time (Owen and Zelaya 2005; Nandula et al. 2012). Glyphosate-resistant crops are grown in several countries with the most prevalent use in the United States, Canada, Argentina, and Brazil (Nandula et al. 2012; James 2014). Due to the widespread adoption of glyphosate-resistant crops and subsequent overuse of glyphosate, GR weed populations have become problematic (Nandula et al. 2012; Webster and Sosnoskie 2010). Worldwide, 35 weed species are glyphosate-resistant (Heap 2016). Glyphosate-resistant Palmer amaranth was first reported in 2005 in Georgia and has been confirmed in 25 other states since that time (Culpepper et al. 2006; Heap 2016). Nearly 680,000 hectares across the Southern United States were infested with GR Palmer amaranth by 2009 (Nichols et al. 2009).

The entire Mid-South along with the Southeastern U.S. has confirmed cases of GR Palmer amaranth with many of these states also having Palmer amaranth resistant to multiple sites of action (SOA) such as acetolactate synthase (ALS) inhibitors and photosystem II (PSII) inhibitors (Heap 2016). Cotton cultivars that are resistant to glyphosate, glufosinate, and dicamba were available for the 2015 growing season; however, in-crop application of dicamba is still prohibited in 2016. Bollgard II® XtendFlex® (Monsanto Company, St. Louis, MO) cotton cultivars provide producers with cotton resistant to three modes of action (MOA). While applications in-season of multiple MOA can be made, timing of herbicide applications will continue to be critical. Timely herbicide applications will increase efficacy and potential crop yield (Gower et al. 2002; Sosnoskie et al. 2010).

Since commercialization of glyphosate in 1974, glyphosate has been extensively used in both crop and noncrop areas; however, due to its nonselective nature, glyphosate use was initially limited to preplant, posdirected, preharvest, and postharvest applications (Nandula et al. 2012). Once glyphosate-resistant crops were introduced in 1996, glyphosate use greatly increased due to crop safety and broad spectrum weed control (Owen and Zelaya 2005; Nandula et al. 2012).

Glufosinate is a non-selective, contact herbicide that inhibits the glutamine synthetase enzyme which converts glutamic acid and ammonia into glutamine (Everman et al. 2007). Glufosinate-resistant cotton was introduced in 2004 as LibertyLink®. Glufosinate provides excellent weed control when timely applications are made (Culpepper et al. 2009; Gardner et al. 2006). Many cotton producers faced with GR weeds have adopted cultivars which allow for POST application of glufosinate (USDA-AMS 2015). During the 2015 growing season, four of the top five cotton cultivars planted in the U.S. were glufosinate-resistant (USDA-USA 2015).

Dicamba is a benzoic acid herbicide and is a part of the synthetic auxin class of herbicides (Senseman 2007). Dicamba has been traditionally used for control of annual, biennial and perennial broadleaf weeds and was applied preplant in cotton. Dicamba can be applied at up to 0.3 kg ae ha^{-1} to control emerged broadleaf weeds prior to planting (Anonymous 2014). Dicamba has been shown to effectively control several weed species when tank mixed with glyphosate or glufosinate. Dicamba provided 30 to 65% greater control of GR Palmer amaranth when mixed with glyphosate as opposed to sequentially applied glyphosate (Johnson et al. 2010). In addition, tank mixing dicamba with glufosinate provided up to 15% greater control of Palmer amaranth one week after application compared to glufosinate alone (Chafin et al. 2010).

Many factors such as weather and timeliness play a role in successful weed management. The likelihood of weed escapes due to failed herbicide applications are becoming more common due to weed resistance. The robust growth and prolific seed production of Palmer amaranth has made control very troublesome when herbicide applications are not timed properly. In addition, weather conditions can potentially delay herbicide applications. Therefore, this research was conducted to evaluate sequential herbicide timing for control of GR Palmer amaranth at two separate initial application timings that were larger than a recommended height of 10-cm (L. Steckel, personal communication) at the time of initial herbicide application.

Materials and Methods

Two separate field studies were conducted at Hood Farms in Dundee, MS in 2014 and 2015. Additional field studies were conducted in 2015 at the Mississippi State University Delta Research and Extension Center in Stoneville, MS. The two field studies consisted of two different initial application targets of 20- to 25-cm as well as 40- to 50-cm Palmer amaranth. Treatment combinations and experimental design were the same for both studies. Treatments were arranged in a factorial arrangement of treatments within a randomized complete block design with four replications. Factor A consisted of two herbicide tank mix combinations which included glyphosate (Roundup PowerMAX- Monsanto Company, St. Louis, MO) at 0.8 kg ae ha^{-1} plus dicamba (Clarity- BASF Corporation, Research Triangle Park, NC) at 0.6 kg ae ha^{-1} and glufosinate (Liberty 280 SL- Bayer CropScience, Research Triangle Park, NC) at 0.6 kg ai ha^{-1} plus dicamba

(Clarity- BASF Corporation, Research Triangle Park, NC) at 0.6 kg ae ha^{-1}. An untreated check was included for comparison. Factor B consisted of one of five sequential herbicide application timings which occurred one, two, three, four, and five weeks after the initial application. Studies were conducted in fields with no crop present and with heavy natural infestations of GR Palmer amaranth. S-metolachlor (Dual Magnum- Syngenta Crop Protection, Greensboro, NC) was applied to all plots in a separate application at the time of initial herbicide application at 1.4 kg ai ha^{-1} to prevent subsequent Palmer amaranth germination. Applications were made with a CO_2 – powered backpack sprayer at 317 kPa of pressure and an application volume of 140 L ha^{-1}. All herbicides were applied using Turbo Teejet Induction 110015 tips.

Plots consisted of three 97-cm rows that were 12.2 m in length in Dundee and four 76-cm rows that were 12.2 m in length in Stoneville. Untreated check rows were utilized between each plot for comparison purposes at each location.

