ABSTRACT

WHITAKER, JARED ROSS. Distribution, Biology, and Management of Glyphosate-resistant Palmer amaranth in North Carolina. (Under the direction of Alan C. York and David L. Jordan).

The introduction of glyphosate-resistant (GR) crops allowed for topical applications of the herbicide glyphosate. This herbicide revolutionized weed control and crop management. Widespread adoption of this technology and extensive use of glyphosate led to intense selection pressure for evolution of GR weeds.

In 2005, GR Palmer amaranth was suspected in North Carolina. A survey detected GR populations in 49 of 290 fields sampled. Resistance to herbicides that inhibit acetolactate synthase (ALS) was also detected in 52 fields. Five fields had populations exhibiting multiple resistance to both glyphosate and ALS-inhibitors.

Experiments were conducted to determine the resistance mechanism of GR Palmer amaranth. A GR biotype exhibited a 20-fold level of resistance compared to a glyphosate-susceptible (GS) biotype. Maximum absorption of ^{14}C-glyphosate was observed by 12 hours after treatment (HAT), and was similar among biotypes except at 6 HAT, where GS plants absorbed 67% more than GR plants. Distribution of ^{14}C was similar among biotypes in, above, and below the ^{14}C-glyphosate treated leaf and in roots. This work did not lead to discovery of a resistance mechanism.

Field experiments were conducted to develop management strategies for GR Palmer amaranth in cotton. One evaluated residual control of Palmer amaranth by various herbicides. Of herbicides typically applied PRE or pre-plant, fomesafen, flumioxazin, and pyrithiobac were most effective. Pyrithiobac and *S*-metolachlor were the most effective

postemergence (POST) herbicides. Flumioxazin and prometryn plus trifloxysulfuron were the most effective options for postemergence-directed applications. Integration of these herbicides into glyphosate-based systems would increase Palmer amaranth control.

Another experiment evaluated PRE herbicides in a season-long system. All PRE herbicides increased late-season control. Among individual herbicides, fomesafen and pyrithiobac were most effective. Combinations of fomesafen plus pyrithiobac or diuron and diuron plus pyrithiobac were the most effective PRE applications. Another experiment investigated herbicide systems with residual herbicides applied pre-plant, PRE and POST. Pre-plant applications of flumioxazin and PRE applications of fomesafen increased late-season control, but applications of both were more effective than either herbicide alone. Applications of glyphosate plus pyrithiobac POST were more effective than glyphosate alone. Glyphosate plus *S*-metolachlor was more effective than glyphosate alone when activated by rainfall or irrigation and when Palmer amaranth had not emerged. These data suggest early-season control of GR Palmer amaranth is critical for successful management in cotton.

Glufosinate is another herbicide effective on Palmer amaranth. However, growers were reluctant to plant glufosinate-tolerant cotton cultivars. Widestrike cotton is GR and also contains a glufosinate tolerance gene used as a selectable marker, however glufosinate tolerance in production situations had not been investigated. Experiments were conducted to evaluate Widestrike cotton tolerance to glufosinate. Yield was reduced by glufosinate in only one of 11 trials by 4%, suggesting acceptable tolerance.

Another experiment evaluated weed control with glufosinate and glyphosate in Widestrike cotton. Control of GR Palmer amaranth by glufosinate-based systems was higher than

glyphosate-based systems, which demonstrated that glufosinate-based systems could be used to control GR Palmer amaranth in Widestrike cotton.

In soybean, several glyphosate alternative herbicides could be used to control Palmer amaranth. An experiment was conducted to evaluate control of GS and GR Palmer amaranth from a glyphosate-only system compared to several alternative systems. Glyphosate alone applied once POST was very effective on GS Palmer amaranth and alternative systems with two PREs followed by fomesafen POST provided similar control compared to glyphosate. In fields with GR Palmer amaranth, greater than 80% late-season control was obtained only with systems of two PREs followed by fomesafen POST.

Distribution, Biology, and Management of Glyphosate-resistant Palmer amaranth in North Carolina

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Crop Science

Raleigh, North Carolina

2009

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DEDICATION

This effort is dedicated to my parents, Jerry and Faith Whitaker. Their love and support had made this and everything else I have accomplished possible.

BIOGRAPHY

Jared Ross Whitaker grew up in Cordele, GA. In high school he spent his summers working as a cotton scout. In hindsight, not only did it put money in his pocket, it also most likely kept him out of trouble. Jay Holder, his boss, kindled his appreciation for agriculture and answered question after question about production agriculture. Upon graduating from Georgia Southern University with a Bachelor's degree in biology, he attended the University of Georgia and earned a Master's degree in Agronomy under Dr. Craig Bednarz. Soon afterwards, he left Georgia to attend North Carolina State University to study weed science under Dr. Alan York.

ACKNOWLEDGMENTS

There are quite a few people who deserve credit for my completion of this degree. I would like first thank my family. Their support, guidance, and tolerance have made my journey possible. My parents, Jerry and Faith Whitaker, and my brother Robby have always been supportive and they instilled in me a level of confidence which made me believe that I could achieve any goal. I would also like to thank Shellie Dowdy. She has been a constant supporter of me and I am truly blessed to have her in my life. I won't ever be able to thank her enough for her unwavering commitment to me during these past few years.

I would like to thank Alan York for the opportunity to further my education and for the opportunity to learn from one of the most respected weed scientists in the country. He instilled in me a work ethic that I did not know existed and his tireless support and help will always be appreciated. Also, I would like to thank David Jordan for mentoring me during the last few, but important, months of my education here at NC State. I would also like to thank Randy Wells and Jim Burton for serving on my graduate committee.

I would also like to thank Rick Seagroves, his endless help and friendship were not only were critical to my success, but critical to me maintaining my sanity. I am also thankful for the hard work and advice given to me by Jamie Hinton. I would also like to thank Michael Brewington and Nathan Bennett for their help in completing this research.

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Literature Review

The herbicide glyphosate [*N*-(phosphonomethyl)glycine] was first introduced as Roundup® by Monsanto in 1974 and has been used as a postemergence, non-selective herbicide for control of more than 300 weed species in non-cropland areas and for preplant weed control, including many annuals and perennials (Franz et al. 1997). In the late 1990's, its use was expanded to weed control in genetically modified crops with tolerance to glyphosate (Tsafaris 1996). Glyphosate has good toxicological and environmental profiles and provides broad-spectrum weed control. This herbicide has become the largest-selling single crop protection chemical in the worldwide market (Woodburn 2000).

Glyphosate in the environment. Glyphosate toxicity has been extensively reviewed, and the conclusion of these studies demonstrated that glyphosate toxicity is very low in mammals, birds, and fish (Franz et al. 1997). Glyphosate is tightly adsorbed to soil colloids, making it essentially immobile in the environment (Franz et al. 1997). Adsorption of glyphosate to soil causes inactivation of the herbicide and results in the loss of phytotoxicity upon soil contact (Torstensson and Aamisepp 1977). In a review by Vereecken (2005) the mobility and leaching of glyphosate was stated to be affected by the availability of unoccupied phosphate binding sites and that soil pH can have a small effect on glyphosate binding. Furthermore, since this herbicide is tightly bound to soil, leaching is unlikely to occur and therefore it posses very little concern for ground water pollution (Mosheir and Penner 1978; Sprankle et al. 1975). Degradation of glyphosate is primarily associated with

soil microbial microorganisms (Torstensson and Aamisepp 1977) and completely degraded to CO_2 by microorganisms in soil (Bronstad and Friestad 1985). The half-life of glyphosate in soil is variable, and can range from weeks to years depending on the balance of soil adsorption and microbial activity (Moshier and Penner 1978).

The pathway that glyphosate interferes with is only found in plants and microorganisms rendering glyphosate essentially non-toxic to mammals and birds. Studies on the environmental impact of glyphosate used in non-cropland areas have found that glyphosate has little indirect effect on animal communities other than destruction of habitat by glyphosate applications (Sullivan and Sullivan 2003). Due to commercial formulation of glyphosate, fish and invertebrates are more vulnerable to glyphosate (Carlisle and Trevors 1988). Yet, it has been proposed that applications made to land near water bodies will not affect the populations of fish and invertebrates (Folmar et al. 1979).

Glyphosate toxicity in plants. Glyphosate controls a broad spectrum of broadleaf weeds as well as annual and perennial grasses (Wilcut et al. 1996). Although glyphosate is a broad-spectrum herbicide, there are differences glyphosate susceptibility between weed species (Culpepper et al. 2000; Payne and Oliver 2000; Taylor 1996). Some grasses, such as barnyardgrass and broadleaf signalgrass, are more susceptible to glyphosate than broadleaf weeds, such as morningglory species, velvetleaf, and hemp sesbania (Taylor 1996). Tolerance of glyphosate may be due physiological mechanisms such as low absorption, reduced translocation, or higher enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) activity (Norsworthy et al. 2001) and morphological characteristics of plants

inlcuding, such as leaf orientation and leaf surface properties which affect efficacy due to herbicide runoff, decreased spray retention, and droplet deflection (De Ruiter et al. 1990).

Glyphosate affects many physiological and physiochemical processes in plants, among those are reductions in photosynthesis, degradation of chlorophyll, and inhibition of auxin transport (Baylis 2000). The primary mechanism of action is inhibition of the shikimate pathway of plants, microorganisms, and fungi (Comai et al. 1985; Devine et al. 1993; Siehl 1997). Approximately 20% of the assimilated carbon in plants moves through the shikimate pathway (Haslam 1993) to form chorismate, a precursor of three essential aromatic amino acids and many secondary plant metabolites (Carlisle and Trevors 1988). Glyphosate interacts with the EPSPS, which is involved with the sixth step of the pathway (Amrhein et al. 1980; Jaworski 1972; Steinrucken and Amhrein 1980). In absence of glyphosate, shikimate-3-phosphate binds to EPSPS, after which phosphoenolpyruvate (PEP) is bound to the enzyme-substrate complex and the reaction pathway continues. Glyphosate binds with EPSPS after shikimate-3-phosphate has bound to EPSPS, and subsequently causes inhibition of PEP binding (Baylis, 2000) essentially blocking the shikimate pathway (Schonbrunn et al. 2001).

The actual cause of plant death from glyphosate is not known, yet phytotoxicity from glyphosate applications may due to several things associated with the inhibition of EPSPS. Consequences in most plants include chlorosis, necrosis, and stunted growth of plant parts that are sink tissues (Franz et al. 1997). The toxicity of glyphosate appears to vary within plant species and may be due to one or more of the following mechanisms (Siehl 1997). One possible method of phytotoxicity may be from decreased protein synthesis, due to lack of

aromatic amino acids. Another method may correlate with the depletion of the secondary products formed from products of the shikimate pathway. Substrate buildup from the inhibited reaction may cause plant phytotoxicity. Also, the deregulation of the shikimate pathway may cause plant death.

Glyphosate interferes directly with plant photosynthesis, respiration, or membrane permeability, and effects of glyphosate application often correlate with the reduction of these processes (Geiger et al. 1986). Reduction of stomatal conductance, depletion of ribulose bisphosphate carboxylase (RuBP), disruption of leaf carbon metabolism, and restriction of water availability due to disruption of root processes are possible candidates of the mode of action of glyphosate (Franz et al. 1997). Glyphosate can stimulate phenylalanine-lyase (PAL) activity (Cole et al. 1980). This enzyme is the major regulatory enzyme in secondary phenolic synthesis and is associated with bleaching and chlorosis of glyphosate treated plants. Stimulation of this enzyme is probably due to the depletion of secondary products produced by the shikimate pathway. Hollander and Amrhein (1980) proposed that the stimulation of PAL is not the primary cause of glyphosate toxicity because bacteria, which do not have PAL, are also sensitive to glyphosate.

Glyphosate also increases metabolism of indole-3-acetic acid (IAA), therefore decreasing amounts of free IAA (Lee and Dumas 1985). Growth inhibition has been correlated with increases in IAA metabolism (Lee 1984). Enhancement of ethylene production has been observed in some plant species after glyphosate application, and ethylene production is associated with IAA metabolism (Lee and Dumas 1985). These findings may correlate loss of apical dominance and growth inhibition with glyphosate applications (Lee 1984).

Glyphosate movement in plants. Glyphosate applied to plants must overcome several barriers to reach its target site, EPSPS. The leaf cuticle and plasma membrane pose the first barrier to glyphosate absorption into plants (Riechers et al. 1994). The acid form of glyphosate cannot pass through these barriers easily, and formulation of glyphosate influences efficacy (Leaper and Holloway 2000). Isoproplyamine, diammonium, potassium, and trimesium salts of glyphosate have been commercialized. Adjuvants are also used to enhance biological performance of glyphosate (Leaper and Holloway 2000). Nonionic surfactant is often applied with glyphosate to reduce surface tension of spray liquids allowing more herbicide to come in contact with the leaf (Leaper and Holloway 2000). Efficacy of glyphosate with various adjvants generally does not affect weed control (Li et al. 2005; Ramsdale et al. 2003). Ammonium sulfate increases efficacy of glyphosate in some weeds and is often associated with increased efficacy when carrier water quality is poor (Jordan et al. 1997; Nalewaja and Matyslak 1991). This increase in efficacy has been attributed to increased absorption and translocation as well as greater partitioning of glyphosate out of the treated tissue (Young et al. 2003).

Once glyphosate penetrates through the cuticle and the plasma membrane, translocation occurs in symplastic tissue in a source to sink manner (Arnaud et al. 1994; Gougler and Gieger 1981). Although most translocation of glyphosate is symplastic, apoplastic movement also occurs (Franz et al. 1997; Klevorn and Wyse 1984). Gougler and Geiger (1981) postulated that glyphosate slowly enters and exits phloem, allowing accumulation and transport in the phloem.

Glyphosate-resistant crops. Researchers have actively searched for ways to develop glyphosate-resistant crops (Tsafaris 1996). There have been several mechanisms explored to convey resistance to glyphosate (Pline-Srnic 2006). Mechansims have been associated with overproduction of normal plant EPSPS enzyme, amplification of genes, or reduced EPSPS turnover time and also associated with glyphosate metabolism or conjugation. Researchers have also explored resistance to glyphosate by introducing glyphosate-tolerant EPSPS enzymes.

Amrhein et al. (1983) reported glyphosate resistance in cultured rock harlequin (*Corydalis sempervirens*) cells and found that the cells could survive in the presence of glyphosate. Resistance of these cells was based on increased transcription rate and reduced turnover of EPSPS (Smart et al. 1985). Nafziger et al. (1984) reported increased rates of EPSPS activity in excised carrot (*Daucus carota*) cells, although the EPSPS enzyme remained susceptible to glyphosate. Several plant cell lines expressed resistance to glyphosate as a result of amplification of EPSPS genes (Dyer et al. 1988; Forlani et al. 1992). Although glyphosate-resistant cells were regenerated, plants did not confer resistance either because resistance was not heritable or plants were insufficiently stable after regeneration (Sellin et al. 1992).

Researchers have explored glyphosate resistance from detoxification of glyphosate by *N*-acetylation mediated by microbes (Castle et al. 2004; Siehl et al. 2005). Castle et al. (2004) isolated genes, responsible for glyphosate metabolism and used genetic shuffling to rearrange the DNA of these detoxifying genes. After shuffling, new genes were tested for activity they eventually found one that would produce a very efficient metabolizing enzyme. This gene, glyphosate *N*-acteyltransferase (GAT) that would readily metabolize glyphosate was

introduced into several plant species including corn (*Zea mays*), which was completely tolerant to glyphosate applied at 5 kg/ha (Castle et al. 2004; Siehl et al. 2005).

To date, the only commercialized glyphosate-resistant crops available were generated through transformation of a gene in *Agrobacterium* sp. strain CP4 encoding a glyphosate-tolerant enzyme (Funke et al. 2006). Expression of the tolerant gene allows plant to overcome inhibition of native EPSPS sensitive genes allowing uninhibited growth (Nida et al. 1996). The *Agrobacterium* sp. strain CP4, found in a waste-fed column at a glyphosate production facility, had a glyphosate tolerant EPSPS which did not decrease the enzyme affinity for PEP allowing insertion into plants. Upon successful genetic transfer of the glyphosate-resistant gene, several commercial crops have become available: canola (*Brassica rapa*), corn (*Zea mays*), cotton (*Gossypium hirsutum*), sugar beet (*Beta vulagaris*) and soybean (*Glycine max*) and this technology was referred to as Roundup Ready® (Agbios 2009).

Soybean was the first commercially available Roundup Ready® crop, released in 1996, allowing growers to control grass and broadleaf weeds season-long with a single application of glyphosate in narrow-row plants (18-cm spacing), or with two applications wide-row plantings (76-cm spacing) (Nida et al. 1996; Mulugeta and Boerboom 2000). Glyphosate can be applied to both vegetative and reproductive soybean tissue with no adverse effect on grain yield (Delannay et al. 1995; Elmore et al. 2001). Certain glyphosate formulations can injure soybean and inhibit nodule development, but soybean yield is not affected (Reddy and Zablotowicz 2003).

Glyphosate-resistant cotton, released in 1997, allowed for over-the-top application of glyphosate to cotton with four or fewer leaves (Jones and Snipes 1999; Welch et al. 1997). Topical application of glyphosate after the four-leaf stage reduces pollen viability and could reduce yield under some circumstances (Jones and Snipes 1999; Pline et al. 2003). A second generation of glyphosate-resistant cotton was released in 2006, referred to as Roundup Ready Flex® cotton, which allowed for extended topical glyphosate application timings from cotton emergence throughout the growing season (May et al. 2004). The percentage of cotton planted with glyphosate-resitant cultivars has increased from 4% of US hectarage in 1997 to 80% in 2005 (Sankula 2006).

Glyphosate-resistant technology revolutionized weed management in these crops and has been an important component of reducing use of other herbicides (Shaner 2000; Young 2006). Shaner (2000) proposed that the long-term consequences of glyphosate most likely will cause shifts to weed species that are tolerant to field rates of glyphosate. Additionally, unprecedented adoption of this technology and associated use of glyphosate has resulted in development of resistant biotypes (Heap 2009).

Glyphosate-resistant weed species. Herbicide resistance is defined as the naturally occurring inheritable ability of some weed biotypes within a population to survive a herbicide treatment that would, under normal conditions of use, effectively control that weed population (Heap 1997). Herbicide resistance is often discovered after the repeated use of the same herbicide (Shaner 1995). There are various factors which relate to the evolution of herbicide resistance. These factors include initial frequency of the resistance genes, rate of gene mutation, inheritance of the resistance trait, and weed fitness in the presence or absence

of the resistance trait, therefore different weed species evolve resistance to herbicides at different rates (Gressel and Segel 1990; Jasieniuk 1996).

Glyphosate was considered by some to have a relatively low risk for herbicide resistance since it had not occurred after 20 years of use (Pagette et al. 1995). Bradshaw et al. (1997) suggested that evolution of an EPSPS mutant resistant to glyphosate would either provide little overall plant resistance or its enzymatic activity would be limited, to the extent which fitness and survival would be reduced. Also, since the development of glyphosate-resistant crops proved to be extremely difficult, some believed that glyphosate resistance was unlikely.

Currently, there are 15 weed species expressing resistance to glyphosate have been confirmed (Heap 2009). Only two mechanisms of resistance to glyphosate have been defined and include reduced translocation of glyphosate to meristematic tissues and an insensitive EPSPS enzyme (Powles and Preston 2006). Powles et al. (1998) reported the first case of glyphosate resistance in 1996, in rigid ryegrass (*Lolium rigidum*) expressing seven- to 11-fold resistance when compared to a susceptible population. Feng et al. (1999) suggest altered absorption, translocation, or metabolism was not the mechanism of resistance. Lorraine-Colwill et al. (2001) reported that resistance was transmitted through pollen suggesting resistance is conferred by a single nuclear gene. Bearson et al. (2002a) found that shikimate accumulates in both the resistant and susceptible plants suggesting that resistance was not associated with an insensitive EPSPS enzyme and proposed that resistance may be due to increased flux in the shikimate pathway or reduced herbicide absorption. The mechanism of resistance was finally determined to be associated with reduced translocation of glyphosate in resistant plants (Wakelin et al. 2004). However, glyphosate resistance in rigid ryegrass

occurs by at least two mechanisms, a less-sensitive target site and altered herbicide translocation (Wakelin and Preston, 2006). Glyphosate-resistant rigid ryegrass has been confirmed in the United States, although this biotype is expressed heterozygously, such that rates of glyphosate slightly above the traditional use rate were effective on a portion of the population (Simarmata et al. 2005).

In 1997, glyphosate-resistant populations of goosegrass (*Elusine indica*) were confirmed in Malasia (Lee and Ngim 2000). In the tropical environment of Malaysia, goosegrass produces several generations per year and multiple glyphosate applications were applied routinely to manage goosegrass and other weeds. Lee and Ngim (2000) reported 2- to 4-fold level of resistance of this population to glyphosate. It was determined that glyphosate resistance in goosegrass was associated with an enzyme change, and that two mutations in the EPSPS enzyme could result in failure of glyphosate to occupy the binding site (Baerson et al. 2002b; Ng et al. 2003).

Glyphosate-resistant horseweed (*Conyza canadensis*) was confirmed in Delware during 2001 (VanGessel 2001). This was the first annual dicotyledonous weed exhibiting resistance to glyphosate that occurred in fields with glyphosate-exclusive weed management programs prior to and after planting glyphosate-resistant soybean. Mueller et al. (2003) demonstrated that shikimate accumulated in both resistant and susceptible biotypes. In further studies, Feng et al. (2004) and Koger and Reddy (2005) found that glyphosate resistance was not due to absorption but rather due to reduced translocation of glyphosate in resistant plants. Glyphosate-resistant horseweed is now widespread and is documented in 17 U.S. states (Heap 2009). This discovery identified the possibility that glyphosate-resistant weeds in crop

production systems could risk the long-term sustainability of Roundup Ready crops® (Heap 2009).

Glyphosate-resistant Palmer amaranth. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) was first observed in Georgia during 2005 (Culpepper et al. 2006). Fields with suspected resistance received herbicide applications consisting of paraquat, pendimethalin, and glyphosate for four continuous years. Confirmed resistance of this biotype demonstrated a 6- to 8-fold level of resistance compared to a susceptible biotype. Initial investigations into the mechanism of resistance indicated that there were no differences in absorption or translocation between resistant and susceptible plants. However, resistant plants did not accumulate shikimate, an indicator for inhibition of EPSPS after exposure to glyphosate, indicating an altered target site (Culpepper et al. 2006).

Palmer amaranth is the most troublesome weed for cotton producers in North Carolina (Webster 2005) and has historically been difficult to control in cotton and soybean production systems. The photosynthetic capacity of Palmer amaranth is extremely high relative to other C_4 plants, and its leaves are able to solar track (Ehleringer 1983). Solar tracking allows leaves to remain perpendicular to the sun's direct rays in order to maximize solar energy use. Ehleringer (1983) also provided evidence to show that Palmer amaranth has effective drought tolerance mechanisms that allows survival and growth during dry conditions. Palmer amaranth is also a prolific seed producer, which enables it to develop tremendous seed banks (Keely et al. 1987). Palmer amaranth can also reach a height of more than two meters in height (Sellers et al. 2003). Vigorous growth allows this weed to establish a competitive dominance for light and space with crops (Morgan et al. 2001). Palmer

amaranth has been shown to decrease cotton yield by 13% for a single plant per 9.1 row m and up to 54% at a density of 10 per 9.1 row m (Morgan et al. 2001). Soybean yield reductions of 17 to 68% were reported with densities of 0.33 to 10 plants per m of row (Klingaman and Oliver 1994). Palmer amaranth present at harvest also interferes with mechanical harvesting of cotton (Smith et al. 2000).

Glyphosate is extremely effective in controlling glyphosate-susceptible Palmer amaranth (Bond et al. 2006; Corbett et al. 2004; Culpepper et al. 2000; Scott et al. 2002) and glyphosate-resistant technology has allowed growers to effectively manage Palmer amaranth in cotton and soybean with glyphosate-only herbicide systems (Culpepper and York 1998; Culpepper et al. 2000; Scott et al. 2002). Therefore selection for glyphosate-resistant biotypes has minimized utility of glyphosate-resistant crops.

Pyrithiobac controls Palmer amaranth in cotton (Dotray et al. 1996; Smith et al. 1997). Pyrithiobac inhibits acetolactate synthase (ALS), curtailing biosynthesis of three branched-chain amino acids valine, leucine, and isoleucine (Shimizu et al. 1994). Pyrithiobac applied both preemergence (PRE) and postemergence (POST) is effective for Palmer amaranth control, although effectiveness is more consistent when applied PRE (Dotray et al. 1996; Kaloumenos et al. 2005). Bond et al. (2006) reported that topical applications of pyrithiobac were less effective than glyphosate, and pyrithiobac effectiveness varied widely among Palmer amaranth accessions across the southern United States. The inconsistent control of weeds provided by pyrithiobac POST can be related to several factors including temperature and size of Palmer amaranth at application, where susceptibility increases when applied to

smaller plants, thus making the window narrow for effective Palmer amaranth control (Corbett et al. 2004; Light et al. 2001).

Although ALS-inhibiting herbicides typically control Palmer amaranth well (Mayo et al. 1995; Gossett and Toler, 1999), reliance on ALS inhibitor herbicides has resulted in widespread resistance to this herbicidal mode of action (Heap 2009). Ninety-five confirmed weeds have developed resistance to ALS inhibitors (Heap 2009). Palmer amaranth is one of these weeds with widespread resistance (Horak and Peterson 1995; Sprague et al. 1997). Sprague et al. (1997) reported cross resistance of Palmer amaranth to chlorimuron, imazethapyr, and thifensulfuron. Cross resistance of Palmer amaranth to multiple ALS inhibitors has also been documented with other ALS herbicides (Gaeddert et al. 1997). Specifically, Palmer amaranth resistant to multiple ALS inhibitors has been documented to be cross resistant to pyrithiobac (Wise et al. 2009) and the presence of Palmer amaranth populations with multiple resistance to both pyrithiobac and glyphosate has been reported (Sosnoskie et al. 2009) presenting a situation where effective topical herbicide options in cotton would be dramatically diminished.

Glyphosate-resistant Palmer amaranth has been found in North Carolina. Presence of this weed may dramatically affect weed management and endanger the livelihood of cotton and soybean producers in this state. Little is known about the distribution of this pest and what management strategies will need to be adopted to effectively control Palmer amaranth. In cotton and soybean, growers are heavily dependent on glyphosate for the control of many weeds, including Palmer amaranth. In 2005 alone, North Carolina growers planted over 326,000 ha of cotton and over 602,000 ha of soybean (NADACS 2009) and over 99% of

cotton and a large portion of soybean hectares were planted in Roundup Ready® varieties (USDA-AMS 2005). Palmer amaranth likely infests a large portion of the state in which these crops are produced and glyphosate resistance would undoubtedly have a tremendous impact on weed management.

Distribution of Palmer amaranth resistant to glyphosate needs to be accessed. Since ALS inhibitors will likely be part of management strategies to combat this weed, knowledge about the distribution of ALS resistance also needs to be determined. A survey of the distribution of glyphosate- and ALS inhibitor-resistant Palmer amaranth is needed to alert producers to the severity of the problem and encourage the adoption of resistance management strategies. Evolution of glyphosate-resistant Palmer amaranth will undoubtedly force growers to change production practices and management strategies to sustain cotton production. Herbicide systems need to be evaluated to develop management strategies to combat this pest in cotton and soybean. Additionally, herbicides with other modes of action must be integrated into glyphosate-based management systems to avoid or delay resistance in fields currently infested with glyphosate susceptible Palmer amaranth.

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CHAPTER II

Distribution of Glyphosate- and Thifensulfuron-resistant Palmer amaranth (*Amaranthus palmeri*) in North Carolina

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Abstract

Glyphosate-resistant Palmer amaranth was first suspected in North Carolina during 2005 and confirmed in 2006. Palmer amaranth resistant to acetolactate synthase (ALS)-inhibiting herbicides has also been widely documented across the southern U.S., including North Carolina. A survey was conducted to determine distribution of these resistant biotypes within North Carolina. Information pertaining to distribution of resistance could alert producers to the severity of the problem and encourage the adoption of resistance management strategies. Palmer amaranth seed were collected from 290 fields in North Carolina in 29 counties during 2005. Seed from each field were greenhouse grown and treated with glyphosate applied at 280, 560, and 840 g ae/ha or thifensulfuron applied at 4.4, 18, and 70 g ai/ha. Resistance to glyphosate was detected in 49 fields distributed across 11

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counties located in the southeastern portion of the state. Resistance to thifensulfuron was detected in 52 fields across 15 counties. Most of these counties were located across the eastern portion of the state, along with two counties in western North Carolina. Five fields had populations resistant to both glyphosate and thifensulfuron.

Nomenclature: *Amaranthus palmeri* S. Wats.

Key words: Herbicide resistance, multiple resistance.

Introduction

Resistance to glyphosate has been reported in 15 weed species worldwide (Heap 2009). Glyphosate-resistant Palmer amaranth was confirmed in Georgia during 2005 (Culpepper et al. 2006). This was the world's first conformation of glyphosate resistance in an *Amaranthus* species. Soon after conformation in Georgia, researchers from North Carolina, South Carolina, Tennessee, and Arkansas were all studying populations of Palmer amaranth suspected of being glyphosate-resistant. By the end of the 2008 production season, glyphosate-resistant Palmer amaranth was confirmed or being confirmed in Arkansas, North Carolina, South Carolina, and Tennessee (Heap 2009; Norsworthy et al. 2008; Steckel et al. 2008).

Palmer amaranth resistant to glyphosate poses a serious problem for producers across the midsouth and southeastern United States. This weed was already one of the most troublesome weeds of agronomic crops in the southern United States (Webster 2005). Glyphosate resistance only exacerbates problems associated with managing this pest. This weed is a summer annual that can grow an average of 4.3 cm per day and reach over two

meters in height (Elmore 1990; Horak and Loughin 2000). It has an extremely high photosynthetic capacity and utilizes the C_4 photosynthetic pathway (Ehleringer 1983). There is also evidence that Palmer amaranth has effective drought tolerance mechanisms that allow it to survive and grow during dry conditions, along with mechanisms to readily adapt to shading (Ehleringer 1983; Jha et al. 2008). Palmer amaranth is also a prolific seed producer, which enables it to replenish seed banks quickly (Keely et al. 1987; Sellers et al. 2003).

Palmer amaranth effectively competes with crops for light and space (Monks and Oliver 1988). It is a very competitive weed in cotton (*Gossypium hirsutum*) and soybean (*Glycine max*), as evidenced by cotton yield reductions of 13% with one plant in 9.1 m row and 17% soybean yield reductions with one plant in 3 m row. Yield losses up to 79% in soybean have occurred with densities of eight plants per m row and 54% in cotton with densities of 10 plants per 9.1 m row (Bensch et al. 2003; Klingaman and Oliver 1994; Morgan et al. 2001). Additionally, Palmer amaranth present at harvest may interfere with cotton harvest due to stoppages to remove lodged weeds (Smith et al. 2000).

Researchers are actively searching for resistance management strategies to avoid or delay further evolution of resistance along with strategies to combat resistance where it already occurs. A key component of these strategies is the use of herbicides with different modes of action. Some ALS-inhibiting herbicides typically control Palmer amaranth well (Mayo et al. 1995; Gossett and Toler, 1999; Kaloumenos et al. 2005). Pyrithiobac, an ALS-inhibiting herbicide applied both preemergence and postemegence to cotton, is effective for Palmer amaranth control (Dotray et al. 1996; Jordan et al. 1993; Smith et al. 1997).

There are currently 97 documented weeds that have developed resistance to ALS inhibitors, including Palmer amaranth (Heap 2009; Horak and Peterson 1995; Sprague et al. 1997). Management of ALS-resistant biotypes, including Palmer amaranth, is exacerbated due to widespread cross resistance (Heap 2009; Gaeddert et al. 1997; Sprague et al. 1997). In Georgia, Palmer amaranth is confirmed to have multiple resistance to both glyphosate and pyrithiobac (Sosnoskie et al. 2009), which greatly minimizes effective topical herbicide options in cotton.

The objectives of this study were to document distribution of glyphosate- and ALS-resistant biotypes of Palmer amaranth in North Carolina. Knowledge about the distribution of this pest is important in providing producers the best recommendations to manage these resistant biotypes and to potentially reduce spread to non-infested areas. Additionally this survey will provide essential information for researchers to use in the future, helping to access changes in distribution or spread of resistance over time.

Materials and Methods

Sampling. In 2005, glyphosate resistance was initially suspected in seven fields in Hoke, Robeson, and Wayne counties in North Carolina (Figure 1). Palmer amaranth at these locations survived growers' typical glyphosate applications and additional applications of glyphosate applied at 3.0 kg ae/ha. Palmer amaranth seedheads were collected from 290 fields in 29 counties across North Carolina (Figure 2). Collection of seed in the fall of 2005 was concentrated in the two general areas where resistance was suspected, but an effort was made to sample all counties in the eastern portion of the state plus four counties with

significant cotton production in southwestern North Carolina. Palmer amaranth was not found in three additional counties surveyed in northeastern North Carolina. The counties sampled represent the predominate areas where Palmer amaranth is found, although not found in the western part of the state, it is beginning to become a problem in southern areas of the Piedmont.

Fields were randomly selected after visual conformation of Palmer amaranth from public roadways. Sampling technique included random harvesting a minimum of 30 seedheads from female plants spaced at least 10 m apart in infested soybean and cotton fields while walking in a zig-zag pattern over the field. Cropping history and herbicide use was not known at the time of the survey. Glyphosate-resistant cultivars comprised 99% of the cotton and over 80% of the soybean planted in North Carolina during 2005 (USDA-AMS 2005) hence there was a high probability that each field had been treated at least once with glyphosate. Seedheads were placed in paper bags and the GPS coordinates of each sampled field recorded. Each sample was dried, threshed, and cleaned and then stored at 1 C until use.

