OCCURRENCE AND MANAGEMENT OF GLYPHOSATE RESISTANT *AMARANTHUS* WEED SPECIES IN CENTRAL TEXAS COTTON AND EVALUATION OF SPRAY DROPLET SIZE SPECTRA AS AFFECTED BY NOZZLE DESIGN AND HERBICIDE FORMULATION

A Dissertation

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JOSHUA ALLAN MCGINTY

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Chair of Committee,	Paul Baumann
Committee Members,	Gaylon Morgan
	Peter Dotray
	Robert Lyons
Head of Department,	David Baltensperger

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ABSTRACT

With the recent confirmation of glyphosate-resistant *Amaranthus* species in many important agronomic regions of the United States, a study was initiated to identify and document the occurrence of glyphosate resistant common waterhemp in East-central Texas. Accessions of several suspected glyphosate-resistant biotypes of common waterhemp were grown in a greenhouse before receiving rates of glyphosate from 434 to 3468 g ae ha^{-1}. Dose-response analyses were conducted to provide LD_{50} values for each accession. LD_{50} values ranged from 387 to 4549 g ae ha^{-1} glyphosate.

A study evaluating the efficacy of twelve different weed control programs for common waterhemp and Palmer amaranth control in cotton was conducted in Burleson County, TX in 2012 and 2013. The study was conducted in cotton possessing stacked glyphosate-, glufosinate-, and dicamba-tolerant technologies. Preplant and preemergence treatments included fomesafen, pendimethalin, prometryn, pyrithiobac, *S*-metolachlor, and trifluralin. These treatments were followed by a variety of early- and mid-postemergence treatments. Preplant and preemergence treatments resulted in 81 to 100% control of Palmer amaranth and common waterhemp with the exception of pyrithiobac, which provided only 29 to 60% control of these species. Following early- and mid-postemergence applications, 92 to 100% control of these species was obtained. Applications of pendimethalin PRE followed by pyrithiobac EPOST and glufosinate MPOST in 2013 provided lower control of both species (92 to 93 %) than all other treatments evaluated in the study 14 days after MPOST applications.

With the potential commercialization of synthetic auxin-tolerant crops, there is an increased need for understanding of the influence of spray nozzle design and herbicide formulation on physical spray drift reduction. A study was conducted in a low speed wind tunnel utilizing laser diffraction technology to analyze the droplet size spectra produced by different spray nozzles and herbicide formulations. Nozzles utilizing a pre-orifice design or a combination of pre-orifice and air-inclusion design were observed to produce significantly larger spray droplets than those without these features. Herbicide formulations were shown to have a significant influence on droplet size as well. Different herbicide formulations were observed to decrease the production of drift-prone fine droplets by as much as 64%.

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NOMENCLATURE

DAT	Days after treatment
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
GR	Glyphosate-resistant
GRC	Glyphosate-resistant crop
GS	Glyphosate-susceptible
POST	Postemergence
PPI	Preplant incorporated
PRE	Preemergence

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CHAPTER I

INTRODUCTION AND OBJECTIVES

Plants of the genus Amaranthus are troublesome weeds in many agricultural systems of the United States. Two dioecious species of this genus, Palmer amaranth (*Amaranthus palmeri* S. Watson) and common waterhemp (*Amaranthus rudis* Sauer), have become the subject of intense study since glyphosate resistance was first identified in these species in 2005 (Culpepper et al. 2006; Legleiter and Bradley 2008). Confirmation of the presence of new glyphosate resistant (GR) populations of these species as well as the development of management strategies for GR weeds in agronomic systems continues to be of upmost importance to preserve crop yield.

To help combat the spread of GR weeds, 2,4-D and dicamba tolerant traits are being developed for commercial release in several major agronomic crops. Maximizing the efficacy of herbicide applications while also decreasing the potential for off-target movement continues to be a major concern in agriculture, particularly in light of the recent development of these new crop technologies. Off-target herbicide movement, or drift, may occur through the unintentional movement of spray droplets before deposition or the movement of volatilized herbicide after deposition. To minimize the potential for vapor drift of herbicides such as 2,4-D and dicamba, manufacturers such as BASF, Dow AgroSciences, and Monsanto have developed extremely low volatility formulations of these herbicides (Armstrong et al. 2012; Johnson et al. 2012; Thomas et al. 2012). Although the potential for vapor movement of these herbicides may be reduced by these formulations, physical drift onto susceptible plants remains a major concern and requires further research.

The objectives of this study were to (1) evaluate the effect of glyphosate on suspected glyphosate-resistant common waterhemp accessions in East-central Texas, (2) to evaluate the efficacy of several different herbicide regimes utilizing multiple modes of action for weed control in central Texas cotton with stacked glyphosate-, glufosinate-, and dicamba-tolerant technologies, and (3) to evaluate the effect of spray nozzle type and herbicide formulation on the droplet size spectra of herbicide sprays.

CHAPTER II

LITERATURE REVIEW

Glyphosate-Resistant *Amaranthus* Weeds in Cotton

Palmer Amaranth. Palmer amaranth is a member of the subgenus Acnida, which contains only dioecious species. It is native to the arid regions of the southwestern United States, but now occurs on disturbed sites throughout the midsouth and southeastern U.S. (Sauer 1957; Steckel 2007).

Palmer amaranth is characterized as a herbaceous C_4 annual with erect branched stems growing to 2 m in height (Bryson and Defelice 2009). Roots may be red in color and are fibrous from a central taproot (Halvorson and Guertin 2003). Cotyledons are narrowly lanceolate, green above and reddish below, and glabrous (DiTomaso and Healy 2006; Bryson and Defelice 2009). The first true leaf is often ovate with a slight indentation at the apex (DiTomaso and Healy 2007). Mature leaves are simple, 2.0 to 15.0 cm in length and 1.0 to 7.0 cm wide, lanceolate to ovate in shape, and arranged alternately (Bryson and Defelice 2009; Whitson et al. 2009). Leaf petioles are equal to or longer than the leaf blade, 1.0 to 15.0 cm in length (Halvorson and Guertin 2003). Inflorescences are terminal panicles comprised of several spikes growing to 20 cm in length. Pistillate flowers are subtended by stiff bracts 4.0 to 8.0 mm in length (Bryson and Defelice 2009). Bracts subtending staminate flowers are spine-like and 2.5 to 6.0 mm in length (DiTomaso and Healy 2007). Sepals of pistillate flowers are 5-merous and 3.0 to 4.0 mm long and obtuse in shape, with a mucronulate apex (DiTomaso and Healy

2007; Bryson and Defelice 2009). Staminate flowers are also 5-merous with 2.5 to 6.0 mm long pointed sepals (DiTomaso and Healy 2007). Both pistillate and staminate flowers are without petals (Halvorson and Guertin 2003). Fruit is a utricle 1.5 to 2.0 mm in length with a rough, wrinkled appearance. Seeds are lenticular, dark brown to black, glossy in appearance, and 1.0 to 1.2 mm in diameter (Bryson and Defelice 2009).

Palmer amaranth seed germination appears to begin at 18^{o}C (Keeley et al. 1987). Germination success appears to be temperature-dependent as shown by Keeley et al. (1987), where germination increased with temperature to a maximum of 57 to 73% germination at a 38/32^{o}C (day/night) temperature regime. Similar results were found by Guo and Al-Khatib (2003), who found peak germination to occur at 35/30^{o}C. Palmer amaranth seeds do not appear to be particularly long-lived in the soil as reported by Sosnoskie et al. (2012), where nearly 80% seed death occurred after three years of soil burial in Georgia.

Palmer amaranth possesses many unique characteristics that allow it to be extremely competitive, particularly in warm, moisture-limited environments. It is very heat-tolerant, and has been shown to resist high temperature exposures better than redroot pigweed (*Amaranthus retroflexus* L.) and common waterhemp (Guo and Al-Khatib 2003). Leaves of mature Palmer amaranth plants have shown the ability to exhibit diaheliotropism (solar tracking by orienting leaves perpendicular to the direction of sunlight) allowing it to maintain high photosynthetic rates throughout the day (Ehleringer 1983). Palmer amaranth has been observed to reach its peak photosynthetic efficiency at approximately 42^{o}C (Ehleringer 1983). Palmer amaranth has been shown to produce significantly greater biomass than either redroot pigweed or common waterhemp when grown under temperature regimes of 25/20 and 35/30^{o}C (Guo and Al-Khatib 2003). Under conditions of decreased leaf water potential, the leaves have been observed to increase solute concentration, allowing continued stomatal opening longer into drought conditions than many other plant species (Ehleringer 1983). Like many problematic weed species, this species has been shown to produce vast quantities of seed, as many as 600,000 per female plant (Keeley et al. 1987).

Common Waterhemp. Common waterhemp, also a member of the dioecious subgenus Acnida, is native to North America and is currently found in the Midwestern United States from Illinois and southern Michigan in the north, to Texas and Louisiana in the south (Horak et al. 1994; Pratt et al. 1999; Steckel 2007). It can be found growing on stream banks, lakeshores, floodplains, and cultivated areas (Sauer 1957; DiTomaso and Healy 2007; Bryson and DeFelice 2009).

It is characterized as a herbaceous C_4 annual with ascending or erect stems green to red in color, which may be simple or highly branched and growing to 0.5 to 3.0 m tall (McGregor et al. 1986; Steckel 2007; Bryson and DeFelice 2009). Roots are fibrous with a well-developed taproot (McGregor et al. 1986; Bryson and DeFelice 2009). Cotyledons are egg- or oar-shaped, green to red in color, and glabrous (Pratt et al. 1999; Bryson and DeFelice 2009). Stems and leaves of both immature and mature plants are glabrous and glossy in appearance (Horak et al. 1994; Franssen et al. 2003). Mature leaves are alternate and variable in size and shape, but are most often lanceolate and 1.0 to 15.0 cm long and 0.5 to 3.0 cm wide with petioles generally shorter than leaf blades

(McGregor et al. 1986; Baumann 2006; Steckel 2007). Inflorescence is commonly a terminal spike ranging from 3 to 35 cm long, which can be simple or highly branched (Steckel 2007; Bryson and DeFelice 2009). Both pistillate and staminate flowers are subtended by bracts 0.5 to 2.8 mm long (Pratt et al. 1994). Pistillate flowers have rudimentary or absent sepals, while staminate flowers have five green sepals 2.5 to 3.0 mm long (Bryson and DeFelice 2009). Fruits are 1.5 to 2.0 mm long utricles, dehiscing irregularly. Seeds are lenticular in shape, dark red to black, and 0.8 to 1.0 mm in diameter (Bryson and DeFelice 2009).