Visual estimates of weed control were collected at the time of each sequential application as well as two and four weeks after sequential applications (WASA) using a scale from 0 to 100 with 0 being no control and 100 being complete plant death (Frans et al. 1986). Palmer amaranth heights were collected at the initiation of the experiment, as well as at the time of sequential application and at two and four WASA by measuring the tallest point of five plants within a one m^2 quadrat in each plot. Palmer amaranth densities were collected at the same rating periods by counting the total number of live Palmer amaranth plants in the same one m^2 quadrat for each plot. The one m^2 area from which heights and densities were collected was established prior to herbicide application and maintained in the same location for the duration of the experiment. Height and density reductions were determined by comparing initial Palmer amaranth height and density to height and density within each plot at each rating period. Visual estimates of weed control, plant height, plant height reduction, density per square meter, and density reduction data were analyzed using the PROC MIXED procedure in SAS v9.4 with site year and replication (nested within site year) as random effect parameters (Blouin et al. 2011). All data were subjected to analysis of variance and means were separated using Fisher's Protected LSD the 0.05 level of significance.

Results and Discussion

20- to 25-cm treated Palmer amaranth Study

Palmer amaranth height and percent height reduction was affected by herbicide treatment (p=<.0001) (p=0.0065) and sequential application timing (p=0.0002) (p=<.0001) at the time of a sequential application (Tables 3.1 and 3.2). Application of glyphosate plus dicamba resulted in shorter Palmer amaranth plants and greater percent height reduction compared to height and percent height reduction following application of glufosinate plus dicamba when pooled across sequential application timing (Table 3.1). When pooled across herbicide treatment, delaying a sequential application until four weeks after initial treatment (WAIT) resulted in the shortest Palmer amaranth plants compared to Palmer amaranth height following applications of other timing intervals (Table 3.2). Applications made ≥ 3 WAIT resulted in less height reduction than one and two WAIT.

Herbicide treatment had a significant effect on Palmer amaranth density at the time of sequential application (p=0.0344) (Table 3.1). Application of glufosinate plus dicamba resulted in reduced Palmer amaranth density at 3 plants m^2 compared to 5 plants

m^2 following application of glyphosate plus dicamba (Table 3.1). Average percent density reduction was not affected by herbicide program or sequential application timing at the time of sequential application.

Palmer amaranth control was affected by sequential application timing (p=0.0365) at the time of a sequential application (Table 3.2). Palmer amaranth control ranged from 56 to 73%. When sequential applications were made one WAIT, greater control was observed (73%) at the time of sequential application compared to all other intervals (56 to 62%) when pooled across herbicide treatment (Table 3.2).

An interaction between herbicide treatment and sequential application timing (p=0.0276) was present for Palmer amaranth height two WASA. Palmer amaranth heights ranged from 9- to 25-cm. Application of glyphosate plus dicamba resulted in similar Palmer amaranth height to those following application of glufosinate plus dicamba at all timing intervals, excluding delaying sequential application for four and five WAIT (Figure 3.1). Palmer amaranth plants were taller when sequential applications of glufosinate plus dicamba were made four and five WAIT compared to both tank mixes at all other timing intervals (Figure 3.1). Herbicide treatment had an effect on percent height reduction of Palmer amaranth at two WASA (p=0.0012) (Data not shown). Glyphosate plus dicamba provided greater height reduction (49%) compared to glufosinate plus dicamba which provided 18% (Data not shown).

Average Palmer amaranth density was affected by herbicide treatment at two WASA (p=0.0357) (Table 3.3). Application of glufosintate plus dicamba resulted in fewer Palmer amaranth plants m^2 when pooled across sequential application timings (Table 3.3). Higher Palmer amaranth densities following sequential applications of glyphosate plus dicamba compared to sequential applications of glufosinate plus dicamba were also observed by Cahoon et al. (2015). Neither herbicide treatment nor sequential application timing had an effect on density two WASA.

Average Palmer amaranth height at four WASA was affected by herbicide treatment (p=0.0103) and sequential application timing (p=<.0001) (Tables 3.3 and 3.4). Application of glyphosate plus dicamba resulted in shorter Palmer amaranth plants at four WASA compared to Palmer amaranth height following application of glufosinate plus dicamba four WASA when pooled across sequential application timing (Table 3.3). Average Palmer amaranth height pooled across herbicide treatment ranged from 6- to 20-cm two and four weeks after sequential application, respectively (Table 3.4). Sequential applications made ≥ 2 WAIT resulted in taller plants compared to heights when sequential applications were made one WAIT four WASA (Table 3.4). Height reduction was also affected by sequential application timing following the same trend as average height at four WASA. Sequential applications made one WAIT resulted in 62% height reduction which was greater than height reduction at all other sequential application timing intervals (Data not shown).

Herbicide treatment had a significant effect on Palmer amaranth density (p=0.0075) at four WASA (Table 3.3). When pooled across sequential application timing, application of glufosinate plus dicamba resulted in less Palmer amaranth plants compared to glyphosate plus dicamba. Whitaker et al. (2011) observed similar results with glufosinate increasing Palmer amaranth control by 10% to that of glyphosate alone. Percent density reduction was not affected by herbicide treatment or sequential application timing four WASA.

Palmer amaranth control four WASA was affected by sequential application timing (p=0.0357) (Table 3.4). Visual control ranged from 77 to 90% depending on rating period. Sequential applications made three WAIT resulted in 90% Palmer amaranth control which was greater than control from applications made four and five WAIT (Table 3.4). Sequential applications made ≤ three WAIT provided similar Palmer amaranth visual control five WASA (Table 3.4).

40- to 50-cm treated Palmer amaranth Study

An interaction between herbicide treatment and sequential application timing for average Palmer amaranth height at the time of sequential application was present (Figure 3.2). Application of glyphosate plus dicamba resulted in shorter Palmer amaranth plants ≤ three WAIT as well as at five WAIT compared to Palmer amaranth height following glufosinate plus dicamba application. No height difference due to application of glufosinate plus dicamba or glyphosate plus dicamba was observed when the sequential application was delayed four WAIT (Figure 3.2). Herbicide treatment (p=0.0003) and sequential application timing (p=0.0025) both affected percent height reduction at the time of sequential application (Tables 3.5 and 3.6). Glyphosate plus dicamba provided greater height reduction at 56% compared to 33% provided by glufosinate plus dicamba when pooled across sequential application timing (Table 3.5). Sequential applications one WAIT resulted in less percent height reduction than when compared to height reductions from sequential applications made after one WAIT (Table 3.6).