Glyphosate screening. The glyphosate screening was conducted during the fall and winter of 2005, 2006, and 2007. Samples were screened in a greenhouse where temperatures were maintained at 32 ± 5 C, and natural light was supplemented for 12 to 14 h each day by metal halide lamps (400 µmol per m^2/s). Due to the large number of samples, all locations could not be screened simultaneously. During each screening, previously confirmed glyphosate-resistant and –susceptible biotypes were included for comparison. Seed from each sample were planted into two trays containing six individual flats (10 cm by 15 cm, 7 cm deep) filled

with a commercial growing medium[1]. Seedlings were thinned to approximately eight plants per flat. Each flat was fertilized with 12 g of Peter's Professional Blend 20-20-20 water soluble fertilizer[2] and irrigation was applied with automatic sprinklers to maintain optimum soil moisture.

Seedlings 10 to 14 cm in height were treated with the potassium salt of glyphosate[3] applied at 0, 280, 560, and 840 g ae/ha. In a preliminary study, it was determined that glyphosate at 280 g/ha was at least 95% effective on glyphosate-susceptible Palmer amaranth grown under similar greenhouse conditions. Glyphosate applied at 840 g/ha is a normally recommended rate for postemergence applications to cotton under field conditions to control Palmer amaranth (Anonymous 2008). Plants were sprayed using an enclosed track sprayer applied with a single even-spray flat-nozzle[4] calibrated to deliver 140 L/ha at 165 kPa. Plants were not irrigated for 24 h after herbicide application to prevent herbicide washoff.

The experimental design was a randomized complete block with treatments replicated three times, blocking against Palmer amaranth height, and the experiment was conducted three times for each biotype. Visible estimates of Palmer amaranth control were recorded 14 d after glyphosate application using a scale of 0 to 100, where 0 = no control and 100 = death of all plants (Frans et al. 1986). Foliar chlorosis, necrosis, and plant stunting were considered when making visual estimates. Shoot fresh weight was also recorded immediately following visual estimates of control. Results were pooled across runs within location because of a lack of interaction. The following criteria were used to define resistance: plants controlled less than 50% by glyphosate at 560 and 840 g/ha were considered highly resistant; plants controlled less than 50% by glyphosate at 280 g/ha were

considered to express a low level of resistance. Several samples had one or two plants poorly controlled while the remainder died; such samples were considered to be a mixed population.

ALS-inhibitor screening: The ALS screening was conducted in fall and winter of 2006 and 2007. Screening of the Palmer amaranth for ALS resistance was originally intended to be conducted with pyrithiobac. Pyrithiobac is applied topically to cotton for Palmer amaranth control (Jordan et al. 1993; Smith et al. 1997). However, preliminary research revealed several difficulties associated with using pyrithiobac. The spray chamber used to in this experiment was located approximately 100 m from the greenhouse where plants were grown. Special care was taken to ensure that plants were not exposed to environmental conditions different from the greenhouse during transport. Despite measures taken, variable results were seen within the plants from the same location sprayed at different dates. Variability in control of Palmer amaranth with pyrithiobac has been documented in other research due to differences in temperature surrounding time of application (Mahan et al. 2004). Another difficulty associated with pyrithiobac was based on soil activity of the herbicide. Pyrithiobac enters plants through roots and foliage (Mitchell et al. 1992). Organic matter can cause adsorption of pyrithiobac in soils (Veletza et al. 2005). The extremely high organic matter content of the growing media used in this experiment would likely interrupt root absorption. It is typical for ALS-resistant weeds to be cross-resistant to at least one if not many other ALS-inhibiting herbicides; therefore ALS-resistant Palmer amaranth likely has cross resistance to more than one herbicide (Gaeddert et al. 1997; Horak and Peterson 1995). Therefore, plants were screened for ALS resistance using thifensulfuron[5], another ALS inhibiting herbicide that effectively controls Palmer amaranth when applied topically (Mayo

et al. 1995). Preliminary research with two biotypes previously determined to be highly resistant to pyrithiobac were also found to be highly resistant to thifensulfuron and imazethapyr. Thifensulfuron is effective only when applied postemergence (Anonymous 2006); therefore growing media would not likely play a large role in determining resistance.

In this experiment, Palmer amaranth populations from all locations were screened simultaneously. Seed from each sample were grown in the same manner in the glyphosate resistance screen. Seedlings 7 to 10 cm tall were treated with thifensulfuron applied at 4.4, 18, and 70 g ai/ha. The lower rate, 4.4 g/ha, is the rate recommended for postemergence application in soybean (Anonymous 2006). The intermediate rate, 18 g/ha, is the maximum rate recommended for postemergence application in sulfonylurea-tolerant soybean (STS) varieties. Preliminary research showed that the lowest rate, 4.4 g/ha was at least 95% effective on susceptible Palmer amaranth grown under similar greenhouse conditions (Anonymous 2006). Plants were sprayed as previously described with the aforementioned rates combined with nonionic surfactant[6] at 0.25% (v/v). The experimental design was a randomized complete block, blocking against Palmer amaranth height, with four treatments replicated three times, and the experiment was conducted three times. Visible Palmer amaranth control was estimated 21 d after thifensulfuron application and shoot fresh weight was recorded after visual evaluation. Results were pooled across runs within location because of a lack of interaction. The following criteria were used to determine resistance: plants controlled less than 90% by thifensulfuron at 4.4 and 17.5 g/ha were considered have a low level of resistance; plants controlled less than 90% by thifensulfuron at 70 g/ha were

considered highly resistance. If samples had one or two plants poorly controlled while the remainder died, such samples were considered to be a mixed population.

Results and Discussion

Populations of glyphosate-resistant Palmer amaranth were found in 49 fields scattered over 11 counties, or 17% of the fields sampled (Figure 3). Most of the fields with resistant biotypes were located near locations initially suspected to have resistance (Figure 1). Of the 49 fields with Palmer amaranth expressing glyphosate resistance, 10 fields had populations expressing high levels of resistance, 30 fields had populations with a low level, and 9 fields had mixed populations. Populations of ALS-resistant Palmer amaranth were found in 52 fields, or 18% of the fields sampled (Figure 4). These fields were scattered over 17 counties and was more widely distributed than fields with glyphosate-resistant Palmer amaranth. Of the 52 fields, 15 had populations expressing a low level of resistance, 22 expressed a high level of resistance, and 15 had mixed populations. Five of the fields had populations of Palmer amaranth which were resistant to both glyphosate and thifensulfuron. These fields were scattered over four counties in the southeastern portion of the state (Figure 5).

The level of resistance within these fields has not been quantified for all locations. However, one of the biotypes expressing a high level of glyphosate resistance, based upon I_{50} values (rate necessary for 50% inhibition) for shoot fresh weight reduction, required 20 times more glyphosate to reduce shoot fresh weight by 50% when compared to a known susceptible population (Whitaker et al. 2007). In two of the populations most resistant to

thifensulfuron, the I_{50} for fresh weight reduction was at least 500 times greater than for a known susceptible population (Whitaker 2009).

Cotton is produced primarily in the eastern part of the state in the Coastal Plain (Figure 6) and Palmer amaranth resistant to glyphosate and thifensulfuron is widespread throughout this part of North Carolina. In 2005, North Carolina growers planted over 326,000 ha of cotton and over 602,000 ha of soybean (NADACS 2009). Over 99% of cotton and large portion of soybean hectares were planted in Roundup Ready varieties (USDA-AMS 2005). Soybean production in North Carolina is more widespread across the state, although 80% or 480,000 ha are produced in the eastern portion of the state (Figure 7). Counties which have fields with glyphosate-resistant Palmer amaranth produced 31 and 32% of the cotton and soybean hectares, respectively, in 2005 (NADACS 2009). The counties which have fields with populations of ALS inhibitor-resistant Palmer amaranth produced 50 and 43% of the cotton and soybean hectares, respectively, in North Carolina during 2005 (NADACS 2009). At the end of the 2008 growing season, glyphosate resistance was suspected in 20 or more counties based on the authors' observations.

Results from this survey will be incorporated into recommendations and educational programs to increase growers' awareness to the presence of glyphosate- and ALS-resistant biotypes within the state and to encourage proactive resistance management programs to reduce further selection pressure.

Sources of Materials

[1] Metro Mix 200®, Scotts-Sierra Horticultural Products Company, Marysville, OH.

[2] Peters Professional® Water Soluble 20-20-20 Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH.

[3] Roundup® WEATHERMAX herbicide, Monsanto Company, St. Louis, MO 63167.

[4] TeeJet TP8003E even-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

[5] Harmony GT XP® herbicide, Dupont Agricultural Products, Crop Protection, Wilmington DE.

[6] Induce®, blend of alkylarylpolyoxylkane ether, free fatty acids, and isopropyl (90%), and water and formulation acids (10%). Helena Chemical Corporation, 225 Schilling Blvd., Suite 300, Collierville, TN 38017.

Acknowledgements

The authors would like to thank Rick Seagroves and Jamie Hinton for technical support. Funding for this research was provided by Cotton Incorporated and Monsanto Company.

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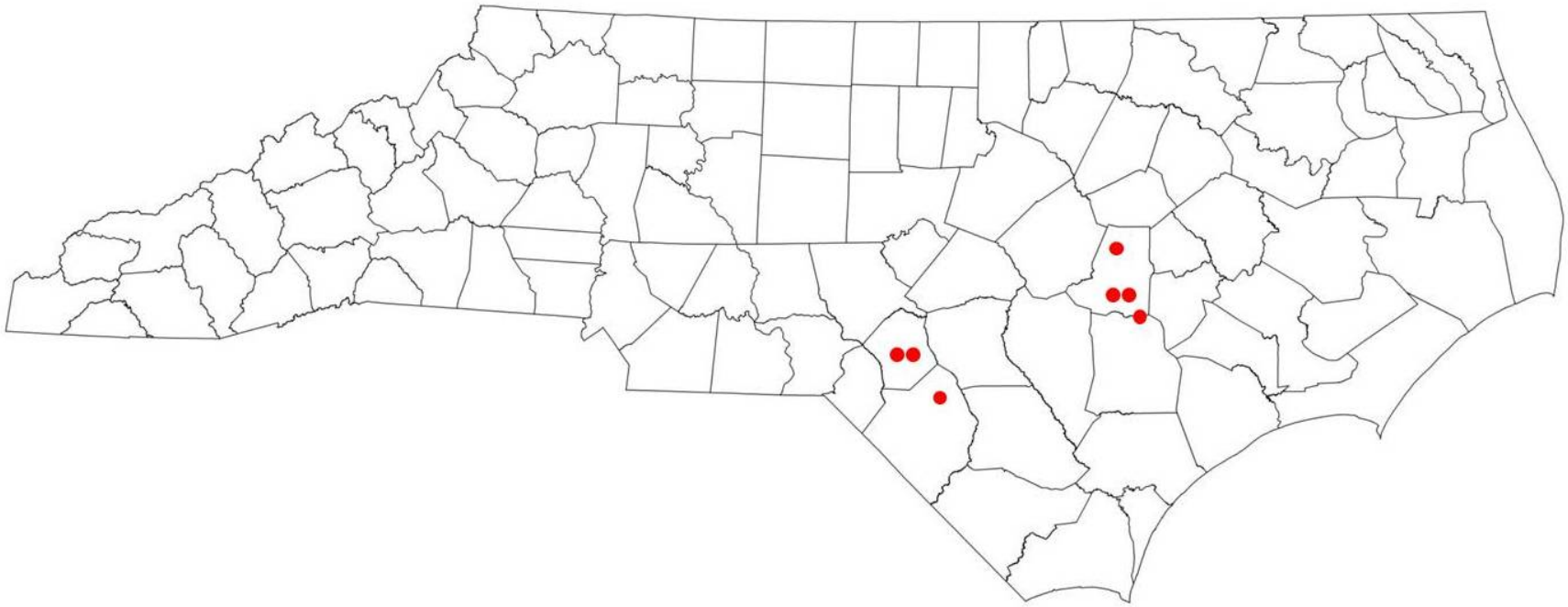

Figure 1. Location of seven fields in Hoke, Roberson, and Wayne counties in North Carolina during 2005 where glyphosate-resistant Palmer amaranth was initially suspected.

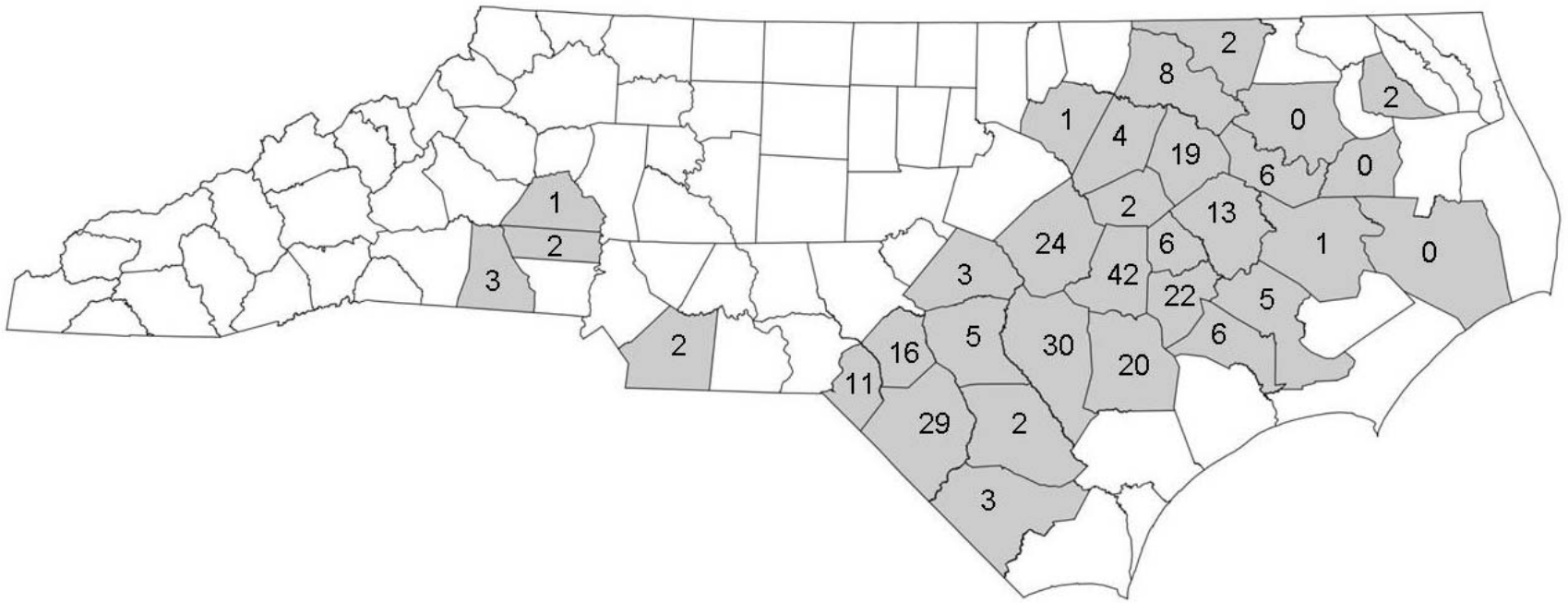

Figure 2. Distribution of North Carolina counties surveyed in the fall of 2005 (shaded grey) and the number of fields in each county where Palmer amaranth seedheads were collected.

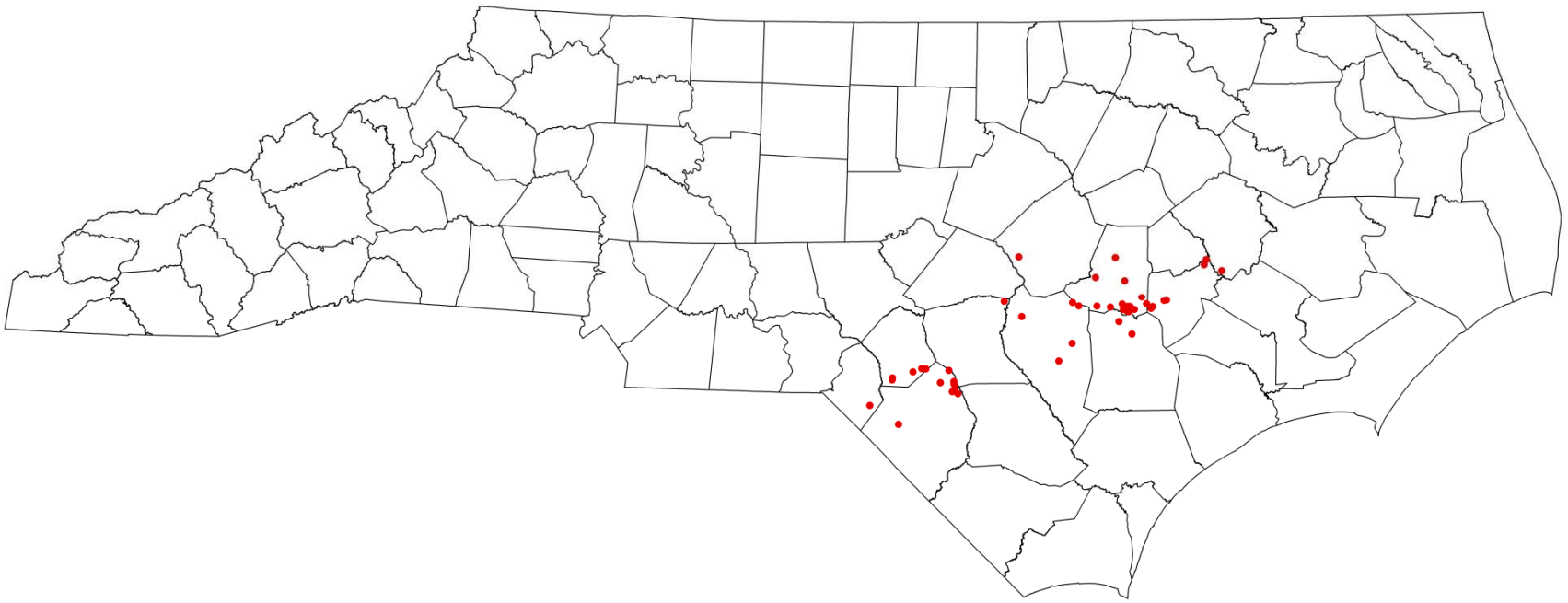

Figure 3. Location of 49 fields in North Carolina with glyphosate-resistant populations of Palmer amaranth during 2005.

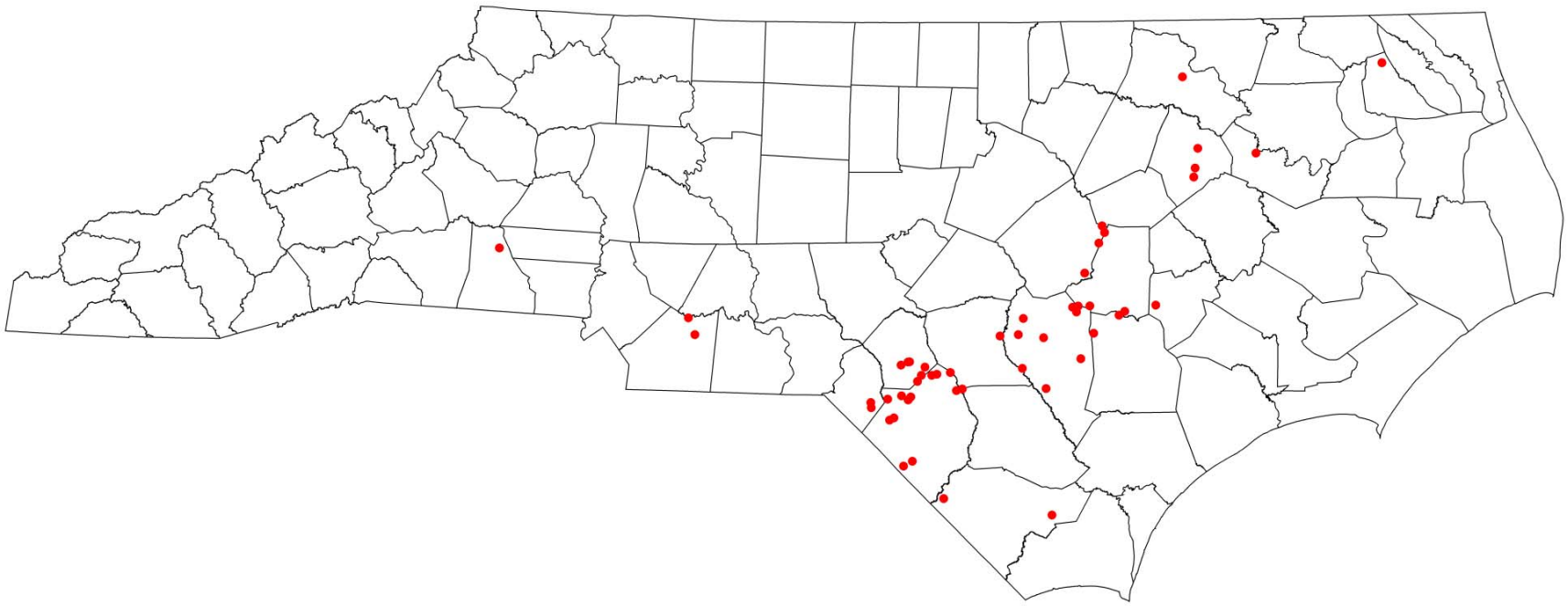

Figure 4. Location of 52 fields in North Carolina with ALS inhibitor-resistant populations of Palmer amaranth during 2005.

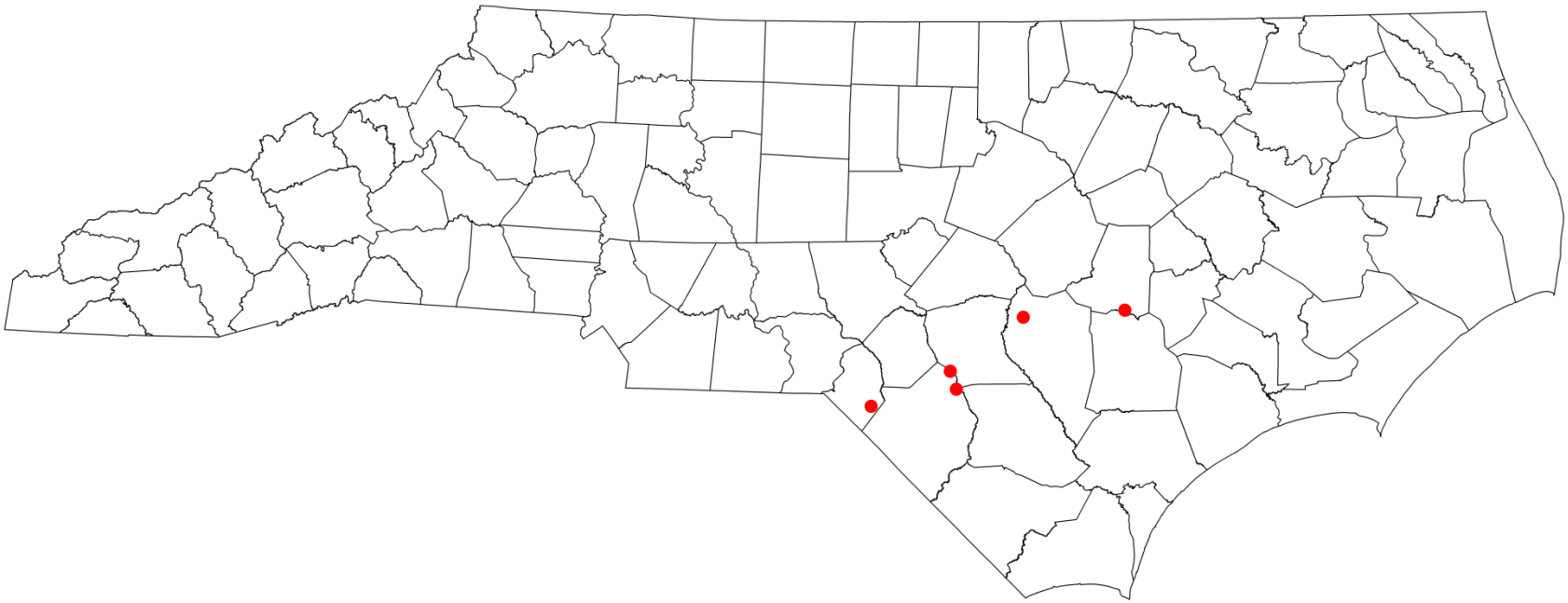

Figure 5. Locations of five fields with Palmer amaranth populations resistant to both glyphosate and thifensulfuron.

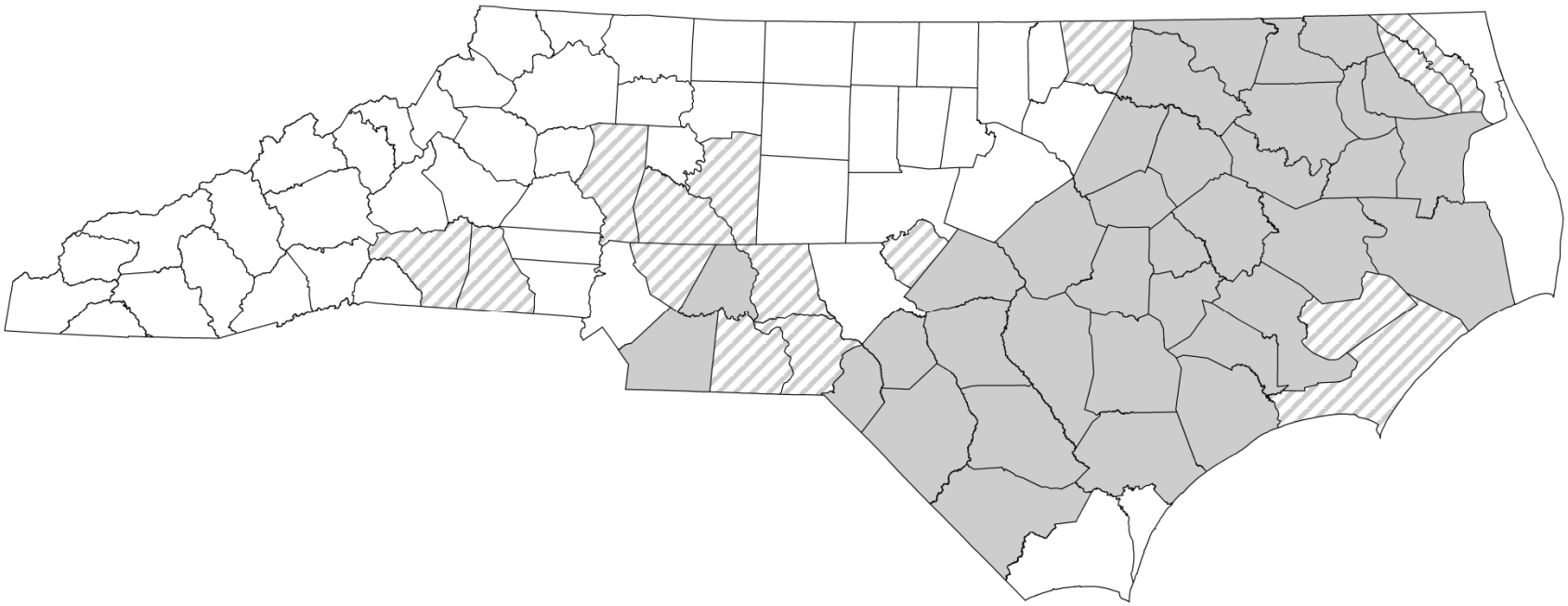

Figure 6. Cotton production in North Carolina during 2005. Counties with hatched lines had between 100 and 2000 ha of cotton; grey shaded counties had greater than 2000 ha planted in cotton.

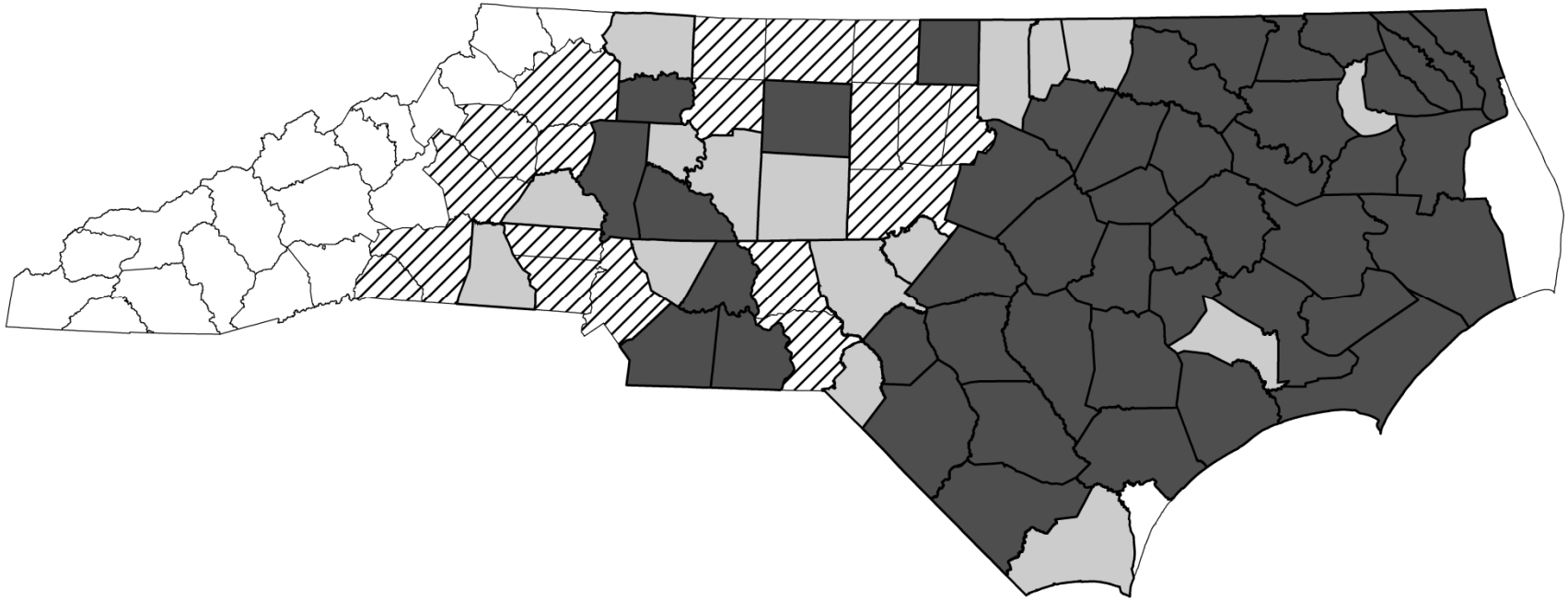

Figure 7. Soybean production in North Carolina during 2005. Counties with hatch marks had between 100 and 2000 ha of soybean, light grey shaded counties had 2001 to 4000 ha, and dark grey shaded counties had greater than 4001 ha planted in soybean.

CHAPTER III

Physiology of Glyphosate Resistance in Palmer amaranth (*Amaranthus palmeri*) from North Carolina

Jared R. Whitaker, James D. Burton, Alan C. York and David L. Jordan*

Abstract

Glyphsoate-resistant (GR) Palmer amaranth biotypes have been confirmed in five southern U.S. states. Experiments were conducted to characterize physiological differences between GR and glyphosate–susceptible (GS) biotypes from North Carolina. Glyphosate rate required to reduce fresh weight of the GR biotype by 50% was 20 times greater than the rate needed for the GS biotype. Absorption and translocation of ^{14}C-glyphosate was studied in both biotypes when oversprayed with commercial unlabeled glyphosate (840 g ae/ha) or

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not oversprayed immediately prior to a ^{14}C-glyphosate application to the uppermost fully expanded leaf. Plants were harvested at 6, 12, 24, 48, and 72 h after treatment (HAT) and sectioned into the treated leaf, tissue above the treated leaf, tissue below the treated leaf and roots. Maximum absorption was observed by 12 HAT, and was similar between biotypes at all timings except 6 HAT, where GS plants absorbed 65% more ^{14}C than GR plants. Oversprayed plants absorbed 33 and 61% more ^{14}C than plants not oversprayed by 48 and 72 HAT. Glyphosate distribution was similar among biotypes in the treated leaf (40 to 43%), tissue above the treated leaf (30 to 31%), and in tissue below the treated leaf (22%). Both GS and GR plants not oversprayed with glyphosate had similar ^{14}C distribution in the roots, but oversprayed GS plants had much less ^{14}C in roots compared to GR plants at 48 and 72 HAT. When unlabeled glyphosate was applied at a sub-lethal rate, GS plants absorbed 43% less ^{14}C and moved less ^{14}C out of the treated leaf than GR plants.

Nomenclature: glyphosate, Palmer amaranth, *Amaranthus palmeri* S. Wats.

Key words: absorption, herbicide resistance, shikimate, translocation.

Abbreviations: GR, glyphosate-resistant; GS, glyphosate-susceptible; HAT, hours after treatment.

Introduction

Palmer amaranth is the most troublesome weed for cotton producers in North Carolina (Webster 2005). However, glyphosate has traditionally been extremely effective in controlling Palmer amaranth (Bond et al. 2006; Corbett et al. 2004) and glyphosate-resistant technology has allowed growers to effectively manage Palmer amaranth in cotton with

glyphosate-only herbicide systems (Culpepper and York 1999; Culpepper et al. 2000; Scott et al. 2002). Along with excellent control of *Amaranthus* species, growers have readily adopted this technology because of broad-spectrum weed control, convenience of overtop application, increased rotational flexibility, and reductions in labor and time required for weed management activities (Askew et al. 2002; Culpepper and York 1998, 1999; Faircloth et al. 2001; Gianessi 2008; Young 2006). The percentage of cotton planted with GR cultivars has increased from 4% of U.S. hectares in 1997 to 80% in 2005 (Sankula 2006). During 2008, cotton was planted on over 2.07 million ha in Alabama, Georgia, North Carolina, South Carolina and Tennessee combined and greater than 99% of the crop was devoted to glyphosate-resistant cultivars (USDA-AMS 2008).