Common waterhemp seeds have been observed to be highly viable, ranging from 74 to 83% (Hartzler et al. 1999), but viability has been observed to decrease dramatically after 4 years in the soil (Buhler and Hartzler 2001; Steckel et al. 2007). Hartzler et al. (1999) found that while common waterhemp seeds were highly viable, a very small portion of these seeds germinate in a given season. Buhler and Hartzler (2001) found that germination never exceeded 7% in a year over a four year period. The study by Hartzler et al. (1999) also found that the duration of emergence was much longer for common waterhemp than for the other weed species studied (*Setaria faberi* Herrm., *Abutilon theophrasti* Medik., and *Eriochloa villosa* (Thunb.) Kunth). The delayed and prolonged emergence of common waterhemp seed likely confers an advantage for survival under reduced cultivation and reduced use of residual herbicides. Seeds have been observed to mature as soon as 9 days after pollination (DAP) and are capable of optimum germination beginning 10 to 12 DAP (Bell and Tranel 2010). It is important to note, however, that common waterhemp seed germination and dormancy have been observed to exhibit great variation among biotypes as well as under differing temperatures and soil moisture levels (Leon et al. 2006). Germination appears to begin at temperatures of 10 to 20^o C, and reaches its maximum at temperatures of 30 to 35^o C (Guo and Al-Khatib 2003; Leon et al. 2004; Steckel et al. 2004; Steckel et al. 2007). Germination appears to be enhanced in no-till cropping systems, which results in a more rapid decrease of seeds in the soil seed-bank if emerged plants are controlled before producing additional seed (Steckel et al. 2007).

Following germination, common waterhemp seedling emergence and growth varies based on temperature and moisture availability. A field study by Hartzler et al. (1999) found that common waterhemp emergence appeared to be opportunistic based on moisture, with the majority (70%) of the total emergence in one year occurring immediately after one significant rainfall event. Growth appears to be greatest under temperatures of 20 to 35^o C (Guo and Al-Khatib 2003). A study by Horak and Loughlin (2000) comparing growth rates of four *Amaranthus* species (Palmer amaranth, common waterhemp, redroot pigweed, and tumble pigweed (*Amaranthus albus* L.)) across two years revealed that the growth rate of common waterhemp (0.16 and 0.11 cm growing degree day^{-1} (GDD) was second only to Palmer amaranth (0.21 and 0.18 cm GDD^{-1}). This study also revealed that common waterhemp growth was greater than redroot and tumble pigweeds in terms of plant volume, specific leaf area (SLA) and primary branch number measured at final harvest. Common waterhemp is a prolific producer of seed, having been observed to produce in excess of one million seeds per female plant under ideal growing conditions (Steckel et al. 2003), and is capable of producing nearly 300,000 seed per female plant under field conditions (Sellers et al. 2003).

Interference in Agronomic Crops. Plants of the genus *Amaranthus* are often very problematic weeds in agronomic crops due to their ability to germinate under a wide range of conditions, grow rapidly, and produce large numbers of seed, all while competing with the crop for sunlight, moisture, and nutrients. In cotton (*Gossypium hirsutum* L.), Palmer amaranth has been shown to reduce cotton lint yield by 57% when growing at a density of 10 plants 9.1 m row^{-1} (Morgan et al. 2001). Additionally, Palmer amaranth densities greater than six plants 9.1 m row^{-1} of cotton may not be harvestable due to the potential for damage to harvesting equipment (Morgan et al. 2001). A study by Smith et al. (2000) found that Palmer amaranth densities of 650 to 3260 ha^{-1} in dryland stripper-harvested cotton increased harvesting time by 2 to 3.5-fold. Fast et al. (2009) found that the critical timing of Palmer amaranth removal in Oklahoma cotton was approximately 19 days after cotton emergence. If removal was delayed beyond this point, economically significant yield losses should be expected. In soybean (*Glycine max* L. Merr.), common waterhemp that emerged with the crop at a density of 8 plants m^{-1} of row reduced yield by as much as 56% (Bensch et al. 2003). Palmer amaranth growing at a density of 10 plants m row^{-1}of soybean has been shown to reduce yield by nearly 70% (Klingaman and Oliver 1994). Early-emerged common waterhemp in corn (*Zea mays* L.) has been observed to reduce yields from 11 to 74% (Steckel and Sprague 2004). Palmer amaranth emerging with corn has been shown to reduce yield by as much as 91% when present at a density of 8 plants m row^{-1} (Massinga et al. 2001).

Glyphosate. Glyphosate (N-(phosphonomethyl)glycine) is a widely used broad-spectrum postemergence herbicide (Baylis 2000). It is a derivative of the amino acid glycine and was first created in 1950 by Dr. Henri Martin, a Swiss chemist working for a pharmaceutical company (Dill et al. 2010). Its herbicidal properties were not discovered until 1970 by Dr. John Franz, a Monsanto chemist (Dill et al. 2010).

Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, which is only present in the shikimate biosynthesis pathway of plants (Green and Castle 2010). This enzyme plays an important role in the synthesis of aromatic amino acids by catalyzing the transfer of phosphoenolpyruvate to shikimate-3-phosphate to form 5-enolpyruvyl-shikimate-3-phosphate (Dill et al. 2010). In plants affected by glyphosate, an increase in shikimate levels is indicative of injury, as identified by Singh and Shaner (1998) in a study analyzing glyphosate-treated plant tissues by high performance liquid chromatography. In addition to interfering with the synthesis of aromatic amino acids, glyphosate can also affect other physiological processes, resulting in decreases in photosynthesis, degradation of chlorophyll, inhibition of auxin transport, and enhancement of auxin oxidation (Baylis 2000). Visible external effects of glyphosate in treated plants are generally slow to appear (Singh and Shaner 1998), while physiological effects have been observed to occur much earlier (Baylis 2000). Stasiak et al. (1992) found that within 24 h of application of sublethal rates of glyphosate to young (3 month) white birch trees (*Betula papyrifera* Marsh.), ethylene evolution and shikimic acid production were higher than in nontreated controls. In a study by Abu-Irmaileh and

Jordan (1978), significant decreases in the chlorophyll level of purple nutsedge plants (*Cyperus rotundus* L.) were observed within 24 h of glyphosate application.

Outside of its uses as a herbicide, glyphosate can be used for many other purposes (Baylis 2000). Glyphosate has been used as an effective preharvest desiccant for soybean, grain sorghum (*Sorghum bicolor* (L.) Moench), and alfalfa (*Medicago sativa* L.) (Bovey et al. 1975; Bennett and Shaw 2000; May et al. 2003). Glyphosate can be used as a ripener in sugarcane (*Saccharum* spp.) to increase sucrose content by suppressing acid invertase activity (Su et al. 1992). Hormesis, the stimulation of plant growth by low levels of chemical stress, has been observed in some plant and algae species exposed to low rates of glyphosate (Schabenberger et al. 1999; Streibig et al. 2007; Velini et al. 2008). A study in Australia by Hill et al. (1996) found that glyphosate applied at low rates in the spring to bentgrass (*Agrostis castellana* Bois. & Reut.) pastures led to decreased seedhead numbers and increased digestible dry matter and crude protein by the summer.

Herbicide-Resistant Cotton. In 1996, the first glyphosate resistant crop (GRC) was introduced as Roundup Ready® soybean (Monsanto, St. Louis, MO) (Feng et al. 2010). This crop was engineered by transforming the plant with a variant of EPSPS that was less susceptible to binding by glyphosate. The variant, referred to as CP4 EPSPS, was obtained from *Agrobacterium tumefaciens*, a soil bacterium found living in a glyphosate manufacturing facility waste stream in Louisiana (Nida et al. 1996; Green and Castle 2010). The CP4 EPSPS gene was later used to create several other GRC's of cotton, corn, canola (*Brassica napus* L. and *Brassica rapa* L.), alfalfa, and sugarbeet

(*Beta vulgaris* L.). The first glyphosate resistant crops had some issues; limitations for rate, timing, and number of glyphosate applications, as well as a report of decreased yield in GR soybean when compared to sister glyphosate-susceptible (GS) crops (Elmore et al. 2000; Pline-Srnic 2005; Dill et al. 2008). The first Roundup Ready event in cotton, designated MON1445, experienced insufficient expression of the CP4 EPSPS gene after the four-leaf stage of growth (Pline-Srnic et al. 2004; Green and Castle 2010). Applications of glyphosate after this stage of growth could potentially affect cotton yield (Pline-Srnic et al. 2004). The CP4 EPSPS gene in MON1445 was expressed by using the viral promoter FMV from figwort mosaic virus (Feng et al. 2010). The next generation of GR cotton, MON88913 (Roundup Ready® Flex, Monsanto), was made available in 2006 and utilized two CP4 EPSPS genes expressed by stronger chimeric promoters. This resulted in increased expression of the gene in the four to twelve-leaf stages of growth, allowing greater flexibility for applications of glyphosate.

Glufosinate (2-amino-4-[hydroxy(methyl)phosphinyl] butanoic acid) is a common nonselective herbicide that inhibits glutamine synthetase activity, resulting in a destructive accumulation of ammonia in plant tissues (Senseman 2007). Two genes, the bar gene of *Streptomyces hygroscopicus* and the pat gene from *Streptomyces viridochromogenes*, are responsible for the production of an enzyme that deactivates the glufosinate ammonium (Thompson et al. 1987; Wohlleben et al. 1988). These genes have both been successfully used to produce commercially available glufosinate tolerant soybean, cotton, corn, canola, and rice (*Oryza sativa* L.).

Currently, new technologies are being investigated and are scheduled to be released in cotton to provide tolerance to additional herbicide modes of action (Feng et al. 2010). Researchers from Dow Agrosciences (Indianapolis, IN) identified three aryloxyalkanoate dioxygenase enzymes (AAD) of soil bacteria (TfdA-*Ralstonia eutropha*, RdpA-*Sphingobium herbicidivorans*, and SdpA- *Delftia acidovorans*) that effectively cleave 2,4-D ((2,4-dichlorophenoxy)acetic acid) into dichlorophenol and glyoxylate, both of which have no herbicidal activity (Wright et al. 2010). Two of these enzymes also have the ability to act upon herbicide compounds other than 2,4-D. The RdpA enzyme has been shown to cleave herbicides belonging to the grass selective aryloxyphenoxypropionates, while SdpA has the ability to degrade herbicides of the pyridine carboxylic acid family. The genes responsible for the enzymes RdpA and SdpA are commonly referred to as AAD-1 and AAD-12, respectively. The authors successfully transformed corn and soybean plants with the genes responsible for these enzymes and demonstrated that effective tolerance to 2,4-D could be achieved through the use of these transgenes. Commercial release of 2,4-D-tolerant corn with the AAD-1 gene and soybean and cotton with the AAD-12 gene is currently pending (Stagg et al. 2012; Craigmyle et al. 2013; Dow AgroSciences 2013).

Monsanto researchers identified an enzyme called dicamba O-demethylase in a soil bacterium (*Pseudomonas maltophila*) that converts dicamba (3,6-dichloro-2-methylbenzoic acid) to 3,6-dichlorosalicylic acid (DCSA)(Behrens et al. 2007). The enzyme DCSA has no significant herbicidal properties. The gene responsible for this enzyme is known as DMO (dicamba monooxygenase). The authors were able to successfully insert the DMO gene into *Arabidopsis thaliana*, tomato (*Solanum lycopersicum* L.), and tobacco (*Nicotiana tabacum* L.) and provide these plants with effective tolerance to foliar applications of dicamba. Commercial release of dicamba-tolerant cotton and soybean by Monsanto is currently pending regulatory approval.