Average Palmer amaranth density was affected by both herbicide treatment (p=0.0068) and sequential application timing (p=0.0462) at the time of sequential application (Tables 3.5 and 3.6). Application of glufosinate plus dicamba resulted in an average of 3 plants m^2 when data were pooled across sequential application timing which was less than the 5 plants m^2 present following application of glyphosate plus dicamba (Table 3.5). These results are similar with Cahoon et al. (2015) where two applications of glufosinate plus dicamba resulted in lower Palmar amaranth densities compared to two applications of glyphosate plus dicamba. Average Palmer amaranth densities ranged from 3 to 7 m^2 when pooled across herbicide treatment. Delaying a sequential application ≥ two WAIT resulted in fewer Palmer amaranth plants m^{-2} at the time of sequential application compared to delaying sequential application by one WAIT (Table 3.6).

Sequential application timing (p=0.0124) affected visual control at the time of sequential application (Table 3.6). Control varied from 45 to 65% depending on timing interval between sequential applications. Delaying sequential applications two, three, and five WAIT provided greater Palmer amaranth control compared to delaying sequential applications by one WAIT. No differences in Palmer amaranth control were observed when delaying a sequential application one and four WAIT with respect to visual control pooled across herbicide treatment (Table 3.6).

An interaction was present between herbicide treatment and sequential application timing for average Palmer amaranth height two WASA (Figure 3.3). Application of glyphosate plus dicamba resulted in shorter Palmer amaranth plants when sequential applications were made ≥ three WAIT compared to Palmer amaranth height when glufosinate plus dicamba applications were made at the same intervals (Figure 3.3). No difference in Palmer amaranth height was found due to herbicide tank mix combinations when sequential applications were made one and two WAIT for data collected at two

WASA (Figure 3.3). Glyphosate plus dicamba provided a reduction in average Palmer amaranth height of 50 % whereas glufosinate plus dicamba provided a 27% reduction in height. Sequential applications made one and two WAIT provided greater percent reduction in height than sequential applications made four and five WAIT at two WASA. Sequential applications made three WAIT were not different than any other timing intervals with respect to percent height reduction.

Herbicide treatment (p=0.0060) had a significant effect on average Palmer amaranth density two WASA (Table 3.7). Treatments containing glufosinate plus dicamba resulted in fewer Palmer amaranth plants m^{-2} than treatments containing glyphosate plus dicamba (Table 3.7). Sequential application timing affected percent density reduction two WASA (Data not shown). Sequential applications made one WAIT resulted in less percent density reduction compared to all other timing intervals pooled across herbicide treatment. No differences were observed among sequential applications made ≥ two WAIT with respect to percent density reduction two WASA (Data not shown).

Palmer amaranth control two WASA was affected by an interaction between herbicide treatment and sequential application timing (Figure 3.4). Application of glufosinate plus dicamba resulted in increased control compared to control following application of glyphosate plus dicamba when sequential applications were made one and two WAIT (Figure 3.4). Sequential applications of glyphosate plus dicamba or glufosinate plus dicamba three and five WAIT provided greater visual control than sequential applications of glyphosate plus dicamba made one and four WAIT two WASA

(Figure 3.4). No difference in control was observed between herbicide tank mix combinations ≥ three WAIT (Figure 3.4).

Average Palmer amaranth height four WASA was affected by herbicide treatment (p=0.0150) (Table 3.7). When pooled across sequential application timing, application of glyphosate plus dicamba resulted in shorter plants (27-cm) compared to plant height following application of glufosinate plus dicamba (33-cm) (Table 3.7).

Herbicide treatment had a significant effect on Palmer amaranth density at four WASA (p=0.0105) (Table 3.7). The same trend that has remained constant throughout this study of applications containing glufosinate resulting in fewer Palmer amaranth densities was present four WASA. Application of glufosinate plus dicamba resulted in fewer plants per m^2 than treatments containing glyphosate plus dicamba (Table 3.7).

Control four WASA was affected by sequential application timing (p=0.0004) (Table 3.8). Palmer amaranth control ranged from 71 to 90%. Sequential applications made one and three WAIT resulted in greater control than sequential applications made four and five WAIT (Table 3.8). Sequential applications made two WAIT resulted in no difference in control compared to applications made one, four, and five WAIT (Table 3.8).

In conclusion, rescue applications incorporating multiple applications are practical. Control is not adequate; however, a rescue treatment may facilitate crop harvest. These data suggest applications made earlier to Palmer amaranth increases control and reduces height. Sequential applications should be made no later than three WAIT to maximize rescue efforts.

Table 3.1 Height, density, and control of 20- to 25-cm Palmer amaranth at the time of sequential application pooled over data collected at five sequential timings in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Herbicide Treatment	Rate	Height	Density	Height Reduction	Density Reduction	Control
	kg ae ha^{-1}	cm	m^{-2}		%	
Glyphosate + Dicamba	0.8 0.6	11 b	5 a	66 a	50 a	60 a
Glufosinate[b] + Dicamba	0.6 0.6	16 a	3 b	49 b	49 a	66 a

Data were pooled over sequential application timing at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Rates of this herbicide are expressed in kg ai ha^{-1}.

Table 3.2 Height, density, and control of 20- to 25-cm Palmer amaranth at the time of sequential application pooled over data collected at five sequential timings in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Sequential Application Timing	Height	Density	Height Reduction	Density Reduction	Control
	cm	m^{-2}		%	
1 WAIT[b]	17 a	3 a	85 a	75 a	73 a
2 WAIT[b]	13 b	5 a	74 a	50 a	60 b
3 WAIT[b]	15 ab	5 a	33 b	33 a	62 b
4 WAIT[b]	10 c	4 a	53 b	48 a	62 b
5 WAIT[b]	13 b	3 a	44 b	41 a	56 b

Data were pooled over herbicide treatment at three locations in two years.
[a]Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Abbreviation: WAIT, weeks after initial treatment.