In the late 1990s, weed resistance to glyphosate was considered unlikely because of unique properties of the herbicide, such as its mechanism of action, absence of metabolic degradation in plants, and lack of residual activity in soil (Bradshaw et al. 1997). However, by then end of 2008 resistance to glyphosate had been confirmed in 15 weed species (Heap 2009). The first confirmed case of glyphosate resistance in Palmer amaranth was documented in Georgia during 2005 (Culpepper et al. 2006). By 2009, GR Palmer amaranth was confirmed in North Carolina, South Carolina, Tennessee, and Arkansas (Heap 2009; Norsworthy et al. 2008; Steckel et al. 2008; York et al. 2007). Presence of glyphosate-resistant biotypes has increased rapidly in Georgia and North Carolina due to the reproductive characteristics of this weed and to weed management practices (Culpepper et al. 2008; Sosnoskie et al. 2007).

Resistance to glyphosate is due to at least two mechanisms in plants to avoid phytotoxicity. Horseweed (Feng et al. 2004) and rigid ryegrass (Wakelin et al. 2004) have resistance mechanisms that involve altered glyphosate movement and distribution. Rigid ryegrass (Baerson et al. 2002b) and goosegrass (Baerson et al. 2002a) have each developed a resistance mechanism due to a single amino acid change in the target enzyme, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS, E.C.2.5.1.19), that allows plants to survive commercial rates of glyphosate. Italian ryegrass biotypes from Oregon have resistance mechanisms including both altered movement and an alteration in the target site (Perez-Jones et al. 2007). Alternatively, resistance in a rigid ryegrass from Australia may involve the transient increase in expression of EPSPS mRNA and enhanced enzyme activity (Baerson et al. 2002a).

Research (Culpepper et al. 2006) suggests that altered absorption and translocation is not associated with glyphosate resistance in Palmer amaranth. In susceptible plants, glyphosate competes with the substrate phosphoenolpyruvate for a binding site on EPSPS, resulting in unregulated flow of carbon into the shikimate pathway and a characteristic accumulation of shikimate in sensitive tissues (Amrhein et al. 1980). The Georgia biotype of GS Palmer amaranth accumulated shikimate after exposure to glyphosate, compared with no accumulation in the resistant biotype (Culpepper et al., 2006). Gaines et al. (2008) reported an altered moiety of EPSPS in the resistant biotype, but the amino acid change was not predicted to confer resistance. Further research has suggested resistance due to gene amplification (Gaines et al. 2009). A 60- to 120-fold increase in gene copy number was noted in resistant plants and the increase in gene copy number was correlated with the level

of resistance. Resistance levels differ considerably among biotypes of Palmer amaranth collected in North Carolina and Georgia. For example, different GR biotypes from Georgia have levels of resistance ranging from 3- to 8-fold higher than susceptible biotypes, whereas the resistant biotypes from North Carolina range from 3- to 22-fold higher (Culpepper et al. 2008).

Comparing absorption and translocation between resistant and susceptible biotypes can be important in defining possible mechanisms of resistance. Additionally, methodology can vary among researchers and can contribute to variation in conclusions used in developing plausible explanations of mechanisms of resistance. Therefore, research was conducted to determine the level of resistance in a North Carolina GR biotype and to compare absorption and translocation of ^{14}C-glyphosate in a GR and GS biotype from North Carolina in the presence and absence of commercial application rates of non-labeled glyphosate.

Materials and Methods

Seed collection and methods common to both experiments. Palmer amaranth seed were collected from a known GS population at the Central Crops Research Station near Clayton, NC. Glyphosate-resistant Palmer amaranth seed were also collected from a field near Parkton, NC where plants survived multiple applications of glyphosate during the 2006 growing season. Seed from female plants in both fields were planted in a greenhouse. Glyphosate[1] was applied at 1.2 kg ae/ha to 7- to 10-cm tall plants with a track sprayer equipped with a single even-spray flat fan nozzle[2] delivering 140 L/ha. Preliminary research determined that glyphosate applied at 0.28 kg/ha was completely effective on the GS biotype.

Plants from the GR population which survived the glyphosate application were grown to maturity and allowed to cross-pollinate. Flowering was induced by covering 40- to 60-cm tall plants with black plastic for 14 h for five consecutive nights. Male plants were interspersed among female plants, and cross pollination was facilitated by shaking the inflorescence of male and female plants at least three times weekly during pollination. After seed development and maturation, seed were gathered and the process was repeated two additional times, although greater than 95% of plants grown from field-collected seed survived glyphosate applications. Seed from both GR and GS populations were threshed by hand and stored at 1 C until use.

In each experiment, GR and GS Palmer amaranth seeds were planted in round pots (10 cm diameter, 12 cm deep) and thinned to one plant per pot upon emergence. Plants were watered with an overhead irrigation system with automatic sprinklers to maintain optimum soil moisture. The greenhouse was maintained at 32 ± 5 C, and natural lighting was supplemented for 12 to 14 h each day with metal halide lighting (400 μmol m^2/s). Plants were fertilized with Peter's Professional Blend 20-20-20 water soluble fertilizer[3] as needed to maintain good growth. Glyphosate was applied using the track sprayer previously described. Following applications, plants were returned to the greenhouse where irrigation was withheld for 24 h.

Glyphosate dose response. Seeds of both GR and GS biotypes were planted in pots containing a commercial potting medium[4]. Plants, 7 to 10 cm in height, were treated with fifteen rates of glyphosate[1]. Each biotype received a different set of rates, based on preliminary research, to determine the glyphosate rate required to reduce plant fresh weight

by 50%. The susceptible plants received glyphosate applied at 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.075, 0.09, 0.12, 0.15, 0.195, 0.24, 0.36, and 0.48 kg ae/ha while the resistant plants received glyphosate at 0, 0.12, 0.24, 0.36, 0.54, 0.72, 0.96, 1.2, 1.44, 1.68, 1.98, 2.28, 2.64, 3.0, and 3.36 kg/ha.

The experimental design was a randomized complete block design with treatments replicated six or seven times, blocking against plant size, and the experiment was conducted a total of three times. Visible Palmer amaranth control was estimated 14 d after glyphosate application using a scale of 0 to 100, where 0 = no control and 100 = death of all plants (Frans et al. 1986). Shoot fresh weight was recorded after visual evaluation.

Absorption and translocation. Glyphosate-resistant and -susceptible seed were planted in pots containing a sandy soil with low organic matter. Plants 10 to 14 cm tall with 9 to 11 true leaves were selected for the experiment. The experiment was conducted as a randomized complete block design with five treatments replicated four or six times, blocking against plant size, and the experiment was repeated once. Four treatments had a factorial arrangement based on biotype (GR and GS) and glyphosate overspray (oversprayed with glyphosate[1] applied at 0.84 kg/ha or not oversprayed immediately before ^{14}C-glyphosate[5] application). Entire plants were oversprayed using the track sprayer described in the dose response experiment. The uppermost fully expanded leaf was then spotted with 10 µl of ^{14}C-glyphosate solution using a microapplicator. Technical grade phosphono-methyl-^{14}C-glyphosate[5] with 2.035 GBq/mmole specific activity and 99% radiochemical purity was used. The spotting solution contained 330 µl of ^{14}C-glyphosate diluted in 920 µl of deionized water with 0.125% non-ionic surfactant[6]. Glyphosate dose from 10 µl of spotting solution equaled

0.14 kg/ha based on a 10-cm^2 leaf (approximate size of treated leaf) and contained 6.5 kBq of radioactivity.

One additional treatment was included in the experiment where an approximate I_{50} glyphosate dose (0.09 kg/ha) was applied to GS plants. This treatment was included to allow comparisons between resistant and susceptible plants oversprayed with a non-lethal glyphosate dose (in resistant plants 0.84 kg/ha was not lethal). In this treatment, prior to the overspray, the uppermost fully expanded leaf was covered with aluminum foil. After overspray, the foil was removed from the leaf and spotted with a ^{14}C-glyphosate solution. The ^{14}C treated leaf in these plants was covered because the dose which would be applied on top of the overspray would more than double the glyphosate rate on the treated leaf compared to the rest of the plant. In the other treatments, the spotting solution only increased the rate of glyphosate on the treated leaf of oversprayed plants by 16%. The solution for this treatment was prepared separately and contained 60 µl of ^{14}C-glyphosate[5] diluted in 320 µl of deionized water with 0.125% nonionic surfactant. Total glyphosate dose from 10 µL of spotting solution equaled 0.1 kg/ha based on a 10-cm^2 leaf and contained 4 kBq of radioactivity.

Plants were harvested at 6, 12, 24, 48, and 72 HAT and divided into four regions: (1) treated leaf, (2) above the treated leaf, (3) below the treated leaf, and (4) roots. The treated leaf was removed at the point of attachment to the stem. This point of attachment was the basis for division of plant parts. Roots were washed over wire mesh to remove soil. Foliar absorption of glyphosate was determined by washing the treated leaf in 20 ml of 50:50 mixture of methanol and deionized water with 0.25% non-ionic surfactant for 1 m to remove

herbicide remaining on the leaf surface. One-ml aliquots of the leaf wash were added to 15 ml of scintillation cocktail[7] and radioactivity was quantified with liquid scintillation spectrometry[8] (LSS). Plant parts were dried for 72 hours at 45 C, weighed, and combusted with a biological sample oxidizer[9]. Radioactivity was quantified by LSS. Absorption was expressed as a percentage of total recovered ^{14}C in leaf washes divided by total ^{14}C recovered from all plant parts plus ^{14}C in leaf washes). Distribution of ^{14}C in each plant part was expressed as a percentage of the ^{14}C in each plant part divided by total ^{14}C recovered from all plant parts minus the ^{14}C recovered from the leaf wash.

Statistical Analysis. Data from the dose response experiment were subjected to ANOVA using the PROC MIXED procedure of SAS[10] and nonlinear regression. Each experimental run was considered a random effect. Palmer amaranth shoot fresh weight and visual control, expressed as a percentage of non-treated plants were regressed against the $\log_{10}$ of the glyphosate rate to obtain log-logistic dose response curves (Equation 1), where C = lower limit, D = upper limit, b = slope, and I_{50} = dose giving 50% response I_{50} according to Seefeldt et al. (1995).

$$y = C + \frac{D-C}{1+\left({}^{x}/_{I_{50}}\right)^{b}} \qquad [1]$$

This log-logistic curve has been used determine rates glyphosate rates required to reduce shoot fresh weight by 50% in GR and GS Palmer amaranth in Georgia (Culpepper et al. 2006). For presentation, Palmer amaranth fresh weight reduction is plotted against glyphosate rate, with sigmodial response curves fitted using SigmaPlot[11].

Absorption and translocation data were subjected to ANOVA with sums of squares partitioned appropriately for the factorial arrangement of four treatments. An additional analysis was conducted where all treatments were subjected to ANOVA to make comparisons with the non-factorial treatment. Experimental run was considered a random effect. Significant interaction and main effect means were separated with Fisher's Protected LSD test at $P \leq 0.05$.

Results and Discussion

Glyphosate dose response. A glyphosate rate by experimental run interaction was not observed with either biotype. Log-logistic dose response curves described both visual estimates of control and fresh weight reduction of the GR and GS biotypes (Seefeldt et al. 1995). The I_{50} parameter for percent visual control of the susceptible biotype was 0.089 kg/ha while I_{50} for the resistant biotype was 1.769 kg/ha (data not shown). The I_{50} parameters for fresh weight reduction of the susceptible biotype was 0.097 and 1.963 kg/ha for the resistant biotype (Figure 1). A 19.9-fold and a 20.2-fold increase in glyphosate rate was necessary to achieve 50% visual control and 50% shoot fresh weight reduction, respectively, in the GR biotype compared with the GS biotype.

Levels of resistance have varied in previous reports on GR Palmer amaranth. Culpepper et al. (2006) reported that GR Palmer amaranth in Georgia had a 6- to 8-fold level of resistance. In Arkansas, a GR biotype was reported to have a resistance level between 79- to 115-fold (Norsworthy et al. 2008), and Steckel et al. (2008) reported a 1.5- to 5-fold level of glyphosate resistance in Palmer amaranth from Tennessee. These results suggest that

resistance likely evolved independently across geographical regions and that mechanisms of resistance may vary among populations. Differences in resistance levels among states may also reflect differences in methodology. In Arkansas, resistance levels were based upon the amount of glyphosate needed to cause 50% mortality as opposed to resistance levels in Georgia, North Carolina, and Tennessee being based on visual or shoot fresh weight reduction. Other factors, including the sensitivity of the susceptible biotypes and the methodology regarding GR seed selection also may have played a role in determining the level of resistance. In the Arkansas study, GS Palmer amaranth seed were collected from a South Carolina field with no history of glyphosate use (Norsworthy et al. 2008). Seed of the GS biotype in North Carolina was from a field that had been treated with glyphosate at least once per year for several consecutive years, but glyphosate had consistently controlled the Palmer amaranth completely.

Absorption and Translocation. Absorption and translocation studies with ^{14}C-glyphosate have been conducted both with and without an overspray of non-labeled glyphosate (Feng et al. 2004). **S**ink tissue in plants is more sensitive to glyphosate than source tissue (Fuchs et al. 2002), thus the glyphosate dose to the whole plant could impact the transport or accumulation profile. Because of interest in possible differences regarding absorption or translocation in the resistant and susceptible biotypes, glyphosate overspray could impact results. Absorption and translocation was, therefore, observed over time in GR and GS biotypes in both the presence and absence of a glyphosate overspray.

Approximately 86% of the total applied ^{14}C was recovered from leaf washes and oxidized plant parts. Data were averaged over runs as there were no run by treatment interactions.

The interactions of biotype by time and glyphosate overspray by time were significant (Table 1). Maximum absorption was observed in the GR and GS biotypes at 6 and 12 HAT, respectively, and absorption was similar between biotypes thereafter (25 to 35%). These results are similar to two studies of Palmer amaranth in Georgia where Culpepper et al. (2006) reported 36.4 to 31.2% glyphosate absorption in GR and GS plants, respectively, 48 h after application and from Grey et al. (2008) who reported 44% absorption by both resistant and susceptible plants 24 h after application.

Although absorption in resistant and susceptible plants was similar from 12 to 72 HAT, susceptible plants absorbed 67% more glyphosate than resistant plants 6 HAT (Table 1). The opposite of this response was noted by Grey et al. (2008) as absorption in resistant Palmer amaranth reached 41% within 6 h of application compared to only 28% absorption in susceptible plants. Although a difference in absorption at 6 HAT may not play a role in the resistance mechanism, differences in speed of absorption may have an impact on overall susceptibility of plants to glyphosate. Satchivi et al. (2000) reported higher glyphosate absorption (38%, 24 hours after application) in giant foxtail (*Setaria faberi*), a relatively glyphosate sensitive species, compared to the more tolerant species, velvetleaf (*Abutilon theophrasti*) (23%). Young et al. (2003) demonstrated that increasing absorption of glyphosate into velvetleaf by tank mixing ammonium sulfate resulted in increased glyphosate efficiency.

Overspraying plants also affected ^{14}C-glyphosate absorption. Plants treated with glyphosate at 0.84 kg/ha absorbed 38 and 60% more glyphosate 48 and 72 HAT than plants not oversprayed with glyphosate (Table 1). This difference in absorption may have been

associated with the adjuvant composition in the commercial glyphosate[1] used to overspray plants before ^{14}C-glyphosate application compared to 0.125% non-ionic surfactant included in the ^{14}C spotting solution. Li et al. (2005) reported that glyphosate absorption by common waterhemp (*Amaranthus rudis*) was affected by glyphosate formulation. In our experiment glyphosate used to spray over the plants was a potassium salt with adjuvants in the commercial formulation.

Distribution of ^{14}C of glyphosate in the treated leaf, above the treated leaf, and below the treated leaf was not affected by biotype, glyphosate overspray, or time. In resistant and susceptible plants, approximately 40% of ^{14}C applied remained in the treated leaf, 30% was translocated above the treated leaf, and 22% was translocated below the treated leaf (Table 2). There also was no difference in amount of ^{14}C in the roots at 6 to 48 HAT due to biotype or overspraying (Table 3). Less ^{14}C at 72 HAT was noted in roots of GS plants oversrayed with glyphosate compared to GR plants, but there was no difference between biotypes not oversprayed with glyphosate.

One documented mechanism of resistance is associated with limited translocation of glyphosate to the mersitematic sinks (Feng et al. 2004). Our results suggest that is not the case with this resistant Palmer amaranth biotype, but could be associated with self-limiting glyphosate translocation in plants, as movement of glyphosate plays is important pertaining to phytotoxicity (Geiger et al. 1999). As glyphosate toxicity occurs, reductions in photoassilimate products and translocation occur due to disruption of the shikimate pathway (Geiger et al. 1999). These results also demonstrate that methodology of an experiment can play a significant role in determination of glyphosate distribution of resistant and susceptible

plants. Feng et al. (2004) treated entire resistant and susceptible plants with a non-lethal dose of ^{14}C-glyphosate to examine distribution of glyphosate in resistant horseweed along with an I_{50} dose to each biotype.

In our experiment, to further examine whole plant responses to glyphosate without the lethal effects of glyphosate affecting distribution, one additional treatment was included in this study. Susceptible plants were oversprayed with glyphosate at 0.09 kg/ha, to compare glyphosate movement in susceptible plants oversprayed with a non-lethal dose compared to resistant plants oversprayed with a non-lethal dose (0.84 kg/ha). These two treatments had differences which did not appear in other treatments. These GS plants absorbed 50% less ^{14}C than GR plants (Table 4). Translocation of glyphosate was also affected by biotype in these treatments. Over 75% more ^{14}C remained in the treated leaf of GS plant compared to GR plants, and susceptible plants translocated less than half the percentage of ^{14}C above the treated leaf than resistant plants.

These differences in translocation from the sub-lethal overspray treatments do not seem consistent with known resistance mechanisms, where moving twice as much glyphosate above the treated leaf including the meristem, would not conceptually result in reduced phytotoxicity. The mechanism for resistance may not be evident in these results, but they do point out potential differences in the translocation patterns of resistant and susceptible biotypes.

Shikimate accumulation in plants is a chemical diagnostic tool for glyphosate activity (Hollander and Amrhein 1980; Singh and Shaner 1998). Preliminary research demonstrated that accumulation of shikimate differed between the North Carolina GR and GS biotypes

(data not shown). Shikimate levels in both leaves and meristematic tissue in GS plants increased as the rate of glyphosate increased. Low amounts of shikimate were detected in GR plants, but increasing rates of glyphosate did not cause an increase in shikimate compared to non-treated plants. Accumulation of shikimate in susceptible plants in this study, and lack of accumulation of shikimate in resistant plants is similar to results observed in GR and GS Palmer amaranth from Georgia (Culpepper et al. 2006). Different from North Carolina and Georgia biotypes, Steckel et al. (2008) reported that shikimate accumulated in both GR and GS Tennessee populations. This may indicate that Tennessee populations have a different mechanism of resistance, similar to that observed in GR horseweed (Mueller et al. 2003). Also, the mechanism of resistance in GR Palmer amaranth from North Carolina could be similar to the Georgia GR biotype (Culpepper et al. 2006). It has been proposed that the mechanism of glyphosate resistance in the Georgia GR Palmer amaranth is due to gene amplification and a resulting over expression of EPSPS (Gaines et al. 2009). Preliminary work has suggested that this phenomenon also occurs in North Carolina GR Palmer amaranth (Todd Gaines, personal communication).

Our results suggest that the GR Palmer amaranth biotype in North Carolina has a resistance level somewhat different from other reports. This biotype did not accumulate shikimate after glyphosate application, similar to reports from Georgia, but different from Tennessee (Culpepper et al. 2006; Steckel et al. 2008). In this study, GS plants absorbed more glyphosate quicker than GR plants, but overall absorption was similar. This research also demonstrated that overspraying plants with glyphosate can affect distribution of ^{14}C-glyphosate, especially in GS plants.

Source of Materials

[1] Roundup WEATHERMAX® herbicide, 660 g ae glyphosate per liter, Monsanto Company, St. Louis, MO 63167.

[2] TeeJet TP8003E even-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

[3] Peters Professional® Water Soluble 20-20-20 Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH.

[4] Metro Mix 200®, Scotts-Sierra Horticultural Products Company, Marysville, OH.

[5] ^{14}C-Glyphosate, Sigma Chemical Co., 3050 Spruce Street, St. Louis, MO 63103.

[6] Induce®, blend of alkylarylpolyoxylkane ether, free fatty acids, and isopropyl (90%), and water and formulation acids (10%). Helena Chemical Corporation, 225 Schilling Blvd., Suite 300, Collierville, TN 38017.

[7] ScintiVerse® BD cocktail, Scintanalyzed, Fisher Scientific, 1 Reagent Lane, Fairlawn NJ 07410.

[8] Packard TRI-CARB 2100TR Liquid Scintillation Spectrometer, Packard Instrument Company, 2200 Warrenville Road, Downers Grove, IL 60515.

[9] Model OX-500 Biological Material Oxidizer, R.J. Harvey Instrument Corp., 123 Patterson Street, Hillsdale, NJ 07642.

[10] Statistical Analysis Systems®, version 9.1, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

[11] SigmaPlot®, version 11.0, Systat Software, Inc. 1735 Technology Dr., Suite 430, San Jose, CA 95110.

Acknowledgement

The authors would like to thank Rick Seagroves, Jamie Hinton, and Jared Wilcut for technical support. Partial funding for this research was provided by the North Carolina Cotton Growers Association and Cotton Incorporated.

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Table 1. Absorption of ^{14}C affected by biotype and glyphosate overspray.[a]

	Biotype[b]		Glyphosate overspray[c]	
Hours after treatment	Resistant	Susceptible	Not oversprayed	Oversprayed
	%			
6	18 c	30 ab	22 d	25 cd
12	35 a	30 ab	36 ab	29 bcd
24	32 ab	25 bc	26 cd	31 abc
48	32 ab	31 ab	26 cd	36 ab
72	32 ab	33 ab	25 cd	40 a

[a] Absorption expressed as percentage of total ^{14}C recovered. Means within the main effect of biotype or glyphosate overspray followed by the same letter are not different according to Fisher's Protected LSD at $P \leq 0.05$.

[b] Data pooled over both glyphosate overspray options.

[c] Data pooled over glyphosate-resistant and –susceptible biotypes. Glyphosate applied at 0.84 kg/ha.

Table 2. Distribution of ^{14}C in glyphosate-resistant and –susceptible Palmer amaranth.[a]

	Distribution[b]		
Biotype	Treated Leaf	Above treated leaf	Below treated leaf
	%		
Resistant	40 a	31 a	22 a
Susceptible	43 a	30 a	22 a

[a] Data pooled over time and glyphosate overspray options. Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $P \leq 0.05$.

[b] Distribution expressed as percentage of absorbed ^{14}C.

Table 3. Percentage of ^{14}C in the roots of Palmer amaranth affected by biotype and glyphosate overspray over time.[a]

Hours after treatment	Roots			
	Resistant		Susceptible	
	Not oversprayed	Oversprayed[b]	Not oversprayed	Oversprayed
	%			
6	5 a	4 a	3 a	3 a
12	8 a	3 a	4 a	3 a
24	10 a	5 a	9 a	6 a
48	8 a	7 ab	7 ab	3 b
72	11 a	9 a	12 a	1 b

[a] ^{14}C in roots expressed as percentage of absorbed ^{14}C. Means in the same time period followed by the same letter are not different according to Fisher's Protected LSD at $P \leq 0.05$.

[b] Oversprayed plants received a topical application of glyphosate at 0.84 kg/ha immediately prior to ^{14}C-glyphosate treatment.

Table 4. Absorption and distribution of ^{14}C in Palmer amaranth in susceptible and resistant plants oversprayed with a sublethal dose of glyphosate.[a]

Treatment			Distribution[b]			
				Above	Below	
Biotype	Glyphosate overspray	Absorption[b]	Treated leaf	treated leaf	treated leaf	Roots
		%	%			
Susceptible	Oversprayed (0.09 kg/ha)	23 b	63 a	17 c	14 c	6 d
Resistant	Oversprayed (0.84 kg/ha)	33 a	36 b	38 b	21 c	5 d

[a] Data pooled over time.

[b] Means regarding absorption followed by the same letter are not different according to Fisher's Protected LSD at $P \leq 0.05$.

[c] Distribution expressed as percentage of absorbed ^{14}C. All distribution means Means followed by the same letter are not different according to Fisher's Protected LSD at $P \leq 0.05$.

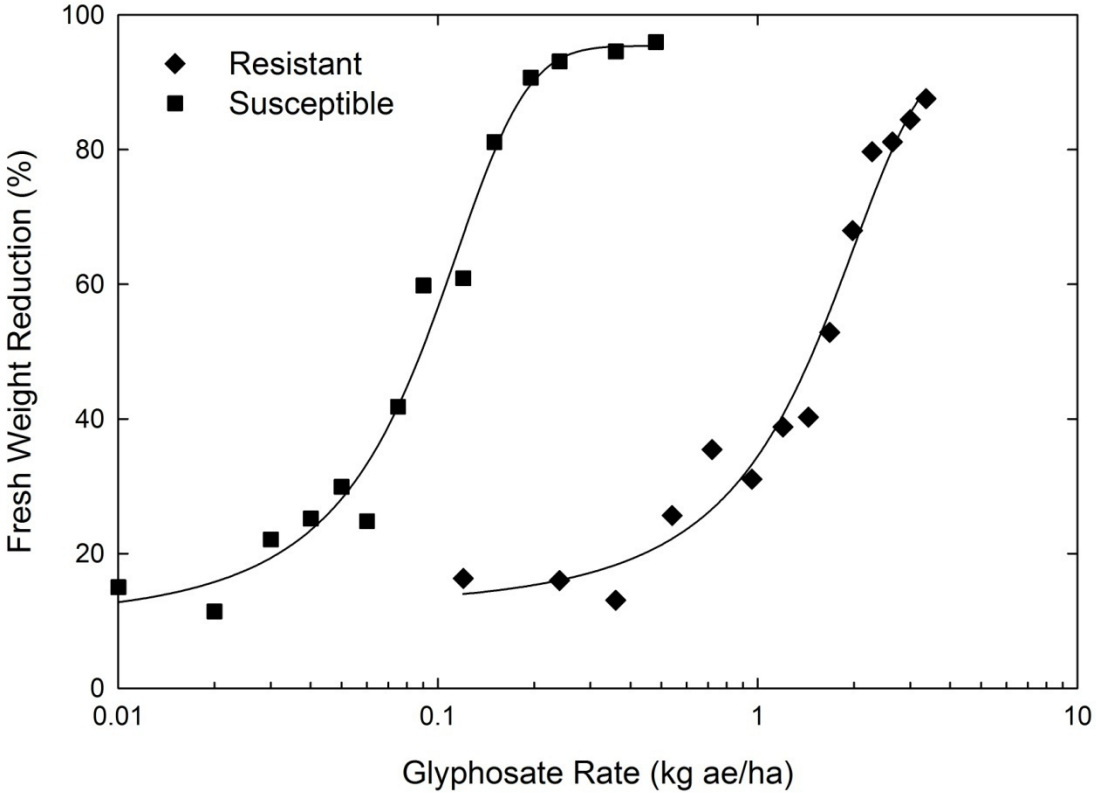

Figure 1. Fresh weight reduction of glyphosate-resistant and –susceptible Palmer amaranth 14 d after glyphosate application. Log-logistic dose-response curves:

Susceptible: (I_{50} = 0.098 kg/ha)

$$y = 16.11 + \frac{99.45 - 16.11}{1 + \left({}^{x}/_{97.9}\right)^{-28.9}}$$

Resistant: (I_{50} = 1.96 kg/ha)

$$y = 17.44 + \frac{111.81 - 17.44}{1 + \left({}^{x}/_{1963}\right)^{-39.4}}$$

CHAPTER IV

TITLE: **Evaluation of Residual Herbicides for Control of Glyphosate-resistant Palmer Amaranth**

DISCIPLINE: Weed Science

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ABBREVIATIONS: PRE, preemergence; POST, postemergence; POST-DIR, postemergence directed.

KEY WORDS: diuron; flumioxazin; fluometuron; linuron; metolachor; pendimethalin; prometryn; pyrithiobac; resistance management; *S*-metolachlor; trifloxysulfuron.

ABSTRACT

Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a major problem in the southeastern U.S. Extension personnel are actively promoting resistance management strategies, including integration of herbicides with other modes of action into glyphosate-based programs, to reduce selection pressure on glyphosate. This field experiment, conducted in five environments in North Carolina and Georgia during 2006 and 2007, evaluated residual control of Palmer amaranth by 13 herbicides registered for use in cotton (*Gossypium hirsutum* L.). Treatments consisted of a factorial arrangement of the 13 residual herbicides applied at 1X rates (manufacturer's suggested use rates) and 1.5X rates. Herbicides were applied preemergence (PRE), regardless of intended use, to evaluate residual effectiveness. Herbicides applied at 1.5X rates were an average of 9% more effective 20 d after application compared with 1X rates. Of the herbicides typically applied PRE or preplant, fomesafen, flumioxazin, and pyrithiobac were most effective. Fluometuron and diuron were intermediately effective, and pendimethalin and prometryn were least effective. Pyrithiobac and *S*-metolachlor were the most effective herbicides which would be applied postemergence (POST) to cotton. Pyrithiobac was more effective than trifloxysulfuron, and *S*-metolachlor was more effective than metolachlor. Flumioxazin and

prometryn plus trifloxysulfuron were the most effective options for postemergence-directed (POST-DIR) application to cotton. These POST-DIR herbicides were more effective than diuron, linuron, linuron plus diuron, or prometryn. Integration of effective residual herbicides into glyphosate-based management systems will help sustain cotton production in areas infested with Palmer amaranth.

INTRODUCTION

Palmer amaranth is one of the most troublesome weeds for cotton producers in the southeastern U.S. (Webster, 2005). It grows very rapidly and can reach 2 m or more in height (Horak and Loughin, 2000). It has an extremely high photosynthetic capacity and utilizes the C_4 photosynthetic pathway (Ehleringer, 1983). Along with rapid growth, Palmer amaranth has effective drought tolerance mechanisms that allow it to survive and grow during dry conditions, and it readily adapts to shading (Ehleringer, 1983; Jha et al., 2008). These characteristics allow Palmer amaranth to establish a competitive dominance for light and space with crops (Monks and Oliver, 1988). A single Palmer amaranth per 9.1 m of row in cotton or 3 m of row in soybean [*Glycine max* (L.) Merr.] can reduce yield 13 and 17%, respectively (Morgan et al., 2001; Klingaman and Oliver, 1994). Losses as high as 78% in soybean with Palmer amaranth densities of eight plants per m of row and 54% in cotton with densities of 10 plants per 9.1 m of row have been documented (Bensch et al., 2003; Morgan et al. 2001). Palmer amaranth may also interfere with mechanical harvesting efficiency of cotton (Smith et al., 2000).

Once established in fields, Palmer amaranth can be difficult to control due to its rapid growth, competitive ability, and prolific seed production (Bensch et al., 2003; Horak and Loughin, 2000; Keely et al., 1987). Continued emergence throughout the season, coupled with prolific seed production, allows Palmer amaranth to quickly replenish seed banks if control is not season-long (Keely et al., 1987; Sellers et al., 2003). Glyphosate typically is very efficacious on Palmer amaranth (Bond et al., 2006; Corbett et al., 2004; Norsworthy and Grey, 2004), but multiple applications are often needed for season-long control (Culpepper and York, 1998, 2000; Everitt et al., 2003; Grichar et al. 2004; Scott et al., 2002).

Cotton was planted on over 1.5 million combined acres in North Carolina, South Carolina, and Georgia in 2008 (USDA-NASS, 2009), greater than 99% of which were planted with glyphosate-resistant cultivars (USDA-AMS, 2008) and this cotton routinely receives multiple applications of glyphosate. Management programs consisting of only glyphosate have effectively controlled Palmer amaranth and other weeds in cotton (Culpepper and York, 1999; Culpepper et al., 2000; Scott et al., 2002). However, extensive reliance on glyphosate has lead to selection for glyphosate-resistant biotypes of weeds (Heap, 2009). Glyphosate-resistant Palmer amaranth was first suspected in Georgia in 2004 and confirmed in 2005 (Culpepper et al., 2006), and it was first noted in North Carolina in 2005 (York et al., 2007). Currently, an estimated 120,000 ha in Georgia and 75,000 ha in North Carolina are thought to be infested with the resistant biotype (Culpepper et al., 2008). Glyphosate-resistant Palmer amaranth also occurs in Arkansas, South Carolina, and Tennessee (Heap, 2009; Norsworthy et al., 2008; Steckel et al., 2008; York et al. 2007)

Failure to adopt a strategy that effectively controls glyphosate-resistant Palmer amaranth can result in total crop failure (Whitaker, 2009). A key component of an effective management strategy will be integration of herbicides with different modes of action and residual activity. Herbicides applied PRE reduce early season weed interference and often improves season-long control of Palmer amaranth (Culpepper and York, 1998; Keeling and Abernathy, 1989; Reddy, 2001; Toler et al., 2002; Whitaker et al., 2008). Herbicides such as diuron, fluometuron, fomesafen, pendimethalin, prometryn, and pyrithiobac can be applied PRE to cotton for residual control of Palmer amaranth and other weeds; flumioxazin can be used as an early preplant surface-applied treatment (York and Culpepper, 2009). Pyrithiobac and trifloxysulfuron applied POST control small Palmer amaranth (Corbett et al., 2004; Dotray et al., 1996; Porterfield et al., 2003) although the manufacturer of trifloxysulfuron does not claim control and the manufacturer of pyrithiobac claims only suppression of this weed (Anonymous, 2009a; 2009b). Both trifloxysulfuron and pyrithiobac can be applied with glyphosate to provide residual control of Palmer amaranth (Branson et al., 2002; Burke and Wilcut, 2004). Metolachlor and *S*-metolachlor may be mixed with glyphosate and applied POST to cotton (York and Culpepper, 2009). These herbicides do not have POST activity on Palmer amaranth, but the residual activity of metolachlor and *S*-metolachlor have been documented to increase effectiveness of glyphosate applied POST to Palmer amaranth in cotton (Clewis et al., 2006; Grichar and Minton, 2007).