Glyphosate Resistance in Weeds. The widespread adoption of GRC's by growers and the resulting changes in weed management strategies utilized by those growers has brought about significant changes in weed populations. Shifts away from soil-applied residual herbicides to POST herbicide programs became an increasingly attractive option for growers who chose to utilize GRC's (Culpepper and York 1998; 1999). In 1996, the first documented case of evolved glyphosate resistance in a weed species was reported in Australia by Pratley et al. (1996) in rigid ryegrass (*Lolium rigidum* Gaud.). Since then, glyphosate resistance has been reported in twenty-four weed species of eighteen genera in twenty countries (Heap 2014). In 2005, glyphosate resistance in an *Amaranthus* species was first documented in a biotype of Palmer amaranth growing in a Georgia cotton field, where six- to eightfold levels of resistance to glyphosate were observed (Culpepper et al. 2006). Studies conducted in 2006 and 2007 by Legleiter and Bradley (2008) confirmed glyphosate resistance in a biotype of common waterhemp found in a Missouri soybean field following multiple glyphosate applications. Culpepper (2006) evaluated surveys sent to weed scientists across the United States and revealed that all of the responders felt that significant weed population changes had occurred in their areas due to the adoption of GRC's. Of those responders, 50% indicated that weeds of the genus *Amaranthus* had significantly increased in cotton, along with weeds of the genera *Ipomoea*, *Commelina*, and *Cyperus*. Currently, GR Palmer amaranth and common waterhemp have been reported in 22 and 15 U.S. states, respectively (Heap 2014). In Texas, the presence of glyphosate-resistance in a common waterhemp biotype collected in Wharton County was confirmed in 2006 by Light et al. (2011). In 2011, GR Palmer amaranth was found in the Texas panhandle (Heap 2014).

Two possible mechanisms for evolved glyphosate resistance in weeds have been demonstrated; target site-based and non-target site-based resistance (Powles and Preston 2006). Target site-based resistance can involve an alteration of the site of action resulting in reduced herbicide affinity for that site, or an over-production of the target enzyme as a result of gene amplification (Perez-Jones and Mallory-Smith 2010). Non target site-based resistance can involve differential uptake or translocation, sequestration, or metabolic detoxification (Powles and Preston 2006; Yuan et al. 2006). Leigleiter and Bradley (2008) suggested that an insensitive EPSPS enzyme may have been the mechanism of resistance in one biotype included in their study since those plants exhibited much higher levels of glyphosate resistance than that of other documented biotypes; however, this was not confirmed. A study by Gaines et al. (2011) investigating the mechanism of glyphosate resistance in Palmer amaranth found that gene amplification resulting in increased production of EPSPS was likely the cause of resistance, not an altered EPSPS enzyme, as EPSPS of glyphosate susceptible (GS) and GR plants in the study were equally inhibited by glyphosate.

Management. An article by Gressel and Segel (1990) described four tactics for delaying or preventing the appearance of herbicide resistant weeds: using herbicides with minimum selection pressure that may not give total weed control and leave enough susceptible individuals behind to “dilute” out resistant plants, using mixtures of herbicides that act at different sites of action, rotating herbicides with different sites of action and modes of degradation, and employing mechanical cultivation. In a survey of weed scientists by Culpepper (2006), respondents provided the following four recommendations for managing glyphosate-induced weed species shifts: tank-mix combinations of other herbicides with glyphosate for postemergence (POST) applications, rotating with non-GR crops (though there was some disagreement among responders), use of POST herbicides other than glyphosate, and using preplant incorporated (PPI) or preemergence (PRE) soil-applied herbicides.

Several residual herbicides are commercially available for the control of Palmer amaranth and common waterhemp in cotton. Prometryn (N,N’ –bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine) plus metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide) applied PRE has been shown to provide 100% control of Palmer amaranth (Grichar et al. 2004). Culpepper and York (1998) found that PPI-applied trifluralin (2,6-dinitro-N,N-dipropyl-4-(tifluoromethyl)benzamine) plus PRE-applied fluometuron (N,N-dimethyl-N’-[3-(trifluoromethyl)phenyl]urea) provided 81% late-season control of Palmer amaranth without the use of any POST herbicides. A study in corn by Vyn et al. (2005) found that pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) applied PRE provided 71 to 94% control of common waterhemp. Pendimethalin plus fluometuron applied PRE has been shown to provide 58% control of Palmer amaranth without including any POST herbicide applications, while the addition of pyrithiobac (2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid) early- and mid-POST applications to the pendimethalin/fluometuron PRE increased control to 88% (Culpepper and York 2000). Fomesafen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzenamide) applied PRE at a rate of 280 g ha^{-1} has been shown to provide 95% control of redroot pigweed and Palmer amaranth, while also providing some control of common lambsquarters (*Chenopodium album* L.) (80%) and annual grasses (90%) (Gardner et al. 2006). Pyrithiobac applied PRE has been shown to provide 91 to 97% control of Palmer amaranth (Dotray et al. 1996; Gardner et al. 2006). A study by Sweat et al. (1998) in soybean found that acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide) applied PRE at 1790 g ha^{-1} provided 100% control of both Palmer amaranth and common waterhemp 28 days after treatment. Additionally, the authors found that 94 to 100% control of these two species could be achieved by PRE applications of metolachlor at 1680 g ha^{-1}.

In addition to the several residual herbicides available for *Amaranthus* weed control in cotton, there are multiple options for post-emergence control, particularly in light of the recent development of GRC's. When applied to GS plants, glyphosate offers excellent control (100%) of *Amaranthus* spp. weeds (Corbett et al. 2004). Glufosinate has been shown to offer effective control of Palmer amaranth (93 to 97%) when applied to plants less than 10 cm in height (Corbett et al. 2004). A study by Doherty et al.

(2010) showed that dicamba applied at rates of 0.28 and 0.56 kg ha^{-1} to 7.6 and 15 cm Palmer amaranth provided 99 to 100% control 40 days after treatment. In a two year weed control study in cotton, Branson et al. (2005) found that trifloxysulfuron-sodium (N-[(4,6-dimethoxy-2-pyrimidinyl)carmaboyl]-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium) applied POST at 5.3 g ai ha^{-1} resulted in 95 to 100% control of Palmer amaranth. The same study also found that POST applications of 70 g ai ha^{-1} pyrithiobac provided 100% control of Palmer amaranth.

Physical Spray Drift Reduction

The widespread popularity of existing glyphosate and glufosinate tolerant crop technologies and the probable future acceptance of 2,4-D and dicamba-tolerant crops has increased the need for understanding the potential for non-tolerant crop injury due to physical spray drift.

Impacts of Herbicide Drift on Crop Growth and Yield. Thomas et al. (2005) found that non-tolerant cotton yields were reduced by glyphosate rates of 35 g ha^{-1} or higher in one year; however, this was not the case the following year, indicating that cotton susceptibility to glyphosate drift may be influenced by environmental conditions. Ellis and Griffin (2002) found that sublethal rates of 140 g ha^{-1} glyphosate and 53 g ha^{-1} glufosinate applied at several growth stages from 3-leaf to early bloom resulted in maximum visual injury values of 16 and 39% to non-tolerant cotton, respectively, but had no effect on yield. Soybean appears to be able to recover adequately from sublethal applications of glyphosate as indicated by Al-Khatib and Peterson (1999) and Ellis and

Griffin (2002). The results of these two studies showed that although significant visual injury was induced by application rates as high as 370 g ha^{-1} glyphosate made at several crop growth stages, no reductions in yield were reported. Ellis et al. (2003) found that rates of 35 g ha^{-1} glyphosate and 53 g ha^{-1} glufosinate reduced corn yields by 22 and 13%, respectively. In wheat, decreases in yield have been reported following glyphosate rates of 84 g ae ha^{-1} (Deeds et al. 2006). Significant yield increases in peanut have been observed following glyphosate rates of 35 g ae ha^{-1}, but yield reductions begin to occur at rates of 280 g ae ha^{-1} or higher (Lassiter et al. 2007).

Sufficient data from simulated drift studies exist supporting that 2,4-D and dicamba drift can also have a significant impact on the growth and yield of susceptible crops. A study by Marple et al. (2008) investigating the effect of simulated drift of 2,4-D and dicamba on cotton found that rates of 2.8 g ae ha^{-1} 2,4-D and dicamba applied to 3- to 4-leaf cotton resulted in visible injury as great as 88 and 41%, respectively. These rates of 2,4-D and dicamba applied at this stage also reduced cotton yield by approximately 30 and 10%, respectively. Crop injury and yield losses were significantly increased by repeated simulated drift applications. Everitt and Keeling (2009) found that a simulated drift rate of 28 g ae ha^{-1} 2,4-D resulted in 45 to 68% reductions in cotton yield when applied to 2-leaf, 4- to 5-leaf, and pinhead square growth stages. Similar rates of dicamba applied at the same cotton growth stages resulted in less yield reduction (<15%) than rates of 2,4-D. In soybean, rates as low as 1.3 g ha^{-1} of a dimethylamine (DMA) salt of dicamba have been shown to result in a 10% reduction in yield under droughty conditions, when plants are less able to recover following injury caused by dicamba (Weidenhamer et al. 1989). Al-Khatib and Peterson (1999) found that dicamba applications of 56.1 and 185 g ha^{-1} at the 2 to 3 trifoliate stage decreased soybean yield by 45 and 92%, respectively.

Schroeder et al. (1983) found that rates of 140, and 280 g ha^{-1} of 2,4-D DMA (dimethylamine salt) had no impact on sugarbeet yield, regardless of crop growth stage at application, but did have a negative effect on sugarbeet quality as expressed by percent sucrose, sugarbeet purity, and extractable sucrose. This study also found that dicamba DMA at 140 g ha^{-1} resulted in a yield reduction of 15% as well as significantly lower sugarbeet quality. Hemphill and Montgomery (1981) examined the effect of sublethal applications of 2,4-D on a variety of vegetable crops and found that 2.1 g ha^{-1} of 2,4-D DMA reduced yield of pepper and radish. Applications of 20.8 g ha^{-1} reduced yield of tomato and resulted in 100% unmarketable roots of carrot, radish, rutabaga, and turnip. Yields of broccoli, cabbage, carrot, cauliflower, cucumber, lettuce, onion, radish, and turnip were all decreased by applications of 208 g ha^{-1}.

The potential for physical drift of agricultural sprays is influenced by several factors which can be arranged into three major groups. First are environmental factors such as wind speed, air temperature, humidity, and atmospheric stability. Next are application equipment factors such as operating pressure, nozzle orifice size, spray boom height, and application speed. Finally, the characteristics of the spray solution as affected by formulation, such as surface tension, density, and viscosity, may influence the potential for spray drift to occur (Maybank et al. 1974; Nuyttens et al. 2009).