Table 3.3 Height, density, and control of 20- to 25-cm Palmer amaranth two and four weeks after sequential application (WASA) based on herbicide treatment in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Herbicide Treatment	Rate	2 WASA			4 WASA		
		Height	Density	Control	Height	Density	Control
	kg ae ha^{-1}	cm	m^{-2}	%	cm	m^{-2}	%
Glyphosate	0.8	11 b	4 a	88 a	14 b	3 a	84 a
+ Dicamba	0.6						
Glufosinate[b]	0.6	18 a	2 b	88 a	18 a	1 b	84 a
+ Dicamba	0.6						

Data were pooled over sequential application timing at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Rates of this herbicide are expressed in kg ai ha^{-1}.

Table 3.4 Height, density, and control of 20- to 25-cm Palmer amaranth two and four weeks after sequential application (WASA) based on sequential application timing in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015[be].

Sequential Application Timing	2 WASA			4 WASA		
	Height	Density	Control	Height	Density	Control
	cm	m^{-2}	%	cm	m^{-2}	%
1 WAIT[b]	12 b	3 a	91 a	6 b	3 a	87 ab
2 WAIT[b]	12 b	4 a	84 a	16 a	1 a	88 ab
3 WAIT[b]	11 b	3 a	88 a	20 a	1 a	90 a
4 WAIT[b]	20 a	2 a	92 a	19 a	3 a	77 c
5 WAIT[b]	18 a	2 a	83 a	18 a	2 a	79 bc

Data were pooled over herbicide treatment at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Abbreviation: WAIT, weeks after initial treatment.

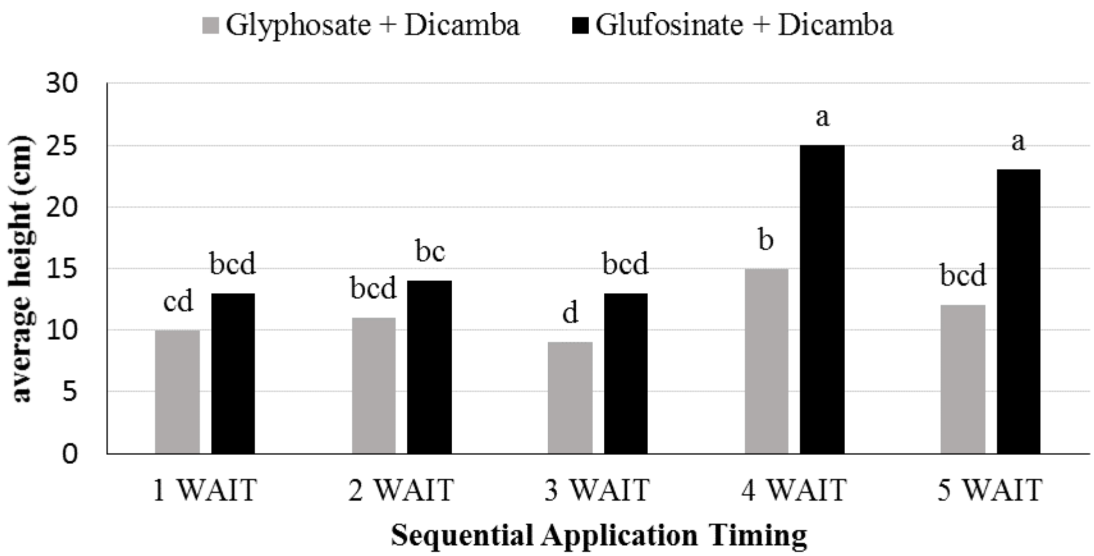

Figure 3.1 Average height at two weeks after sequential application (WASA) as affected by an interaction between herbicide treatment and sequential application timing for 20- to 25-cm initially treated Palmer amaranth.

Table 3.5 Height, density, and control of 40- to 50-cm Palmer amaranth at the time of sequential application pooled over data collected at five sequential timings in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Herbicide Treatment	Rate	Height	Density	Height Reduction	Density Reduction	Control
	kg ae ha^{-1}	cm	m^{-2}		%	
Glyphosate + Dicamba	0.8 0.6	22 b	5 a	56 a	1 a	55 a
Glufosinate[b] + Dicamba	0.6 0.6	36 a	3 b	33 b	29 a	59 a

Data were pooled over sequential application timing at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at p≤0.05.
[b]Rates of this herbicide are expressed in kg ai ha^{-1}.

Table 3.6 Height, density, and control of 40- to 50-cm Palmer amaranth at the time of sequential application pooled over data collected at five sequential timings in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Sequential Application Timing	Height	Density	Height Reduction	Density Reduction	Control
	cm	m^{-2}		%	
1 WAIT[b]	32 a	7 a	69 a	39 a	45 b
2 WAIT[b]	30 a	3 b	36 b	21 a	65 a
3 WAIT[b]	30 a	3 b	33 b	-14 a	64 a
4 WAIT[b]	25 b	4 b	43 b	36 a	54 ab
5 WAIT[b]	30 a	4 b	41 b	-6 a	60 a

Data were pooled over herbicide treatment at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Abbreviation: WAIT, weeks after initial treatment.