Several herbicides can be applied to cotton as postemergence-directed (POST-DIR) sprays when a height difference exists between cotton and weeds (Wilcut et al., 1997; York and

Culpepper, 2009). These herbicides not only control small emerged weeds but also provide residual control (Askew et al., 2002; Price et al., 2008; Porterfield et al. 2003). Residual herbicides are being actively promoted to improve management of glyphosate-resistant Palmer amaranth and to delay further evolution of resistance (York and Culpepper, 2009; Steckel, 2009; Stephenson et al., 2008). This research was conducted to evaluate residual control of Palmer amaranth by various herbicides available to cotton producers. Information of this nature will be essential in developing sustainable management systems for glyphosate-resistant cotton in the southeastern U.S.

MATERIALS AND METHODS

The experiment was conducted at sites near Oglethorpe, Georgia and Mount Olive, North Carolina during both 2006 and 2007 and near Parkton, NC during 2006. Each site was selected based on dense infestations of Palmer amaranth in which portions of the populations were glyphosate-resistant. Soil information, application dates, and Palmer amaranth densities are provided in Table 1. These soils are typical of those where glyphosate-resistant Palmer amaranth has been most problematic in North Carolina and Georgia.

The experimental design was a randomized complete block with treatments replicated three or four times depending upon location. Plots consisted of four 96-cm rows 9 m long. Cotton was planted in a conventional tillage system in early May. Cultivars were PHY 485 WRF (PhytoGen Cottonseed, Dow AgroSciences LLC; Indianapolis, IN) and DP 555 BG/RR (Delta Pine and Land Co.; Scott, MS) at Oglethorpe during 2006 and 2007, respectively, and

DG 2100 B2RF (Dyna-Gro seed; United Agri Products, Inc.; Greely, CO) at Mt. Olive and Parkton during 2006 and ST 4357 B2RF (Stoneville Cotton, Bayer CropScience.; Research Triangle Park, NC) at Mount Olive during 2007.

Treatments consisted of a factorial arrangement of 13 herbicides applied at a normal application rate for each soil series (1X rate) and at a 1.5X rate. Residual herbicides and their respective 1X rates included diuron (Direx 4L; Dupont Crop Protection Co., Inc.; Wilmington, DE) at 1120 g a.i. ha^{-1}; flumioxazin (Valor SX; Valent U.S.A. Corp.; Walnut Creek, CA) at 54 g a.i. ha^{-1}; fluometuron (Cotoran 4L; Griffin LLC; Valdosta, GA) at 1120 g a.i. ha^{-1}; fomesafen (Reflex; Syngenta Crop Protection, Inc.; Greensboro, NC) at 280 g a.i. ha^{-1}; linuron (Linex 4L; Dupont Crop Protection Co., Inc.) at 1120 g a.i. ha^{-1}; linuron plus diuron (Layby Pro; Dupont Crop Protection Co., Inc.) at 560 + 560 g a.i. ha^{-1}; metolachlor (Stalwart ; Sipcam Agro USA, Inc.; Roswell, GA) at 1120 g a.i. ha^{-1}; pendamethalin (Prowl H_2O; BASF Ag Products; Research Triangle Park, NC) at 1064 g a.i. ha^{-1}; prometryn (Caparol 4L; Syngenta Crop Protection, Inc.) at 1120 g a.i. ha^{-1}; prometryn plus trifloxysulfuron (Suprend; Syngenta Crop Protection, Inc.) at 888 + 8 g a.i. ha^{-1}; pyrithiobac (Staple LX; Dupont Crop Protection Co., Inc.) at 48 g a.i. ha^{-1}; *S*-metolachlor (Dual Magnum; Syngenta Crop Protection, Inc.) at 1067 g a.i. ha^{-1}; and trifloxysulfuron (Envoke; Syngenta Crop Protection, Inc.) at 5.3 g ha^{-1}. A non-treated control was also included.

Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (TeeJet XR 11002 nozzles; Spraying Systems Co.; Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 160 kPa. Some of the herbicides evaluated are not intended for PRE application

on cotton, but the objective of this study was to determine residual control obtained from each herbicide. Therefore, all herbicides were applied PRE regardless of intended application timing.

Weed control was visually estimated 20, 40, and 60 d after application using a scale of 0 to 100, where 0 = no weed control and 100 = complete weed control (Frans et al., 1986). In Oglethorpe during 2007, no rainfall occurred until 18 d after application, and the initial flush of Palmer amaranth was not controlled by any herbicide. Immediately after the initial rainfall, glyphosate was applied at 1.2 kg ae/ha over the entire study and visual ratings were recorded at 20-day intervals thereafter. The initial flush of Palmer amaranth, fewer than 3 plants per m^2, were not considered in visual ratings. Data were subjected to analyses of variance with partitioning appropriate for the factorial treatment arrangement. Because of heterogeneity of variance, weed control data were arcsine square root transformed before analysis (Ahrens et al., 1990). Analyses were preformed with the PROC MIXED procedure of SAS (version 9.1; SAS Institute Inc.; Cary, NC). Each year and location was considered an environment (McIntosh, 1983), and environments and replications were treated as random experimental effects. Data were averaged over environments, herbicides, and herbicide rates as appropriate, and means of significant main effects and interactions were separated with Fisher's Protected LSD at $P \leq 0.05$. Non-transformed means are reported with interpretation based on transformed data.

RESULTS AND DISCUSSION

Data from each environment were analyzed separately due to treatment by environment interactions. The herbicide by rate interaction was not significant except for the 60-d evaluation at Oglethorpe in 2006 and Mount Olive in 2007. The main effect of application rates was significant at most evaluation periods and environments, and the main effect of herbicides was significant at all evaluations and environments.

Herbicide rates affected Palmer amaranth control similarly across all environments 20 d after application. Control, averaged over herbicides, varied among environments from 53 to 89% with the 1X rate and 65 to 93% with the 1.5X rate (Table 2). Averaged over environments, herbicides applied at 1.5X rates were 9% more effective than when applied at 1X rates. Except for Oglethorpe in 2007, control decreased 10 to 59 percentage points by 40 d, and control continued to decrease between 40 and 60 d at each environment. Greater control was noted with the 1.5-X rate at 40 d (5 to 15 percentage points) and 60 d (10 to 13 percentage points) after application at Oglethorpe in both years and Parkton. Herbicide rate did not affect control at 40 or 60 d in either year at Mount Olive. This was likely due to poor control regardless of the herbicides or rates applied. Control at Mount Olive was 10% or less by 40 d in 2006 and 35% or less in 2007. Control at Mount Olive in 2007 further declined to 10% or less by 60 d.

Irrigation was not available at any site, and rainfall patterns were likely a major contributor to variation in control among environments. Greatest control at 40 and 60 d was achieved at Oglethorpe in 2006 (Table 2). At this location, nearly 13 cm of rainfall occurred

within the first 10 d after herbicide application to adequately activate the herbicides (Table 3). No rainfall occurred during the subsequent 20 d, and only 1.2 cm of rainfall was received during the period of 11 to 50 d after herbicide application. Dry soil conditions following the first 2 wk of the evaluation period greatly reduced weed seed germination. Overall Palmer amaranth control was least at Mount Olive in 2006 (Table 2), where adequate rainfall was received throughout the evaluation period. These rainfall events lead to continued Palmer amaranth germination during the evaluation period.

In this study, all herbicides were applied PRE in order to better observe residual activity; however, not all of the herbicides are intended to be applied in this manner to cotton. Herbicides in this study which are typically applied PRE include diuron, fluometuron, fomesafen, pendimethalin, prometyrn, and pyrithiobac (York and Culpepper, 2009). Flumioxazin is applied 14 to 30 d ahead of planting, depending upon rate and tillage system (Anonymous, 2009c).

Although some differences occurred among environments, flumioxazin, fomesafen, and pyrithiobac were generally the most effective of the herbicides typically applied preplant or PRE. Flumioxazin, fomesafen, and pyrithiobac were similarly effective 20 d after treatment at Oglethorpe in 2006, controlling Palmer amaranth 97 to 100% (Table 4). Control by pyrithiobac declined to 87 and 69 to 78% by 40 and 60 d, respectively, but flumioxazin and fomesafen still controlled Palmer amaranth 99% at 40 d and 95 to 98% at 60 d (Tables 5 and 6). Pyrithiobac was the most effective herbicide 20 d after treatment in both years at Mount Olive, controlling Palmer amaranth 93 to 97% (Table 4). Flumioxazin and fomesafen were

the next most effective herbicides, controlling Palmer amaranth 74 to 83% in 2006 and 89 to 93% in 2007 at 20 d. Control by all herbicides declined rapidly after 20 d at Mount Olive in both years. Control by flumioxazin, fomesafen, and pyrithiobac at Mount Olive declined to 18 to 27% at 40 d in 2006 and 42 to 69% at 40 d in 2007 (Table 5). Flumioxazin and fomesafen controlled Palmer amaranth 3% or less at 60 d in both years at Mount Olive (Table 6). Pyrithiobac controlled Palmer amaranth only 1% at 60 d at Mount Olive in 2006. At this location in 2007, pyrithiobac at 1 and 1.5X rates controlled the weed 14 and 53%, respectively, at 60 days. Flumioxazin was most effective at Parkton, where it controlled Palmer amaranth 96, 90, and 57% 20, 40, and 60 d after treatment, respectively. Fomesafen and pyrithiobac were similarly effective at Parkton, controlling Palmer amaranth 87 to 88% at 20 d, 77% at 40 d, and 27 to 28% at 60 d. Flumioxazin, fomesafen, and pyrithiobac were similarly effective (75 to 82% control) 20 d after treatment at Oglethorpe in 2007. By 40 d, flumioxazin controlled the weed 85% compared with 73% by fomesafen and pyrithiobac. At 60 d, flumioxazin, fomesafen, and pyrithiobac were also similarly effective (68 to 81% control).

Diuron and fluometuron were generally intermediately effective among the preplant and PRE herbicides while pendimethalin and prometryn tended to be least effective. At Parkton in 2006 and Oglethorpe in 2007, control by diuron and fluometuron was similar at 20 and 40 d (Tables 4 and 5). At each of these locations, control by diuron and fluometuron usually exceeded control by pendimethalin and prometryn at 20 and 40 d. By 60 d at Oglethorpe in 2007, control by diuron, fluometuron, and pendimethalin was similar and greater than control

by prometryn (Table 6). At Parkton, control by all of these herbicides declined to 24% or less by 60 d. At Oglethorpe in 2006, Palmer amaranth control at 20 and 40 d was similar with fluometuron, pendimethalin, and prometryn but less than control by diuron (Tables 4 and 5). A herbicide by herbicide rate interaction was noted at 60 d at Oglethorpe in 2006 (Table 6). Diuron was more effective than fluometuron, pendimethalin, or prometryn at the 1X rates, but diuron and pendimethalin were similarly effective when applied at 1.5X rates and more effective than fluometuron. At Mount Olive in 2006, fluometuron, pendimethalin, and prometryn were similarly effective at 20 d but less effective than diuron (Table 4). Control by all of these herbicides declined to 4% or less by 40 d (Table 5). Control by diuron, fluometuron, pendimethalin, and prometryn was generally similar at 20 and 40 d at Mount Olive in 2007. Control by these herbicides declined to 3% or less by 60 d (Table 6).

Metolachlor, *S*-metolachlor, pyrithiobac, and trifloxysulfuron can be applied POST to cotton (York and Culpepper, 2009). Pyrithiobac and trifloxysulfuron exhibit both PRE and POST activity on weeds when applied POST. Although metolachlor and *S*-metolachlor have little to no POST activity on emerged weeds, the residual activity from these herbicides applied POST can be beneficial in management programs for Palmer amaranth (Clewis et al. 2006).

Greater control of Palmer amaranth at all evaluation dates was observed with *S*-metolachlor than with metolachlor at Parkton and at Oglethorpe in both years (Tables 4, 5, and 6). Greater control by *S*-metolachlor also was noted in both years at Mount Olive at 20 d. At both Mount Olive locations, however, control declined greatly by 40 d, and no

differences were noted between metolachlor and *S*-metolachlor. *S*-metolachlor controlled Palmer amaranth 90 to 96% at 20 d and 76 to 86% at 40 d at Parkton and Oglethorpe in 2006. Control at Parkton declined to 24% by 60 d and at Oglethorpe to 58% with the 1X rate. Control by the 1.5X rate remained at 90% at 60 d at Oglethorpe. Control was less at Oglethorpe in 2007, but the control remained relatively constant over time. At this location, *S*-metolachlor controlled Palmer amaranth 57, 60, and 46% at 20, 40, and 60 d, respectively. Metolachlor has four stereoisomers. Previous research has shown that on a gram for gram basis, products containing metolachlor (equal mixture of *R* and *S* isomer pairs) are about 65% as effective on weeds as products containing predominately *S*-metolachlor (O'Connell et al., 1998). However, when application rates are adjusted to account for this difference in activity, metolachlor and *S*-metolachlor are equally effective (Shaner et al., 2006). In our study, control by metolachlor at the 1.5X rate was very similar to control by *S*-metolachlor at the 1X rate (data not shown).

Pyrithiobac and trifloxysulfuron were similarly effective at Parkton. These two herbicides controlled Palmer amaranth 86 to 88%, 73 to 77%, and 23 to 26% at 20, 40, and 60 d, respectively (Tables 4, 5, and 6). At the other four environments, however, pyrithiobac was more effective than trifloxysulfuron. At Oglethorpe in 2006 and at Mount Olive in 2007, pyrithiobac controlled Palmer amaranth 97% at 20 d and 69 to 87% at 40 d compared with 79 to 88% control by trifloxysulfuron at 20 d and 37 to 64% at 40 d. A herbicide by herbicide rate interaction was noted at both locations at 60 d, but regardless of rate, pyrithiobac was more effective than trifloxysulfuron. Pyrithiobac at 1.5X controlled Palmer amaranth 53 to

78% compared with 10 to 41% control by trifloxysulfuron at 60 d. At Mount Olive in 2006, pyrithiobac controlled Palmer amaranth 93% at 20 d compared with 34% control by trifloxysulfuron. Control had declined greatly by 40 d, but pyrithiobac was still the more effective herbicide. Pyrithiobac was less effective at 20 d at Oglethorpe in 2007 compared with the other locations. However, control by pyrithiobac remained relatively constant over time at this location. Regardless of the evaluation date, pyrithiobac was more effective than trifloxysulfuron at this location. The 1X rate of pyrithiobac chosen for this study was primarily the manufacturer's recommended rate for PRE applications. Pyrithiobac can be applied POST at rates approximately twice the 1X rate in this study (Anonymous, 2009b), and one would anticipate a greater difference in control between pyrithiobac and trifloxysulfuron is pyrithiobac had been applied at normal POST application rates.

Palmer amaranth was more effectively controlled by pyrithiobac than *S*-metolachlor at 20 and 40 d at Oglethorpe in 2007 and Mount Olive in both years (Tables 4 and 5). The same observation was made at 60 d at Oglethorpe in 2007 and with the 1.5X rates at Mount Olive in 2007 (Table 6). Neither herbicide controlled Palmer amaranth at 60 d at Mount Olive in 2006. Pyrithiobac and *S*-metolachlor were similarly effective at 20 and 40 d at Oglethorpe in 2006 and at Parkton. At 60 d, both herbicides were similarly effective at Parkton and with 1X rates at Oglethorpe in 2006, but *S*-metolachlor at the 1.5X rate was more effective than pyrithiobac at 60 d at Oglethorpe in 2006. Pyrithiobac was more effective than metolachlor at all environments and evaluation dates except the 60-d evaluation at Mount Olive in 2006 where no control was noted with either herbicide.

Every herbicide evaluated in this study can be applied as a POST-DIR spray in cotton. However, only diuron, flumioxazin, linuron, linuron plus diuron, prometryn, and prometryn plus trifloxysulfuron are typically applied in this manner in North Carolina and Georgia. As POST-DIR sprays to cotton, these herbicides are usually mixed with either MSMA or glyphosate (York and Culpepper, 2009). These combinations control emerged weeds, and the diuron, flumioxazin, linuron, linuron plus diuron, prometryn, and prometryn plus trifloxysulfuron in the mixtures can provide additional residual control. In this study, all herbicides were applied PRE to the weeds, so only the residual effects of these POST-DIR herbicides were evaluated.

Flumioxazin was among the most effective herbicides at each evaluation at each environment although prometryn plus trifloxysulfuron and flumioxazin were similarly effective at four of the five environments at 20 d and three of the five environments at 40 and 60 d (Tables 4, 5, and 6). Diuron and diuron plus linuron were similarly effective in most cases, but linuron was often less effective than diuron. In many cases, prometryn was the least effective of these POST-DIR herbicides. Prometryn was always less effective than prometryn plus trifloxysulfuron or flumioxazin.

The purpose of this experiment was to determine the most effective residual herbicides that could be integrated into a glyphosate-based system for control of glyphosate-resistant Palmer amaranth. Previous research has clearly demonstrated that good residual control, beginning with preplant or PRE herbicides, is critical to manage glyphosate-resistant Palmer amaranth (Culpepper et al., 2008; Whitaker et al., 2008). The most effective PRE herbicides

were found to be fomesafen and pyrithiobac. Flumioxazin, which could be applied 2 to 4 wk ahead of planting, was also effective. In a normal production system, flumioxazin applied early preplant might be less effective, relative to fomesafen, than was observed in this study due to herbicide dissipation during the interval between application and cotton planting. However, fomesafen or other herbicides applied PRE must receive timely rainfall for activation; lack of timely activation leads to poor control (Culpepper et al., 2007; Whitaker, 2009). Early preplant application of a herbicide such as flumioxazin would increase the chances of receiving rainfall for activation prior to cotton planting or weed emergence. Among the residual herbicides applied POST in cotton, pyrithiobac was more effective than *S*-metolachlor or trifloxysulfuron. However, wide-spread resistance to ALS-inhibiting herbicides limits the areas where pyrithiobac would be effective. Among the POST-DIR herbicides, flumioxazin and prometryn plus trifloxysulfuron were most effective. Glyphosate-resistant Palmer amaranth can be effectively controlled in glyphosate-based management systems by integration of these residual herbicides (Whitaker, 2009; Whitaker et al., 2008)

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Table 1. Description of soils, planting dates, and Palmer amaranth density at experiment sites.

Year	Site	Application date	Soil series	Soil pH (units)	Soil humic or organic matter (%)	Palmer amaranth density (no. m^{-2})
2006	Mt. Olive, NC	5 May	Wagram[w]	6.3	0.51[y]	300
2006	Oglethorpe, GA	1 May	Dothan[x]	6.3	2.00[z]	195
2006	Parkton, NC	24 May	Wagram	6.0	0.56[y]	180
2007	Mt. Olive, NC	14 May	Wagram	5.5	0.60[y]	150
2007	Oglethorpe, GA	1 May	Dothan	6.3	2.00[z]	70

[w] Wagram is a loamy, kaolinitic, thermic Arenic Kandiudults.

[x] Dothan is a fine-loamy, siliceous, thermic Plinthic Paleudults.

[y] Soil humic matter was determined as described by Mehlich (1984).

[z] Soil organic matter was determined according to a modification of the method of Walkley and Black (1934).

Table 2. Palmer amaranth control as affected by herbicide rate 20, 40, and 60 d after herbicide application.

Residual herbicide rate	Palmer amaranth control (%)[y]					
	2006			2007		Pooled
	Oglethorpe	Mount Olive	Parkton	Oglethorpe	Mount Olive	across environments[z]
	20 d after application					
1 X	89	53	77	54	82	71
1.5 X	93*	67*	87*	65*	86*	80*
	40 d after application					
1 X	69	10	62	59	30	--
1.5 X	80*	8	77*	64*	35	--
	60 d after application					
1 X	52	0	17	39	8	--
1.5 X	64*	0	30*	49*	10	--

[y] Data averaged over 13 herbicides. Means for the 1.5X rate within an evaluation period followed an asterisk are different from the means of the 1X rate at $P \leq 0.05$.

[z] Data pooled across environments due to lack of rate by environment interaction.

Table 3. Rainfall at experiment sites.

Interval after application (d)	Rainfall (cm)[z]				
	Mount Olive 2006	Oglethorpe 2006	Parkton 2006	Mount Olive 2007	Oglethorpe 2007
0 to 5	2.24	0.0	0.15	0.97	0.0
6 to 10	1.22	12.7	0.03	0.00	0.0
11 to 15	0.00	0.0	3.81	0.00	0.0
16 to 20	1.70	0.0	4.26	4.22	1.2
21 to 25	0.79	0.0	7.49	0.00	0.0
26 to 30	2.54	0.0	5.72	0.03	0.0
31 to 40	6.76	0.8	3.53	1.55	6.3
41 to 50	6.02	0.4	3.40	1.47	3.5
51 to 60	3.74	2.8	1.37	3.02	0.2

[z] Rainfall data corresponds to the amount which occurred within each five day interval.

Table 4. Palmer amaranth control 20 d after herbicide application.

	Control (%)[y]				
	2006			2007	
Residual herbicides[z]	Oglethorpe	Mt. Olive	Parkton	Oglethorpe	Mt. Olive
Diuron	91 de	71 c	81 cde	55 de	86 cde
Flumioxazin	100 a	83 b	96 a	82 a	93 b
Fluometuron	86 fg	49 ef	83 cde	61 bcd	79 ef
Fomesafen	99 ab	74 bc	87 bcd	78 a	89 bc
Linuron	92 cd	58 de	73 fg	81 a	87 cd
Linuron plus diuron	91cd	68 cd	81 de	71 abc	85 cde
Metolachlor	86 ef	32 g	79 ef	36 fg	68 g
Pendimethalin	82 fg	49 ef	61 h	44 ef	73 fg
Prometryn	79 g	39 fg	70 g	30 g	79 ef
Prometryn plus trifloxysulfuron	97 ab	75 bc	95 a	48 def	93 b
Pyrithiobac	97 ab	93 a	88 bc	75 ab	97 a
S-metolachlor	96 bc	57 de	90 b	57 de	81 de
Trifloxysulfuron	88 def	34 g	86 cde	57 cde	79 ef

[y] Data averaged over 1 and 1.5 times normal use rates for each residual herbicide. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[z] Residual herbicides and their respective 1X rates are diuron (1120 g ha^{-1}); flumioxazin (54 g ha^{-1}); fluometuron (1120 g ha^{-1}); fomesafen (280 g ha^{-1}); linuron (1120 g ha^{-1}); linuron + diuron (560 + 560 g ha^{-1}); metolachlor (1120 g ha^{-1}); pendimethalin (1064 g ha^{-1}); prometryn (1120 g ha^{-1}); prometryn + trifloxysulfuron (888 + 8 g ha^{-1}); pyrithiobac (48 g ha^{-1}); *S*-metolachlor (1067 g ha^{-1}); and trifloxysulfuron (5.3 g ha^{-1}).

Table 5. Palmer amaranth control 40 d after herbicide application.

	Control (%)[y]				
	2006			2007	
Residual herbicides[z]	Oglethorpe	Mt. Olive	Parkton	Oglethorpe	Mt. Olive
Diuron	76 cd	4 def	72 bcd	64 cde	15 gh
Flumioxazin	99 a	18 abc	90 a	85 a	42 cd
Fluometuron	65 e	0 f	69 b-e	61 de	24 efg
Fomesafen	99 a	19 ab	77 b	73 bc	61 ab
Linuron	62 e	4 c-f	61 ef	80 ab	25 efg
Linuron plus diuron	69 de	4 def	65 cde	73 bc	19 fg
Metolachlor	60 e	6 c-f	63 def	42 g	20 fg
Pendimethalin	64 e	0 f	44 g	46 fg	6 h
Prometryn	57 e	4 e	51 fg	27 h	18 gh
Prometryn plus trifloxysulfuron	80 cd	13 a-d	87 a	58 ef	53 bc
Pyrithiobac	87 bc	27 a	77 b	73 bcd	69 a
S-metolachlor	86 b	4 def	76 bc	60 e	32 def
Trifloxysulfuron	64 e	11 b-e	73 bcd	58 ef	37 cde

[y] Data averaged over 1 and 1.5 times normal use rates for each residual herbicide. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[z] Residual herbicides and their respective 1X rates are diuron (1120 g ha^{-1}); flumioxazin (54 g ha^{-1}); fluometuron (1120 g ha^{-1}); fomesafen (280 g ha^{-1}); linuron (1120 g ha^{-1}); linuron + diuron (560 + 560 g ha^{-1}); metolachlor (1120 g ha^{-1}); pendimethalin (1064 g ha^{-1}); prometryn (1120 g ha^{-1}); prometryn + trifloxysulfuron (888 + 8 g ha^{-1}); pyrithiobac (48 g ha^{-1}); *S*-metolachlor (1067 g ha^{-1}); and trifloxysulfuron (5.3 g ha^{-1}).

Table 6. Palmer amaranth control 60 d after herbicide application.

	Control (%)[x]						
	2006				2007		
Residual	Oglethorpe					Mt. Olive	
herbicides[y]	1 X	1.5 X	Mt. Olive[z]	Parkton[z]	Oglethorpe[z]	1 X	1.5 X
Diuron	53 g-j	72 cde	1 a	19 c-f	55 bc	0 g	1 fg
Flumioxazin	95 ab	97 a	1 a	57 a	81 a	8 def	14 b-e
Fluometuron	33 mno	44 i-m	1 a	24 cde	46 c	0 g	3 efg
Fomesafen	95 ab	98 a	2 a	28 c	73 ab	23 bc	22 b
Linuron	38 k-n	47 h-l	0 a	16 def	38 cd	4 efg	1 fg
Linuron plus diuron	50 hij	56 ghi	0 a	11 f	39 cd	0 g	9 efg
Metolachlor	25 o	48 h-k	0 a	14 ef	28 de	3 efg	10 c-f
Pendimethalin	26 no	62 efg	0 a	12 f	41 cd	0 g	0 g
Prometryn	35 l-o	29 no	0 a	10 f	8 f	1 fg	0 g
Prometryn plus trifloxysulfuron	64 d-g	75 cd	0 a	41 ab	30 de	15 bcd	16 bcd
Pyrithiobac	69 c-f	78 c	1 a	27 bc	68 ab	14 bcd	53 a
S-metolachlor	58 fgh	90 b	0 a	24 cd	46 c	11 b-e	10 b-e
Trifloxysulfuron	38 k-n	41 j-m	1 a	23 cde	19 e	3 fg	10 b-e

[x] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[y] Residual herbicides at 1X rates are diuron (1120 g ha^{-1}); flumioxazin (54 g ha^{-1}); fluometuron (1120 g ha^{-1}); fomesafen (280 g ha^{-1}); linuron (1120 g ha^{-1}); linuron + diuron (560 + 560 g ha^{-1}); metolachlor (1120 g ha^{-1}); pendimethalin (1064 g ha^{-1}); prometryn (1120 g ha^{-1}); prometryn + trifloxysulfuron (888 + 8 g ha^{-1}); pyrithiobac (48 g ha^{-1}); *S*-metolachlor (1067 g ha^{-1}); and trifloxysulfuron (5.3 g ha^{-1}).

[z] Data averaged over 1 and 1.5 times the labeled rates for each residual herbicide.

CHAPTER V

Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) Management in Cotton

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Abstract

Two field experiments were conducted in North Carolina during 2007 and 2008 to evaluate control of glyphosate-resistant (GR) Palmer amaranth in cotton. In one experiment, various PRE herbicides and herbicide combinations were evaluated in a system that included glyphosate plus *S*-metolachlor applied early POST, glyphosate mid-POST, and prometryn plus trifloxysulfuron plus MSMA POST-directed at lay-by. Pyrithiobac, fomesafen, and diuron were more effective than fluometuron while pendimethalin was least effective. Combinations of herbicides were often more effective than individual herbicides. Palmer amaranth was controlled 92 to 98% late in the season in systems containing diuron plus

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pyrithiobac, fomesafen plus diuron, or fomesafen plus pyrithobac. Yields were increased an average of 70 to 72% by PRE herbicides. The second experiment focused on combinations of flumioxazin applied pre-plant, fomesafen applied PRE, and glyphosate, glyphosate plus *S*-metolachlor, and glyphosate plus pyrithiobac applied early POST. Each system included glyphosate mid-POST and diuron plus MSMA at lay-by. Fomesafen PRE was more effective than flumioxazin pre-plant, but greatest control was achieved with flumioxazin followed by fomesafen. Fomesafen or flumioxazin increased yield up to 300%. Pyrithiobac early POST increased control and increase yield 16% whereas *S*-metolachlor was of limited value. Systems with flumioxazin pre-plant, fomesafen PRE, and pyrithiobac early POST controlled Palmer amaranth greater than 90% late in the season. These results demonstrate that residual pre-plant or PRE herbicides are critical in managing GR Palmer amaranth in GR cotton.

Nomenclature: diuron; flumioxazin; fluometuron; fomesafen; glyphosate; MSMA; pendimethalin; prometyrn; pyrithiobac; *S*-metolachlor; trifloxysulfuron; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; cotton, *Gossypium hirsutum* L.

Key words: diuron; flumioxazin; fluometuron; fomesafen; glyphosate-resistant cotton; herbicide-resistant weeds; pendimethalin; pyrithobac; *S*-metolachlor.

Introduction

Glyphosate-resistant (GR) cultivars revolutionized weed management in cotton. The technology has been widely adopted by growers, with greater than 99% of the cotton planted in North Carolina, South Carolina, and Georgia being GR (USDA-AMS 2008). Reasons for

the extensive adoption include broad-spectrum weed control, reductions in time and labor inputs, and less complicated weed management strategies (Askew et al. 2002; Culpepper and York 1998, 1999; Young 2006).

Palmer amaranth is the most troublesome weeds for cotton producers in the southeastern U.S. (Webster 2005). This weed can grow over 2 m in height, and it can reach a height of 10 cm within 2 wk after planting (Ehleringer 1983; Horak and Loughin 2000; Sellers et al. 2003). Palmer amaranth is a prolific seed producer. Continued emergence throughout the season, coupled with prolific seed production, allows quick replenishment of the soil seed bank (Keely et al. 1987; Sellers et al. 2003). If not controlled, very high populations are common (Culpepper and York 1998; Gardner et al. 2006). Palmer amaranth is very competitive with cotton, as evidenced by 13% yield reduction from a single plant in 9.1 m of row and 54% yield reduction with densities of 10 plants in 9.1 row m (Morgan et al. 2001). Palmer amaranth present at harvest may also interfere with mechanical harvesting of cotton (Smith et al. 2000).

Glyphosate is very effective on glyphosate-susceptible (GS) Palmer amaranth although multiple applications are needed to address continued emergence during the season (Bond et al. 2006; Corbett et al. 2004; Culpepper and York 1998, 1999). With multiple applications, herbicide programs consisting only of glyphosate have effectively controlled Palmer amaranth and other weeds in cotton (Culpepper and York 1998, 2000; Toler et al. 2002).

Widespread planting of GR cotton and extensive use of glyphosate has placed intensive selection pressure on weed populations. Glyphosate-resistant Palmer amaranth was first found in Georgia during 2004 (Culpepper et al. 2006) and North Carolina in 2005 (York et

al. 2007). The resistant biotype infested at least 120,000 and 75,000 ha in Georgia and North Carolina, respectively, in 2008 (Culpepper et al. 2008b). Resistant populations were found in South Carolina, Arkansas, and Tennessee in 2006 (Griffith et al. 2007; Heap 2009; Main and Jones 2007; Norsworthy et al. 2008; Steckel et al. 2008).

Glyphosate-resistant Palmer amaranth will force growers to change management practices; in fact, changes are already occurring (Culpepper 2009). Effective herbicide systems integrated with cultural practices must be developed. Additionally, herbicides with other modes of action must be integrated into glyphosate-based systems to avoid or delay resistance in fields currently infested with GS Palmer amaranth. The objective of our research was to develop effective herbicide programs for GR Palmer amaranth in cotton utilizing residual herbicides with varying modes of action.

Materials and Methods

Methods common to all experiments. Two experiments were conducted in a commercial production field near Mount Olive, NC during 2007 and 2008 with a natural population of Palmer amaranth exceeding 170 plants per m^2. Previous experiments indicated this population consisted of a mixture of GR and GS biotypes, with approximately one-fourth to one-third of the population made up of the resistant biotype. The soil was a Wagram loamy sand (loamy, kaolinic, thermic, Arenic, kandiudults) with a pH of 5.1 and 6.1 (2007 and 2008, respectively) and humic matter content of 0.66 and 0.51% (2007 and 2008, respectively). Soil humic matter was determined by the North Carolina Department of Agriculture and Consumer Services, Agronomic Services Division, according to Mehlich

(1984). Cotton cultivar 'PHY 485 WRF'[1] was planted on May 15, 2007 and May 1, 2008 in plots with four rows spaced 96 cm apart by 9 m. Insect control, fertilization, growth regulation, and defoliation practices were standard for the area.

Herbicides were applied with a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles[2] calibrated to deliver 140 L/ha at 160 kPa. Percent Palmer amaranth control and cotton injury were estimated visually prior to early POST and mid-POST herbicide applications in Experiment 1, prior to EPOST and lay-by applications in Experiment 2, and in mid-September (late-season) in both experiments using a scale of 0 to 100%, where 0 = no Palmer amaranth control or no cotton injury and 100 = complete Palmer amaranth control or cotton death (Frans et al. 1986). Cotton was harvested with a spindle picker modified for small-plot harvesting in mid-October. Data for Palmer amaranth control, cotton injury, and cotton yield were subjected to ANOVA appropriate for the treatment structure using the PROC MIXED procedure of Statistical Analysis System[3]. Non-treated checks were included in each experiment but were excluded from the analysis. In both experiments, years and replications were considered random effects (McIntosh 1983). Means were separated with Fisher's Protected LSD ($P \leq 0.05$). Estimates of Palmer amaranth control and cotton injury were arcsine square root transformed prior to analysis (Ahrens et al. 1990); non-transformed data are presented with statistical interpretation based upon transformed data.