Effect of Droplet Size on Physical Drift Potential. The size of droplets exiting the spray nozzle has a large effect on the potential for physical spray drift. Larger droplets have been shown to have greater droplet velocities than small droplets. Lower droplet velocities translate into an increased amount of time between exiting the spray nozzle and deposition on the target site (Nuyttens et al. 2009). The length of this interval is directly related to the potential for off-target movement of the droplet. Further complicating this effect is the decrease in droplet size after exiting the nozzle due to evaporation. Smaller droplets have higher surface area to volume ratios, resulting in an increased rate of evaporation of water in the spray solution (Akesson and Yates 1964). This evaporation of water can result in a rapid decrease in droplet size before deposition takes place, exacerbating the risk for drift of spray droplets off-target. Both smaller droplet size and increased time to droplet deposition create more vulnerability to wind that will cause increased lateral movement of droplets.

Effect of Droplet Size on Herbicide Efficacy. The efficacy of foliar herbicide sprays appears to be the result of two factors; (1) the leaf area contacted by the herbicide, and (2) the rate of diffusion of the herbicide into plant tissues. If the applied spray solution volume is held constant, smaller droplets result in greater coverage of leaf area. This was shown in a study by Liu et al. (1996), where application of 326 μm droplets resulted in twice the leaf area coverage compared to 977 μm droplets. A study by McKinlay et al. (1972) investigating the effect of spray droplet size of 2,4-D on sunflower control found that 100 μm droplets were more effective than 200 or 400 μm droplets. Herbicide concentration certainly appears to affect herbicide diffusion into plant tissues as shown by Liu et al. (1996); however, when herbicide concentration is held constant, larger droplets appear to negatively affect diffusion. Larger droplets of herbicide solution have been observed to result in greater localized tissue death directly under the droplet, potentially hindering further diffusion of the herbicide in the plant (McKinlay et al. 1972; Prasad and Cadogan 1992). A meta-analysis by Knoche (1994) of studies investigating the effect of droplet size on herbicide efficacy found that in more than 70% of experiments, efficacy was improved as droplet size decreased.

Effect of Nozzle Characteristics on Droplet Size. There are three main categories of agricultural spray nozzles: (1) conventional nozzles where the spray solution simply passes through an orifice and exits the nozzle, (2) low-drift nozzles that typically utilize a pre-orifice of smaller size than the terminal orifice to slow the flow of the spray solution prior to reaching the terminal orifice, and (3) air-inclusion nozzles that draw air into the spray solution before exiting the nozzle. Spray nozzles advertised as drift-reducing nozzles typically utilize either of the latter two designs or a combination of these. If all other factors are held constant, larger orifice sizes result in a larger droplet size spectra and decreases in the proportion of spray volume made up of small droplets. Nuyttens et al. (2007) found that air inclusion nozzles had the greatest drift reduction potential, followed by nozzles with a pre-orifice and conventional flat-fan nozzles. This trend has been observed by several others (Etheridge et al. 1999; Nuyttens et al. 2009; Klein et al. 2011).

Effect of Formulation on Droplet Size. The properties of the spray solution have been shown to significantly influence droplet size spectra. The viscosity, density, and surface tension of the spray solution have all been shown to greatly influence the spray characteristics of a nozzle; however, surface tension appears to be the main factor influencing droplet size (Ellis et al. 2001). Spray solutions tend to exit flat-fan nozzles as a sheet that then breaks up as the sheet expands due to oscillations produced by sinuous waves in the sheet (Fraser et al. 1962). The fragments of the broken sheet become droplets. The addition of surfactants to a spray solution reduces surface tension, delaying the breakup of the sheet by suppressing these oscillations (Ellis et al. 2001). This generally results in a decrease in droplet size spectra when sprayed through a conventional hydraulic flat-fan nozzle. Air-inclusion nozzles appear to be sensitive to other changes in the characteristics of the spray solution and occasionally do not follow the trend of sprays produced by conventional nozzles (Miller and Ellis 2000.) Commercial herbicide products and adjuvants are often highly complex, since the physical characteristics of the product are a result of many components. As a result, most investigations of the effect of herbicide formulations and adjuvants are conducted on a case-by-case basis (Chapple et al. 1993; Hanks 1995; Ellis and Tuck 1999; Stainier et al. 2006).

CHAPTER III

MATERIALS AND METHODS

Confirmation of Glyphosate-Resistant Common Waterhemp

To document and confirm the presence of glyphosate-resistant common waterhemp in East Central Texas, seeds were collected from common waterhemp accessions at various locations in East-central Texas that were suspected to be glyphosate-resistant. Seed from a confirmed glyphosate-susceptible individual was also included. Seeds were brought to the Texas A&M Institute for Plant Genomics and Biotechnology greenhouse in College Station, TX to be grown and treated with glyphosate. This experiment was set up as a completely randomized design (CRD) with 4 replications. Seeds from each plant were planted 0.3 cm deep in Sunshine LC1 growing mix (Sun Gro Horticulture, Vancouver, BC, Canada) in 10 by 10 cm square pots. Pots were placed into 25 by 51 cm plastic flats and covered with a transparent plastic lid until emergence. Treatments of glyphosate were applied once plants reached approximately 12 cm in height. Treatments included an nontreated control, 434 g ae ha^{-1} (0.5X), 867 g ae ha^{-1} (1X), 1734 g ae ha^{-1} (2X), and 3468 g ae ha^{-1} (4X) of Roundup PowerMax® (Monsanto Company, St. Louis, MO), a potassium salt of glyphosate. Plants were removed from the greenhouse for treatment to prevent injury to other studies in the greenhouse. Applications were made with a CO_2-pressurized backpack sprayer through a single TeeJet 8002E spray nozzle (Spraying Systems Co., Wheaton, IL). Total spray volume was 187 L ha^{-1}. After application, plants were returned to the greenhouse.

At 7, 14, 21, and 28 days after treatment (DAT), plant height (cm) and mortality (100% necrosis = dead) were recorded. After the final evaluation at 28 DAT, above-ground biomass of each plant was harvested, dried at 55°C for 48 h, and weighed.

Post-treatment plant biomass data were analyzed by analysis of variance (ANOVA) to determine if an effect of accession exists. Mean separation of these data were conducted using Tukey's honestly significant difference (HSD). Logistic regression of mortality data was utilized to construct dose-response curves and determine LD_{50} values (the lethal dose of a chemical which kills 50% of the sample population) for each accession (Seefeldt et al. 1995). All analyses were conducted using JMP 10 (SAS Institute 2012a; 2012b).

Glyphosate-Resistant Weed Management in Cotton

A two-year field research trial was established at the Texas A&M Agrilife Research Farm in Burleson County, Texas in 2012 to evaluate the efficacy of twelve different herbicide regimes for control of common waterhemp and Palmer amaranth with an emphasis on the weed control provided by PPI and PRE applications. This study was conducted in cotton possessing glyphosate-, glufosinate-, and dicamba-tolerant technologies. The study was conducted on a furrow-irrigated field dedicated for the purpose of growing this currently federally-regulated cotton technology. Soil at this site is characterized as a Weswood silty clay loam. The field typically has large populations of common waterhemp and Palmer amaranth, as well as a variety of other dicot and monocot weed species. In 2012, weed control data were recorded for Palmer amaranth, common waterhemp, and red sprangletop (*Leptochloa filifomis* (Lam.)). In 2013, weed control data were recorded for Palmer amaranth, common waterhemp, junglerice (*Echinochloa colona* (L.) Link), and sharppod morningglory (*Ipomoea trichocarpa* Elliot var. *trichocarpa*). The study was conducted as a randomized complete block (RCBD) for two consecutive years. Individual plots were 4.0 m wide and 9.1 m in length with cotton planted on a 1.0 m row spacing. Blocks were separated by 4.5 m buffers to allow for lateral movement of equipment between blocks. Cotton was planted May 22th in 2012 and May 8th in 2013.

Preplant incorporated and PRE applications included the sodium salt of fomesafen, pendimethalin, prometryn, pyrithiobac sodium, *S*-metolachlor, and trifluralin. PPI applications of trifluralin were incorporated with two passes of a rolling cultivator at 9.5 Km h^{-1}. Postemergence applications included acetochlor, diglycolamine salt of dicamba, glufosinate-ammonium, potassium salt of glyphosate, pyrithiobac-sodium, and trifloxysulfuron-sodium. Postemergence applications were split into two application timings; early-postemergence (EPOST) and mid-postemergence (MPOST). The common names, trade names, application rates, and manufacturers of the herbicides used are listed in Table 1, while the applications within each treatment are listed in Table 2. Applications were made with a CO_2-pressurized backpack sprayer with eight nozzles spaced 50 cm apart. Preplant incorporated and PRE applications were applied using TeeJet 11003 DG nozzles, while POST applications were applied with TeeJet TTI110015 nozzles. Total spray volume for all applications was 140 L ha^{-1}. A metronome was utilized to keep walking speeds consistent at 4.8 Km h^{-1}. Environmental conditions were recorded at the time of herbicide applications and are reported in Table 3.

Weed control was evaluated by obtaining visual control ratings of 0 to 100% (0% = no control, 100% = complete control) recorded prior to each application timing as well as after the last POST application. Standard crop management practices common for this region were followed throughout the season. Cotton yields were collected, recorded, and analyzed. Data for weed control and cotton yield were analyzed by ANOVA and means were separated by Tukey's HSD test in JMP 10 (SAS Institute 2012a).

Table 1. Herbicides and application rates.

Common Name	**Trade Name**	**Application Rate**	**Manufacturer**
acetochlor	Warrant	1.26 kg ai ha^{-1}	Monsanto Company, St. Louis, MO 63167
dicamba	Clarity	0.56 kg ae ha^{-1}	BASF Corporation, Research Triangle Park, NC 27709
fomesafen	Reflex	0.28 kg ai ha^{-1}	Syngenta Crop Protection, Greensboro, NC 27419
glufosinate	Liberty 280 SL	0.59 kg ai ha^{-1}	Bayer CropScience LP, Research Triangle Park, NC 27709
glyphosate (A)	Roundup PowerMAX	1.26 kg ae ha^{-1}	Monsanto Company, St. Louis, MO 63167
glyphosate (B)	Touchdown Total	0.88 kg ae ha^{-1}	Syngenta Crop Protection, Greensboro, NC 27419
pendimethalin	Prowl H_2O	1.60 kg ai ha^{-1}	BASF Corporation, Research Triangle Park, NC 27709
prometryn	Caparol 4L	0.56 kg ai ha^{-1}	Syngenta Crop Protection, Greensboro, NC 27419
pyrithiobac	Staple LX	58.84 g ai ha^{-1} PRE 72.86 g ai ha^{-1} POST	E.I. du Pont de Nemours and Company, Wilmington, DE 19898
S-metolachlor	Dual Magnum	1.07 kg ai ha^{-1}	Syngenta Crop Protection, Greensboro, NC 27419
trifloxysulfuron	Envoke	5.25 g ai ha^{-1}	Syngenta Crop Protection, Greensboro, NC 27419
trifluralin	Treflan 4L	1.12 kg ai ha^{-1}	Loveland Products Inc., Greeley, CO 80632

Table 2. Herbicide components and applications within each treatment.