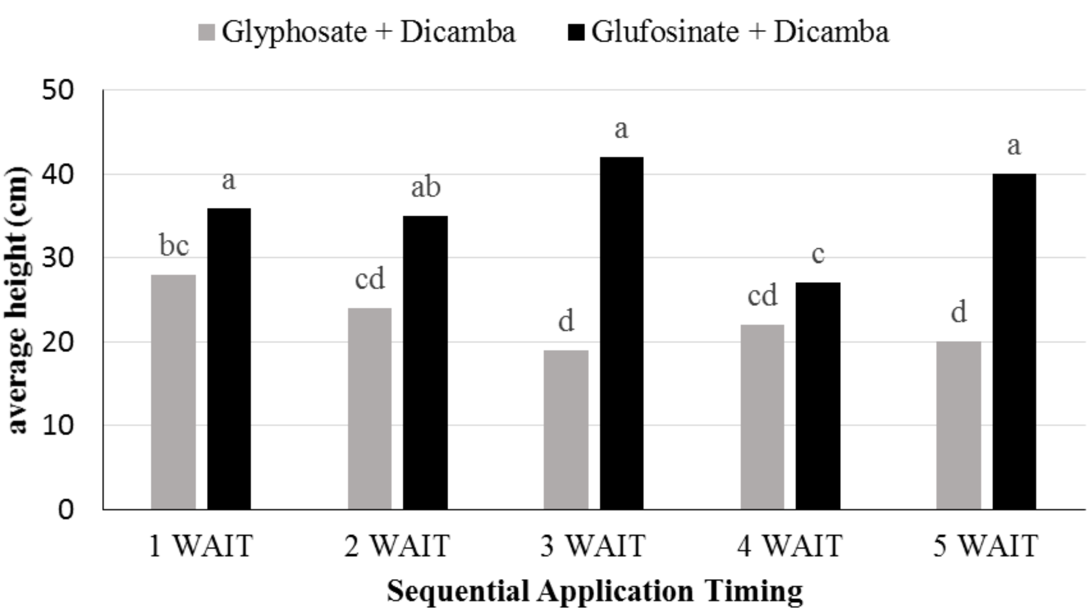

Figure 3.2 Average height at time of sequential application as affected by an interaction between herbicide treatment and sequential application timing for 40- to 50-cm initially treated Palmer amaranth.

Table 3.7 Height, density, and control of 40- to 50-cm Palmer amaranth two and four weeks after sequential application (WASA) based on herbicide treatment in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Herbicide		2 WASA			4 WASA		
Treatment	Rate	Height	Density	Control	Height	Density	Control
	kg ae ha^{-1}	cm	m^{-2}	%	cm	m^{-2}	%
Glyphosate + Dicamba	0.8 0.6	22 b	3 a	77 b	27 b	2 a	77 a
Glufosinate[b] + Dicamba	0.6 0.6	33 a	1 b	90 a	33 a	1 b	83 a

Data were pooled over sequential application timing at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at $p \le 0.05$.
[b]Rates of this herbicide are expressed in kg ai ha^{-1}.

Table 3.8 Height, density, and control of 40- to 50-cm Palmer amaranth two and four weeks after sequential application (WASA) based on sequential application timing in Stoneville, MS in 2015 and Dundee, MS in 2014 and 2015.

Sequential Application Timing	2 WASA			4 WASA		
	Height	Density	Control	Height	Density	Control
	cm	m^{-2}	%	cm	m^{-2}	%
1 WAIT[b]	23 b	2 a	80 bc	28 a	1 a	86 ab
2 WAIT[b]	23 b	2 a	85 ab	24 a	1 a	79 bc
3 WAIT[b]	26 b	3 a	88 a	36 a	1 a	90 a
4 WAIT[b]	33 a	2 a	77 c	31 a	2 a	72 c
5 WAIT[b]	34 a	2 a	87 ab	30 a	2 a	71 c

Data were pooled over herbicide treatment at three locations in two years.
[a]Means within a column followed by the same letter are not different based on Fisher's protected LSD at $p \leq 0.05$.
[b]Abbreviation: WAIT, weeks after initial treatment.

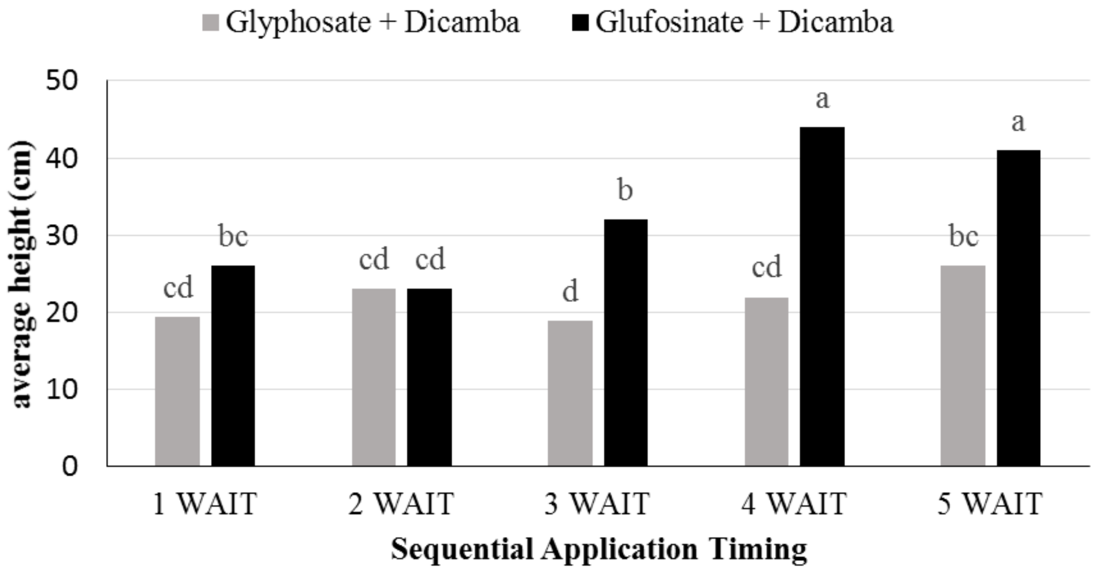

Figure 3.3 Average height at two WASA as affected by an interaction between herbicide treatment and sequential application timing for 40-50 cm initially treated Palmer amaranth.

