ABSTRACT

CHANDI, AMAN. Characterization and Management of Selected Herbicide Resistant Weed Populations. (Under the direction of Drs. David L. Jordan and James D. Burton).

Weed populations or biotypes expressing resistance to glyphosate and other herbicides have become more wide spread and in some cases more difficult to manage in agricultural systems. Determining genotypic and phenotypic variation among populations can increase knowledge that may be useful in developing weed management strategies. Assessment of genetic diversity among and within eight Palmer amaranth biotypes collected from North Carolina and Georgia using Amplified Fragment Length Polymorphism (AFLP) markers revealed levels of genetic diversity to be high, ranging in value from 0.0773-0.8605. Cluster and Principal Coordinate (PCO) analyses grouped individuals mostly by geographic origin irrespective of either resistance or susceptibility to glyphosate or gender of individuals. Analysis of Molecular Variance (AMOVA) results indicated that within biotype contribution towards total variation was always higher and significant ($P \leq 0.0001$) than among biotypes. Resistance in a Palmer amaranth biotype from North Carolina was found to be incompletely dominant, nuclear inherited, and might not be consistent with a single gene mechanism of inheritance.

Recovery of fifteen Palmer amaranth biotypes collected across North Carolina following exposure to different periods of moisture stress indicated that percent reduction in height for the glyphosate resistant (GR) group was less compared with acetolactate synthase resistant (ALSR) and susceptible (ALSS/GS) groups 24 DAE. Dry weight of both ALSR and GR groups was affected less by moisture stress than the ALSS/GS group. Early season

interference of corn, cotton, peanut, snap bean, and soybean growth by eight Palmer amaranth biotypes (4 GR and 4 GS) in greenhouse revealed that when pooled over crops the percent reduction for fresh weight was 26% in presence of GS biotypes and 20% in presence of GR biotypes. Differential interference of soybean by seven Palmer amaranth biotypes (4 GR and 3 GS) in the field was observed.

Research was conducted to determine if differences in control existed among Palmer amaranth biotypes when treated with herbicides with different modes of action. In the greenhouse, when GR and GS biotypes were grouped, minor differences in control were observed with herbicides other than glyphosate. No clear conclusion could be made relative to differences in control of GR and GS biotypes of Palmer amaranth by herbicides representing major modes of action.

Populations of Italian ryegrass collected from six fields from two counties in North Carolina where mesosulfuron was no longer effective were used to confirm resistance to ALS-inhibiting herbicides and to determine if resistance to the acetyl-CoA carboxylase (ACCase) inhibitors diclofop and pinoxaden was present in these populations. All populations were resistant to diclofop and cross resistant to pinoxaden. Five of the six populations displayed multiple resistance to the ACCase inhibitors and the ALS inhibitor. An additional study with two biotypes confirmed cross resistance to the ALS inhibitors imazamox, mesosulfuron, and pyroxsulam. Resistance to mesosulfuron was shown to be heritable.

Research was conducted in order to confirm common ragweed resistance to the ALS-inhibiting herbicide diclosulam in a field with suspected resistance and to compare herbicide programs in that field designed to control ALS-resistant common ragweed in corn, cotton, peanut, and soybean. In greenhouse experiments, I_{50} values following postemergence application of diclosulam for mortality of plants, visual estimates of percent control, and percent reduction in plant fresh weight were 557 to 653 fold higher for the suspected ALS-resistant biotype compared with a suspected ALS-susceptible biotype. Herbicides with modes of action different from ALS-inhibiting herbicides, including: atrazine, dicamba, and glyphosate in corn; fomesafen, glyphosate, MSMA, and prometryn in cotton; bentazon, flumioxazin, and lactofen in peanut; and flumioxazin, glyphosate, and lactofen in soybean controlled common ragweed more effectively in many instances than programs relying on cloransulam (soybean), diclosulam (peanut), thifensulfuron (corn), and trifloxysulfuron (cotton) that typically control common ragweed populations not resistant to ALS-inhibiting herbicides. Applying tank-mix or sequential applications of herbicides with different modes of action were effective in controlling ALS-resistant common ragweed in all crops.

Characterization and Management of Selected Herbicide Resistant Weed Populations

by
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DEDICATION

This dissertation is dedicated to my husband and parents. Their love and support have made this possible.

BIOGRAPHY

Aman Chandi grew up in Amritsar, in Punjab, the largest grain producing state of India. She was raised up in an environment that inculcated in her love for plants quite spontaneously. Although she did not grow up on a farm, but during weekend visits to family farm, her father, who himself was an aquatic weed scientist, used to explain to her the basics of botany along with botanical names of crops around them. Her mother, a science teacher, did the same every time family would eat at dinner table. Thus plants fascinated her and she developed an urge to learn more about them.

She pursued her undergraduate degree in Agriculture from Guru Nanak Dev University with distinction and then Master's in Agronomy from Punjab Agricultural University. After completion of her Master's degree she worked as a research fellow for a year and a half under an Indian Council of Agricultural Research project on the behavior of sulfonylureas in sub-soil and their effect on subsequent crops under different cropping systems. This project involved managing field experiments and working in lab with High Performance Liquid Chromatograph. In August 2007, Aman accepted graduate research assistantship at North Carolina State University for pursuing PhD under the direction of Dr. David Jordan. During her PhD she engaged in weed biology and ecology studies. Her projects included assessing genetic diversity of Palmer amaranth biotypes using Amplified Fragment Length Polymorphisms and studying inheritance of glyphosate resistance. She also worked on recovery of various glyphosate and ALS resistant and susceptible Palmer amaranth biotypes from drought stress. One of her projects included studying early season interference of glyphosate resistant and susceptible Palmer amaranth biotypes in corn, cotton, peanut, snap bean, and soybean and also studying full season interference of these biotypes in soybean in the field. Another project involved testing the efficacy of various PRE and POST herbicides on the control of glyphosate resistant and susceptible biotypes of Palmer amaranth. Her work with ryegrass under Dr. York's direction, involved documentation of cross and multiple resistant biotypes of Italian ryegrass to ACCase and ALS inhibitors. Another experiment included evaluation of various alternate herbicide systems to manage ALS resistant ragweed in corn, cotton, peanut, and soybean. During her graduate studies at NC State, Aman also assisted Dr. Spears in teaching Seeds, Societies and Biotechnology course.