Treatment	**Herbicide**	**Application**
1	Nontreated	N/A
2	*S*-metolachlor	PRE
	glyphosate (B)	MPOST
	trifloxysulfuron	MPOST
3	*S*-metolachlor	PRE
	prometryn	PRE
	glyphosate (B)	MPOST
	trifloxysulfuron	MPOST
4	fomesafen	PRE
	glyphosate (B)	MPOST
	trifloxysulfuron	MPOST
5	fomesafen	PRE
	S-metolachlor	PRE
	glyphosate (B)	MPOST
	trifloxysulfuron	MPOST
6	trifluralin	PPI
	glyphosate	EPOST
	dicamba	EPOST
	glyphosate (A)	EPOST
7	trifluralin	PPI
	glufosinate	EPOST
	glufosinate	EPOST
8	trifluralin	PPI
	glyphosate (A)	EPOST
	dicamba	EPOST
	acetochlor	EPOST
9	trifluralin	PPI
	dicamba	EPOST
	glufosinate	EPOST
	glyphosate (A)	MPOST
	dicamba	MPOST
10	pendimethalin	PRE
	glyphosate (A)	EPOST
	dicamba	EPOST
	acetochlor	EPOST
11	pyrithiobac	PRE
	glyphosate (A)	EPOST
	dicamba	EPOST
	acetochlor	EPOST
12	pendimethalin	PRE
	pyrithiobac	EPOST
	glufosinate	MPOST
13	pendimethalin	PRE
	glyphosate (A)	EPOST

Table 3. Environmental conditions at herbicide applications.

----------------------------------2012 Applications----------------------------------				
Application	PPI	PRE	EPOST	MPOST
Date	5/7/12	5/22/12	6/21/12	7/4/12
Time	12:00 PM	7:00 PM	5:30 PM	9:00 AM
Air Temperature (^oC)	27	31	35	31
Soil Temperature at 12 cm depth (^oC)	26	29	32	29
Relative Humidity (%)	64	45	58	47
Cloud Cover (%)	5	5	15	20
Dew?	No	No	No	No
Soil Surface	Dry	Dry	Dry	Moist
Soil Moisture	Good	Fair	Good	Excellent

----------------------------------2013 Applications----------------------------------				
Application	PPI	PRE	EPOST	MPOST
Date	5/8/13	5/9/13	6/7/13	6/16/13
Time	1:00 PM	11:00 AM	11:30 AM	7:00 PM
Air Temperature (^oC)	28	26	32	36
Soil Temperature at 12 cm depth (^oC)	26	22	27	33
Relative Humidity (%)	33	63	35	41
Cloud Cover (%)	5	100	5	75
Dew?	No	No	No	No
Soil Surface	Dry	Dry	Dry	Dry
Soil Moisture	Good	Good	Good	Fair

Spray Droplet Size Spectra Experiment

A low-speed wind tunnel was utilized for analyzing the effect of herbicide formulation and spray nozzle on droplet size spectra. This tunnel is operated by United States Department of Agriculture, Agricultural Research Service in College Station, TX. The tunnel is 1.2 X 1.2 m in cross-section and 14.6 m in length. Airflow from a fan at the upstream end of the tunnel pushes air through flow straighteners to produce a laminar flow in the tunnel. Air speed was 6.7 m sec^{-1}. Spray droplet sizing was conducted with a Helos/KR laser diffraction sensor (Sympatec GmbH, Clausthal, Germany). The sensor consists of two portions, an emitter and a receiver. The emitter houses a 623 nm helium-neon laser that is aligned with the receiver. The receiver is fitted with a lens with 32 sizing bins that can measure droplet sizes from 0.5 to 3500 µm (denoted as an R7 lens by the manufacturer). The Helos sensor is positioned such that the laser fires horizontally across the center of the downstream end of the tunnel. A single spray nozzle is affixed to a vertically-mounted traverse system that allows the nozzle to travel from the top to the bottom of the tunnel over a 1-m length. The traverse system is positioned such that the spray nozzle is 30.5 cm from the Helos sensor. Three replications were conducted for each combination of herbicide, spray nozzle, and operating pressure. Each replication consisted of traversing the vertically-aligned spray nozzle from the top to the bottom of the tunnel so that the entire spray pattern travelled across the Helos sensor laser. Spray solutions were prepared in 11 L samples placed into 19 L stainless steel containers pressurized by a pressure-regulated supply of compressed air. A quarter-turn ball valve on the container was used to start and stop the flow of spray solution to the spray nozzle. A portable air scrubber was positioned at the downstream end of the tunnel to capture airborne spray solution.

Spray nozzles for this study included XR 11002 Extended Range, DG 11002 Drift Guard, AI 11002 Air Induction, AIXR11002 Air Induction, and TTI 11002 Turbo TeeJet Induction flat spray tips (TeeJet Technologies, Wheaton, Illinois). The numerical designation of spray nozzles denotes the angle of the spray plume and the flow when operated at a pressure of 275 kPa (40 psi). In the case of the nozzles included in this study, all produce a 110^{o} spray plume and flow 0.757 L min^{-1} (0.2 GPM) at 275 kPa. Nozzles, recommended operating pressures, and the manufacturer-estimated droplet sizes produced at a given pressure are shown in Table 4. The XR nozzle is a conventional flat-fan nozzle with a single orifice. The DG nozzle utilizes a -02 pre-orifice with a larger terminal orifice. The AI, AIXR, and TTI nozzles are all air-inclusion nozzles that also utilize a pre-orifice. Operating pressures for this study were 207 and 414 kPa. New, unused nozzles were utilized for this study. Before droplet size analyses were conducted, nozzles were tested to verify that their flow rate is within manufacturer specifications. TeeJet 8079 50-mesh strainers were used for all nozzles to prevent any contaminant from altering the spray pattern characteristics or droplet sizes produced by the nozzles.

Table 4. Spray nozzle specifications as supplied by the manufacturer.

Nozzle	Operating Pressure Range	Manufacturer-estimated droplet size	
	kPa	µm at 207 kPa	µm at 414 kPa
XR	103-414	136-177	136-177
DG	207-414	177-218	177-218
AIXR	103-620	349-428	218-349
AI	207-689	428-622	349-428
TTI	103-689	>622	>622

Herbicides included in this study are shown in Table 5. The products MON 76832, GF-2726, Roundup PowerMAX, Durango DMA, and Liberty 280 SL do not require the addition of a surfactant. A non-ionic surfactant, Activator 90 (Loveland Products, Greeley, CO), was added at 0.25 % v/v to the products Clarity and 2,4-D Amine 4, as recommended by the product labels. Additionally, solutions of water alone and water + 0.25 % v/v Activator 90 were included in this study. Spray solutions were prepared based on the assumption of a 140 L ha^{-1} total application volume.

Table 5. Active ingredients and rates for herbicide products.

Product	**Active Ingredient(s)**	**Acid Equivalent**	**Product Rate**
		g L^{-1}	**mL ha^{-1}**
MON76832	diglycolamine salt of dicamba monoethylamine salt of glyphosate	120 240	1893
GF-2726	choline salt of 2,4-D dimethylamine salt of glyphosate	195 205	1656
Clarity	diglycolamine salt of dicamba	480	473
2,4-D Amine 4	dimethlyamine salt of 2,4-D	455	698
Roundup PowerMAX	potassium salt of glyphosate	540	840
Durango DMA	dimethylamine salt of glyphosate	480	710
Liberty 280 SL	glufosinate ammonium	N/A	858

For each nozzle/formulation/pressure combination, the Helos sensor system records $D_v0.1$, $D_v0.5$, and $D_v0.9$ values, which are the droplet sizes in μm for which 10, 50, and 90% of the spray volume is made up of droplets less than or equal to that size. Relative span, a dimensionless measure was calculated to give a relative measure of the spread of the droplet size distribution of the spray volume. Relative span is calculated by subtracting the $D_v0.1$ from the $D_v0.9$ and dividing this by the $D_v0.5$. Also, the percentage of the total spray volume made up of droplets less than or equal to a given size (30, 50, 80, 100, 141, 150, 200, 730 microns) was recorded (referred to as Q_i30, Q_i50, Q_i80, Q_i100, etc.). The Q_i100 value is used for this experiment as an indicator of the "driftable" portion of the total spray volume. This study was designed as a 2-factor (main factors nozzle and product) experiment, with data analyzed via a full-factorial analysis of variance (ANOVA) separated by the two operating pressures. Data were analyzed separately for the main effects of nozzle and product using one-way ANOVA.

Means were separated with Tukey’s honestly significant difference (HSD). Statistical analyses were conducted using JMP 10 (SAS Institute 2012a).

CHAPTER IV

RESULTS

Confirmation of Glyphosate-Resistant Common Waterhemp

Estimated LD_{50} values for the seventeen accessions ranged from 387 to 4548 g ae ha^{-1}. LD_{50} values of accessions 3a and 4 could not be estimated as there was no mortality of these plants following any treatment. When the LD_{50} value for each accession is compared to that of the chosen susceptible population (accession 6), relative glyphosate resistance ranged from 0.8- to 9.0-fold. Estimated LD_{50} values and the level of glyphosate resistance of each accession are shown in Table 6

Table 6. Estimated LD_{50} values and levels of relative glyphosate resistance.

Accession	**Location**	**estimated LD_{50} g ae ha^{-1}**	**level of resistance**
1	Brazos Co., TX	1028	2.0
2	Brazos Co., TX	387	0.8
3a	Brazos Co., TX	N/A	>9.0
3b	Brazos Co., TX	2496	5.0
4	Brazos Co., TX	N/A	>9.0
5	Burleson Co., TX	4450	8.8
6	Burleson Co., TX	503	1.0
7a	Burleson Co., TX	2101	4.2
7b	Burleson Co., TX	1719	3.4
7c	Burleson Co., TX	1484	2.9
8	Burleson Co., TX	2470	4.9
9	Robertson Co., TX	2496	5.0
10	Robertson Co., TX	3240	6.4
11	Milam Co., TX	4549	9.0
12a	Fort Bend Co., TX	2238	4.4
12b	Fort Bend Co., TX	2196	4.4
13	Wharton Co., TX	1735	3.4

When the dried biomass harvested 28 days after treatment (DAT) of glyphosate-treated plants were compared to that of nontreated plants of the same accession, biomass reductions in response to glyphosate ranged from 0 to 92.0% following the 0.5X rate, 0 to 100% following the 1.0X rate, 0 to 100% following the 2.0X rate, and 13.7 to 100% following the 4.0X rate. Biomass reduction data failed to meet the assumption of homogeneity of variances for ANOVA and were arcsine transformed for analyses. The nontransformed means are reported. Biomass reduction of accessions in response to glyphosate treatments are shown in Table 7. Following the 0.5X rate, plants of accession 11 exhibited less biomass reduction (0%) than accessions1, 2, 3b, 6, 7a, 7b, 7c, 9, 12b, and 13. Also after the 0.5X rate, biomass reduction of accession 3a (6.2%) was less than that of accessions 6, 7b, and 12b. Following the 1.0X rate, accession 3a exhibited less biomass reduction (0%) than accessions 2, 3b, 6, 7a, 7b, 7c, 9, and 13. Biomass reduction of accessions 5 and 11 (14.4 and 22.1%, respectively) was less than that of accessions 2 and 6 following the 1.0X rate. Following the 2.0X rate, biomass reduction of accession 3a (0%) was less than that of all others with the exception of accessions 4, 5, and 11 (32.4, 39.3, and 25.1%, respectively). The aforementioned accessions also exhibited less biomass reduction than several other accessions at the 2.0X rate. Following the 4.0X rate, plants of accession 3a exhibited less biomass reduction (13.7%) than accessions other than 4, 5, 7a, 10, 11, and 12a.