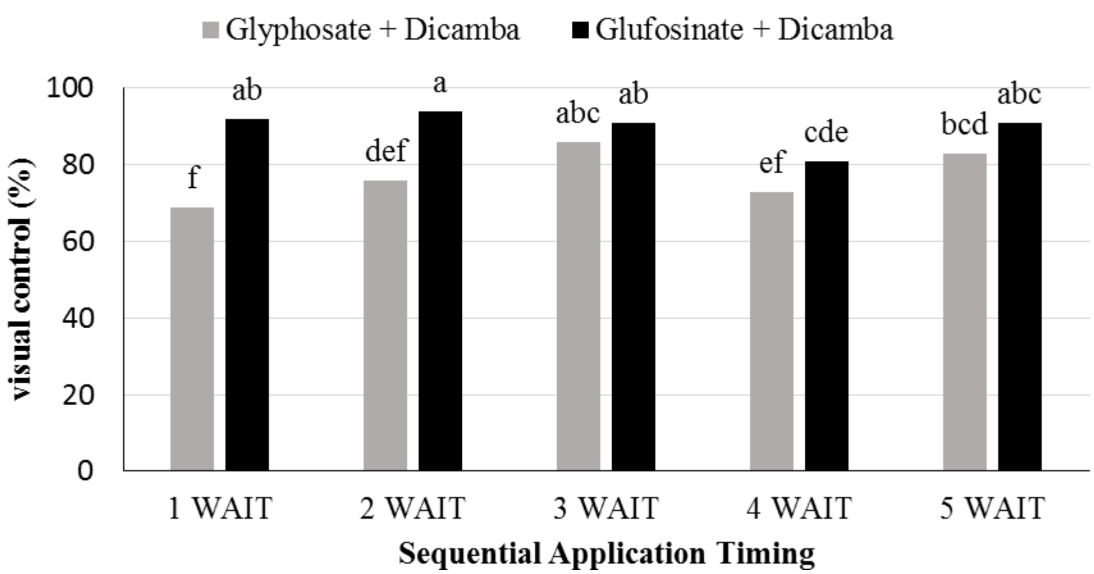

Figure 3.4 Visual control at two WASA as affected by an interaction between herbicide treatment and sequential application timing for 40-50 cm initially treated Palmer amaranth.

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CHAPTER IV

DETERMINING THE NUMBER OF APPLICATIONS NEEDED FOR CONTROL OF GLYPHOSATE-RESISTANT PALMER AMARANTH

Abstract

An experiment was conducted at Hood Farms in Dundee, MS and at the Delta Research and Extension Center in Stoneville, MS in 2015 to determine the effect of multiple herbicide applications and timings on glyphosate-resistant (GR) Palmer amaranth control. The experiment was initiated in fields with heavy natural infestations of GR Palmer amaranth. Applications were initiated when Palmer amaranth plants were 20- to 25-cm in height. Additional treatments were initiated two and four weeks after the original application timing. Herbicide programs in which two applications were made, the second application was made two weeks or four weeks after the initial application regardless of when treatments were initiated. Herbicide programs in which three applications were made, the third application was made two weeks after the second application regardless when treatments were initiated. Treatments utilized in this experiment included: glyphosate plus dicamba at 0.8 kg ae ha^{-1} and 0.6 kg ae ha^{-1}; glufosinate plus dicamba at 0.6 kg ai ha^{-1} and 0.6 kg ae ha^{-1}, glyphosate plus 2, 4-D at 0.8 kg ae ha^{-1} and 1.1 kg ae ha^{-1}; glufosinate plus 2, 4-D at 0.6 kg ai ha^{-1} and 1.1 kg ae ha^{-1}. Multiple applications increased Palmer amaranth control compared to single application programs two weeks after the final application when sequential applications were made at

two week intervals. Multiple applications of any herbicide combination were needed to control 20- to 25-cm GR Palmer amaranth.

Introduction

Palmer amaranth [*Amaranthus palmeri* (S. Wats.)], is a fast growing broadleaf weed that is problematic in agriculture throughout the Mid-South and Southeastern U.S. (Ward et al. 2013; Webster 2013). Being native to the Sonoran desert, Palmer amaranth is very adapted to the heat of the southern United States (Ehleringer 1983). The presence of Palmer amaranth as a major agronomic weed pest is somewhat recent (Ward et al. 2013). In 2009, Palmer amaranth was ranked as the most troublesome weed in cotton in the southern United States (Webster and Nichols 2012). Palmer amaranth is the most common and troublesome weed in *Gossypium hirsutum* (L.) for the state of Mississippi (Webster 2013).

Glyphosate-resistant (GR) crops were introduced in 1996 and glyphosate has been widely used for in-season weed control since that time (Owen and Zelaya 2005; Nandula et al. 2012). Due to the widespread adoption of glyphosate-resistant crops and subsequent overuse of glyphosate, GR weed populations have become problematic (Nandula et al. 2012; Webster and Sosnoskie 2010). Worldwide, 35 weed species are glyphosate-resistant (Heap 2016). Glyphosate-resistant Palmer amaranth was first reported in 2005 in Georgia and has been confirmed in 25 other states since that time (Culpepper et al. 2006; Heap 2016). Nearly 680,000 hectares across the Southern United States were infested with GR Palmer amaranth by 2009 (Nichols et al. 2009).

Glufosinate is a non-selective, contact herbicide that inhibits the glutamine synthetase enzyme which converts glutamic acid and ammonia into glutamine (Everman et al. 2007). Glufosinate-resistant cotton was introduced in 2004 as LibertyLink®, and was created by the insertion of the bialaphos resistance (bar) gene isolated from *Streptomycyes viridochromogenes*, a soil fungus, which encodes for phosphinothricin-acetyl-transferase (PAT) (Culpepper et al. 2009; Gardner et al. 2006). In glufosinate resistant cotton, the *bar* gene expresses the PAT enzyme which makes the plant resistant to glufosinate (Culpepper et al. 2009; Gardner et al. 2006). Glufosinate provides excellent weed control when timely applications are made (Culpepper et al. 2009; Gardner et al. 2006). Many cotton producers facing glyphosate-resistant weeds have incorporated genetically modified (GM) cultivars such as LibertyLink®, Glytol® plus LibertyLink®, and Widestrike® (Anonymous 2013) which are all glufosinate-resistant. During the 2015 growing season, four out of the top five cotton cultivars planted were glufosinate-resistant (USDA-AMS 2015).