Experiment 1. Cotton was planted using conventional tillage in 2007 and strip-tillage into cotton stubble in 2008. The strip-tillage operation, conducted 2 d prior to planting, consisted of in-row subsoiling and tilling a seed bed 20 cm wide (Meijer et al. 2009). Winter vegetation was controlled in 2008 by the potassium salt of glyphosate[4] at 840 g ae/ha plus the

isooctyl (2-ethylhexyl) ester of 2,4-D[5] at 538 g ae/ha applied April 17. Treatments included the following herbicides and herbicide combinations applied PRE: no PRE herbicide; diuron[6] at 900 g ai/ha; fluometuron[7] at 1120 g ai/ha; fomesafen[8] at 280 and 420 g ai/ha; pendimethalin[9] at 1120 g ai/ha; pyrithiobac[10] at 50 g ai/ha; diuron at 900 g/ha plus pendimethalin at 1120 g/ha; diruon at 900 g/ha plus pyrithiobac at 50 g/ha; fomesafen at 280 g/ha plus diuron at 900 g/ha; fomesafen at 280 g/ha plus pendimethalin at 1120 g/ha; and fomesafen at 280 g/ha plus pyrithiobac at 50 g/ha. All treatments, except the non-treated check, received the commercial mixture of the potassium salt of glyphosate plus *S*-metolachlor[11] at 788 plus 1050 g/ha, respectively, applied early POST to cotyledonary to one-leaf cotton followed by the potassium salt of glyphosate[12] at 1240 g ae/ha applied mid-POST to 4- to 5-leaf cotton, and a POST-directed lay-by application of MSMA[13] at 2220 g ai/ha plus the commercial mixture of prometryn plus trifloxysulfuron[14] at 1110 plus 10 g ai/ha. Crop oil concentrate[15] was included in the lay-by application at 0.5% (v/v). Cotton was 35 and 50 cm tall at time of the lay-by application in 2007 and 2008, respectively.

Experiment 2. Cotton was planted with no-tillage in 2007 and strip-tillage in 2008 in a desiccated wheat (*Triticum aestivum*) cover crop. Treatments consisted of a factorial arrangement of four preplant or PRE herbicides (hereafter referred to as soil-applied herbicides) by three early POST herbicides or herbicide combinations. Pre-plant herbicides included glyphosate[4] at 840 g/ha plus 2,4-D at 270 g/ha alone or with flumioxazin[16] at 71 g ai/ha applied April 19, 2007 and April 17, 2008. The PRE options included no residual herbicide or fomesafen at 280 g/ha applied immediately after planting. Paraquat[17] at 840 g ai/ha plus non-ionic surfactant[18] at 0.25% (v/v) was included with the PRE herbicides. The

early POST options, applied to one-leaf cotton, included glyphosate[4] applied at 840 g /ha alone or with *S*-metolachlor[19] at 1070 g/ha or pyrithiobac at 73 g/ha. All plots, except non-treated checks, received a mid-POST application of glyphosate[4] at 840 g/ha applied to five-leaf cotton and a POST-directed lay-by application of diuron at 1120 g/ha plus MSMA at 2470 g/ha with 0.25% (v/v) non-ionic surfactant applied when cotton was 38 to 43 cm tall. The checks received only glyphosate plus 2,4-D pre-plant and paraquat PRE.

Results and Discussion

Experiment 1. Data are presented by year as the interaction of year by treatment was significant for cotton injury, Palmer amaranth control, and cotton yield. Prior to early POST application in 2007, no injury was noted with either diuron or pendimethalin (Table 1). Fluometuron and fomesafen injured cotton 4 to 6%. Injury by combinations of diuron plus pendimethalin, fomesafen plus diuron, and fomesafen plus pendimethalin was similar to the injury by the herbicides applied individually. Pyrithiobac injured cotton 24%. This injury appeared primarily as stunting and chlorosis. Combinations of diuron plus pyrithiobac and fomesafen plus pyrithiobac were no more injurious than pyrithiobac alone.

Diuron, fluometuron, fomesafen at 280 g/ha, and pendimethalin injured cotton 3% or less prior to early POST application in 2008 while fomesafen at 420 g/ha and pyrithiobac injured cotton 10 and 8%, respectively (Table 1). Injury by combinations of diuron plus pendimethalin, fomesafen plus diuron, and fomesafen plus pendimethalin was no greater than injury by the individual herbicides applied alone. Injury by diuron plus pyrithiobac was similar to injury by pyrithobac alone whereas fomesafen plus pyrithiobac injured cotton 18%

compared to 8% injury by pyrithiobac alone. Prior to the mid-POST application in each year, cotton was injured 2% or less by all treatments not containing pyrithiobac. Pyrithiobac alone and combinations of diuron plus pyrithiobac or fomesafen plus pyrithiobac injured cotton 4 to 7%. No cotton injury was noted late in the season.

Adequate rainfall for PRE herbicide activation was received each year. Rainfall during the first 7 d after PRE herbicide application totaled 1.5 and 2.1 cm in 2007 and 2008, respectively (data not shown). Pyrithiobac was the most effective individual PRE herbicide prior to early POST application in 2007, controlling Palmer amaranth 93% (Table 2). Diuron and fomesafen at either rate were intermediately effective, controlling Palmer amaranth 53 to 67%, while fluometuron and pendimethalin were least effective. Fluometuron and pendimethalin controlled Palmer amaranth only 19 and 6%, respectively. Control by combinations of diuron plus pyrithiobac and fomesafen plus pyrithiobac was similar to control by pyrithiobac alone. Control by diuron plus pendimethalin and fomesafen plus pendimethalin was similar to control by diuron and fomesafen alone, respectively, and greater than control by pendimethalin alone. Control by fomesafen plus diuron was greater than control by fomesafen or diuron applied alone.

With the exception of treatments containing pyrithiobac, greater Palmer amaranth control prior to early POST herbicide application was observed in 2008 compared with 2007 (Table 2). Greater rainfall in the first 7 d after PRE herbicide application in 2008 (2.1 cm in 2008, 1.5 cm in 2007) may have been a contributing factor. However, tillage systems probably played a greater role. The site was in conventional tillage in 2007 and strip-tillage into a wheat cover crop in 2008. Culpepper et al. (2008a) noted better Palmer amaranth control

with various herbicide systems in cotton planted no-till into a wheat cover crop as compared with conventional tillage, and they attributed the difference to weed suppression from the cover crop.

In 2008, diuron, fomesafen, and pyrithiobac were similarly effective on Palmer amaranth prior to early POST herbicide application. These herbicides controlled Palmer amaranth 97 to 99% (Table 2). Diuron, fomesafen, and pyrithiobac were more effective than fluometuron, which in turn was more effective than pendimethalin. Fluometuron and pendimethalin controlled Palmer amaranth 86 and 72%, respectively. In other research, pendimethalin applied PRE was less effective on Palmer amaranth than pyrithiobac, fomesafen, fluometuron, or diuron (Whitaker et al. 2008). Control by mixtures of diuron plus pendimethalin, fomesafen plus diuron, and fomesafen plus pendimethalin was similar to control by the more effective component of the mixture applied alone. Additionally, there was little to no advantage to adding diuron or fomesafen to pyrithiobac compared with pyrithiobac applied alone.

Palmer amaranth was controlled 69 to 71% following the early POST application of glyphosate plus *S*-metolachlor and the mid-POST application of glyphosate but prior to lay-by application (Table 2). This level of control is consistent with previous observations of about one-fourth to one-third of Palmer amaranth population in this field consisting of a GR biotype. Glyphosate applied twice would have controlled GS Palmer amaranth completely (Culpepper and York 2000; Main et al. 2007). All PRE herbicides except pendimethalin in 2008 and fluometuron and pendimethalin in 2007 increased Palmer amaranth control prior to lay-by herbicide application. Palmer amaranth was controlled at least 82% in both years in

systems that included diuron, fomesafen at 280 g/ha, diuron plus pendimethalin, and fomesafen plus pendimethalin. Systems containing pyrithiobac, fomesafen at 420 g/ha, diuron plus pyrithiobac, fomesafen plus diuron, and fomesafen plus pyrithiobac controlled Palmer amaranth at least 91% in both years. Except for fomesafen plus pyrithiobac in 2007, no herbicide combination was more efficacious than the more effective component of the combination. In 2007, greater control was noted with fomesafen plus pyrithiobac than with either fomesafen or pyrithiobac applied alone.

In systems without a PRE herbicide, late-season control in 2007 was similar to the control noted prior to the lay-by application (Table 2). In contrast, late-season control in the absence of a PRE herbicide declined to only 23% in 2008. This difference between years was at least partially due to the size of the weeds when the lay-by application was made. In systems with less effective PRE herbicides or no PRE herbicide, the Palmer amaranth was larger when the lay-by herbicides were applied in 2008. Adequate spray coverage could not be obtained on larger weeds with the directed spray. Differences in rainfall between the years following lay-by application did not appear to contribute to differences in control. Cumulative rainfall during the first 4, 6, and 8 wk following lay-by application in 2007 totaled 10.6, 11.1, and 17.9 cm, respectively; rainfall totaled 10.0, 13.8, and 18.4 cm during the same periods in 2008 (data not shown).

Greatest Palmer amaranth control late in the season was obtained in systems that included fomesafen at 420 g/ha, diuron plus pyrithiobac, fomesafen plus diuron, and fomesafen plus pyrithiobac (Table 2). Systems with these herbicides applied PRE controlled Palmer amaranth greater than 90% late in the season in both years. Systems that included fomesafen

at 280 g/ha, pyrithiobac, and fomesafen plus pendimethalin controlled Palmer amaranth greater than 90% in one year and 83% or greater in both years.

Soil variability at the 2007 site resulted in variable seed cotton yields, thus limiting statistical separation of treatments. Yields in 2007 did not always correlate well with weed control, but PRE herbicides increased cotton yield in most cases (Table 3). Averaged over all treatments with a PRE herbicide, cotton yield was increased 72% by PRE herbicides. Seed cotton yield in 2008 generally followed trends in Palmer amaranth control. All PRE herbicides except pendimethalin increased yield (Table 3). Pendimethalin was less effective on Palmer amaranth in 2008 than any of the other PRE herbicides (Table 2). Greatest yields were obtained with fomesafen at both rates, diuron plus pendimethalin, diuron plus pyrithiobac, fomesafen plus diuron, fomesafen plus pendimethalin, and fomesafen plus pyrithobac (Table 3). Yield from cotton receiving these herbicides applied PRE in 2008 averaged 2870 kg/ha, or 70% more than cotton not receiving a PRE herbicide. Yield of cotton receiving diuron plus pendimethalin exceeded the yield of cotton receiving either of these herbicides alone. However, yield of cotton receiving combinations of fomesafen plus diuron, fomesafen plus pendimethalin, and fomesafen plus pyrithiobac did not exceed yield of cotton receiving only fomesafen, and yield of cotton receiving diuron plus pyrithiobac did not exceed yield of cotton receiving only pyrithiobac.

Experiment 2. *2007*. Treatment by year interactions were significant for Palmer amaranth control at all evaluations and for cotton yield, therefore data are presented by year. An interaction of soil-applied herbicides and early POST herbicides was not observed in 2007. However, main effects of both soil-applied herbicides and early POST herbicides were

significant for Palmer amaranth control at all evaluations and for cotton yield. No crop injury was observed.

At all evaluations in 2007, greater control was obtained with fomesafen applied PRE than with flumioxazin applied pre-plant (Table 4). Control late in the season was similar with fomesafen PRE and flumioxazin pre-plant followed by fomesafen PRE. At the earlier evaluations, however, the combination of flumioxazin pre-plant followed by fomesafen PRE was more effective than either herbicide applied alone. Palmer amaranth was controlled 96, 98, and 95% in systems with flumioxazin pre-plant followed by fomesafen PRE prior to early POST application, prior to lay-by application, and late in the season, respectively.

Prior to lay-by herbicide application, Palmer amaranth was controlled only 78% in systems without a soil-applied herbicide in 2007 (Table 4). This evaluation followed early POST application of glyphosate alone or mixed with *S*-metolachlor or pyrithiobac and mid-POST application of glyphosate. Lack of more effective control by glyphosate applied early POST and mid-POST is consistent with the field having a mixed population of GR and GS biotypes.

Averaged over soil-applied herbicides, pyrithiobac and *S*-metolachlor included with glyphosate applied early POST increased Palmer amaranth control (Table 5). Pyrithiobac and *S*-metolachlor were similarly effective when evaluated prior to lay-by herbicide application. By late in the season, greater control was noted with pyrithiobac than with *S*-metolachlor. Averaged over soil-applied herbicides, *S*-metolachlor and pyrithiobac increased late-season Palmer amaranth control 12 and 16%, respectively.

Seed cotton yields were numerically greater in systems with either flumioxazin applied pre-plant or fomesafen applied PRE, but a statistically significant difference was noted only with the combination of flumioxazin pre-plant followed by fomesafen PRE (Table 4). Averaged over early POST herbicides, flumioxazin pre-plant followed by fomesafen PRE increased cotton yield 25%. Averaged over soil-applied herbicides, *S*-metolachlor applied with glyphosate early POST did not impact seed cotton yield (Table 5). Pyrithobac applied with glyphosate early POST increased cotton yield 16%.

2008. Main effects of both soil-applied herbicides and early POST herbicides were significant for Palmer amaranth control at all evaluations and seed cotton yield. Additionally, a soil-applied herbicide by early POST herbicide interaction was noted for Palmer amaranth control prior to lay-by and for seed cotton yield. No crop injury was observed.

Similar to results in 2007, a difference in Palmer amaranth control between flumioxazin applied pre-plant and fomesafen applied PRE was noted in 2008. Prior to early POST herbicide application, fomesafen applied PRE controlled Palmer amaranth greater than flumioxazin applied pre-plant, and the combination of flumioxazin pre-plant and fomesafen PRE was more effective than either herbicide applied alone (Table 4). The combination controlled Palmer amaranth 92% prior to early POST herbicide application.

Control by both flumioxazin and fomesafen prior to EPOST herbicide application tended to be less in 2008 compared with 2007 (Table 4). However, the relative difference between years was greater for flumioxazin than for fomesafen. This may have been related to tillage systems. Cotton in 2007 was planted no-till with little to no soil disturbance whereas the

field was strip-tilled 2 d prior to planting in 2008. Strip-tilling after flumioxazin application can lead to reduced weed control in the tilled strip. To compensate for this impact of soil disturbance on flumioxazin activity, a PRE herbicide banded over the cotton row is recommended when the strip-tillage operation occurs after flumioxazin application (York and Culpepper 2009).

The soil-applied herbicide by early POST herbicide interaction for Palmer amaranth control prior to lay-by herbicide application occurred because pyrithiobac increased control only when used in the absence of a residual soil-applied herbicide (Table 6). Prior to lay-by, Palmer amaranth was controlled only 55% by glyphosate in systems without flumioxazin pre-plant or fomesafen PRE compared with 91 to 98% control in systems with a residual soil-applied herbicide. With the high degree of control by soil-applied herbicides, pyrithiobac applied early POST did not increase control. In systems without a soil-applied residual herbicide, pyrithoibac increased Palmer amaranth control 33%. *S*-metolachlor did not increase control, regardless of the soil-applied herbicides. *S*-metolachlor is effective on Palmer amaranth only when applied and activated prior to emergence of the weed. Palmer amaranth was emerged at the time of early POST application in systems without a residual soil-applied herbicide, and *S*-metolachlor would be expected to have no effect on the GR biotype which was not controlled by glyphosate. In contrast, pyrithiobac has both PRE and POST activity on Palmer amaranth (Dotray et al. 1996).

Flumioxazin applied pre-plant and fomesafen applied PRE were similarly effective on Palmer amaranth late in the season. Averaged over early POST herbicides, flumioxazin and fomesafen increased late-season Palmer amaranth control 35 to 38% (Table 4). The

combination of flumioxazin pre-plant followed by fomesafen PRE was 16 to 19% more effective than either herbicide alone. Averaged over soil-applied herbicides, pyrithiobac early POST increased late-season Palmer amaranth control 22% while *S*-metolachlor had no effect on control (Table 5). A greater response to *S*-metolachlor in 2007 may have been related to rainfall following application. In 2007, 4.2 cm of rainfall occurred in the week following early POST application compared with 1.4 cm during the same period in 2008.

A soil-applied herbicide by early POST herbicide interaction was noted for seed cotton yield in 2008 (Table 6). Plots that received no soil-applied herbicide and glyphosate or glyphosate plus *S*-metolachlor early POST could not be harvested. These plots, along with the non-treated checks, were completely decimated by weeds, and yields were assumed to be zero. Visual observation indicated no seed cotton was produced. Cotton in the system with no residual soil-applied herbicide and pyrithiobac early POST yielded 1610 kg/ha of seed cotton. Yield of cotton receiving this treatment was similar to that from cotton receiving fomesafen PRE and glyphosate early POST but less than with flumioxazin pre-plant or flumioxazin pre-plant followed by fomesafen PRE and glyphosate early POST. Yields were similar in systems with fomesafen PRE and flumioxazin pre-plant. Yields also were similar in systems with flumioxazin pre-plant and flumioxazin pre-plant followed by fomesafen PRE. However, yield was greater in systems with flumoxazin pre-plant plus fomesafen PRE compared with only fomesafen PRE. Pyrithiobac early POST did not increase yield in systems with a residual soil-applied herbicide, and *S*-metolachlor did not increase yield in any system. Numerically, the greatest yield was obtained in the system with flumioxazin pre-plant, fomesafen PRE, and pyrithobac early POST. Compared with that system, similar

yields were noted with either soil-applied herbicide alone followed by pyrithiobac early POST, whereas lesser yields were noted with systems of flumioxazin or fomesafen followed by *S*-metolachlor.

The objective of these experiments was to develop a management system for GR Palmer amaranth in GR cotton. Our results demonstrate the challenge presented by GR Palmer amaranth in cotton. Nearly complete control of this weed is necessary to avoid cotton yield losses and harvesting difficulties, but systems with two topical applications of glyphosate followed by a residual lay-by herbicide application controlled Palmer amaranth only 60% and 14% late in the season in 2007 and 2008, respectively (data not shown). These systems would control GS Palmer amaranth very well (Culpepper and York 2000; Main et al. 2007). Residual herbicides, applied pre-plant or PRE, are critical in managing the resistant biotype. Based upon these and other experiments (Whitaker et al. 2008), the most effective residual herbicides for pre-plant or PRE application would be flumioxazin, fomesafen, and pyrithiobac. Systems that include a pre-plant and PRE herbicide or two PRE herbicides are often more effective than systems with only a pre-plant or one herbicide applied PRE. *S*-metolachlor mixed with glyphosate and applied POST can increase residual control of Palmer amaranth if applied before weed emergence and activated timely (Clewis et al. 2006), but it is no replacement for pre-plant or PRE herbicides. Pyrithiobac applied POST is more effective than *S*-metolachlor because pyrithiobac provides residual control and also kills emerged weeds. However, resistance to acetolactate synthase (ALS)-inhibiting herbicides is wide-spread in the Southeast and Mid-South regions of the U.S. cotton belt (Heap 2009), and continued reliance on pyrithiobac will only lead to additional problems with resistance to

ALS inhibitors. Sustainable production of GR cotton in the presence of GR Palmer amaranth will require not only intensive herbicide programs in cotton but also integration of cultural practices such as cover crops or cultivation and crop rotation with intensive management systems in the rotational crops (Culpepper 2009).

Sources of Materials

[1] PHY 485 WRF cotton, Phytogen Seed Company L.L.C., Indianapolis, IN 46268.

[2] TeeJet XR11002 flat-fan spray nozzles, Spraying Systems Co., Wheaton, IL 60189.

[3] Statistical Analysis Systems, version 9.1, SAS Institute Inc., Cary, NC, 27513.

[4] Roundup WEATHERMAX herbicide, Monsanto Company, St. Louis, MO 63167.

[5] Weedone LV4 herbicide, Nufarm, Inc., Burr Ridge, IL 60527.

[6] Direx 4L herbicide, Dupont Crop Protection Co., Wilmington, DE 19898.

[7] Cotoran 4L herbicide, Griffin L.L.C., Valdosta, GA 31603.

[8] Reflex herbicide, Syngenta Crop Protection Inc., Greensboro, NC 27419.

[9] Prowl H_2O herbicide, BASF Ag Products, Research Triangle Park, NC 27709-3528.

[10] Staple herbicide, Dupont Crop Protection Co., Wilmington, DE 19898.

[11] Sequence herbicide, Syngenta Crop Protection Inc., Greensboro, NC 27419.

[12] Touchdown Total herbicide, Syngenta Crop Protection Inc., Greensboro, NC 27419.

[13] MSMA 6 plus herbicide, Drexel Chemical Co., Memphis, TN 38113-0327.

[14] Suprend herbicide, Syngenta Crop Protection Inc., Greensboro, NC 27419.

[15] Agridex crop oil concentrate, Helena Chemical Co., Collierville, TN 38017.

[16] Valor SX herbicide, Valent U.S.A. Corporation, Walnut Creek, CA 94596-8025.

[17] Gramoxone Inteon herbicide, Syngenta Crop Protection Inc., Greensboro, NC 27419.

[18] Induce, nonionic low foam wetter/spreader adjuvant, Helena Chemical Co., Collierville, TN 38017.

[19] Dual Magnum herbicide, Syngenta Crop Protection Inc., Greensboro, NC 27419.

Acknowledgment

The authors would like to thank Rick Seagroves and Jamie Hinton for technical support. Partial funding for this research was provided by the cotton growers of North Carolina through a grant from Cotton Incorporated.

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Table 1. Cotton injury by PRE herbicides. Experiment 1.[a]

		Cotton injury			
	Application	Prior to early POST		Prior to mid-POST	
Herbicides[b]	rates	2007	2008	2007	2008
	g/ha	%			
No PRE		0[c]	0[c]	0 b	0 b
Diuron	900	0 c	3 d	1 b	0 b
Fluometuron	1120	4 bc	0 d	0 b	0 b
Fomesafen	280	4 bc	3 d	0 b	0 b
Fomesafen	420	6 bc	10 b	0 b	1 b
Pendimethalin	1120	0 c	0 d	0 b	0 b
Pyrithiobac	50	24 a	8 bc	4 a	6 a
Diuron plus pendimethalin	900 + 1120	5 bc	1 d	1 b	0 b
Diuron plus pyrithiobac	900 + 50	19 a	10 b	5 a	6 a
Fomesafen plus diuron	280 + 900	5 bc	4 cd	0 b	2 b
Fomesafen plus pendimethalin	280 + 1120	2 c	3 d	1 b	1 b
Fomesafen plus pyrithiobac	280 + 50	20 a	18 a	5 a	7 a

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] All plots, except non-treated check, received glyphosate plus *S*-metolachlor at 788 + 1050 g/ha applied early POST to cotyledonary to one-leaf cotton and glyphosate at 1240 g/ha applied mid-POST to 4- to 5-leaf cotton.

[c] This treatment was assigned a value of 0 at the evaluation prior to early POST and was not included in the ANOVA.

Table 2. Palmer amaranth control by PRE herbicides. Experiment 1.[a]

		Palmer amaranth control					
	Application	Prior to early POST		Prior to lay-by		Late-season	
Herbicides[b]	rates	2007	2008	2007	2008	2007	2008
	g/ha	%					
No PRE	--	0[c]	0[c]	71 f	69 f	70 e	23 g
Diuron	900	53 e	97 bc	84 cde	86 e	88 cd	70 e
Fluometuron	1120	19 f	86 d	76 ef	87 de	83 d	68 e
Fomesafen	280	67 de	97 bc	87 b-e	98 ab	83 d	91 abc
Fomesafen	420	66 de	98 bc	95 ab	98 ab	95 ab	90 bc
Pendimethalin	1120	6 g	72 e	82 def	72 f	73 e	46 f
Pyrithiobac	50	93 abc	99 b	91 bcd	95 abc	96 ab	83 cd
Diuron plus pendimethalin	900 + 1120	65 e	95 c	82 def	89 cde	83 d	74 de
Diuron plus pyrithiobac	900 + 50	99 a	100 a	95 ab	99 a	98 a	98 a
Fomesafen plus diuron	280 + 900	91 bc	99 b	95 ab	98 ab	96 ab	92 abc
Fomesafen plus pendimethalin	280 + 1120	82 cd	98 bc	89 bcd	95 bcd	92 bc	88 c
Fomesafen plus pyrithiobac	280 + 50	97 ab	100 a	98 a	99 a	98 a	97 ab

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

Table 2. continued.

[b] All plots, except the non-treated check, received glyphosate plus *S*-metolachlor at 788 + 1050 g/ha applied early POST to cotyledonary ton one-leaf cotton, glyphosate at 1240 g/ha applied mid-POST to 4- to 5-leaf cotton, and prometryn plus trifloxysulfuron plus MSMA at 1110 + 10 + 2220 g/ha applied at lay-by.

[c] This treatment was assigned a value of 0 at the evaluation prior to early POST and was not included in the ANOVA.

Table 3. Seed cotton yield as affected by PRE herbicides. Experiment 1.[a]

Herbicides[b]	Application rates	Seed cotton yield	
		2007	2008
	g/ha	kg/ha	
No PRE	--	740 b	1690 d
Diuron	900	1160 ab	2470 c
Fluometuron	1120	1200 a	2610 bc
Fomesafen	280	1270 a	3120 a
Fomesafen	420	1440 a	2770 abc
Pendimethalin	1120	1200 a	1850 d
Pyrithiobac	50	1190 ab	2610 bc
Diuron plus pendimethalin	900 + 1120	1030 ab	2740 abc
Diuron plus pyrithiobac	900 + 50	1560 a	3040 ab
Fomesafen plus diuron	280 + 900	1180 ab	2690 abc
Fomesafen plus pendimethalin	280 + 1120	1410 a	2720 abc
Fomesafen plus pyrithiobac	280 + 50	1290 a	2990 ab

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] All plots, except the non-treated check, received glyphosate plus *S*-metolachlor at 788 + 1050 g/ha applied early POST to cotyledonary to one-leaf cotton, glyphosate at 1240 g/ha applied mid-POST to 4- to 5-leaf cotton, and prometryn plus trifloxysulfuron plus MSMA at 1110 + 10 + 2220 g/ha applied at lay-by.

Table 4. Palmer amaranth control and seed cotton yield as affected by soil-applied herbicides. Experiment 2.[a]

Soil-applied herbicides[b]		Palmer amaranth control					Seed cotton yield
		Prior to early POST		Prior to Lay-by[e]	Late-season[e]		
Pre-plant[c]	PRE[d]	2007	2008	2007	2007	2008	2007
		%					kg/ha
None	None	0[f]	0[f]	78 d	76 c	28 c	1670 b
None	Fomesafen	93 b	83 b	95 b	92 a	66 b	1770 b
Flumioxazin	None	81 c	62 c	89 c	83 b	63 b	1870 ab
Flumioxazin	Fomesafen	96 a	92 a	98 a	95 a	82 a	2080 a

[a] Data pooled over early POST herbicides. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] All treatments, except the non-treated check, received glyphosate at 840 g/ha at mid-POST and a lay-by application of diuron at 1120 g/ha plus MSMA at 2470 g/ha.

[c] Flumioxazin was applied at 71 g/ha. All treatments included glyphosate at 840 g/ha plus 2,4-D at 270 g/ha.

[d] Fomesafen was applied at 280 g/ha. All treatments included paraquat applied PRE at 840 g/ha.

[e] Data averaged over early POST herbicides.

[f] This treatment was assigned a value of 0 at the evaluation prior to early POST and was not included in the ANOVA.

Table 5. Palmer amaranth control and seed cotton yield as affected by early POST herbicides. Experiment 2.[a]

	Palmer amaranth control			Seed cotton
	Prior to lay-by	Late-season		yield
Early POST herbicides[b,c]	2007	2007	2008	2007
	%			kg/ha
Glyphosate	86 b	77 c	52 b	1710 b
Glyphosate + pyrithiobac	94 a	93 a	74 a	1990 a
Glyphosate + *S*-metolachlor	91 a	89 b	53 b	1840 ab

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] Glyphosate, pyrithiobac, and *S*-metolachlor were applied at 840, 73, and 1070 g/ha, respectively.

[c] Data pooled over soil-applied herbicides. All plots received glyphosate applied at 840 g/ha mid-POST and a lay-by application of diuron at 1120 g/ha plus MSMA at 2470 g/ha.

Table 6. Palmer amaranth control prior to lay-by herbicide application and seed cotton yield as affected by soil-applied and early POST herbicides in 2008. Experiment 2.[a]

Herbicides[b]			Palmer amaranth control prior to lay-by	Seed cotton yield
Soil-applied				
Pre-plant[c]	PRE[d]	Early POST[e]		
			%	kg/ha
None	None	Glyphosate	55 d	0 e
		Glyphosate + pyrithiobac	88 c	1610 d
		Glyphosate + *S*-metolachlor	48 d	0 e
None	Fomesafen	Glyphosate	94 abc	1900 cd
		Glyphosate + pyrithiobac	96 ab	2200 abc
		Glyphosate + *S*-metolachlor	92 abc	1900 cd
Flumioxazin	None	Glyphosate	91 bc	2130 bc
		Glyphosate + pyrithiobac	97 ab	2430 ab
		Glyphosate + *S*-metolachlor	88 c	1960 cd
Flumioxazin	Fomesafen	Glyphosate	98 a	2440 ab
		Glyphosate + pyrithiobac	97 a	2570 a
		Glyphosate + *S*-metolachlor	98 a	2260 abc

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] All treatments, except the non-treated check, received glyphosate 840 g/ha applied mid-POST and a lay-by application of diuron at 1120 g/ha plus MSMA at 2470 g/ha.

[c] Flumioxazin was applied at 71 g/ha. All treatments included glyphosate plus 2,4-D at 840 + 270 g/ha, respectively.

[d] Fomesafen was applied at 280 g/ha. All treatments included paraquat applied PRE at 840 g/ha.

[e] Glyphosate, pyrithiobac, and *S*-metolachlor applied early POST at 840, 73, and 1090 g/ha, respectively, to one-leaf cotton.

CHAPTER VI

Widestrike Cotton Tolerance to Glufosinate

Jared R. Whitaker, Alan C. York, and David L. Jordan*

Abstract

Three field experiments were conducted in North Carolina to evaluate 'PHY 485 WRF' cotton response to glufosinate applied postemergence. This glyphosate-resistant cotton contains a gene, used as a selectable marker, for glufosinate resistance allowing use of glufosinate to control certain broadleaf weeds. Weed-free experiments were conducted in North Carolina during 2007 and 2008 to evaluate PHY 485 WRF tolerance to glufosinate applied postemergence. In one experiment, glyphosate and glufosinate were applied alone, mixed together, or tank mixed with other herbicides three times during early cotton growth (less than 12-leaf cotton). Visual injury of cotton was 6 and 15% by glufosinate at 470 and 940 g/ha when applied sequentially. Mixing glyphosate or trifloxysulfuron with glufosinate did not increase injury. Pyrithiobac and *S*-metolachlor increased injury by at least four percentage points. Glufosinate applied at 940 g/ha reduced cotton height 16 to

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31% 10 d after the third application and reduced open boll percent at harvest by 14%. Glufosinate plus glyphosate at 840 g/ha, pyrithiobac, or *S*-metolachlor also reduced percent open boll. However, no glufosinate treatment adversely affected yield or fiber quality. In another experiment, glufosinate applied twice at 600 and 900 g/ha injured cotton 5 and 16%. Including ammonium sulfate (AMS) at 340 g/ha with glufosinate slightly increased injury and reduced cotton height and percent open boll at harvest. Glufosinate plus AMS reduced yield in only one of four locations. In a third experiment, co-applying insecticides with glufosinate applied twice at 470 g/ha did not exacerbate effects of glufosinate injury and no treatment affected yield.

Nomenclature: Glufosinate; glyphosate; pyrithiobac; *S*-metolachlor; trifloxysulfuron, ammonium sulfate, acephate, dicrotophos, dimethoate, imidacloprid, lambda-cyhalothrin, oxamyl, thiamethoxam.

Key words: crop injury, crop tolerance.

Abbreviations: AMS, ammonium sulfate; glyp, glyphosate; GR, glyphosate-resistant; gluf, glufosinate; POST, postemergence; pyri, pyrithiobac; S-met, *S*-metolachlor.

Introduction

Glyphosate-resistant (GR) cotton (trade name Roundup Ready®) is widely planted throughout the southeastern United States and growers have readily adopted this technology because of broad-spectrum weed control, convenience of overtop application, increased rotational options, and reductions in labor and time inputs (Askew et al. 2002; Culpepper and York 1998, 1999; Faircloth et al. 2001; Gianessi 2008; Young 2006). The percentage of

cotton planted with GR cultivars has increased from 4% of U.S. hectares in 1997 to 80% in 2005 (Sankula 2006). During 2008, cotton was planted on over 2.07 million ha in Alabama, Georgia, North Carolina, South Carolina, and Tennessee combined and greater than 99% of the crop was devoted to GR cultivars (USDA-AMS 2008).

Extensive use of glyphosate in GR cultivars has placed intense selection pressure on the evolution of weed resistance. Glyphosate-resistant horseweed, first reported in Deleware (VanGessel 2001) is now widespread and found in 17 U.S. states (Heap 2009). More recently, glyphosate-resistant Palmer amaranth has been confirmed in Arkansas, Georgia, North Carolina, South Carolina, and Tennessee (Culpepper et al. 2006, 2008; Heap 2009; Norsworthy et al. 2008; Steckel et al. 2008; York et al. 2007). Additionally, common weed species in cotton including common ragweed (*Ambrosia artemisiifolia*), giant ragweed (*Ambrosia trifida*), common lambsquarters (*Chenopodium album*, Italian ryegrass (*Lolium perenne* L. spp. muliflorum), and johnsongrass (*Sorghum halapense*), have developed resistance to glyphosate (Heap 2009).