Table 7. Biomass reduction of accessions in response to glyphosate 28 DAT.

Accession	0.5 X	1.0 X	2.0 X	4.0 X
	---------------------% biomass reduction---------------------			
1	57.8 ab[1]	42.7 abc	100.0 a	100.0 a
2	34.5 ab	100.0 a	100.0 a	100.0 a
3a	6.2 bc	0.0 c	0.0 e	13.7 b
3b	65.0 ab	65.7 ab	87.1 abc	97.3 a
4	35.3 abc	56.3 abc	32.4 de	82.6 ab
5	31.3 abc	14.4 bc	39.3 bcde	64.4 ab
6	92.0 a	100.0 a	100.0 a	100.0 a
7a	70.9 ab	92.7 ab	96.1 ab	75.6 ab
7b	86.1 a	89.0 ab	98.3 a	100.0 a
7c	65.9 ab	90.3 ab	95.1 ab	100.0 a
8	54.6 abc	54.4 abc	56.4 abcd	100.0 a
9	60.0 ab	70.7 ab	73.1 abcd	92.1 a
10	17.7 abc	53.0 abc	76.8 abcd	72.8 ab
11	0.0 c	22.1 bc	25.1 cde	70.5 ab
12a	32.9 abc	33.7 abc	50.9 abcd	76.2 ab
12b	75.5 a	56.7 abc	95.2 ab	96.6 a
13	68.8 ab	61.9 ab	86.1 ab	87.4 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Glyphosate-Resistant Weed Management in Cotton

Data from 2012 and 2013 are presented separately here since there was a significant interaction between treatment and year for all weed control and cotton yield data. Weed control data are reported at three times: early (ratings taken prior to EPOST application timing), mid (ratings taken prior to MPOST application timing), and late (ratings taken two weeks after MPOST application timing). In order to meet the assumption of homogeneity of variances for ANOVA, weed control data were arcsine transformed for analyses (Ahrens et al. 1990). Nontransformed means are reported in the following tables. Cotton yield data did not require transformation prior to ANOVA and mean separation.

Control of Palmer amaranth in 2012 in treated plots ranged from 29 to 99% at the early weed control rating, 93 to 100% at the mid rating, and 99 to 100% at the late rating. Palmer amaranth control data from 2012 are shown in Table 8. Palmer amaranth control provided by treatment 11 (pyrithiobac PRE) prior to EPOST applications (29%) was lower than all other herbicide treatments. No differences among herbicide treatments were detected at the mid and late ratings.

Table 8. Effect of treatment on mean 2012 Palmer amaranth control.

Treatment	**Early**	**Mid**	**Late**
		------------------control (%)------------------	
1	0 c[1]	0 b	0 b
2	96 a	99 a	100 a
3	81 a	99 a	100 a
4	88 a	93 a	100 a
5	85 a	99 a	100 a
6	97 a	100 a	100 a
7	86 a	100 a	100 a
8	96 a	100 a	100 a
9	99 a	100 a	100 a
10	83 a	100 a	100 a
11	29 b	99 a	100 a
12	84 a	95 a	99 a
13	83 a	99 a	99 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Common waterhemp control provided by herbicide treatments in 2012 ranged from 55 to 100% at the early rating, 89 to 100% at the mid rating, and 98 to 100% at the final rating. These data are shown in Table 9. Early common waterhemp control was lower from treatment 11 (pyrithiobac PRE) (55%) compared to all herbicide treatments other than 3, 12, and 13. Following EPOST applications, common waterhemp control was lower for treatment 5 (fomesafen+*S*-metolachlor PRE) (85%) than for treatments 6 and 8 through 11. There were no differences in common waterhemp control among herbicide treatments following MPOST applications.

Table 9. Effect of treatment on mean 2012 common waterhemp control.

Treatment	Early	Mid	Late
	------------------	control (%)	------------------
1	0 d[1]	0 c	0 b
2	95 ab	97 ab	100 a
3	89 abc	91 ab	100 a
4	92 ab	89 ab	100 a
5	91 ab	85 b	100 a
6	100 a	100 a	100 a
7	98 ab	99 ab	100 a
8	100 a	100 a	100 a
9	100 a	100 a	100 a
10	95 ab	100 a	100 a
11	55 c	100 a	100 a
12	79 abc	89 ab	100 a
13	73 bc	99 ab	98 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Red sprangletop control among herbicide treatments in 2012 ranged from 45 to 100% at the early rating, 34 to 100% control at the mid rating, and 90 to 100% at the late rating. Red sprangletop control data are shown in Table 10. Early red sprangletop control provided by treatment 4 (fomesafen PRE) (45%) was lower than that of treatments 3 and 6 through 13. Treatments 3 and 5 also resulted in reduced control (70 and 68%, respectively) compared to several other treatments. At the mid rating, treatments 2 through 5 provided lower control of red sprangletop than treatments 6 through 13. Following MPOST applications, no differences among herbicide treatments were detected.

Table 10. Effect of treatment on mean 2012 red sprangletop control.

Treatment	Early	Mid	Late
	------------------	control (%)	------------------
1	0 f[1]	0 e	0 b
2	92 de	54 c	99 a
3	70 d	58 c	95 a
4	45 e	34 d	99 a
5	68 de	59 c	93 a
6	98 ab	100 a	100 a
7	100 a	100 a	100 a
8	98 ab	100 a	100 a
9	99 a	100 a	100 a
10	81 cd	99 a	98 a
11	70 cd	98 a	95 a
12	83 cd	89 b	90 a
13	91 bc	100 a	99 a

[1] Within a column, means followed by different letters are significantly different at P<0.05.

In 2013, mean Palmer amaranth control in treated plots ranged from 63 to 100% following PPI and PRE applications, 82 to 100% following EPOST applications, and 92 to 100% following MPOST applications (see Table 11). Similar to Palmer amaranth control in 2012, treatment 11 (pyrithiobac PRE) provided less control (63%) than all other herbicide treatments at the early rating. Also at the early rating, treatments with pendimethalin PRE (10, 12, and 13) provided lower control of Palmer amaranth (88 to 90%) than several other treatments. At the mid rating, control from treatment 2 (82%) was lower than all other treatments except for treatment 12 (86%), which was lower than treatments other than 2 and 3 (93%). At the late rating, all herbicide treatments provided 99 to 100% control of Palmer amaranth, with the exception of treatment 12 (92%).

Table 11. Effect of treatment on mean 2013 Palmer amaranth control.

Treatment	Early	Mid	Late
	------------------	control (%)	------------------
1	0 f[1]	0 e	0 c
2	93 bcd	82 d	99 a
3	99 abc	93 bc	100 a
4	100 a	98 ab	99 a
5	100 a	100 a	100 a
6	99 abc	99 ab	99 a
7	99 abc	99 a	100 a
8	98 abc	100 a	100 a
9	99 ab	100 a	100 a
10	88 d	99 a	100 a
11	63 e	99 a	100 a
12	90 cd	86 cd	92 b
13	88 d	100 a	100 a

[1] Within a column, means followed by different letters are significantly different at P<0.05.

Common waterhemp control in 2013 ranged from 60 to 100% at the early rating, 81 to 100% at the mid rating, and 93 to 100% at the late rating. These data are shown in Table 12. Control of common waterhemp following PPI and PRE applications was again lower from treatment 11 (pyrithiobac PRE) (60%) than all other treatment with the exception of treatment 10. Early control was numerically lower from treatments 10, 12, and 13 (79 to 92%) than many other herbicide treatments. At the mid rating, control from treatments 2 and 11 (81 and 86%, respectively) was lower than all other herbicide treatments at that time. At the final rating, common waterhemp control provided by treatment 12 (93%) was lower than that provided by all other herbicide treatments.

Table 12. Effect of treatment on mean 2013 common waterhemp control.

Treatment	Early	Mid	Late
	------------------control (%)------------------		
1	0 f[1]	0 c	0 c
2	91 bcd	81 b	98 a
3	97 abc	96 a	99 a
4	100 a	100 a	100 a
5	100 a	100 a	100 a
6	99 ab	100 a	100 a
7	99 a	100 a	100 a
8	96 abc	100 a	100 a
9	98 ab	100 a	100 a
10	79 de	100 a	100 a
11	60 e	99 a	100 a
12	92 bcd	86 b	93 b
13	88 cd	100 a	99 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Herbicidal control of junglerice in 2013 ranged from 65 to 100% following PPI and PRE applications, 76 to 100% following EPOST applications, and 84 to 100% following MPOST applications (see Table 13). At the early rating, control provided by treatment 11 (pyrithiobac PRE) (65%) was lower than herbicide treatments other than 4 and 10 (80 and 92%, respectively). Junglerice control from treatment 4 at the mid rating (76%) was lower than that of herbicide treatments other than 2, 5, and 11. At the final rating, control with treatment 12 (84%) was lower than all other treatments, with the exception of treatment 4 (97%).

Table 13. Effect of treatment on mean 2013 junglerice control.

Treatment	Early	Mid	Late
	------------------	Control (%)	------------------
1	0 e[1]	0 c	0 c
2	91 abc	88 ab	100 a
3	96 abc	95 a	99 a
4	80 cd	76 b	97 ab
5	98 ab	93 ab	99 a
6	99 a	100 a	100 a
7	99 a	100 a	100 a
8	99 a	99 a	100 a
9	100 a	100 a	100 a
10	92 bcd	96 a	99 a
11	65 d	95 a	100 a
12	98 abc	91 ab	84 b
13	91 abc	100 a	99 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Sharppod morningglory control data from 2013 revealed several differences among herbicide treatments. Control ranged from 68 to 95% at the early rating, 70 to 97% at the mid rating, and 88 to 99% at the late rating. These data are shown in Table 14. No differences among herbicide treatments were detected at the early rating. At the mid rating, control from treatment 4 (70%) was lower than all other treatments, with the exception of treatments 2 and 12 (78 and 85%, respectively). At the late rating, sharppod morningglory control provided by treatments 2 and 4 (88%) was lower than that provided by treatments 3, 7, and 13.

Table 14. Effect of treatment on mean 2013 sharppod morningglory control.

Treatment	Early	Mid	Late
	------------------Control (%)------------------		
1	0 b[1]	0 c	0 d
2	86 a	78 bc	88 c
3	85 a	94 a	99 a
4	75 a	70 c	88 c
5	94 a	92 ab	95 abc
6	93 a	96 a	97 abc
7	91 a	96 a	99 ab
8	88 a	97 a	95 abc
9	95 a	96 a	96 abc
10	68 a	96 a	94 abc
11	83 a	95 a	89 bc
12	85 a	85 abc	94 abc
13	89 a	97 a	97 ab

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Seed cotton yields ranged from 1823 to 4002 kg ha^{-1} in 2012 and from 254 to 4209 kg ha^{-1} in 2013. These data are shown in Table 15. No differences in seed cotton yield were detected among herbicide treatments, however for both years; yields were greater for all herbicide treatments compared to the nontreated control.