Given the increased incidence of GR weeds, cotton cultivars resistant to dicamba along with glyphosate and glufosinate were deregulated and available for commercial use for the 2015 planting season; however, in-season application of dicamba is still prohibited (USDA-APHIS 2015a). Dicamba, a benzoic acid herbicide, belongs to the synthetic auxin class of herbicides (Senseman 2007). Dicamba has traditionally been used for control of annual, biennial and perennial broadleaf weeds and was applied preplant in cotton. Dicamba has been shown to effectively control several weed species when tank mixed with glyphosate or glufosinate. Dicamba provided 30 to 65% greater control of GR Palmer amaranth when mixed with glyphosate as opposed to sequentially applied glyphosate (Johnson et al. 2010). In addition, tank mixing dicamba with glufosinate provided up to 15% greater control of Palmer amaranth one week after application as opposed to glufosinate alone (Chafin et al. 2010).

Cotton resistant to 2,4-dichlorophenoxyacetic acid (2,4-D), another synthetic auxin, is available for commercial use for the 2016 planting season; however, in-season application of 2,4-D is still prohibited (USDA-APHIS 2015b). This new technology will be known as Enlist™ and allow for the application of Enlist™Duo (Dow AgroSciences LLC, Indiapolis, IN), a pre-mix herbicide of glyphosate and 2,4-D to be broadcast on 2,4-D-resistant crops. Enlist™ cotton cultivars will also be resistant to glufosinate. Symptomology following 2,4-D application is similar to that of dicamba and is characterized by epinasty with bending and twisting of the stems and petioles along with leaf cupping and curling (Senseman 2007). 2,4-D has been used for many decades to control broadleaf weeds (Bayley et al. 1992).

Timing of herbicide application will be a primary consideration with both the Enlist™ and Xtend® technologies. Timely applications at an optimal growth stage will increase efficacy and potential yield (Gower et al. 2002; Sosnoskie et al. 2010). Multiple herbicide applications will be required for optimum weed control. Sequential herbicide applications have been shown to be effective at reducing weed biomass and increasing yield compared to single herbicide applications (Jha et al. 2008). Palmer amaranth's robust growth and prolific seed production make weed control very problematic when timely applications are not made. Therefore, this research was conducted to evaluate application timing along with multiple applications for control of GR Palmer amaranth that was larger than a recommended height of 10-cm (L. Steckel, personal communication) at the time of initial herbicide application.

Materials and Methods

Studies were conducted in 2015 at Hood Farms in Dundee, MS and at the Mississippi State University Delta Research and Extension Center in Stoneville, MS. Treatments were arranged in a factorial arrangement of treatments within a randomized complete block design with four replications. Factor A consisted of four herbicide tank mix combinations which included glyphosate (Roundup PowerMAX- Monsanto Company, St. Louis, MO) at 0.8 kg ae ha^{-1} plus dicamba (Clarity- BASF Corporation, Research Triangle Park, NC) at 0.6 kg ae ha^{-1}, glufosinate (Liberty 280 SL- Bayer CropScience, Research Triangle Park, NC) at 0.6 kg ai ha^{-1} plus dicamba , glyphosate at 0.8 kg ae ha^{-1} plus 2,4-D (Opti-Amine- Helena Chemical Company, Collierville, TN) at 1.1 kg ae ha^{-1}, and glufosinate at 0.6 kg ai ha^{-1} plus 2,4-D at 1.1 kg ae ha^{-1}. An untreated check was included for comparison. Factor B consisted of herbicide application program and included the following: single application initiated when Palmer amaranth plants were 20- to 25-cm in height; two additional single application programs where initial application was delayed two or four weeks after the 20- to 25-cm application timing; two applications in which the second application was made two or four weeks after the initial 20- to 25-cm application, two applications in which the second application was made two weeks after an initial application that was delayed two weeks after the 20- to 25-cm application timing; and a herbicide program in which three applications were made with the initial application being made to 20- to 25-cm Palmer amaranth, followed by a second application made two weeks following the initial application, followed by a third application made two weeks following the second application. Experiments were conducted in fields with heavy natural infestations of GR Palmer amaranth and no crop present. Height and density reductions were determined by comparing initial height and density to height and density within each plot at each rating period. S-metolachlor (Dual Magnum- Syngenta Crop Protection, Greensboro, NC) was applied to all plots in a separate application at the time of initial herbicide application at 1.4 kg ai ha^{-1} to prevent subsequent Palmer amaranth germination. Applications were made with a CO_2 – powered backpack sprayer at 317 kPa of pressure and an application volume of 140 L ha^{-1}. All herbicides were applied using Turbo Teejet Induction 110015 tips.

Plots consisted of three 97-cm rows that were 12.2 m in length in Dundee and four 76-cm rows that were 12.2 m in length in Stoneville. Untreated check rows were utilized between each plot for comparison purposes at each location.

Visual estimates of weed control were collected at two and four weeks after application (WAA) using a scale from 0 to 100 with 0 being no control and 100 being complete plant death (Frans et al. 1986). Palmer amaranth heights were collected at the initiation of the experiment and at two and four WAA by measuring five plants within a one m^2 quadrat. Palmer amaranth densities were collected at the same rating period by counting the total number of Palmer amaranth plants in the same one m^2 quadrat for each plot. The one m^2 area from which heights and counts were collected was established prior to herbicide application and maintained in the same location for the duration of the experiment. Visual estimates of weed control, plant height, plant height reduction, densities per square meter, and density reduction data were analyzed using the PROC MIXED procedure in SAS v9.4 with site year and replication (nested within site year) as random effect parameters (Blouin et al. 2011). All data were subjected to analysis of variance and means were separated using Fisher's Protected LSD the 0.05 level of significance.

Results and Discussion

A significant difference in Palmer amaranth height was observed at the initiation of the study (p=0.0006) (Table 4.1). Programs in which an application was delayed for two or more weeks resulted in taller Palmer amaranth plants. Delaying an application four weeks resulted in 56 cm Palmer amaranth plants (Table 4.1). Palmer amaranth initial density followed the same trend as initial height with Palmer amaranth densities increasing as initial applications were delayed. Application programs where applications were delayed two and four weeks beyond the 20- to 25-cm target resulted in Palmer amaranth densities of 21, 27, and 23 plants m^{-2} (Table 4.1). Allowing Palmer amaranth density to increase has been shown to reduce cotton yields and significantly increase cotton harvest time (Smith et al. 2000).