Glufosinate is a non-selective herbicide which can be applied POST on glufosinate-resistant (trade name Liberty Link®) cotton (Blair-Kerth et al. 2001). This cotton was created by the insertion and expression of a bialaphos resistance (*bar*) gene isolated from the soil bacterium *Streptomycyes hygroscopius* which encodes for the phosphinothricin acetyl transferase (PAT) enzyme, which detoxifies the L-isomer of glufosinate into an inactive form (Devine et al. 1993; Tsaftaris 1996). Liberty Link® cotton has excellent tolerance to glufosinate applied POST from emergence until the early bloom stage to control broadleaf weeds and grasses when applied timely (Anonymous 2008; Blair-Kerth et al. 2001).

Glufosinate-based herbicide systems have been used to effectively control common ragweed, common lambsquarters, and johnsongrass (Beyers et al. 2002; Culpepper et al. 2000; Everman et al. 2007). Glufosinate is somewhat more effective than glyphosate on giant ragweed (Hoss et al. 2003) and is more effective than glyphosate on GR horseweed (Steckel et al. 2006). Glufosinate is typically less effective than glyphosate in controlling glyphosate-susceptible Palmer amaranth and some annual grass spp. (Corbett et al. 2004; Price et al. 2007). However, when applied timely, glufosinate can control Palmer amaranth (Culpepper et al. 2000; Gardner et al. 2006; Norsworthy et al. 2008). Glufosinate-based herbicide systems have been more effective than glyphosate-based systems on GR Palmer amaranth (Culpepper et al. 2008; MacRae et al. 2008). Liberty Link cultivars have not been adopted by growers in the southeastern United States (USDA-AMS 2008) likely because of traditionally higher yield potential from Roundup Ready® cultivars (NCSU 2008; UGA 2008; UT 2008) coupled with difficulty in controlling *Amaranthus* spp. and annual grasses with glufosinate (Gardner et al. 2006).

Widestrike™ cotton has two genes which confer resistance to lepidopteron pests (Thompson et al. 2005). During transformation of this cotton, both inserted genes contained a phosphinothricin acetyl transferase (*pat*) gene. The *pat* gene was isolated from *Streptomyces viridochromogenes* (Agbios 2005). Liberty Link® cotton contains a *bar* gene, however, both the pat gene and the *bar* genes code for a phosphinothricin acetyl transferase enzyme which are very similar at the nucleotide level and have similar functional characteristics (Wehrmann et al. 1996). The pat gene in Widestrike™ cotton was used as a

selectable marker to detect successful transformation of the lepidopteron insect resistant genes (Agbios 2005).

Bayer Cropscience has developed a GR cotton technology referred to as Glytol® cotton. Glytol cotton has season-long tolerance to glyphosate and is expected to be released in 2009 (Trolinder et al. 2008). Stacked cultivars with both Glytol® and Liberty Link® traits are expected to be released in 2010 (Henniger et al. 2009). In these cultivars, it may be possible to use both glyphosate and glufosinate for weed control. Moreover, these varieties could be used to reduce selection pressure for glyphosate-resistance. However, yield potential of these varieties may not differ from traditional Liberty Link® varieties.

Several cultivars are available now with Widestrike™ traits stacked with Roundup Ready and Roundup Ready Flex technologies. These cultivars produce consistently greater yields than Liberty Link® cultivars currently available (NCSU 2008; UGA 2008; UT 2008). In these cultivars, both glufosinate and glyphosate may be used as in Glytol Liberty Link cultivars; however, the tolerance of Widestrike Roundup Ready Flex cotton to glufosinate has not been thoroughly examined. Therefore, the objectives of this study were to tolerance, growth, development, and yield of a Widestrike Roundup Ready Flex cultivar, PHY 485 WRF[1], following various POST glufosinate applications including glufosinate applied with other herbicides, ammonium sulfate, and various insecticides which could likely be co-applied with glufosinate in a typical cotton production system.

Materials and Methods

Three weed-free experiments were conducted in North Carolina during 2007 and 2008 with PHY 485 WRF cotton[1] planted into conventionally prepared seedbeds in late April or early May. Plots consisted of four rows spaced 0.9 m apart by 9 m in length. The experimental design for each experiment was a randomized complete block with treatments replicated four times. Soils, soil characteristics, and planting dates, at experiment sites are described in Table 1.

Cotton was maintained weed-free by pendimethalin[2] at 1100 g ai/ha plus fluometuron[3] at 1100 g ai/ha applied preemergence and a postemergence-directed lay-by application of the potassium salt of glyphosate[4] at 860 g ae/ha. Aldicarb[5] insecticide at 0.84 kg ai/ha was applied in-furrow during planting to control early season insects and nematodes. Acephate[6] insecticide was applied as a foliar spray as needed for additional control of thrips (*Frankliniella* spp.) in experiments 1 and 2. Herbicides were applied with a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles[7] calibrated to deliver 140 L/ha at 160 kPa.

Experiment 1. This experiment was designed to determine PHY 485 WRF cotton tolerance to glufosinate applied alone, combinations of glufosinate plus glyphosates, and combinations of glufosinate or glyphosate plus pyrithiobac, S-metolachlor, or trifloxysulfuron. The experiment was conducted at the Upper Coastal Plain Research Station near Rocky Mount in 2007 and 2008, the Peanut Belt Research Station near Lewiston in 2007, and the Central Crops Research Station near Clayton in 2008. Postemergence treatments, outlined in Table 2, were applied to 1- to 2-leaf cotton (POST 1), 6- to 7-leaf cotton (POST 2), and 10-to 12-leaf cotton (POST 3) and consisted of the following: the ammonium salt of glufosinate[8] at

410 and 820 g ae/ha or the potassium salt of glyphosate[4] at 860 g ae/ha applied at each application timing; glufosinate at 410 g/ha or glyphosate at 860 g/ha mixed with pyrithiobac[9] at 36 g ai/ha applied POST 1 and POST 2 followed by glufosinate or glyphosate POST 3; glufosinate at 410 g/ha or glyphosate at 860 g/ha mixed with *S*-metolachlor[10] at 1090 g ai/ha applied POST 1 followed by glufosinate or glyphosate at POST 2 and POST 3; glufosinate at 410 g/ha or glyphosate at 860 g/ha applied POST 1 and POST 2 followed by glufosinate or glyphosate plus trifloxysulfuron[11] at 5.25 g ai/ha applied POST 3; and all possible combinations of glufosinate at 205 and 410 g/ha mixed with glyphosate at 430 and 860 g/ha and applied at each of the three application timings. Three additional treatments, with glufosinate at 410 g/ha and glyphosate at 860 g/ha, consisted of glufosinate applied POST 1 followed by glyphosate at POST 2 and POST 3, glufosinate at POST 2 and glyphosate at POST 1 and POST 3, and glyphosate at POST 1 and POST 2 followed by glufosinate at POST 3. The treatment receiving glyphosate applied at 860 g/ha at POST 1, POST 2, and POST 3 was considered as the check in this experiment.

Experiment 2. This experiment was designed to evaluate PHY 485 WRF cotton response to glufosinate and ammonium sulfate (AMS) applied POST. Locations included the Upper Coastal Plain Research Station near Rocky Mount in 2008, the Tidewater Research Station near Plymouth in 2007, and the Central Crops Research Station near Clayton in 2007 and 2008. Treatments consisted of a factorial arrangement of glufosinate at 545 and 805 g/ha by AMS[12] at 0 and 3360 g/ha applied twice to 1- to 2-leaf cotton (POST 1) and six-leaf cotton (POST 2). An additional treatment, glyphosate at 860 g/ha applied POST 1 and POST 2, served as the check in this experiment.

Experiment 3. This experiment was designed to evaluate response of PHY 485 WRF cotton to glufosinate co-applied with insecticides that might be used for early season insect control. The experiment was conducted at the Peanut Belt Research Station near Lewiston in 2007, the Upper Coastal Plain Research Station near Rocky Mount in 2008, and the Central Crops Research Station near Clayton in 2008. Treatments consisted of a factorial arrangement of glufosinate at 0 and 410 g/ha by seven insecticide options. Treatments were applied twice to one-leaf (POST 1) and four-leaf (POST 2) cotton. Insecticide options included no insecticide, acephate[6] (280 g ai/ha), dicrotophos[13] (280 g ai/ha), dimethoate[14] (280 g ai/ha), imidacloprid[15] (50 g ai/ha), lambda-cyhalothrin[16] (30 g ai/ha), oxamyl[17] (500 g ai/ha), and thiamethoxam[18] (50 g ai/ha). A check, which did not receive glufosinate or insecticides, also was included.

Data collected and analysis. Cotton injury was estimated visually throughout the season using a scale of 0 to 100%, where 0 = no cotton injury and 100 = cotton death (Frans et al. 1986). Height of 30 consecutive plants in each plot was recorded 10 d after POST 3 in Experiment 1 and 10 d after POST 2 in Experiments 2 and 3. At least one week after cotton defoliation and before harvest, 10 consecutive plants in each of the two center rows of each plot were plant mapped. Plant mapping consisted of recording the presence of bolls based on main-stem node and sympodial fruiting position. Both open and green bolls were recorded and used to determine the percentage of open bolls as a measure of maturity. The height of each plant was also recorded. Boll production was segregated into four main-stem node zones, including nodes 4 to 7, nodes 8 to 11, nodes 11 to 13, and node14 and higher. Bolls on fruiting positions 2 and 3 were grouped together. The center two rows of each plot were

mechanically harvested and approximately 200 g of mechanically harvested seed cotton was collected from each plot and used for fiber quality determinations. Fiber length, length uniformity, fiber strength, and micronaire were determined by high-volume instrumentation testing (Sasser 1981).

Data were subjected to ANOVA using the PROC MIXED procedure of Statistical Analysis System[19]. Plots treated with glyphosate only were considered as checks and were excluded from the cotton injury data analysis. In Experiments 2 and 3, treatment sum of squares were partitioned to reflect factorial treatment arrangement. In all experiments, location and replication were considered random effects (McIntosh 1983). Means were separated with Fisher's Protected LSD at $P \leq 0.05$. Cotton injury data were arcsine square root transformed prior to analysis (Ahrens et al. 1990); non-transformed data are presented with statistical interpretation based upon transformed data.

Results and Discussion

Current glufosinate labeling allows for 412 to 543 g/ha to be applied three times per season to glufosinate-resistant cotton designated as Liberty Link cultivars, with a seasonal maximum rate of 1454 g/ha (Anonymous 2009). Alternatively, glufosinate can be applied at 561 to 805 g/ha followed by a second application of 412 to 543 g/ha, for a seasonal maximum rate of 1347 g/ha. Current labeling does not prohibit application to cultivars not designated as Liberty Link, but the label does warn of potential severe injury or crop death when glufosinate is applied to cotton cultivars not designated as Liberty Link (Anonymous 2009).

Experiment 1. Cotton injury, cotton yield, percentage open bolls, and plant mapping data were pooled over locations due to lack of a treatment by location interaction. A treatment by location interaction was observed for cotton height 10 d after the POST 3 application. Cotton injury was monitored daily after the initial glufosinate application. Greatest injury by glufosinate usually occurred 5 d after application, hence that time was selected for the initial evaluations. Glufosinate injury appeared as necrosis on cotton leaves exposed at the time of application. Severely injured leaves sometimes abscised, but leaves emerging after application showed no injury. Similar responses have been noted previously (Culpepper et al. 2009).

Glufosinate at 410 g/ha injured PHY 485 WRF cotton 6, 5 and 3% at 5 d after POST 1, POST 2, and POST 3 applications, respectively (Table 2). Injury was 5 to 9 percentage points greater when glufosinate at 820 g/ha was applied three times. However, glufosinate at 820 g/ha applied three times, a rate that is almost twice the seasonal maximum rate on Liberty Link cotton (Anonymous 2009), injured PHY 485 WRF cotton only 8% following the third application. Glufosinate at 410 g/ha applied once at the POST 1, POST 2, or POST 3 application timings injured cotton only 2% or less at 5 d after the POST 3 application timing.

Injury from combinations of glufosinate at 410 g/ha plus glyphosate at 430 or 860 g/ha was not different from injury by glufosinate applied alone (Table 2). Pyrithiobac at 36 g/ha mixed with glufosinate at 410 g/ha and applied POST 1 and POST 2 caused a minor increase (4 percentage points) in cotton injury 5 d after the POST 1 application but no increase in injury at 5 d after the POST 2 or POST 3 applications. However, injury by glufosinate plus

pyrithiobac exceeded the injury noted with glyphosate plus pyrithiobac at each evaluation. *S*-metolachor mixed with glufosinate at the POST 1 application increased injury 7 percentage points at 5 d after POST 1, but injury was similar with glufosinate alone and glufosinate plus *S*-metolachlor at 5 d after the POST 2 and POST 3 timings. Injury by glufosinate plus *S*-metolachlor exceeded the injury from glyphosate plus *S*-metolachlor at each evaluation. *S*-metolachlor caused little to no injury when mixed with glyphosate. Injury by glufosinate plus trifloxysulfuron applied at POST 3 exceeded the injury by glyphosate plus trifloxysulfuron, but injury by glufosinate plus trifloxysulfuron did not differ from the injury by glufosinate alone. Regardless of the treatment, cotton recovered from glufosinate injury. Injury at 28 d after POST 3 was less than 3% for all treatments (data not shown).

No treatment affected cotton height 10 d after the POST 3 application at Rocky Mount in 2008 (Table 2). Glufosinate at 820 g/ha applied three times reduced cotton height 13 to 18% compared to glufosinate at 410 g/ha and 14 to 24% compared to glyphosate at 860 g/ha at the three other locations. This is in agreement with the greater cotton injury observed previously. However, compared to glyphosate at 860 g/ha applied three times, glufosinate at 410 g/ha applied once at POST 1, POST 2, or POST 3 or applied at each of the three application timings did not affect cotton height. Cotton height also was unaffected by glufosinate at 410 g/ha mixed with glyphosate at 430 or 860 g/ha compared to either glufosinate alone at 410 g/ha or glyphosate at 860 g/ha. Pyrithiobac or *S*-metolachlor mixed with either glufosinate or glyphosate also did not impact cotton height compared to glufosinate or glyphosate applied alone. Trifloxysulfuron mixed with either glufosinate at

410 g/ha or glyphosate at 860 g/ha at POST 3 reduced cotton height 10 and 13%, respectively, at Rocky Mount in 2007 but had no effect at the other three locations.

No differences in seed cotton yield (Table 3) or percentage of lint (data not shown) were observed among treatments. Compared to glyphosate alone, pyrithiobac or *S*-metolachlor added to glyphosate did not affect cotton maturity measured as the percentage of open bolls (Table 3). Compared to glyphosate applied alone, glufosinate at 820 g/ha applied three times reduced the percentage of open bolls by 11 percentage points. However, glufosinate at 410 g/ha applied alone did not affect maturity relative to that with glyphosate alone. And, compared to glufosinate alone at 410 g/ha, mixing glyphosate, pyrithiobac, *S*-metolachlor, or trifloxysulfuron with glufosinate had no impact on crop maturity.

Cotton treated with glyphosate had 89% open bolls (Table 3). Most glufosinate treatments did not affect percent open boll; however, glufosinate applied at 940 g/ha, and glufosinate applied at 470 g/ha mixed with glyphosate at 840 g/ha, pyrithiobac, and trifloxysulfuron reduced open boll percentage similarly by 10 to 14%. Cotton treated with glyphosate produced 1.94 first position bolls per plant in nodes four through seven (Table 3). Cotton treated with glufosinate applied at 470 or 940 g/ha produced 0.24 and 0.33 fewer bolls per plant than cotton treated with glyphosate. All tank mixtures of glyphosate plus glufosinate, except when applied at 840 plus 240 g/ha, had 0.2 fewer bolls per plant in nodes 4-7. Cotton treated with pyrithiobac mixed with either glufosinate or glyphosate and glufosinate plus *S*-metolachlor had 0.21 to 0.31 fewer bolls in nodes four through seven. The number of first position bolls per plant in nodes eight to ten and eleven to thirteen was not affected by herbicide treatment. Glyphosate treated plants had 0.14 first position bolls per

plant on node14 and higher. Cotton treated with glufosinate applied at 940 g/ha or glufosinate at 470 g/ha plus pyrithiobac, *S*-metolachlor, or trifloxysulfuron had 0.2 to 0.31 more bolls per plant than cotton treated with glyphosate. Although glufosinate consistently injured cotton and some glufosinate treatments affected open boll percentage at harvest and may have shifted first position boll production higher in the plant, no glufosinate treatment adversely affected cotton yield (Table 3). Seed cotton yield averaged across all locations was 2510 to 2830 kg/ha. Glufosinate also did not adversely affect percent lint and fiber quality (data not shown).

Experiment 2. A treatment by location interaction was not observed in the factorial analysis of glufosinate rate by ammonium sulfate application regarding cotton injury, height and plant mapping data, therefore data were pooled. Regarding cotton injury and height, the main effects of glufosinate rate and ammonium sulfate were often significant; however the interaction of glufosinate rate and ammonium sulfate was not. Pooled over applications made with or without ammonium sulfate, glufosinate applied at 600 g/ha injured cotton 9% 5 days after POST 1, 5% 14 days after POST 2 and 4% 28 days after POST 3 (Table 4). Increasing glufosinate rate to 900 g/ha increased injury by at least six percentage points at each evaluation and reduced cotton height by 4 cm 10 days after POST 2, but no difference in height between glufosinate rates was observed at harvest. Averaged over glufosinate rates, combining ammonium sulfate with glufosinate did not increase cotton injury five days after POST 1, but did increase injury by four and two percentage points 14 and 28 days after POST 2 (Table 4). Mixing ammonium sulfate with glufosinate did not affect cotton height 10 days after POST 2 or at harvest.

Compared to glyphosate treated plants, cotton height accessed ten days after POST 2 was not affected by glufosinate applied alone at 600 g/ha, however it was reduced similarly by 32 to 57% when treated with glufosinate at 600 g/ha with ammonium sulfate and glufosinate applied 900 g/ha with or without ammonium sulfate (Table 5). At harvest plant height followed a similar trend, as glufosinate applied at 600 g/ha did not reduce height but the other glufosinate treatments reduced height by 8%.

Number of bolls produced per plant was affected by glufosinate (Table 6). Glyphosate treated plant had 7.5 bolls per plant. Plants treated with glufosinate at 600 g/ha with or without ammonium sulfate had 0.6 to 0.7 fewer bolls per plant compared to glyphosate treated plants. Glufosinate at 900 g/ha without ammonium sulfate also decreased boll number per plant by 0.5, but cotton treated with glufosinate at 900 g/ha plus ammonium sulfate had a similar number of bolls per plant as glyphosate treated plants. Cotton treated with glyphosate had 84% open bolls at harvest. Glufosinate at 900 g/ha with or without ammonium sulfate reduced open boll percentage by 12% compared to glyphosate treated plants. All glufosinate treatments decreased the number of vegetative bolls per plant compared to glyphosate. Also, glufosinate treated plants had few bolls on positions 2 and 3 in nodes four to seven compared to glyphosate treated plants.

No glufosinate treatment adversely affected cotton yield compared to glyphosate in Plymouth, Rocky Mount, and in Clayton during 2008 (Table 5). However, in Clayton during 2007 cotton treated with glyphosate yielded 4120 kg/ha. Glufosinate at 600 g/ha applied alone did not significantly reduce yield; however, mixing ammonium sulfate with glufosinate at 600 g/ha reduced yield by 24%. Glufosinate applied at 900 g/ha alone or mixed with

ammonium sulfate similarly decreased yields by 18 to 30%. At all locations, glufosinate and ammonium sulfate also did not adversely affect percent lint and fiber quality of samples taken at harvest (data not shown).

Experiment 3. Cotton injury data were pooled across locations since no location interaction was significant. At each evaluation, the main effect of glufosinate application was significant, but the main effect of insecticide along with the interaction of glufosinate and insecticide was not. Injury from glufosinate applied at 470 g/ha, averaged over seven insecticide options, was 6, 6, and 3% five days after POST 1 and five and 28 days after POST 2, respectively (Table 7). No injury was observed at any evaluation period averaged over insecticides when glufosinate was not applied. The main effect of insecticide on cotton injury was also not significant at any evaluation period (Table 7).

Analysis of cotton height ten days after POST 2 revealed no glufosinate by insecticide interaction. In Lewiston, the main effects of glufosinate application or insecticide treatment did not affected cotton height. In Clayton and Rocky Mount the main effect of glufosinate was significant, but the main effect of insecticide was not. Averaged over insecticides, glufosinate reduced cotton height by 32 and 5% in Clayton and Rocky Mount, respectively (Table 7).

At harvest cotton height was not affected by insecticides at any location. The effect of glufosinate was not significant in Lewiston or Rocky Mount, but in Clayton, averaged over insecticides, cotton height was reduced by 9% from glufosinate compared to no glufosinate at harvest (Table 7). Seed cotton yield was not affected by insecticides at any location (Table 8). Yield was not affected by glufosinate in Clayton or Rocky Mount, but glufosinate

reduced cotton yield by 4% in Lewiston. Additionally, no treatment affected number of bolls per plant, percent open boll at harvest, or any fiber quality parameter accessed in this experiment.

Blair-Kerth et al. (2001) reported that glufosinate applied to Liberty Link® cotton did not adversely affect plant height, total number of nodes, bolls per plant or boll positions. In this experiment glufosinate occasionally reduced plant height, percent open bolls, bolls per plant, number of vegetative bolls per plant. Glufosinate also may have reduced boll in the lower part of the plant, however, cotton likely compensated by either producing more bolls higher in the canopy or through some other mechanism not accessed in this study. Injury from glufosinate was increased when mixed with *S*-metolachlor, pyrithiobac and ammonium sulfate; however mixing insecticides with glufosinate did not additionally affect injury or yield. Although glufosinate applied POST consistently injured PHY 485 WRF cotton, it did not adversely affect fiber quality and generally did not affect yield. Until better performing Liberty Link® cultivars are released, or growers adopt cotton with Glytol® and Liberty Link traits, this cotton could fill a void in current management systems to combat or delay glyphosate-resistant weeds. Currently, neither of the companies producing glufosinate or Widestrike cotton recommends the practice of applying glufosinate POST to Widestrike Roundup Ready cotton.

Sources of Materials

[1] PHY 485 WRF cotton. Phytogen Seed Company L.L.C., 9330 Zionsville Road, Indianapolis, IN 46268.

[2] Prowl H2O herbicide, BASF Ag Products, 26 Davis Dr., Research Triangle Park, NC 27709-3528.

[3] Cotoran 4L herbicide, Griffin L.L.C., P.O. Box 1847, Valdosta, GA 31603.

[4] Roundup WEATHERMAX herbicide, Monsanto Company, St. Louis, MO 63167.

[5] TEMIK 15G pesticide, Bayer Cropscience, P.O. Box 12014, 2 T.W. Alexander Dr., Research Triangle Park, NC 27709.

[6] Orthene 97 soluble insecticide, Valent U.S.A. Corporation, Walnut Creek, CA 94596-8025.

[7] TeeJet XR11002 flat-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

[8] Ignite 280 SL herbicide, Bayer Cropscience, P.O. Box 12014, T.W. Alexander Dr., Research Triangle Park, NC 27709.

[9] Staple herbicide, Dupont Crop Protection Co., Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898.

[10] Dual Magnum herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[11] Envoke herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[12] Ammonium sulfate, Fisher Scientific, 1 Reagent Lane, Fair Lawn, NJ 07410.

[13] Bidrin 8 water miscible insecticide, AMVAC Chemical Co., 4100 E. Washington Blvd., Los Angeles, CA 90023.

[14] Dimethoate systemic insecticide – miticide, Drexel Chemical Co., P.O. Box 13327, Memphis, TN 38113-0327.

[15] Trimax PRO insecticide, Bayer Cropscience, P.O. Box 12014, 2 T.W. Alexander Dr., Research Triangle Park, NC 27709.

[16] Karate with Zeon Technology insecticide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[17] Vydate C-LV insecticide/nematicide, Dupont Crop Protection Co., Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898.

[18] Centric insecticide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[19] Statistical Analysis Systems, version 9.1, SAS Institute Inc., SAS Campus Drive, Cary, NC, 27513.

Acknowledgment

The authors would like to thank Rick Seagroves and Jamie Hinton for technical support.

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Table 1. Soil information and planting dates at experiment sites.

Experiment and location	Soil series	Soil texture	Soil humic matter[d]	Soil pH	Planting date
			%		
Experiment 1					
Lewiston, 2007	Lynchburg[a]	Sandy loam	0.97	5.5	4/30
Rocky Mount, 2007	Norfolk[b]	Loamy sand	0.41	5.8	5/1
Clayton, 2008	Norfolk	Loamy sand	0.51	5.4	5/14
Rocky Mount, 2008	Norfolk	Loamy sand	0.36	5.9	5/6
Experiment 2					
Clayton, 2007	Norfolk	Loamy sand	1.08	5.8	4/27
Plymouth, 2007	Cape fear[c]	Loam	3.37	5.9	5/22
Clayton, 2008	Norfolk	Loamy sand	1.31	5.6	5/14
Rocky Mount, 2008	Norfolk	Loamy sand	0.36	5.9	5/6
Experiment 3					
Lewiston, 2007	Lynchburg	Sandy loam	0.97	5.5	4/30
Clayton, 2008	Norfolk	Loamy sand	0.76	5.5	5/14
Rocky Mount, 2008	Norfolk	Loamy sand	0.36	5.9	5/6

[a] Fine-loamy, siliceous, semiactive, thermic Aeric Paleaquults.

[b] Fine-loamy, Koalinitic, thermic, Typic Kandiudults

[c] Fine, mixed, semiactive, thermic, Typic Umbraquults

[d] Soil humic matter was determined by the North Carolina Department of Agriculture and Consumer Services, Agronomic Division, as described by Mehlich (1984).

Table 2. Cotton injury 5 d after POST 1, POST 2, and POST 3, applications and cotton height 10 d after POST 3 application. Experiment 1.[a]

Herbicides, application rates, and application times[b,c]			Cotton injury			Cotton height			
						Clayton	Lewiston	Rocky Mount	
POST	POST 2	POST 3	POST 1	POST 2	POST 3	2008	2007	2007	2008
g/ha			%			cm			
Glyp, 860	Glyp, 860	Glyp, 860	--[d]	--	--	62 abc	43 a-d	48 a-e	36 a
Gluf, 410	Gluf, 410	Gluf, 410	6 cd	5 bc	3 bcd	57 bcd	43 a-d	45 c-g	35 a
Gluf, 820	Gluf, 820	Gluf, 820	15 a	12 a	8 a	47 e	37 e	39 h	36 a
Gluf, 410	Glyp, 860	Glyp, 860	5 de	2 ef	1 ef	56 b-e	41 bcd	48 a-d	34 a
Glyp,860	Gluf,410	Glyp, 860	--	3 de	1 ef	56 b-e	43 a-d	50 abc	36 a
Glyp, 860	Glyp, 860	Gluf, 410	--	--	2 cde	64 ab	44 abc	46 b-f	37 a
Glyp + Gluf, 430 + 205	Glyp + Gluf, 430 + 205	Glyp + Gluf, 430 + 205	3 ef	1 fg	0 ef	62 ab	45 abc	48 abc	36 a
Glyp + Gluf, 430 + 410	Glyp + Gluf, 430 + 410	Glyp + Gluf, 430 + 405	6 cd	6 bc	4 b	53 cde	41 a-d	45 c-g	37 a
Glyp + Gluf, 860 + 205	Glyp + Gluf, 860 + 205	Glyp + Gluf, 860 + 205	4 ef	3 e	1 ef	71 a	43 a-d	51 ab	37 a
Glyp + Gluf, 860 + 410	Glyp + Gluf, 860 + 410	Glyp + Gluf, 860 + 410	8 bc	7 bc	4 b	54 cde	42 a-d	44 d-h	37 a

Table 2. Continued

Glyp + pyri, 860 + 36	Glyp + pyri, 860 + 36	Glyp, 860	3 fg	3 e	1 ef	57 bcd	41 cd	48 a-e	36 a
Gluf + pyri, 410 + 36	Gluf + pyri, 410 + 36	Gluf, 410	10 b	7 bc	3 bc	56 b-e	40 de	43 e-h	33 a
Glyp + S-met, 860 + 1090	Glyp, 860	Glyp, 860	2 g	1 g	0 f	65 ab	45 a	53 a	37 a
Gluf + S-met, 410 + 1090	Gluf, 410	Gluf, 410	13 a	8 b	5 b	59 bcd	42 a-d	41 gh	36 a
Glyp, 860	Glyp, 860	Glyp + trif, 860 + 5.25	--	--	2 cde	57 bcd	45 ab	43 fgh	34 a
Gluf, 410	Gluf, 410	Gluf + trif, 410 + 5.25	5 cde	5 cd	6 ab	51 de	42 a-d	39 h	34 a

[a] Data for cotton injury averaged over four locations. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] Abbreviations: glyp, glyphosate; gluf, glufosinate; pyri, pyrithiobac; S-met, *S*-metolachlor; trif, trifloxysulfuron.

[c] All treatments received pendimethalin (1100 g/ha) and fluometuron (1100 g/ha) applied PRE. POST 1 applied to 1- to 2- leaf cotton. POST 2 applied to 6- to 7-leaf cotton. POST 3 applied to 10- to 12-leaf cotton.

[d] The treatment which received only glyphosate was considered as the check for injury estimates. The check was not included in the analysis

Table 3. Seed cotton yield, percent open bolls, and first position sympodial boll production by PHY 485 WRF cotton as affected by POST applications. Experiment 1.[a]

Herbicides, application rates, and application times[b,c]			Seed cotton	Open	First position sympodial boll production			
					Nodes	Nodes	Nodes	Nodes
POST 1	POST 2	POST 3	yield	bolls	4-7	8-10	11-13	≥ 14
g/ha			kg/ha	%	no./plant			
Glyp, 860	Glyp, 860	Glyp, 860	2680 a	89 a	1.94 a	1.70 a	0.56 a	0.14 d
Gluf, 410	Gluf, 410	Gluf, 410	2690 a	85 ab	1.70 cd	1.93 a	0.65 a	0.29 a-d
Gluf, 820	Gluf, 820	Gluf, 820	2790 a	78 c	1.61 d	1.80 a	0.82 a	0.37 ab
Gluf, 410	Glyp, 860	Glyp, 860	2510 a	89 a	1.79 a-d	1.68 a	0.45 a	0.15 d
Glyp,860	Gluf,410	Glyp, 860	2710 a	88 a	1.89 abc	1.99 a	0.68 a	0.22 bcd
Glyp, 860	Glyp, 860	Gluf, 410	2750 a	86 ab	1.92 ab	1.88 a	0.65 a	0.25 bcd
Glyp + Gluf, 430 + 205	Glyp + Gluf, 430 + 205	Glyp + Gluf, 430 + 205	2720 a	85 ab	1.74 bcd	1.95 a	0.60 a	0.25 bcd
Glyp + Gluf, 430 + 410	Glyp + Gluf, 430 + 410	Glyp + Gluf, 430 + 405	2730 a	86 ab	1.74 bcd	1.74 a	0.55 a	0.17 d
Glyp + Gluf, 860 + 205	Glyp + Gluf, 860 + 205	Glyp + Gluf, 860 + 205	2780 a	85 ab	1.76 a-d	1.92 a	0.76 a	0.27 bcd
Glyp + Gluf, 860 + 410	Glyp + Gluf, 860 + 410	Glyp + Gluf, 860 + 410	2790 a	81 bc	1.74 bcd	1.72 a	0.72 a	0.29 a-d

Table 3. Continued

Glyp + pyri, 860 + 36	Glyp + pyri, 860 + 36	Glyp, 860	2530 a	85 ab	1.63 d	1.76 a	0.67 a	0.16 d
Gluf + pyri, 410 + 36	Gluf + pyri, 410 + 36	Gluf, 410	2830 a	81 bc	1.64 d	1.80 a	0.83 a	0.45 a
Glyp + S-met, 860 + 1090	Glyp, 860	Glyp, 860	2740 a	85 ab	1.87 abc	1.84 a	0.60 a	0.27 bcd
Gluf + S-met, 410 + 1090	Gluf, 410	Gluf, 410	2700 a	84 abc	1.73 bcd	1.89 a	0.71 a	0.34 abc
Glyp, 860	Glyp, 860	Glyp + trif, 860 + 5.25	2720 a	87 ab	1.93 ab	1.85 a	0.71 a	0.20 cd
Gluf, 410	Gluf, 410	Gluf + trif, 410 + 5.25	2760 a	81 bc	1.88 abc	1.96 a	0.83 a	0.36 abc

[a] Means within a location followed by the same letter are not different according to Fisher's Protected LSD test at P = 0.05.

[b] Abbreviations: glyp, glyphosate; gluf, glufosinate; pyri, pyrithiobac; S-met, *S*-metolachlor; trif, trifloxysulfuron.

[c] All treatments received pendimethalin (1100 g/ha) and fluometuron (1100 g/ha) applied PRE. POST 1 applied to 1- to 2- leaf cotton. POST 2 applied to 6- to 7-leaf cotton. POST 3 applied to 10- to 12-leaf cotton.

Table 4. Effect of glufosinate rate and ammonium sulfate on cotton injury and height. Experiment 2.[a]

POST Main effect	Cotton injury			Cotton height	
Glufosinate rate[b]	5 da POST 1	14 da POST 2	28 da POST 2	10 da POST 1	At harvest
	%			cm	
600 g/ha	9 b	5 b	4 b	27 a	83 a
900 g/ha	17 a	16 a	10 a	23 b	79 a
Ammonium sulfate[c]					
None	11 a	9 b	6 b	27 a	81 a
340 g/ha	15 a	13 a	8 a	24 a	80 a

[a] Data pooled across four locations. Means within main effect and evaluation period followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$. POST applications made twice to 1- to 2-leaf and 6-leaf cotton. All plots receieved a lay-by application of glyphosate applied at 840g/ha.