Table 15. Effect of treatment on mean seed cotton yield.

Treatment	**2012**	**2013**
	--------kg ha^{-1}--------	
1	1823 a[1]	254 a
2	3955 b	3733 b
3	3673 b	3881 b
4	3731 b	3986 b
5	3680 b	4003 b
6	3859 b	4122 b
7	3581 b	3983 b
8	4002 b	4209 b
9	3779 b	4207 b
10	3966 b	3838 b
11	3728 b	4034 b
12	3647 b	3526 b
13	3779 b	4084 b

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

Spray Droplet Size Spectra Experiment

Two-way ANOVA analyses of $D_v0.1$, $D_v0.5$, $D_v0.9$ values (the droplet size in μm for which 10, 50, and 90% of the spray volume is made up of droplets of that size or smaller), and Q_i100 data (the percentage of the total spray volume made up of droplets 100 μm or smaller in diameter) obtained at an operating pressure of 202 kPa revealed a significant interaction between spray nozzle and product (F(13,32) = 92.15, *p* = <0.0001, F(13,32) = 173.41, *p* = <0.0001), F(13,32) = 3.04, *p* = <0.0001, F(13,32) = 667.24, *p* = <0.0001, respectively). There was no interaction for relative span data at 207 kPa. At 414 kPa, there was an interaction between nozzle and product for $D_v0.1$, $D_v0.5$, $D_v0.9$, relative span, and Qi100 data (F(13,32) = 355.33, *p* = <0.0001, F(13,32) = 263.94, *p* = <0.0001, F(13,32) = 47.99, *p* = <0.0001, F(13,32) = 27.26, *p* = <0.0001, F(13,32) = 473.24, *p* = <0.0001, respectively). Although significant interactions frequently occurred, these interactions were ordinal in nature, and it will be more useful to analyze the main effects of spray nozzle and product separately. Analyses of the effects of spray nozzle and formulated product on Q_i30, Q_i50, Q_i80, Q_i141, Q_i150, Q_i200, and Q_i730 values are presented in Appendices A and B.

When the effect of spray nozzle type on the droplet size spectra produced at 207 kPa was analyzed, many differences were detected. These data are summarized in Table 16. All nozzles differ significantly from each other for $D_v0.1$, $D_v0.5$, and $D_v0.9$ values. Dv0.5 values range from 233.8 μm with the XR nozzle, to 1022.5 μm with the TTI nozzle. Nozzles ranked in order of increasing droplet size spectra based on D_v values are as follows; XR < DG < AIXR < AI < TTI. The relative span of the droplet size distribution produced by the TTI nozzle (1.034) is lower than that of the other four nozzles (1.117 to 1.185). When Q_i100 data are considered, a much greater portion of the total spray volume produced by the XR nozzle was made up of droplets 100 μm or less in diameter (7.66%). Greater Q_i100 values were also observed from the DG and AIXR nozzles (2.31 and 0.69%, respectively) than from either the AI or TTI nozzles.

Table 16. Effect of spray nozzle on droplet size spectra at 207 kPa.

Nozzle	**$D_v0.1$**	**$D_v0.5$**	**$D_v0.9$**	**Relative Span**	**Q_i100**
XR	112.7 a[1]	233.8 a	388.3 a	1.185 a	7.66 a
DG	170.8 b	347.2 b	568.1 b	1.151 a	2.31 b
AIXR	259.5 c	505.5 c	823.5 c	1.149 a	0.69 c
AI	443.0 d	873.7 d	1448.4 d	1.117 a	0.14 d
TTI	530.2 e	1022.5 e	1586.8 e	1.034 b	0.10 d

[1] Within a column, means followed by different letters are significantly different at $P<0.05$

When the droplet size spectra produced at 414 kPa by the different nozzle types are analyzed, many differences were again observed. These summarized data are presented in Table 17. As was shown before at 207 kPa, all nozzles differ significantly with respect to $D_v0.1$, $D_v0.5$ and $D_v0.9$ values, as well as Q_i100 values. $D_v0.5$ values ranged from 174.1 µm with the XR nozzle to 1190.7 µm with the TTI nozzle. When nozzles are ranked in order of the droplet size spectra they produced, the order is the same as was shown at 207 kPa. When nozzles are ranked in order of increasing relative span of the droplet size distribution, the order is as follows; AIXR < AI <DG and TTI < XR. The XR nozzle again produced a much larger portion of the total spray volume in 100 µm or smaller droplets (17.5%), followed by the DG (7.77%), AIXR (3.06%), AI (1.07%), and TTI (0.34%) nozzles.

Table 17. Effect of spray nozzle on droplet size spectra at 414 kPa.

Nozzle	**$D_v0.1$**	**$D_v0.5$**	**$D_v0.9$**	**Relative Span**	**Q_i100**
XR	80.5 a[1]	174.1 a	300.7 a	1.274 a	17.50 a
DG	114.7 b	251.5 b	423.0 b	1.239 b	7.77 b
AIXR	163.8 c	338.9 c	554.1 c	1.159 d	3.06 c
AI	255.4 d	520.9 d	868.0 d	1.181 c	1.07 d
TTI	333.3 e	685.7 e	1190.7 e	1.251 b	0.34 e

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

When the effect of formulated product on droplet size spectra produced at 207 kPa is analyzed, several differences are detected. These data are summarized in Table 18. Spray solutions of Liberty 280 SL and GF-2726 resulted in lower $D_v0.5$ values (544 and 549.6 µm, respectively) than all other products, followed by MON76832 (582.6

µm), 2,4-D Amine 4 (591.7 µm), water+NIS (600.7 µm), Durango DMA (604.5 µm), Roundup PowerMax and Clarity (610.6 and 610.8 µm, respectively), and water alone (676.0 µm). The relative span of the droplet size distribution produced by GF-2726 (1.047) was smaller than that of Durango DMA, MON76832, or Liberty 280 SL. The portion of the total spray volume produced as droplets 100 um or smaller in diameter by Clarity and water+NIS (1 and 1.06%, respectively) was smaller than that of all other formulated products, followed by GF-2726 (1.31%), 2,4-D Amine 4 (1.69%), water alone (2.16%), Roundup PowerMax (2.58%), Durango DMA and MON76832 (2.99 and 3.03%, respectively), and Liberty 280 SL (3.80%).

Table 18. Effect of formulated product on droplet size spectra at 207 kPa.

Product	**$D_v0.1$**	**$D_v0.5$**	**$D_v0.9$**	**Relative Span**	**Q_i100**
Water	336.4 a[1]	676.0 a	1064.0 a	1.109 bcd	2.16 d
Water + NIS	325.1 b	600.7 c	958.9 abc	1.060 cd	1.06 g
2,4-D Amine 4	306.3 c	590.7 d	927.7 bc	1.065 cd	1.69 e
Durango DMA	292.9 e	604.5 bc	1050.9 a	1.245 a	2.99 b
GF-2726	297.5 de	549.6 f	861.3 c	1.047 d	1.31 f
Clarity	326.6 b	610.8 b	971.0 abc	1.061 cd	1.00 g
Roundup PowerMax	299.5 d	610.6 b	976.3 ab	1.153 abcd	2.58 c
MON76832	285.5 f	582.6 e	952.0 abc	1.177 abc	3.03 b
Liberty 280 SL	259.5 g	544.0 f	905.3 bc	1.228 ab	3.80 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

When spray pressure was increased to 414 kPa, many significant differences were shown among spray droplet size spectra produced by the formulated products. A summary of these data is presented in Table 19. Spray solutions containing Liberty 280 SL resulted in the lowest $D_v0.5$ values (345.7 μm) compared to all other formulated products, followed by MON76832 (373.9 μm), GF-2726 (385.7 μm), Durango DMA and Roundup PowerMax (392.3 and 393.7 μm, respectively), Clarity, water+NIS, and 2,4-D Amine 4 (406.4, 408.3, and 409.2 μm, respectively), and water alone (433.4 μm). Solutions of GF-2726, water+NIS, and Clarity resulted in droplet size distributions with the smallest relative span (1.103, 1.105, and 1.122, respectively). When Q_i100 values are considered, solutions of Liberty 280 SL resulted in a greater portion of the total spray volume in droplets 100 μm or less (10.29%), followed by Roundup PowerMax and MON76832 (7.81 and 7.67%, respectively), Durango DMA (6.57%), water alone (5.59%), 2,4-D Amine 4 (4.73%), Clarity and GF-2726 (3.72 and 3.71%, respectively), and water+NIS (3.46%).

Table 19. Effect of formulated product on droplet size spectra at 414 kPa.

Product	**$D_v0.1$**	**$D_v0.5$**	**$D_v0.9$**	**Relative Span**	**Q_i100**
Water	204.5 c[1]	433.4 a	739.8 a	1.231 c	5.59 d
Water + NIS	211.8 a	408.3 b	665.3 b	1.105 e	3.46 g
2,4-D Amine 4	199.1 d	409.2 b	674.2 b	1.152 d	4.73 e
Durango DMA	182.7 e	392.3 c	668.9 b	1.247 c	6.57 c
GF-2726	200.4 d	385.7 d	624.0 e	1.103 e	3.71 f
Clarity	206.5 b	406.4 b	664.2 bc	1.122 e	3.72 f
Roundup PowerMax	183.1 e	393.7 c	678.3 b	1.286 b	7.81 b
MON76832	172.8 f	373.9 e	642.4 d	1.273 b	7.67 b
Liberty 280 SL	144.9 g	345.7 f	648.7 cd	1.470 a	10.29 a

[1] Within a column, means followed by different letters are significantly different at $P<0.05$.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Discussion

Confirmation of Glyphosate-Resistant Common Waterhemp. The objective of this experiment was to evaluate the effect of glyphosate on several common waterhemp accessions from East-central Texas. The results of this study indicate that glyphosate resistance is present at several locations within this region. Light et al. (2011) identified two categories of glyphosate resistant biotypes of common waterhemp in Texas; one with LD_{50} values of 3.5 to 3.7X the labeled rate, and one with much greater levels of resistance (LD_{50} values of 27.8 to 59.7X the labeled rate). The accessions included in this experiment appear to fall into the former category of lower levels of glyphosate resistance; however, there were two accessions that could not be included in the dose-response analysis (accessions 3a and 4) as they exhibited no mortality 28 DAT for any of the included glyphosate rates. These two may exhibit a much higher level of resistance similar to that observed by Light et al. (2011); however, this cannot be verified based on these data. The inclusion of greater rates of glyphosate in this experiment may have allowed for an estimation of LD_{50} values for these accessions.

Biomass reduction does not appear to be a reliable indicator of the level of glyphosate resistance in this species. For example, LD_{50} values of accessions 7a and 12a differ by only 137 g ae ha^{-1}; however, biomass reduction of these two accessions following the 1.0X rate is very different (92.7% for 7a, 33.7% for 12b), though not significantly so. As previously mentioned, accessions 3a and 4 did not exhibit any mortality following glyphosate treatments; however, they differ greatly in biomass reduction. Biomass reduction of accession 3a did not exceed 14% following any glyphosate treatment, while reductions of accession 4 reached nearly 83% following the 4.0X rate.