Herbicide application program affected Palmer amaranth height two weeks after application (WAA) (p=0.0001) (Table 4.2). Delaying the initial application by two and four weeks resulted in taller plants compared to heights when applications were initiated on 20- to 25-cm Palmer amaranth or two weeks thereafter. Palmer amaranth plants were shorter when receiving more than one herbicide application when applications were made to 20- to 25-cm plants followed by (fb) an application within two weeks (Table 4.2). Delaying the initial application by two weeks fb a sequential application two weeks later resulted in taller plants than that of Palmer amaranth initially treated at 20- to 25-cm fb a sequential application four weeks after. Height reduction was significantly affected two WAA by application program (p=0.0098) (Data not shown). Herbicide programs where the initial application was made to 20- to 25-cm Palmer amaranth plants alone or fb a sequential application within two weeks or where three applications were made at two week intervals resulted in greater height reductions compared to delaying the sequential application by four weeks (Data not shown).

Average Palmer amaranth density was affected by application program two WAA (Table 4.2). Herbicide programs where two or three applications were made resulted in lower densities compared to programs in which one application was made (Table 4.2). Palmer amaranth that was 20- to 25-cm at the time of application fb a sequential application two weeks later resulted in fewer plants m^{-2} compared to delaying the sequential application by four weeks (Table 4.2). Three herbicide applications resulted in the lowest average density of 1 plant m^{-2} two WAA; however, Palmer amaranth density following this program was not different than Palmer amaranth density where two applications were made with the initial application being to 20- to 25-cm Palmer amaranth fb a sequential application two weeks later (Table 4.2).

Palmer amaranth control was affected by application program two WAA ($p=0.0085$) (Table 4.2). Three applications resulted in greater Palmer amaranth control compared to the control observed following programs in which a single application was made (Table 4.2). Sosnoskie et al. (2010) also observed sequential applications to be more effective at controlling GR Palmer amaranth than a single application. There was no difference observed in visual control among multiple application programs when sequential applications were made within two weeks after the previous application (Table 4.2). Although Palmer amaranth control following programs where two applications were made ranged from 83 to 90% two WAA, Palmer amaranth control below 90% has been documented to produce lower crop yields and increase the weed seed bank creating detrimental effects in the future (Fast et al. 2009; Price et al. 2011).

Application program had a significant effect on Palmer amaranth height four WAA (p=0.0002) (Table 4.3). Herbicide applications made to smaller Palmer amaranth plants resulted in a significant advantage following programs utilizing a single application four WAA (Table 4.3). Shorter plants were observed when two applications were made when the initial application made to 20- to 25-cm Palmer amaranth fb an application two weeks after compared to two applications where the initial application was delayed by two weeks fb an application two weeks later (Table 4.3).

Average Palmer amaranth density at four WAA was affected by application program (p=0.0394) (Table 4.3). Palmer amaranth average density was greater following herbicide programs in which a single application was compared to three applications made at two week intervals (Table 4.3). Merchant et al. (2014) noted sequential applications of glufosinate plus 2,4-D to be the most successful herbicide treatment when applied 10 to 15 days apart compared to an 5 day interval between sequential applications. No difference in Palmer amaranth density was observed among multiple application programs. A single application delayed four weeks resulted in greater Palmer amaranth average density compared to programs that received two or three applications (Table 4.3).

In conclusion, multiple applications greatly increased Palmer amaranth control and also decreased Palmer amaranth height and density. Rescue applications should be made as soon as permissible. Smith et al. (2000) reported cotton yield and quality losses along with increased harvest difficulty due to late season Palmer amaranth interference.

When making sequential applications, applications made within two weeks will be more beneficial than a sequential application made four weeks after the initial application, regardless of initial application timing. Although herbicide treatment did not differ, it is important to be cognizant of utilizing all herbicide tools available to control GR Palmer amaranth.

Table 4.1 Initial height and density of Palmer amaranth based on application program in Stoneville and Dundee, MS in 2015.

Application Program	Initial Height[a]	Initial Density[a]
	cm	m^{-2}
1 Application		
Initial Application	21 c	8 b
2 Week Postponement	40 b	21 a
4 Week Postponement	56 a	27 a
2 Applications		
Initial Application fb 2 WAA	20 c	6 b
Initial Application fb 4 WAA	22 c	11 b
2 Week Postponement fb 2 WAA	35 b	23 a
3 Applications		
Initial fb 2 WAA fb 4 WAA	21 c	7 b

Data were pooled over two locations and herbicide program in one year.
[a]Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD at $p \leq 0.05$.

Table 4.2 Height, density, and visual control of Palmer amaranth 2 WAA based on application program in Stoneville and Dundee, MS in 2015.

Application Program	Height[a]	Density[a]	Visual Control[a]
	cm	m^{-2}	%
1 Application			
Initial Application	12 de	9 b	70 cd
2 Week Postponement	26 bc	11 b	68 d
4 Week Postponement	46 a	14 a	71 cd
2 Applications			
Initial Application fb 2 WAA	7 e	2 de	90 ab
Initial Application fb 4 WAA	21 cd	5 c	83 bc
2 Week Postponement fb 2 WAA	33 b	4 cd	87 ab
3 Applications			
Initial fb 2 WAA fb 4 WAA	8 e	1 e	99 a

Data were pooled over two locations and herbicide program in one year.
[a]Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD at $p \leq 0.05$.

Table 4.3 Height, density, and visual control of Palmer amaranth 4 WAA based on application program in Stoneville and Dundee, MS in 2015.

Application Program	Height[a]	Density[a]	Visual Control[a]
	cm	m^{-2}	%
1 Application			
Initial Application	15 d	9 ab	51 a
2 Week Postponement	30 b	8 ab	73 a
4 Week Postponement	50 a	14 a	70 a
2 Applications			
Initial Application fb 2 WAA	14 d	2 bc	88 a
Initial Application fb 4 WAA	21 cd	4 bc	74 a
2 Week Postponement fb 2 WAA	28 bc	4 bc	84 a
3 Applications			
Initial fb 2 WAA fb 4 WAA	8 d	1 c	95 a

Data were pooled over two locations and herbicide program in one year.
[a]Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD at $p \leq 0.05$.

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