[b] Data pooled over two ammonium sulfate options (none and 340 g/ha).

[c] Data pooled over two glufosinate rates (600 and 900g/ha).

Table 5. Cotton height, and boll production at harvest from glufosinate and ammonium sulfate applied POST. Experiment 2.[a]

POST application[b]		Cotton height		Seed cotton yield			
		10 d after	At	2007		2008	
Treatment	Rates	6-leaf	harvest	Clayton	Plymouth	Clayton	Rocky Mount
	g/ha	cm		kg/ha			
Glyphosate	840	33 a	86 a	4120 a	3520 a	4630 a	1950 a
Glufosinate	600	28 ab	84 ab	3870 ab	3240 a	4530 a	1760 a
Glufosinate + ammonium sulfate	600 + 340	25 bc	79 c	3170 c	3400 a	4680 a	1750 a
Glufosinate	900	25 bc	80 bc	3310 bc	3230 a	4640 a	1820 a
Glufosinate + ammonium sulfate	900 + 340	21 c	79 c	3490 bc	2990 a	4710 a	1880 a

[a] Data pooled across four locations. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at P = 0.05.

[b] Treatments applied POST to 1- to 2-leaf and repeated at 6-leaf cotton. All plots receieved a lay-by application of glyphosate applied at 860 g/ha POST-directed to cotton.

Table 6. Boll production affected by glufosinate and ammonium sulfate. Experiment 2.[a]

POST application[b]		Boll production			
Treatment	Rates	Total bolls	Open bolls	Vegetative bolls	Second position nodes 4-7[d]
	g/ha	no./plant	%	—— no./plant ——	
Glyphosate	840	7.5 a	84 a	0.75 a	0.67 a
Glufosinate	600	6.8 b	84 ab	0.36 b	0.52 b
Glufosinate + AMS[c]	600 + 340	6.9 b	78 ab	0.34 b	0.48 b
Glufosinate	900	7.0 b	75 b	0.46 b	0.47 b
Glufosinate + AMS	900 + 340	7.6 a	75 b	0.39 b	0.50 b

[a] Data pooled across four locations. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at P = 0.05.

[b] Treatments applied POST to 1- to 2-leaf and repeated at 6-leaf cotton. All plots receieved a lay-by application of glyphosate applied at 860 g/ha POST-directed to cotton.

Table 7. Cotton injury and seed cotton height affected by glufosinate and insecticides applied POST. Experiment 3.[a]

POST Main effect[b]		Cotton injury			Cotton height				
					10 daPOST 2[f]		At harvest		
Herbicide[c]	Insecticide[d]	5 da POST 1[e]	5 da POST 2[e]	28 da POST 2[e]	Clayton	Rocky Mount	Clayton	Lewiston	Rocky Mount
		%			cm				
None		0 b	0 b	0 b	33 a	19 a	105 a	69 a	68 a
Glufosinate		6 a	6 a	3 a	25 b	18 b	96 b	67 a	68 a
	Acephate	3 a	4 a	1 a	31 a	18 a	100 a	69 a	68 a
	Dicrotophos	3 a	3 a	1 a	31 a	18 a	101 a	68 a	67 a
	Dimethoate	5 a	3 a	1 a	30 a	18 a	103 a	68 a	69 a
	Imidacloprid	3 a	3 a	2 a	27 a	19 a	99 a	65 a	70 a
	Lambda-cyhalothrin	3 a	3 a	2 a	28 a	19 a	103 a	68 a	66 a
	Oxamyl	3 a	3 a	2 a	29 a	20 a	100 a	69 a	90 a
	Thiamethoxam	3 a	3 a	2 a	27 a	18 a	99 a	67 a	69 a

Table 7. Continued

[a] Means within each main effect of herbicide or insecticide followed by the same letter are not different according to Fisher's Protected LSD test at P = 0.05. All treatments received pendimethalin (1.1 kg/ha) and fluometuron (1.1 kg/ha) applied PRE and a lay-by application of glyphosate applied at 0.84 kg/ha.

[b] POST applications made twice to 1-leaf and 4-leaf cotton.

[c] Glufosinate options (none and applied at 0.47 kg/ha) averaged over seven insecticides.

[d] Insecticide option (acephate, dichrotophos, and dimethoate applied at 0.28 kg/ha, imidacloprid at 0.05 kg/ha, lambda-cyhalothrin applied at 0.03 kg/ha, oxamyl applied at 0.5 kg/ha, and thiamethoxam applied at 0.05 kg/ha) averaged over two glufosinate options.

[e] Data pooled over three locations.

[f] Cotton height not recorded in Lewiston 10 days after POST 2.

Table 8. Boll production and cotton yield affected by glufosinate and insecticides applied POST. Experiment 3.[a]

POST Main effect[b]		1st Position Boll Production[e]						
		Nodes 4-7			Nodes 14+	Seed cotton yield		
Herbicide[c]	Insecticide[d]	Clayton	Lewiston	Rocky Mount		Clayton	Lewiston	Rocky Mount
		kg/ha				kg/ha		
None		1.1 b	2.3 a	1.5 a	0.4 b	3920 a	3390 a	1940 a
Glufosinate		1.4 a	2.3 a	1.6 a	0.5 a	4070 a	3260 b	1860 a
	Acephate	1.5 a	2.3 a	1.6 a	0.4 a	3850 a	3380 a	1840 a
	Dicrotophos	1.2 ab	2.3 a	1.6 a	0.5 a	4000 a	3340 a	1810 a
	Dimethoate	1.2 ab	2.3 a	1.4 b	0.5 a	4110 a	3260 a	1900 a
	Imidacloprid	1.4 a	2.3 a	1.7 a	0.5 a	4030 a	3190 a	1890 a
	Lambda-cyhalothrin	1.4 a	2.2 a	1.6 a	0.5 a	4100 a	3230 a	1990 a
	Oxamyl	1.0 b	2.2 a	1.5 ab	0.5 a	4010 a	3430 a	1960 a
	Thiamethoxam	1.4 a	2.4 a	1.7 a	0.5 a	4000 a	3440 a	1920 a

Table 8. Continued.

[a] Means within each main effect of herbicide or insecticide followed by the same letter are not different according to Fisher's Protected LSD test at P = 0.05. All treatments received pendimethalin (1.1 kg/ha) and fluometuron (1.1 kg/ha) applied PRE and a lay-by application of glyphosate applied at 0.84 kg/ha.

[b] POST applications made twice to 1-leaf and 4-leaf cotton.

[c] Glufosinate options (none and applied at 0.47 kg/ha) averaged over seven insecticides.

[d] Insecticide option (acephate, dichrotophos, and dimethoate applied at 0.28 kg/ha, imidacloprid at 0.05 kg/ha, lambda-cyhalothrin applied at 0.03 kg/ha, oxamyl applied at 0.5 kg/ha, and thiamethoxam applied at 0.05 kg/ha) averaged over two glufosinate options.

[e] Boll production in nodes 4-7 presented by location. Boll production in nodes 14 and higher are pooled over three locations.

CHAPTER VII

Weed Management with Glyphosate and Glufosinate in Widestrike Cotton

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Abstract

Glyphosate-resistant (GR) Widestrike™ cotton contains different genes that confer both glyphosate and glufosinate resistance. An experiment was conducted to evaluate weed control and crop tolerance with systems including postemergence treatments of glyphosate and glufosinate applied alone, combined with pyrithiobac or *S*-metolachlor, and tank mixtures of glyphosate and glufosinate followed by a lay-by application of diuron and MSMA. Annual grasses and glyphosate-susceptible Palmer amaranth were controlled more consistently by glyphosate than glufosinate. Co-application of glyphosate and glufosinate was no more effective than glyphosate alone on any weed species, and they reduced control of glyphosate-susceptible Palmer amaranth and annual grasses compared with glyphosate alone. However, co-application of glyphosate and glufosinate was often more effective than glufosinate alone. Control of GR Palmer amaranth by glufosinate-based systems and

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glufosinate plus glyphosate combinations was 13 to 30% greater than glyphosate-based systems. Glufosinate injured cotton 3 to 10% 5 d after application. Pyrithiobac and *S*-metolachlor increased injury by 2 to 11 percentage points compared to glufosinate alone. However, glufosinate alone, or glufosinate mixed glyphosate, pyrithiobac, or *S*-metolachlor did not adversely affect cotton yield or fiber quality.

Nomenclature: Cotton, *Gossypium hirsutum* L. 'PHY 485 WRF'; diuron; Glufosinate; glyphosate; MSMA; Palmer amaranth, *Amaranthus palmeri* S. Wats.; pyrithiobac; *S*-metolachlor.

Key words: herbicide-resistant crops, herbicide tolerance.

Abbreviations: Gluf, glufosinate; glyp, glyphosate; GR, glyphosate-resistant; POST, postemergence; pyri, pyrithiobac; S-met, *S*-metolachlor.

Introduction

Glyphosate-resistant (GR) technology (trade name Roundup Ready®) has revolutionized weed management in cotton. Broadspectrum weed control, connivance of overtop application, increased rotational options, and reductions in labor and time are reasons growers have readily adopted this technology (Askew et al. 2002; Culpepper and York 1998, 1999; Faircloth et al. 2001; Gianessi 2008; Young 2006). The percentage of cotton planted with GR cultivars has increased from 4% of US hectares in 1997 to 80% in 2005 (Sankula 2006). In North Carolina alone, GR cultivars accounted for over 99% of planted cotton in 2008 (USDA-AMS 2008).

Palmer amaranth is the most troublesome weed for cotton producers in the southeastern U.S. (Webster 2005). It has an extremely high photosynthetic rate and can grow more than five cm per day under full light (Ehleringer 1983; Horak and Loughin 2000). Palmer amaranth can reach more than two m in height (Sellers et al. 2003) and can reduce cotton yield dramatically if not controlled (Bensch et al. 2003; MacRae et al. 2008; Morgan et al. 2001; Rowland et al. 1999). Additionally, Palmer amaranth present at harvest impedes mechanical cotton harvest (Smith et al. 2000).

Glyphosate is extremely efficacious on emerged Palmer amaranth (Bond et al. 2006; Corbett et al. 2004). However, if not controlled season-long, the continued emergence of Palmer amaranth throughout the season coupled with its prolific seed production allows it to quickly replenish seed banks (Keely et al. 1987; Sellers et al. 2003). Effective herbicide systems usually require multiple applications of glyphosate (Culpepper et al. 2000; Grichar et al. 2004; Keeling et al. 2004; Kendig and Nichols 2005), but glyphosate-only systems have effectively controlled Palmer amaranth in cotton (Culpepper and York 1998; Scott et al. 2002).

Extensive use of glyphosate in GR cultivars has placed intense selection pressure for the evolution of weed resistance. The first case of glyphosate-resistant Palmer amaranth was reported by Culpepper et al. (2006) in 2005. By 2008, GR Palmer amaranth has been documented in Arkansas, North Carolina, South Carolina, and Tennessee (Heap 2009; Norsworthy et al. 2008; Steckel et al. 2008; York et al. 2007). In Georgia and North Carolina alone, glyphosate-resistant Palmer amaranth infests an estimated 120,000 and 75,000 ha, respectively (Culpepper et al. 2008).

Management of GR Palmer amaranth in cotton will likely be largely dependent on use of preplant or PRE herbicides (Culpepper et al. 2007; Whitaker et al. 2008). Without irrigation to activate these PRE herbicides, control of Palmer amaranth is often erratic, and very few herbicides applied POST control GR Palmer amaranth which escapes PRE herbicide applications. Pyrithiobac, an acetolactate synthase (ALS)-inhibiting herbicide, can be safely applied POST to control Palmer amaranth in cotton (Dotray et al. 1996). However, resistance to ALS-inhibiting herbicides is common throughout the U.S. (Bond and Oliver 2006; Heap 2009; Horak and Peterson 1995; Sprague et al. 1997), and a Palmer amaranth biotype with multiple resistance to glyphosate and pyrithiobac has been documented in Georgia and suspected in North Carolina (Sosnoskie et al. 2009; Whitaker, 2009).

Glufosinate is a non-selective herbicide which can be applied POST on glufosinate-resistant (trade name Liberty Link®) cotton (Blair-Kerth et al. 2001). This genetically modified cotton was created by the insertion and expression of a bialaphos resistance (*bar*) gene isolated from the soil bacterium *Streptomycyes hygroscopius* which encodes for the phosphinothricin acetyl transferase (PAT) enzyme. The PAT enzyme detoxifies the L-isomer of glufosinate into an inactive form (Devine et al. 1993; Tsaftaris 1996). Liberty Link® cotton has excellent tolerance to glufosinate and can be applied POST from emergence until the early bloom stage (Blair-Kerth et al. 2001; Anonymous 2008).

Glufosinate is typically less effective than glyphosate on GS Palmer amaranth (Corbett et al. 2004; Price et al. 2007). However, when applied timely, glufosinate can effectively control Palmer amaranth (Culpepper et al. 2000; Gardner et al. 2006; Norsworthy et al. 2008). Glufosinate-based herbicide systems have been more effective than glyphosate-based

systems on GR Palmer amaranth (Culpepper et al. 2008; MacRae et al. 2008), but Liberty Link cultivars have not been adopted in the Southeast (USDA-AMS 2008). This is likely due to traditionally higher yield potential from Roundup Ready cultivars, coupled with difficulty in controlling *Amaranthus* spp. and annual grasses with glufosinate (Gardner et al. 2006), some of the most common and troublesome weeds in the region (Webster 2005).

Cotton with the Widestrike™ trait has two genes which confer resistance to lepiodopteran pests (Thompson et al. 2005). During transformation of this cotton, both inserted genes contained a phosphinothricin acetyl transferase (*pat*) gene. The *pat* gene was isolated from *Streptomyces viridochromogenes* (Agbios 2005). Liberty Link cotton contains a *bar* gene, however both the *pat* gene and the *bar* genes code for a phosphinothricin acetyl transferase enzyme which are very similar at the nucleotide level and have very similar functional characteristics (Wehrmann et al. 1996). The *pat* gene in Widestrike cotton was used as a selectable marker to detect successful transformation of the lepidopteran insect resistance genes (Agbios 2005).

Bayer Cropscience has developed a GR cotton technology (trade name Glytol®). Glytol cotton, expected to be released in 2009, has season-long tolerance of glyphosate (Trolinder et al. 2008). Stacked cultivars with both Glytol and Liberty Link traits are expected to be released in 2010 (Henniger et al. 2009).

Several cultivars are now commercially available that have the Widestrike technology stacked with Roundup Ready and Roundup Ready Flex technologies. In these cultivars, it may be possible to use both glyphosate and glufosinate for weed control. Due to the limited data regarding potential herbicide systems in these cultivars, coupled with issues surrounding

control of GR Palmer amaranth, research was conducted to evaluate weed control with glufosinate- and glyphosate-based systems along with combinations of glufosinate and glyphosate and to determine tolerance of Widestrike cotton to glufosinate applied POST.

Materials and Methods

The experiment was conducted in a total of six sites in North Carolina during 2007 and 2008. Sites included two fields at the Central Crops Research Station near Clayton (hereafter referred to as North and South Clayton), two fields at the Coastal Plain Research Station near Rocky Mount (hereafter referred to as North and South Rocky Mount), one field at the Tidewater Research Station near Plymouth, and one field on a private farm near Mount Olive. Seedbeds were prepared conventionally at all locations except North Rocky Mount. At this location, cotton was planted in a strip tillage system with paraquat[1] (1.1 kg/ha) applied preemergence (PRE) to desiccate a wheat cover crop (*Triticum aestivum* L.) and emerged weeds. A cultivar containing both the Widstrike and Roundup Ready Flex traits 'PHY 485 WRF'[2], was planted at all locations. Plot size was four rows (0.9 m spacing) by 9 m. Soil information and planting dates at each site are described in Table 1. Except for weed control, cotton was grown using production practices standard for the area.

The experimental design was a randomized complete block with treatments replicated four times. Treatments consisted of nine herbicide systems and a non-treated check. Six treatments consisted of glufosinate- or glyphosate-based systems. These treatments included either glyphosate[3] applied at 0.84 kg ae/ha or glufosinate[4] applied at 0.47 kg ai/ha applied alone, with pyrithiobac[5] (0.05 kg ai/ha), or with *S*-metolachlor[6] (1.07 kg ai/ha) applied to

cotyledon to 2-leaf cotton (POST 1) followed by either glyphosate or glufosinate alone at the same rates applied to 4- to-6-leaf cotton (POST 2). Another set of treatments included tank mixtures of glyphosate plus glufosinate applied at 0.84 plus 0.24 kg/ha, 0.42 plus 0.47 kg/ha, and 0.84 plus 0.47 kg/ha, respectively, applied at POST 1 and POST 2. These rates correspond to labeled rates and one-half of the labeled rates for glyphosate (0.84 kg/ha, 1X) and glufosinate (0.47 kg/ha, 1X), and these treatments represent glyphosate at 1X plus glufosinate at 1/2X rate, glyphosate at 1/2X rate plus glufosinate at 1X rate, and glyphosate at 1X plus glufosinate at 1X. A lay-by application of diuron[7] plus MSMA[8] (1.12 kg ai/ha plus 2.2 kg ai/ha) with crop oil concentrate[9] at 0.5% (v/v) was included in all treatments except the non-treated check and was applied when cotton reached 30- to 45-cm in height.

Postemergence herbicides were applied when weeds were 5- to 8-cm in height (Table 1). Weed species and densities at each location are described in Table 2. Herbicides were applied with a CO_2–pressurized backpack sprayer equipped with flat-fan nozzles[10] calibrated to deliver 140 L/ha at 160 kPa.

Cotton injury was estimated immediately prior to, 5 d, and 14 d after herbicide applications. Cotton treated with glyphosate alone used as check for visually estimating cotton injury. Foliar chlorosis, discoloration, and plant stunting was considered when estimating injury. Weed control was estimated immediately prior to, and two weeks after each herbicide application and late-season. Late-season weed control was estimated in September. Visual estimates were based on a scale of 0 to 100%, where 0 = no cotton injury or no weed control and 100 = cotton death or complete weed control (Frans et al. 1986). The center two rows of each plot were mechanically harvested. A 200-g sample of seed cotton

was collected from each plot and used for fiber quality determinations. Fiber length, length uniformity, fiber strength, and micronaire were determined by high-volume instrumentation testing (Sasser 1981).

Statistical Analyses. Data for cotton injury, weed control, cotton yield, and fiber quality were subjected to ANOVA using the PROC MIXED procedure of Statistical Analysis System[11]. Locations and replications were considered random effects (McIntosh 1983). Data from non-treated plots were excluded from weed control and cotton yield and fiber quality analyses (non-treated plots were unharvestable in all locations). With respect to cotton injury both non-treated plots and glyphosate-only treated plots were excluded from the analysis since glyphosate alone did not injury cotton. Data for weed control and cotton injury were arcsine square transformed prior to analysis (Ahrens et al. 1990); non-transformed data are presented with statistical interpretation based upon transformed data. Means were separated with Fisher's Protected LSD at $P \leq 0.05$. A separate ANOVA with treatment sums of squares partitioned to reflect the factorial treatment arrangement of the glyphosate- and glufosinate-based systems, and means from significant effects were separated with Fisher's Protected LSD at $P \leq 0.05$. Late-season weed control data is presented with reference to early season control as needed. Data were pooled if location-by-treatment effects were not significant; otherwise data are presented by location.

Results and Discussion

Weed Control. *Grasses.* Fall panicum (*Panicum dichotomiflorum*) and large crabgrass (*Digitaria sanguinalis*) were controlled completely by all herbicide systems late in the season

at Plymouth and North Rocky Mount, respectively (data not shown). At North Clayton before POST 2, glufosinate treatments were at least 7% less effective than glyphosate treatments in controlling large crabgrass and goosegrass (*Elusine indica*) (data not shown). Glyphosate plus glufosinate applied at 0.84 plus 0.47 kg/ha was equally effective as glyphosate alone and 10% more effective than glufosinate alone before POST 2. However, control from glyphosate plus glufosinate tank mixtures applied at 0.84 plus 0.24 kg/ha or at 0.42 plus 0.47 kg/ha were 8% less effective than glyphosate applied alone. Late in the season, glyphosate- and glufosinate-based systems were 94 to 100% effective late-season and tank mixtures of glyphosate plus glufosinate were 90 to 99% effective (Table 3). Annual grass control was similar from all treatments, except that glufosinate alone and glyphosate plus glufosinate applied at 0.42 plus 0.47 kg/ha were 6 and 11% less effective than glyphosate plus *S*-metolachlor late-season.

Annual grass control at South Clayton and South Rocky Mount during 2008 was pooled due a lack of treatment-by-location interaction (Table 3). Annual grasses consisted of large crabgrass and goosegrass at South Clayton and consisted of large crabgrass, goosegrass, fall panicum, and crowfootgrass (*Dactyloctenium aegyptium*) at South Rocky Mount. At these two locations, all glufosinate-based systems were at least 5% less effective than glyphosate-based systems. Glufosinate alone was 14% less effective than glyphosate applied alone. Mixing pyrithiobac or *S*-metolachlor with glufosinate increased control by 9% compared to glufosinate applied alone. Including *S*-metolachlor with glyphosate only slightly increased control by 2 percentage points. Tank mixtures of glyphosate plus glufosinate controlled annual grasses 92 to 96% and all were at least 8% more effective than glufosinate alone.

Mixing glufosinate at 0.47 kg/ha with glyphosate at either rate decreased annual grass control compared to glyphosate alone. The tank mixture of glufosinate applied at 0.24 kg/ha with glyphosate at 0.84 kg/ha was equally effective as glyphosate alone.

Broadleaf weeds. Carpetweed (*Mollugo verticillata*), common lambsquarters (*Chenopodium album*), common ragweed (*Ambrosia artemisiifolia*), tall morningglory (*Ipomoea purpurea*), pitted morningglory (*Ipomoea lacunosa*), entireleaf morningglory (*Ipomoea hederacea* var. *integruiscula*), and sicklepod (*Cassia obtusifolia*) were completely controlled late-season by all herbicide programs (data not shown). Redroot pigweed (*Amaranthus retroflexus*) was also entirely controlled by all herbicide systems (data not shown). At South Clayton and North Rocky Mount, glyphosate-based systems were 99 to 100% effective on glyphosate-susceptible Palmer amaranth (Table 4). Applied alone, glufosinate was 28 and 5% less effective than glyphosate at South Clayton and North Rocky Mount, respectively. Mixing pyrithiobac or *S*-metolachlor with glufosinate at North Rocky Mount increased Palmer amaranth control to 99 and 98%, similar to that from glyphosate-based systems. At South Clayton glufosinate plus *S*-metolachlor was not more effective than glufosinate alone and pyrithiobac increased control by 9%, but both were less effective than glyphosate-based systems. Palmer amaranth control from tank mixtures of glyphosate plus glufosinate were equally effective as glyphosate alone and more effective than glufosinate alone at North Rocky Mount. At South Clayton, Palmer amaranth control was reduced at least 7% when glufosinate was tank mixed with glyphosate.

Glyphosate applied alone was only 64% effective in controlling GR Palmer amaranth (Table 4). Within systems, control of GR Palmer amaranth from glufosinate was 13 to 30%

higher than glyphosate-based systems. Mixing pyrithiobac with glyphosate or glufosinate increased GR Palmer amaranth control by 33 and 15%, respectively. Glyphosate or glufosinate plus *S*-metolachlor were similarly effective as glyphosate or glufosinate plus pyrithiobac, but not more effective than glyphosate or glufosinate alone. All three tank mixtures of glyphosate plus glufosinate were at least 30% more effective than glyphosate alone. Glyphosate plus glufosinate applied at 0.84 plus 0.47 kg/ha, controlled GR Palmer amaranth 94% and was 13% more effective than glufosinate alone. Decreasing the glyphosate rate in the tank mixture did not decrease control, however decreasing the glufosinate rate decreased control by 13%.

Cotton Response. A location by treatment interaction prevented cotton injury from being pooled; therefore, data are presented by location. Glufosinate applied alone injured cotton 3 to 10% five days after application (Table 5). Glufosinate injury appeared as necrosis on cotton leaves exposed at the time of application. Leaves which appeared after application showed no injury symptoms. Decreasing cotton size and treatment application while dew is present tended to increase glufosinate injury. Cotton injury observed from glyphosate plus pyrithiobac or *S*-metolachlor was 4% or less in four locations five days after POST 1. At Mount Olive, glyphosate plus pyrithiobac injured cotton 11%; at South Rocky Mount both pyrithiobac and *S*-metolachlor plus glyphosate injured cotton 6 to 7%. In all locations except at North Rocky Mount, mixing pyrithiobac with glufosinate increased injury compared to glufosinate alone by 2 to 7 percentage points. At all locations except North Clayton, mixing *S*-metolachlor with glufosinate increased injury by two to 11 percentage points. At Plymouth and North Rocky Mount, cotton injury from glufosinate plus pyrithiobac was nine percentage

points higher than injury from glufosinate plus *S*-metolachlor and only at North Clayton pyrithiobac increased injury more than *S*-metolachlor. Tank mixtures of glyphosate plus glufosinate applied at 0.84 plus 0.235 kg/ha did not injure cotton more than glufosinate applied at 0.47 kg/ha alone. Glyphosate plus glufosinate at 0.42 plus 0.47 kg/ha increased injury in only two locations and injury from glyphosate plus glufosinate applied at 0.84 plus 0.47 kg/ha was four to six percentage points than glufosinate alone in four locations.

Five days after POST 2, injury from glyphosate-based systems was 3% or less at all locations (Table 6). Glufosinate applied alone injured cotton 3 to 8%. Increased injury for glufosinate plus pyrithiobac or *S*-metolachlor applied at POST 1 was not observed five days after POST 2 except at Plymouth where glufosinate plus *S*-metolachlor was more injurious than glufosinate applied alone. Tank mixtures of glyphosate plus glufosinate did not injury cotton more than glufosinate alone at four locations. At Mount Olive, injury from tank mixtures with glufosinate applied at 0.47 kg/ha were 12 pecentage points higher than glufosinate alone and at North Clayton, injury from glyphosate plus glufosinate applied at 0.84 plus 0.24 kg/ha was three percentage points higher than glufosinate alone. Cotton injury observed at lay-by from glufosinate alone was 1 to 4%, injury from all treatments with glufosinate were 7% or less in all locations (data not shown).

Although injury was observed from glufosinate, it likely did not affect yield, due to no observed differences in seed cotton yield among any treatments at all study locations (Table 8). Average seed cotton yields varied between locations, but cotton in all herbicide systems yielded similarly. Glufosinate also did not negatively affect any of the cotton fiber quality parameters examined (data not shown). At Mount Olive, cotton yield was similar in all

treatments, however Palmer amaranth present at harvest in glyphosate treated cotton should have reduced yield (Morgan et al. 2001). This response was likely not observed likely because of extremely low rainfall accumulation during the growing season at this location.

This research suggests that although injury to PHY 485 WRF cotton is consistently observed from glufosinate, two applications of glufosinate applied at 0.47 kg/ha applied alone or with mixed with glyphosate, pyrithiobac, or *S*-metolachlor does not adversely affect cotton yield or fiber quality. Similar to previous research by Culpepper et al. (2000), Everman et al. (2007) and Gardner et al (2006), the results of this research demonstrate that glyphosate or glufosinate can be used to effectively manage a variety of weeds, but glufosinate-based systems are often marginally effective on glyphosate-susceptible Palmer amaranth and some annual grasses even if applications are made timely. Moreover, this work is consistent with work by Culpepper et al. (2008) demonstrating that glufosinate-based systems are more effective on GR Palmer amaranth in North Carolina than glyphosate-based systems.

These data also indicate that annual grass and glyphosate-susceptible Palmer amaranth control by glyphosate may be reduced by glufosinate co-application and that compared to control from glufosinate alone, may be increased by co-application of glyphosate. Conversely, control of GR Palmer amaranth was increased when glufosinate is tank mixed with glyphosate and glyphosate plus glufosinate applied at 0.84 plus 0.47 kg/ha was more effective than glufosinate alone. Kudsk and Mathiassen (2004) reported antagonism when glyphosate and glufosinate were applied simultaneously in two mustard species, and Everman et al. (2009) demonstrated glyphosate antagonism by glufosinate applied to one

annual grass species. This study also suggests the possibility of weed control antagonism from glyphosate and glufosinate tank mixtures. With the release of stacked Roundup Ready and Liberty Link traits, grower will have the option to use both glyphosate- and glufosinate-based herbicide systems to control a wider spectrum of weeds. However, growers should be hesistant to tank mix glyphosate and glufosinate on weeds where glyphosate is typically effective because of possible weed control anatagonism.

Sources of Materials

[1] Gramoxone Inteon herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[2] PHY 485 WRF cotton. Phytogen Seed Company L.L.C., 9330 Zionsville Road, Indianapolis, IN 46268.

[3] Roundup WEATHERMAX herbicide, Monsanto Company, St. Louis, MO 63167.

[4] Ignite 280 SL herbicide, Bayer Cropscience, P.O. Box 12014, T.W. Alexander Dr., Research Triangle Park, NC 27709.

[5] Staple herbicide, Dupont Crop Protection Co., Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898.

[6] Dual Magnum herbicide, Syngenta Crop Protection Inc., P.O. Box 18300, Greensboro, NC 27419.

[7] Direx 4L herbicide, Dupont Crop Protection Co., Laurel Run Building, Chestnut Run Plaza, Wilmington, DE 19898.

[8] MSMA 6 plus herbicide, Drexel Chemical Co., P.O. Box 13327, Memphis, TN 38113-

0327.

[9] Agridex, a mixture of 83% paraffinic mineral oil and 17% polyoxyethylene sorbitan fatty acid ester, Helena Chemical Co. 225 Schilling Blvd., Suite 300, Collierville, TN 38017.

[10] TeeJet XR11002 flat-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189.

[11] Statistical Analysis Systems, version 9.1, SAS Institute Inc., SAS Campus Drive, Cary, NC, 27513.

Acknowledgments

The authors would like to thank Rick Seagroves and Jamie Hinton for technical support. This research was partially funded by the North Carolina Cotton Producers Association.

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Table 1. Planting dates, herbicide application dates, and soil characteristics at experiment sites.

Site Characteristics	2007			2008		
	North Clayton	Mount Olive	Plymouth	South Clayton	North Rocky Mount	South Rocky Mount
Planting date	4/27	5/14	5/22	5/14	5/6	5/6
Date of POST 1	5/21	5/29	6/10	5/30	5/29	5/29
Date of POST 2	6/9	6/11	6/26	6/11	6/10	6/12
Date of Lay-by	6/25	6/27	7/9	7/2	7/9	7/9
Soil series	Norfolk[a]	Wagram[b]	Cape fear[c]	Wedowee[d]	Aycock[e]	Norfolk
Soil texture	Loamy sand	Loamy sand	loam	sandy loam	Sandy loam	Loamy sand
Soil humic matter (%)	1.08	0.71	3.37	0.71	0.32	0.36
Soil pH	5.8	5.4	5.9	6.0	6.1	5.9

[a] Fine-loamy, Koalinitic, thermic, Typic Kandiudults

[b] Fine, kaolinitic, thermic Typic Kanhapludults.

[c] Fine, mixed, semiactive, thermic, Typic Umbraquults

[d] Fine, kaolinitic, thermic Typic Kanhapludults.

[e] Fine-silty, siliceous, subactive, thermic Typic Paleudults.

Table 2. Weed species and densities at experimental sites.

Species	North Clayton 2007	Mount Olive 2007	Ply-mouth 2007	South Clayton 2008	North Rocky Mount 2008	South Rocky Mount 2008
	no./m^2					
Carpetweed	10	--	7	--	--	--
Common lambsquarters	--	--	12	--	--	25
Common ragweed	--	--	--	--	24	30
Crowfootgrass	--	--	--	--	--	6
Entireleaf morningglory	--	--	2	--	--	4
Fall panicum	--	--	10	--	--	6
Goosegrass	10	--	--	2	--	4
Large crabgrass	43	--	--	16	30	30
Palmer amaranth	--	170[a]	--	190	140	--
Pitted morningglory	4	--	2	--	--	--
Redroot pigweed	--	--	18	--	--	--
Sicklepod	--	8	2	--	--	--
Tall morningglory	8	--	4	--	--	6

[a] Palmer amaranth population in Mount Olive is glyphosate-resistant.

Table 3. Control of annual grasses late-season in Widestrike cotton.[a]

Herbicide applications[b,c]				Annual grasses	
POST 1	Rates	POST 2	Rates	North Clayton[d]	2008[e]
	kg/ha		kg/ha	%	
Glyp	0.84	Glyp	0.84	99 ab	97 bc
Glyp + pyri	0.84 + 0.05	Glyp	0.84	95 ab	98 ab
Glyp + S-met	0.84 + 1.06	Glyp	0.84	100 a	99 a
Gluf	0.47	Gluf	0.47	94 b	85 e
Gluf + pyri	0.47 + 0.05	Gluf	0.47	95 ab	93 d
Gluf + S-met	0.47 + 1.06	Gluf	0.47	96 ab	94 d
Glyp + gluf	0.84 + 0.24	Glyp + gluf	0.84 + 0.24	96 ab	96 c
Glyp + gluf	0.42 + 0.47	Glyp + gluf	0.42 + 0.47	90 b	92 d
Glyp + gluf	0.84 + 0.47	Glyp + gluf	0.84 + 0.47	99 ab	94 d

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] POST 1 applied to 1- to 2-leaf cotton, POST applied to 5- to 6-leaf cotton. All plots received a lay-by application diuron plus MSMA (1.12 plus 2.2 kg/ha).

[c] Abbreviations: glyp, glyphosate; pyri, pyrithiobac; S-met; S-metolachlor; gluf, glufosinate.