Glyphosate-Resistant Weed Management in Cotton. The objective of this experiment was to evaluate the efficacy provided by several different herbicide programs in East-central Texas cotton. The results revealed several important differences among treatments that have potential impacts on effective weed management in light of glyphosate-resistant weeds.

Treatments 2 through 5 included a variety of PRE applications of *S*-metolachlor, prometryn and fomesafen that provided excellent control of both Palmer amaranth and common waterhemp. However, these four treatments relied on a MPOST application alone, and a decrease in the control of the two *Amaranthus* species was occasionally observed in the period of time between PRE applications and the MPOST application. Following MPOST applications, these four treatments resulted in excellent control of both species. When grass weed control is considered, treatments 2 through 5 frequently provided lower levels of preemergence grass control, especially in the case of red sprangletop in 2012. As these treatments did not include an EPOST application, control of grass species was lower in the period between PRE and MPOST applications. The addition of an EPOST application to these four treatments appears to be necessary to ensure sufficient weed control during this period of the season. When pyrithiobac was applied PRE (treatment 11), lower control of Palmer amaranth and common waterhemp was seen in both years compared to the other PPI and PRE treatments. Additionally, treatments of pyrithiobac PRE provided much lower control of grass weeds. In 2013, the lower control of junglerice provided by pyrithiobac PRE may have contributed to the lower level of control seen later in the season after POST applications were made. Control of *Amaranthus* weeds provided by pyrithiobac PRE in this study appears to be lower than that reported by others (Dotray et al. 1996; Branson et al. 2005) for unknown reasons since applications were made at the recommended rate and timing.

Treatments 10, 12, and 13 included pendimethalin PRE, which appeared to provide variable control of the two *Amaranthus* weeds, although not statistically significant. This may be due to the fact that furrow irrigation was utilized for herbicide incorporation into the soil, rather than overhead irrigation or rainfall, as recommended on the product label (BASF 2012). Treatment 12 (pendimethalin PRE followed by pyrithiobac EPOST and glufosinate MPOST) resulted in decreased control of Palmer amaranth and common waterhemp later in the 2013 season. This occurrence is likely due to "escapes" of these two species following the pyrithiobac EPOST application that continued to grow to sizes larger than that specified for control with glufosinate (Bayer CropScience 2013). Treatment 13 (pendimethalin PRE followed by glyphosate EPOST), appeared to provide weed control comparable to that of other treatments, however this treatment poses a significant risk to those who choose to utilize it, since it relies on a single POST herbicide mode of action. In the presence of glyphosate-resistant weeds, this treatment would likely not provide sufficient weed control. Treatments 6 through 9 included trifluralin applied PPI, and consistently provided the highest levels of control of all weed species included in this study, though not always significantly so. Proper incorporation into the soil has been shown to affect the efficacy of trifluralin (Robison and Fenster 1968; Wiese et al. 1969). In this experiment, the excellent weed control provided by PPI trifluralin was likely due in part to the thorough incorporation into the soil by two passes of a rolling cultivator. Keeling et al. (1991) found that trifluralin applied at rates of 1.4 kg ha^{-1} provided control of Palmer amaranth for more than 90 days after planting. This suggests that the high level of weed control observed with treatments 6 through 9 following POST applications may be due in part to the lengthy residual activity of PPI trifluralin treatments.

Spray Droplet Size Spectra Experiment. This experiment was designed to evaluate the effect of several spray nozzles types and herbicide formulations on the droplet size spectra of herbicide sprays. As expected, increased operating pressures resulted in decreased droplet size spectra produced by all nozzles and formulated products. Due to the sensitivity of the equipment used for analysis, many differences among nozzles and formulations were detected.

Nozzles that utilized a pre-orifice (DG, AIXR, AI, and TTI) produced larger mean droplet sizes ($D_v0.5$ values) than the XR nozzle, which does not have a pre-orifice. In addition to larger mean droplet size, pre-orifice nozzles resulted in lower Q_i100 values, indicating a lower potential for physical drift compared to the XR nozzle. Nozzles that utilized an air-inclusion design in addition to a pre-orifice (AIXR, AI, and

TTI) resulted in additional reduction of physical drift potential compared to the DG or XR nozzles. These results agree with those of Nuyttens et al. (2007).

Spray solutions of water alone resulted in the largest median droplet size compared to all other solutions. All other spray solutions contained a surfactant included in the formulated product, or manually added as in the case of the water+NIS, 2,4-D Amine 4, and Clarity solutions. The addition of a surfactant decreases the surface tension of the spray solution, likely resulting in delayed breakage of the fluid sheet produced by flat-fan spray nozzles as shown by Ellis et al. (2001). Delayed breakage of this sheet produces droplets of smaller volume and diameter. Solutions of Liberty 280 SL consistently produced the smallest median droplet size, potentially indicating a stronger surfactant load in this product as prepared by the manufacturer. The highest Q_i100 values were observed with this product, suggesting that physical drift potential when applying this product is quite high compared to the other formulated products. Solutions of GF-2726 also produced smaller median droplet sizes than the other formulated products, but also resulted in the some of the smallest Q_i100 values of this experiment. This indicates that although the median droplet size decreased with solutions of GF-2726, the width of the distribution of droplet sizes (as indicated by small relative span values) decreased as well. This results in decreased production of droplets at either extreme of the distribution. When Q_i100 values are compared, solutions of GF-2726 resulted in a 64% decrease in physical drift potential at 414 kPa compared to solutions of Liberty 280 SL.

Conclusions

Glyphosate-resistant common waterhemp is present and widespread in East-central Texas cotton production as shown by the glyphosate-resistance confirmation study. In order to effectively combat the spread of this glyphosate-resistant weed and prevent the occurrence of new cases of resistance in other species, appropriate weed management practices will need to be implemented by growers in this region. This study has shown that excellent control of *Amaranthus* weed species in cotton can be achieved through the use of several different herbicide programs. The inclusion of soil-applied residual herbicides and the use of diverse POST herbicides will certainly help manage these glyphosate-resistant weeds. Currently, new herbicide-resistant crop technologies are under development to allow for the use of additional POST herbicides such as 2,4-D and dicamba in cotton and other major crops. If there is grower acceptance of these new technologies, problems due to physical drift of these herbicides onto susceptible crops are likely to occur. This study has shown that spray nozzle design and the formulation of herbicide products can have a significant impact on the potential for physical drift to occur. The selection of proper spray nozzles and herbicide formulations by applicators will be vital to help minimize physical drift of these herbicides.

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APPENDIX A

EFFECT OF SPRAY NOZZLE ON Q_i- VALUES

---207 kPa---

Nozzle	**Qi30**	**Qi50**	**Qi80**	**Qi141**	**Qi150**	**Qi200**	**Qi730**
XR	0.41 a[1]	1.07 a	4.14 a	18.26 a	20.82 a	37.93 a	99.94 a
DG	0.11 b	0.29 b	1.18 b	6.40 b	7.44 b	15.62 b	98.69 b
AIXR	0.04 bc	0.10 c	0.36 c	2.02 c	2.37 c	5.29 c	84.82 c
AI	0.02 c	0.04 cd	0.08 d	0.42 d	0.48 d	1.19 d	35.69 d
TTI	0.02 c	0.04 d	0.06 d	0.24 e	0.28 e	0.65 e	23.85 e

---------------------------------------414 kPa---------------------------------------

Nozzle	**Qi30**	**Qi50**	**Qi80**	**Qi141**	**Qi150**	**Qi200**	**Qi730**
XR	1.05 a	3.11 a	10.38 a	35.07 a	39.06 a	60.86 a	99.97 a
DG	0.59 b	1.33 b	4.42 b	17.11 b	19.32 b	33.75 b	99.77 a
AIXR	0.09 c	0.42 c	1.68 c	7.49 c	8.59 c	16.88 c	99.19 b
AI	0.04 c	0.14 d	0.58 d	2.65 d	3.04 d	6.04 d	79.18 c
TTI	0.02 c	0.06 e	0.19 e	0.90 e	1.05 e	2.48 e	55.58 d

[1] Within a column for a given pressure, means followed by different letters are significantly different at $P<0.05$.

APPENDIX B

EFFECT OF FORMULATED PRODUCT ON Q_i- VALUES

207 kPa							
Nozzle	**Q_i30**	**Q_i50**	**Q_i80**	**Q_i141**	**Q_i150**	**Q_i200**	**Q_i730**
Water	0.29 a[1]	0.49 ab	1.27 d	4.89 e	5.56 f	10.44 f	62.59 g
Water+NIS	0.01 d	0.12 f	0.54 g	3.22 g	3.78 h	8.38 i	68.10 d
2,4-D Amine 4	0.13 bc	0.23 de	0.84 e	4.96 e	5.80 e	12.38 e	69.40 c
Durango DMA	0.16 b	0.46 ab	1.68 b	6.84 c	7.76 c	14.10 c	67.11 e
GF-2726	0.07 bcd	0.17 ef	0.64 f	3.94 f	4.62 g	9.87 g	72.70 a
Clarity	0.03 cd	0.07 f	0.43 h	3.32 g	3.93 h	8.93 h	67.41 e
Roundup PowerMax	0.06 bcd	0.30 cd	1.38 c	6.22 d	7.11 d	13.33 d	67.11 f
MON76832	0.15 b	0.40 bc	1.63 b	7.13 b	8.11 b	14.58 b	68.84 c
Liberty 280 SL	0.17 b	0.52 a	2.08 a	8.69 a	9.85 a	17.24 a	71.46 b
414 kPa							
Nozzle	**Q_i30**	**Q_i50**	**Q_i80**	**Q_i141**	**Q_i150**	**Q_i200**	**Q_i730**
Water	0.41 bc	1.01 c	3.28 d	11.65 d	13.05 d	21.90 f	82.32 f
Water+NIS	0.22 d	0.47 f	1.83 f	8.56 g	9.80 g	18.52 h	87.82 bc
2,4-D Amine 4	0.44 ab	0.76 d	2.58 e	11.08 e	12.60 e	22.59 e	85.58 e
Durango DMA	0.30 cd	1.10 c	3.86 c	13.55 c	15.18 c	25.22 d	86.19 d
GF-2726	0.38 bc	0.59 e	1.96 f	9.28 f	10.64 f	19.90 g	90.42 a
Clarity	0.41 bc	0.59 e	1.96 f	9.27 f	10.62 f	19.85 g	87.30 c
Roundup PowerMax	0.24 d	1.26 b	4.64 b	15.57 b	17.33 b	27.55 c	85.08 e
MON76832	0.30 cd	1.26 b	4.52 b	15.54 b	17.34 b	27.84 b	87.62 c
Liberty 280 SL	0.54 a	2.07 a	6.41 a	19.32 a	21.34 a	32.55 a	88.33 b

[1] Within a column for a given pressure, means followed by different letters are significantly different at $P<0.05$.