[d] Annual grasses consisted of large crabgrass and goosegrass in Clayton during 2007

[e] Data pooled over two locations: South Clayton 2008 (consisting of large crabgrass and goosegrass) and South Rocky Mount (consisting of large crabgrass, goosegrass, fall panicum, and crowfootgrass).

Table 4. Control of Palmer amaranth late-season in Widestrike cotton.[a]

Herbicide applications[b,c]				GS Palmer amaranth		GR Palmer amaranth
POST 1	Rates	POST 2	Rates	South Clayton	North Rocky Mount	
	kg/ha		kg/ha	%		
Glyp	0.84	Glyp	0.84	99 a	100 a	64 d
Glyp + pyri	0.84 + 0.05	Glyp	0.84	99 a	100 a	85 bc
Glyp + S-met	0.84 + 1.06	Glyp	0.84	99 a	100 a	75 cd
Gluf	0.47	Gluf	0.47	77 d	95 b	83 bc
Gluf + pyri	0.47 + 0.05	Gluf	0.47	93 b	99 a	96 a
Gluf + S-met	0.47 + 1.06	Gluf	0.47	84 cd	98 a	91 ab
Glyp + gluf	0.84 + 0.24	Glyp + gluf	0.84 + 0.24	92 b	100 a	83 bc
Glyp + gluf	0.42 + 0.47	Glyp + gluf	0.42 + 0.47	88 bc	99 a	87 ab
Glyp + gluf	0.84 + 0.47	Glyp + gluf	0.84 + 0.47	89 bc	100 a	94 a

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] POST 1 applied to 1- to 2-leaf cotton, POST applied to 5- to 6-leaf cotton. All plots received a lay-by application diuron plus MSMA (1.12 plus 2.2 kg/ha).

[c] Abbreviations: GR, glyphosate-resistant; GS, glyphosate-susceptible; glyp, glyphosate; pyri, pyrithiobac; S-met; *S*-metolachlor; gluf, glufosinate.

Table 5. Widestrike cotton injury from glyphosate and glufosinate five days after POST 1 herbicide application.[a]

POST 1 application[b]		North			South	North Rocky	South Rocky
Herbicides	Rates	Clayton	Mount Olive	Plymouth	Clayton	Mount	Mount
	kg/ha	%					
Glyphosate	0.84	--[c]	--	--	--	--	--
Glyphosate + pyrithiobac	0.84 + 0.05	3 d	11 ab	4 e	2 e	2 d	7 d
Glyphosate + *S*-metolachlor	0.84 + 1.06	1 e	1 d	3 f	1 f	2 d	6 d
Glufosinate	0.47	7 bc	8 b	6 de	3 de	10 c	9 cd
Glufosinate + pyrithiobac	0.47 + 0.05	14 a	13 a	8 c	5 bc	11 bc	16 a
Glufosinate + *S*-metolachlor	0.47 + 1.06	8 b	15 a	17 a	5 bc	20 a	19 a
Glyphosate + glufosinate	0.84 + 0.24	5 cd	4 c	6 de	4 cd	9 c	10 bcd
Glyphosate + glufosinate	0.42 + 0.47	8 b	13 a	7 cd	6 ab	12 bc	13 abc
Glyphosate + glufosinate	0.84 + 0.47	11 ab	12 ab	11 b	7 a	15 b	15 ab

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] POST 1 applied to cotyledon to 2-leaf cotton.

[c] The glyphosate treatment was considered as the check when estimating cotton injury from other herbicide treatments, not included ANOVA.

Table 6. Widestrike cotton injury from glyphosate and glufosinate systems five days after POST 2 herbicide application.[a]

Herbicide applications[b,c]				North Clayton	Mount Olive	Plymouth	South Clayton	North Rocky Mount	South Rocky Mount
POST 1	Rates	POST 2	Rates						
	kg/ha		kg/ha	%					
Glyp	0.84	Glyp	0.84	--[d]	--	--	--	--	--
Glyp + pyri	0.84 + 0.05	Glyp	0.84	0 d	0 d	3 c	0 b	0 c	1 b
Glyp + S-met	0.84 + 1.06	Glyp	0.84	0 d	0 d	0 d	1 b	1 b	1 b
Gluf	0.47	Gluf	0.47	6 b	8 c	4 bc	5 a	3 ab	7 a
Gluf + pyri	0.47 + 0.05	Gluf	0.47	6 b	14 bc	4 bc	4 a	2 ab	5 a
Gluf + S-met	0.47 + 1.06	Gluf	0.47	8 ab	11 bc	9 a	5 a	4 a	8 a
Glyp + gluf	0.84 + 0.24	Glyp + gluf	0.84 + 0.24	3 c	11 bc	1 d	1 b	3 ab	5 a
Glyp + gluf	0.42 + 0.47	Glyp + gluf	0.42 + 0.47	7 ab	20 ab	5 bc	6 a	3 a	7 a
Glyp + gluf	0.84 + 0.47	Glyp + gluf	0.84 + 0.47	9 a	24 a	7 ab	4 a	3 ab	7 a

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] POST 1 applied to cotyledon to 2-leaf cotton, POST 2 applied to 4- to 6-leaf cotton.

[c] Abbreviations: glyp, glyphosate; pyri, pyrithiobac; S-met; *S*-metolachlor; gluf, glufosinate.

[d] The glyphosate treatment was considered as the check when estimating cotton injury from other herbicide treatments, not included ANOVA.

CHAPTER VIII

Palmer Amaranth (*Amaranthus palmeri*) Control in Soybean with Glyphosate and Conventional Herbicide Systems

Jared R. Whitaker, Alan C. York, and David L. Jordan*

Abstract

Glyphosate typically controls Palmer amaranth very well. However, glyphosate-resistant (GR) biotypes of this weed are present in several southern states, requiring development of effective alternative management strategies. Field experiments were conducted in seven North Carolina environments to evaluate control of glyphosate-susceptible (GS) and GR Palmer amaranth in soybean by glyphosate and conventional herbicide systems. Conventional systems included either pendimethalin or *S*-metolachlor applied preemergence (PRE) alone or mixed with flumioxazin, fomesafen, or metribuzin plus chlorimuron followed by fomesafen or no herbicide postemergence (POST). *S*-metolachlor was more effective than pendimethalin, and flumioxazin and fomesafen were generally more

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effective than metribuzin plus chlorimuron. Fomesafen applied POST following PRE herbicides increased Palmer amaranth control and soybean yield compared with PRE-only herbicide systems. Glyphosate alone applied once POST controlled GS Palmer amaranth 97% late in the season. Glyphosate was more effective than fomesafen plus clethodim applied POST. Control of GS Palmer amaranth equivalent to control by glyphosate applied once POST was obtained only with pendimethalin or *S*-metolachlor plus flumioxazin, fomesafen, or metribuzin plus chlorimuron applied PRE followed by fomesafen POST. In fields with GR Palmer amaranth, greater than 80% late-season control was obtained only with systems of pendimethalin or *S*-metolachlor plus flumioxazin, fomesafen, or metribuzin plus chlorimuron applied PRE followed by fomesafen POST. Systems of without both flumioxazin, fomesafen, or metribuzin plus chlorimuron applied PRE and fomesafen POST controlled GR Palmer amaranth less than 60% late in the season.

Nomenclature: Chlorimuron; clethodim; flumioxazin; fomesafen; glyphosate; metribuzin; pendimethalin; *S*-metolachlor; Palmer amaranth, *Amaranthus palmeri* S. Wats; soybean, *Glycine max* (L.) Merr.

Key words: Chlorimuron; fomesafen; flumioxazin; glyphosate-resistant weeds; herbicide resistance; metribuzin; pendimethalin; *S*-metolachlor.

Introduction

Palmer amaranth is one of the most troublesome weeds for soybean and other agronomic crop producers in the southeastern U.S. (Webster 2005). It grows very rapidly and can reach 2 m or more in height (Horak and Loughin 2000). It has an extremely high photosynthetic

capacity and utilizes the C_4 photosynthetic pathway (Ehleringer 1983). Along with rapid growth, Palmer amaranth has effective drought tolerance mechanisms that allow it to survive and grow during dry conditions, and it readily adapts to shading (Ehleringer 1983; Jha et al. 2008). These characteristics allow Palmer amaranth to establish a competitive dominance for light and space with crops (Monks and Oliver 1988). Yield losses as great as 79% have been recorded in soybean with Palmer amaranth densities of eight plants per m of row (Bensch et al. 2003). Once established in fields, Palmer amaranth can be difficult to control due to its rapid growth, competitive ability, and prolific seed production (Keely et al. 1987). Continued emergence throughout the season, coupled with prolific seed production, allows Palmer amaranth to quickly replenish seed banks if control is not season-long (Keely et al. 1987; Sellers et al. 2003).

Glyphosate typically is very efficacious on Palmer amaranth (Corbett et al. 2004; Culpepper and York 1998; Parker et al. 2005). Excellent control of problem weeds, such as Palmer amaranth, along with the convenience, simplicity, and economics of glyphosate-based management systems, lead to rapid and wide-spread adoption of glyphosate-resistant soybean, corn (*Zea mays* L), and cotton (*Gosspyium hirsutum* L.) (Culpepper and York 1998; Dill et al. 2008; Gianessi 2005; Parker et al. 2005; Roberts et al. 1999; Thomas et al. 2004). Associated with the wide-spread adoption of the technology has been a major decrease in use of herbicides other than glyphosate (Young 2006). Growers have relied heavily on glyphosate-only weed management systems, and while glyphosate-only programs have controlled weeds very well (Ateh and Harvey 1999; Culpepper and York 1999; Culpepper et al. 2000; Parker et al. 2005), extensive reliance on glyphosate has lead to evolution of GR

weeds (Heap 2009). Glyphosate-resistant Palmer amaranth, first observed in Georgia in 2005 (Culpepper et al. 2006), is now found in Arkansas, Georgia, North Carolina, South Carolina, and Tennessee (Culpepper et al. 2006; Heap 2009; Norsworthy et al. 2008; Steckel et al. 2008; York et al. 2007). Culpepper et al. (2008) estimated that GR Palmer amaranth infested at least 120,000 ha in Georgia and 75,000 ha in North Carolina in 2007.

Palmer amaranth can be controlled in soybean and other crops by acetolactate synthase (ALS)-inhibiting herbicides (Gossett and Toler 1999; Mayo et al. 1995). However, resistance to ALS-inhibiting herbicides has evolved in Palmer amaranth (Heap 2009; Horak and Peterson 1995; Sprague et al. 1997), and resistant biotypes are common in the southeastern U.S. (Wise et al. 2007). Populations of Palmer amaranth with multiple resistance to both glyphosate and ALS-inhibiting herbicides exist in Georgia and North Carolina (Sosnoskie et al. 2009; Whitaker 2009).

Effective alternatives to glyphosate must be found to manage GR Palmer amaranth. Additionally, herbicides with other modes of action must be integrated into glyphosate-based management systems to avoid or delay resistance in fields currently infested with GS Palmer amaranth (York and Culpepper 2009a, 2009b). The objective of our research was to evaluate Palmer amaranth control in soybean using herbicide systems that do not rely on glyphosate or ALS-inhibiting herbicides.

Materials and Methods

The experiment was conducted in North Carolina during 2006 and 2007 in fields with dense infestations of Palmer amaranth. Sites included two fields each year at the Central

Crops Research Station near Clayton (hereafter referred to as Clayton 1 and Clayton 2), a private farm near Mount Olive during 2006 and 2007, and a private farm near Parkton during 2007. Soil descriptions, planting dates, herbicide application dates, and Palmer amaranth densities are presented in Table 1. Palmer amaranth at Clayton was susceptible to glyphosate while the fields at Mount Olive and Parkton contained mixtures of GR and GS biotypes.

Glyphosate-resistant soybean 'AG5905' was planted in 38-cm rows at a seeding rate of approximately 295,000 seed/ha in conventional tillage systems at all sites except in Mount Olive in 2007, where soybean was planted into a desiccated wheat (*Triticum aestivum* L.) cover crop. Plot size was 6 rows by 10 m. The experimental design was a randomized complete block with treatments replicated three or four times.

Treatments consisted of a factorial arrangement of two "PRE grass herbicides", four "PRE broadleaf herbicides", and two POST options. The PRE grass herbicides included pendimethalin[1] (1000 g ai/ha) and *S*-metolachlor[2] (1100 g ai/ha), and PRE broadleaf options included no herbicide, metribuzin plus chlorimuron[3] (270 plus 45 g ai/ha), fomesafen[4] (280 g ai/ha), and flumioxazin[5] (71g ai/ha). The POST options included no herbicide and fomesafen[6] (390 g/ha) plus crop oil concentrate[7] at 0.5% (v/v). Three additional treatments included the potassium salt of glyphosate[8] at 1000 g ae/ha applied POST, a mixture of fomesafen[6] at 390 g/ha plus clethodim[9] at 140 g ai/ha plus crop oil concentrate at 0.5% (v/v) applied POST, and a non-treated check. The POST herbicides were applied when Palmer amaranth was 10 to 15 cm tall. At 6 of the 7 sites, POST herbicides in the absence of PRE herbicides were applied 2 to 14 d earlier than POST herbicides following PRE herbicides

(Table 1). Herbicides were applied with a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles[10] calibrated to deliver 140 L/ha at 160 kPa.

Soybean injury and Palmer amaranth control were estimated visually immediately prior to POST herbicide application. Soybean injury also was estimated 5 and 30 d after POST herbicide application (DAP) while Palmer amaranth control was estimated 30 and 90 DAP. Visual estimates were based on a scale of 0 to 100%, where 0 = no soybean injury or no weed control and 100 = soybean death or complete weed control (Frans et al. 1986). Soybean was mechanically harvested at 5 of the 7 locations and yields were adjusted to 13% moisture. Yield of the non-treated check at all locations and yields of systems without fomesafen POST at Mount Olive in 2006 were assumed to be 0 as these plots were completely overrun by weeds and could not be harvested. Extreme drought during 2007 in Mount Olive and Parkton resulted in negligible soybean yield, regardless of weed control; therefore, yields were not recorded at these sites.

Data for soybean injury and yield and Palmer amaranth control were subjected to ANOVA using the PROC MIXED procedure of SAS[11] with treatment sums of squares partitioned to reflect the factorial treatment arrangement. The non-treated check was not included in the analysis. Each site and year was treated as an environment, and environments and replications were considered random effects (McIntosh 1983). Data for soybean injury and weed control data were arcsine square root transformed (Ahrens et al. 1990). Non-transformed data were presented with statistical information based upon transformation. Means for significant main effects and interactions from the factorial set of treatments were separated with Fisher's Protected LSD at $P \leq 0.05$. A separate ANOVA was conducted to

compare weed control and yield with the glyphosate-only treatment to all other treatments, and significant effects were determined using Dunnett's Procedure at $P \leq 0.05$ (Dunnett 1955).

Results and Discussion

Conventional herbicide systems. Two- and three-way interactions between PRE grass herbicides, PRE broadleaf herbicides, and POST herbicide options were not observed. Data for main effects of PRE grass herbicides and PRE broadleaf herbicides were averaged across environments due to lack of treatment by environment interactions. *S*-metolachlor controlled Palmer amaranth more effectively than pendimethalin. Averaged over the four PRE broadleaf herbicide options, *S*-metolachlor and pendimethalin controlled Palmer amaranth 87 and 82%, respectively, at 0 DAP (Table 2). Similarly, averaged over PRE broadleaf herbicides and POST herbicides, control by *S*-metolachlor was 6 to 7% greater than control by pendimethalin at 30 and 90 DAP. Soybean yield also was 7% greater in systems with *S*-metolachlor as compared to pendimethalin.

The main effect of PRE broadleaf herbicides was also significant for Palmer amaranth control and soybean yield. Averaged over *S*-metolachlor and pendimethalin applied PRE, each of the three PRE broadleaf herbicides increased Palmer amaranth control at 0 DAP (Table 3). Compared to *S*-metolachlor or pendimethalin alone, metribuzin plus chlorimuron applied PRE increased control 0 DAP by 22%. Flumioxazin and fomesafen were similarly effective and increased control 27 to 29%, but both flumioxazin and fomesafen were more effective than metribuzin plus chlorimuron. The same trends were noted with Palmer

amaranth control at 30 and 90 DAP. Averaged over PRE grass herbicides and POST herbicides, Palmer amaranth was controlled only 38% at 90 DAP in systems without a PRE broadleaf herbicide. Metribuzin plus chlorimuron, fomesafen,and flumioxazin applied PRE increased control to 63, 69, and 70%, respectively. The PRE broadleaf herbicides substantially impacted soybean yield. Yields were similar with each of the three PRE broadleaf herbicides and were increased 27%.

A POST herbicide by environment interaction was noted for Palmer amaranth control and soybean yield. Pooled over PRE grass and broadleaf herbicides, fomesafen applied POST increased Palmer amaranth control in 6 of 7 environments at 30 DAP and in all environments 90 DAP (Table 4). Fomesafen applied POST had a greater impact at locations with greater Palmer amaranth densities. At the Clayton sites, where Palmer amaranth densities ranged from 35 to 65 plants/m^2 (Table 1), systems without fomesafen POST controlled Palmer amaranth 50 to 89% at 90 DAP compared with 86 to 95% in systems with fomesafen POST (Table 4). In contrast, at the Mount Olive and Parkton sites, where Palmer amaranth densities ranges from 140 to 180 plants/m^2 (Table 1), systems without fomesafen POST controlled Palmer amaranth only 5 to 20% at 90 DAP compared with 54 to 82% control with systems containing fomesafen POST (Table 4). Soybean canopy development may also have contributed to the greater response to fomesafen POST at Mount Olive and Parkton compared with Clayton. Soybean grew more rapidly and canopy closure was noted to occur more rapidly on the sandy loam soils at Clayton than on the loamy sand soils at Mount Olive and Parkton. Rainfall during the period of 0 to 90 d after POST herbicide

application was 50 and 49% below normal at Mount Olive and Parkton in 2007, and this further delayed canopy closure.

Fomesafen applied POST increased soybean yield in 4 of the 5 environments where yields were recorded (Table 4). Fomesafen POST did not impact soybean yield at Clayton 1 in 2006 where the PRE herbicides alone controlled Palmer amaranth 89% at 90 DAP. However, fomesafen POST increased yield 18 to 48% at the other Clayton environments where PRE herbicides alone controlled Palmer amaranth less than 60% at 90 DAP. Soybean produced on 650 kg/ha yield at Mount Olive in 2006 in systems with fomesafen POST, but no yield was produced in systems without fomesafen POST.

A PRE grass by PRE broadleaf herbicide interaction for soybean injury was noted at the time of POST herbicide application (data not shown). Pendimethalin caused no injury to soybean, and combinations of pendimethalin plus PRE broadleaf herbicides injured soybean 3% or less. *S*-metolachlor mixed with metribuzin plus chlorimuron or fomesafen injured soybean 2% or less while *S*-metolachlor plus flumioxazin injured soybean 7%. The flumioxazin label cautions users on the potential for soybean injury when flumioxazin and chloroacetamide herbicides are applied PRE (Anonymous 2009). Averaged over environments and PRE herbicides, fomesafen POST injured soybean 5% at 5 DAP (data not shown). The injury was transient, with no injury noted 30 DAP.

Conventional herbicide systems compared to glyphosate. Lack of a treatment by environment interaction at the Clayton sites with GS Palmer amaranth allowed data for Palmer amaranth control and soybean yield to be averaged over the four environments. The glyphosate-only treatment controlled Palmer amaranth 100 and 97% at 30 and 90 DAP,

respectively (Table 5). The POST-only treatment of fomesafen plus clethodim was less effective than glyphosate, controlling Palmer amaranth only 92 and 85% at 30 and 90 DAP, respectively. However, soybean yield with the glyphosate and fomesafen plus clethodim treatments was similar.

Pendimethalin or *S*-metolachlor plus one of the PRE broadleaf herbicides plus fomesafen POST were required for GS Palmer amaranth control equivalent to that with glyphosate alone (Table 5). These systems controlled Palmer amaranth 93 to 100% at 30 DAP and 88 to 98% at 90 DAP. Systems that included only pendimethalin plus flumioxazin, fomesafen, or metribuzin plus chlorimuron PRE were 18 to 27% less effective than glyphosate at 30 DAP and 25 to 31% less effective than glyphosate at 90 DAP. Systems with *S*-metolachlor plus one of the PRE broadleaf herbicides were somewhat more effective but still controlled Palmer amaranth 9 to 23% less than glyphosate at 30 DAP and 13 to 28% less at 90 DAP. Systems that included only pendimethalin or *S*-metolachlor plus fomesafen POST were 17 to 21% and 21 to 26% less effective than glyphosate at 30 and 90 DAP, respectively.

Soybean yield was similar with glyphosate only, fomesafen plus clethodim applied POST, and systems that included *S*-metolachlor plus one of the PRE broadleaf herbicides, *S*-metolachlor PRE and fomesafen POST, and pendimethalin plus one of the PRE broadleaf herbicides plus fomesafen POST (Table 5). Soybean yielded 36 to 37% less in systems that included only pendimethalin or *S*-metolachlor PRE compared to the glyphosate systems, and 15 to 19% less in systems that included pendimethalin plus one of the PRE broadleaf herbicides or pendimethalin PRE followed by fomesafen POST.

An environment by treatment interaction was noted for Palmer amaranth control 30 DAP, but not at 90 DAP, at sites with GR Palmer amaranth. The treatment of glyphosate only controlled GR Palmer amaranth only 10 to 23% at 30 DAP and only 4% at 90 DAP (Table 6). At 2 of the 3 sites, fomesafen plus clethodim was more effective than glyphosate at 30 DAP, but control at those two sites was only 69 to 82%. Averaged over the three sites, control by fomesafen plus clethodim declined to only 17% by 90 DAP. Control by pendimethalin alone or *S*-metolachlor alone did not differ from control by glyphosate. At all three sites, systems that included a PRE herbicide plus fomesafen POST were more effective than glyphosate. Systems that included pendimethalin or *S*-metolachlor plus flumioxazin or fomesafen PRE, in the absence of fomesafen POST, controlled GR Palmer amaranth better than glyphosate at 2 of the 3 locations, while systems that included pendimethalin or *S*-metolachlor plus metribuzin plus chlorimuron PRE were more effective than glyphosate at one location. By 90 DAP, only the systems that include pendimethalin plus fomesafen PRE followed by fomesafen POST and *S*-metolachlor plus flumioxazin or fomesafen PRE followed by fomesafen POST controlled the GR Palmer amaranth greater than 80%.

Of the three sites with GR Palmer amaranth, only the site at Mount Olive in 2006 was harvestable. Extreme drought at Mount Olive and Parkton during 2007, along with severe weed competition, resulted in negligible yields regardless of herbicide treatments. Only treatments that included pendimethalin or *S*-metolachlor plus a PRE broadleaf herbicide plus fomesafen POST were harvestable at Mount Olive in 2006. Soybean yield with these treatments was only 770 to 1000 kg/ha (Table 6).

This research demonstrates the excellent control of GS Palmer amaranth that can be achieved with glyphosate. Excellent control of problem weeds, such as Palmer amaranth, along with convenience, simplicity, flexibility, and affordability of glyphosate-based programs, is the primary reason growers in the southeastern U. S. rapidly and widely adopted the GR crop technology. Unfortunately, excessive reliance on glyphosate has lead to evolution of GR biotypes of Palmer amaranth.

Selection of resistant biotypes has dramatically reduced the viability of glyphosate-only herbicide systems. This work indicates that GR Palmer amaranth can be effectively managed in soybean. These results also demonstrate implications for resistance management such that many of the herbicides evaluated in this study could be integrated into glyphosate-based herbicide systems in soybean to reduce selection pressure for ALS inhibitor- and GR Palmer amaranth biotypes.

Sources of Materials

[1] Pendimethalin, Prowl H2O®, BASF Ag. Products, Research Triangle Park, NC 27709.

[2] *S*-metolachlor, Dual Magnum®, Syngenta Crop Protection Inc., Greensboro, NC 27409.

[3] Metribuzin plus chlorimuron, Canopy®, Dupont Crop Protection Co., Inc. Wilmington, DE 19898.

[4] Fomesafen, Reflex®, Syngenta Crop Protection Inc., Greensboro, NC 27409.

[5] Flumioxazin, Valor SX®, Valent U.S.A. Corporation, Walnut Creek, CA 94596-8025.

[6] Fomesafen, Flexstar®, Syngenta Crop Protection Inc., Greensboro, NC 27409.

[7] Crop oil concentrate, Agri-Dex® Spray Adjuvant, Helena Chemical Co., Collierville, TN

38017.

[8] Glyphosate, Roundup Weathermax®, Monsanto Company, St. Louis, MO 63167.

[9] Clethodim, Select®, Valent U.S.A. Corporation, Walnut Creek, CA 94596-8025.

[10] TeeJet XR11002 flat-fan spray nozzles, Spraying Systems Co., Wheaton, IL 60189.

[11] Statistical Analysis Systems®, version 9.1, SAS Institute Inc., Cary, NC 27513.

Acknowledgement

This research was partially funded by the North Carolina Soybean Producers Association. The authors would also like to thank Jamie Hinton and Rick Seagroves for technical assistance.

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Table 1. Herbicide application dates, soil characteristics, and Palmer amaranth densities at experiment sites.

	2006			2007			
Site Characteristics	Clayton 1	Clayton 2	Mount Olive	Clayton 1	Clayton 2	Mount Olive	Parkton
Planting/preemergence date	5/25	5/17	5/25	5/15	5/15	5/18	6/7
Postemergence date (no PRE)[a]	6/16	6/8	6/13	6/6	6/6	6/6	6/22
Postemergence date (with PRE)[b]	6/29	6/22	6/13	6/8	6/9	6/8	6/27
Soil series	Lynchburg[c]	Wedowee[d]	Wagram[e]	Lynchburg	Wedowee	Wagram	Wagram
Soil texture	Sandy loam	Sandy loam	Loamy sand	Sandy loam	Sandy loam	Loamy sand	Loamy sand
Soil humic matter[f] (%)	1.25	0.60	0.51	1.25	0.60	0.66	1.67
Soil pH	5.0	5.6	6.2	5.8	5.6	5.1	5.4
Palmer amaranth density[g] (no./m^2)	35	50	180	35	65	150	140

[a] Date of POST applications for treatments which did not receive a PRE herbicide.

[b] Date of POST applications for treatments which received a PRE herbicide.

[c] Fine-loamy, siliceous, semi-active, thermic Aeric Paleaquults.

[d] Fine, kaolinitic, thermic Typic Kanhapludults.

[e] Loamy, kaolinitic, thermic Arenic Kandiudults.

Table 1. Continued

[f] Humic matter determined according to Mehlich (1994).

[g] Palmer amaranth densities in non-treated checks recorded at time of POST herbicide application.

Table 2. Palmer amaranth control and soybean yield as affected by PRE grass herbicides.[a]

	Palmer amaranth control			Soybean
PRE grass herbicide[b]	0 DAP[c]	30 DAP	90 DAP	Yield
	%			kg/ha
Pendimethalin	82 b	70 b	57 b	1,430 b
S-metolachlor	87 a	77 a	63 a	1,530 a

[a] Data pooled over environments (seven for control, five for yield), four PRE broadleaf herbicides, and two POST herbicide options. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] Pendimethalin and *S*-metolachlor applied PRE at 1000 and 1100 g/ha, respectively.

[c] Abbreviations: DAP, days after POST herbicide application.

Table 3. Palmer amaranth control and soybean yield as affected by PRE broadleaf herbicides.[a]

PRE broadleaf herbicide[b]	Palmer amaranth control			Soybean yield
	0 DAP[c]	30 DAP	90 DAP	
	%			kg/ha
None	65 c	55 c	38 c	1,230 b
Flumioxazin	94 a	84 a	70 a	1,560 a
Fomesafen	92 a	81 a	69 a	1,580 a
Metribuzin + chlorimuron	87 b	76 b	63 b	1,550 a

[a] Data pooled over environments (seven for control, five for yields), two PRE grass herbicides, and two POST herbicide options. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] Flumioxazin, fomesafen, and metribuzin plus chlorimuron applied PRE at 71, 280, and 270 + 45 g/ha, respectively.

[c] Abbreviations: DAP, days after POST herbicide application.

Table 4. Effect of POST herbicides on Palmer amaranth control and soybean yield.[a]

POST	Environment						
	2006			2007			
herbicides[b]	Clayton 1	Clayton 2	Mount Olive	Clayton 1	Clayton 2	Mount Olive	Mount Parkton
	Palmer amaranth control 30 DAP[c] (%)						
None	63	91	29	62	65	42	31
Fomesafen	88*	95	89*	94*	94*	94*	96*
	Palmer amaranth control 90 DAP (%)						
None	59	89	5	49	50	20	6
Fomesafen	87*	95*	54*	85*	86*	82*	79*
	Soybean yield (kg/ha)						
None	2,360	2,370	0	740	1,080	---[d]	---
Fomesafen	2,790*	2,340	650*	900*	1,600*	---	---

[a] Data averaged over four PRE broadleaf herbicides and two PRE grass herbicides. Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $P \leq 0.05$.

[b] Fomesafen applied POST at 390 g/ha.

[c] Abbreviations: DAP, days after POST herbicide application.

[d] Yield not recorded at Mount Olive and Parkton in 2007.

Table 5. Glyphosate-susceptible Palmer amaranth control and soybean yield with glyphosate-only and alternative herbicide systems.[a]

Herbicides[b]			Palmer amaranth control		
PRE Grass	PRE Broadleaf	POST	30 DAP[c]	90 DAP	Soybean yield
			%		kg/ha
Pendimethalin	None	None	36*	24*	1310*
Pendimethalin	None	Fomesafen	79*	71*	1700*
Pendimethalin	Flumioxazin	None	82*	72*	1780*
Pendimethalin	Flumioxazin	Fomesafen	97	93	1940
Pendimethalin	Fomesafen	None	75*	72*	1700*
Pendimethalin	Fomesafen	Fomesafen	93	92	1920
Pendimethalin	Metribuzin + chlorimuron	None	73*	66*	1690*
Pendimethalin	Metribuzin + chlorimuron	Fomesafen	96	88	1940
S-metolachlor	None	None	47*	30*	1340*
S-metolachlor	None	Fomesafen	83*	76*	1870
S-metolachlor	Flumioxazin	None	91*	84*	1890
S- metolachlor	Flumioxazin	Fomesafen	100	97	1880

Table 5. Continued

S-metolachlor	Fomesafen	None	84*	76*	2040
S-metolachlor	Fomesafen	Fomesafen	99	98	2170
S-metolachlor	Metribuzin + chlorimuron	None	77*	69*	1860
S-metolachlor	Metribuzin + chlorimuron	Fomesafen	98	92	1960
None	None	Fomesafen + clethodim	92*	85*	1850
None	None	Glyphosate	100	97	2090

[a] Means within a column followed by an asterisk are different from the glyphosate-only treatment according to Dunnett's procedure at $P \leq 0.05$. Data are averaged over the four Clayton environments with glyphosate-susceptible Palmer amaranth.

[b] Flumioxazin, fomesafen, metribuzin plus chlorimuron, pendimethalin, and *S*-metolachlor applied PRE at 71, 280, 270 + 45, 1000, and 1100 g/ha, respectively. Fomesafen and fomesafen plus clethodim applied POST at 390 and 390 + 140 g/ha, respectively. Glyphosate was applied POST at 1000 g/ha.

[c] Abbreviations: DAP, days after POST herbicide application.

Table 6. Glyphosate-resistant Palmer amaranth control and soybean yield with glyphosate-only and alternative herbicide systems.[a]

Herbicides[b]			Palmer amaranth control				Yield[e]
			30 DAP[c]				
			2006	2007			Mount Olive
PRE Grass	PRE Broadleaf	POST	Mount Olive	Mount Olive	Parkton	90 DAP[d]	2006
			%				kg/ha
Pendimethalin	None	None	10	3	12	0	0
Pendimethalin	None	Fomesafen	79*	68*	90*	35*	0
Pendimethalin	Flumioxazin	None	38*	25	43*	6	0
Pendimethalin	Flumioxazin	Fomesafen	91*	95*	100*	77*	810*
Pendimethalin	Fomesafen	None	34*	63*	22	13	0
Pendimethalin	Fomesafen	Fomesafen	95*	100*	99*	85*	850*
Pendimethalin	Metribuzin + chlorimuron	None	21	55*	17	9	0
Pendimethalin	Metribuzin + chlorimuron	Fomesafen	83*	99*	97*	75*	770*
S-metolachlor	None	None	15	12	12	1	0
S-metolachlor	None	Fomesafen	78*	96*	85*	56*	0
S-metolachlor	Flumioxazin	None	66*	27	83*	26*	0
S-metolachlor	Flumioxazin	Fomesafen	99*	97*	100*	86*	1,000*
S-metolachlor	Fomesafen	None	28	77*	45*	17	0

Table 6. Continued

S-metolachlor	Fomesafen	Fomesafen	97*	100*	100*	82*	850*
S-metolachlor	Metribuzin + chlorimuron	None	19	72*	12	12	0
S-metolachlor	Metribuzin + chlorimuron	Fomesafen	94*	99*	100*	76*	950*
None	None	Fomesafen + clethodim	69*	25	82*	17*	0
None	None	Glyphosate	16	23	10	4	0

[a] Means within a column followed by an asterisk are different from the glyphosate-only treatment according to Dunnett's procedure at $P \leq 0.05$.

[b] Flumioxazin, fomesafen, metribuzin plus chlorimuron, pendimethalin, and ^{S}-metolachlor applied PRE at 71, 280, 270 + 45, 1000, and 1100 g/ha, respectively. Fomesafen and fomesafen plus clethodim applied POST at 390 and 390 + 140 g/ha, respectively. Glyphosate was applied POST at 1000 g/ha.

[c] Abbreviations: DAP, days after POST herbicide application.

[d] Data averaged over Mount Olive 2006, Mount Olive 2007, and Parkton 2007 sites.

[e] Yield not recorded at Mount Olive or Parkton in 2007.