Participation in debate, declamation and dramatics has been Aman's favorite co-curricular activities during undergraduate and graduate studies in India and she has won many awards for her participation. She was actively involved in voluntary activities undertaken by Rotaract Club in her home town under the stewardship of Rotary International. She served as treasurer of Crop Science Graduate Student Association at NC State for the year 2010-11.

Aman has been happily married for five years to Navinderpal who is also a PhD student in the Department of Forest Economics at NC State.

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I am grateful to my parents Harinderjit Kaur and Jagjit Singh Chandi, for upbringing, and for values they have taught me, the most significant of which has been the importance of hard work. Thanks to my brother, Jiteshwar, sister-in-law, Sandeep and nephew, Armin for their affection and support.

Finally, I would like to thank my husband, Navinder, for his love, friendship and patience. His melodious voice has kept me going all these years. I am truly blessed to have him in my life.

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CHAPTER I

Use of AFLP Markers to Assess Genetic Diversity in Palmer Amaranth (*Amaranthus palmeri*) Biotypes from North Carolina and Georgia

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Abstract

Palmer amaranth has become one of the most troublesome weeds in southern cropping systems. This weed has confirmed resistance to herbicides, including glycines, acetolactate synthase inhibitors, dinitroanilines, and photosystem II inhibitors. A great degree of phenotypic variation is observed in Palmer amaranth biotypes with respect to susceptibility to herbicides and plant growth and development and may be related to levels of genetic diversity existing in these biotypes. Research was conducted to assess genetic diversity

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among and within eight Palmer amaranth biotypes collected from North Carolina and Georgia using Amplified Fragment Length Polymorphism (AFLP) markers. Levels of genetic diversity were found to be high, ranging in value from 0.0773 to 0.8605. The highest and the lowest genetic similarities within biotypes were 0.5596 and 0.3569 for Crisp and Sumter counties in Georgia (both susceptible to glyphosate), respectively. Cluster and Principal Coordinate (PCO) analyses grouped individuals mostly by geographic origin irrespective of either resistance or susceptibility to glyphosate or gender of individuals. Analysis of Molecular Variance (AMOVA) results when biotypes were nested within states revealed that significant variation ($P<0.0001$) existed among and within biotypes within states while variation among states was not significant ($P = 0.372$). Variation among and within biotypes within state accounted for 19% and 77% of the total variation, respectively. Variation among states accounted for only 3% of the total variation. The within biotype contribution towards total variation was always higher than among states and among biotypes within states irrespective of either resistance or susceptibility to glyphosate or gender of individuals. These results suggest that genetic variation in the studied biotypes is not discrete and cannot be accounted for by classification of individuals by state, response to glyphosate, or gender.

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

Key words: Glyphosate; herbicide resistance; phenotypic variation.

Abbreviations: AFLP, Amplified Fragment Length Polymorphism; AMOVA, Analysis of Molecular Variance; PCO analysis, Principal coordinate analysis.

Introduction

Palmer amaranth is a dioecious plant belonging to family amaranthaceae (Fernald 1950) and is one of the most troublesome weeds of row crops grown in North Carolina in cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), peanut (*Arachis hypogaea* L.), and soybean [*Glycine max* (L.) Merr.] (Webster 2004, 2005; Webster and Coble 1997). This weed is a prolific seed producer with up to 600,000 seeds per female plant (Keeley et al. 1987), can grow up to 2 m in height (Garvey, 1999), uses C_4 photosynthesis pathway (Ehleringer 1983), and can cause significant reductions in crop yield and harvesting efficiency (Klingaman and Oliver 1994; Smith et al. 2000). An extended period of seedling emergence (Jha et al. 2006) and rapid erect growth (Norsworthy et al. 2008) make it hard to determine the exact timing of postemergence herbicides. Development of confirmed resistance to four major modes of action of herbicides including 5-enolpyruvylshikimate-3-phosphate synthase inhibitors, acetolactate synthase inhibitors, mitotic inhibitors, and photosystem II inhibitors (Heap 2011), contribute to difficulty in controlling this weed.

Among other factors, including continuous use of herbicides with the same mode of action, development of herbicide resistance can be attributed to genetic diversity of weed species (Holt 1992; Holt and LeBaron 1990). A wide range of phenotypic variation with respect to susceptibility to herbicides (Burgos et al. 2001; Gossett et al. 1992; Horak and Peterson 1995) and plant growth and development (Bond and Oliver 2006) can be observed in Palmer amaranth accessions. This variation could be related to genetic diversity existing in these accessions. Genetic variation existing within a weed population and common among populations sharing a geographical area determines their potential for adaptability to different management practices and invasion of new areas (Radosevich et al. 2007). Therefore, characterization of genetic variation existing within selected biotypes of a weed species may provide a baseline contributing to management of evolved resistance (McRoberts et al. 2005).

Several studies have been conducted to assess levels of genetic variation and relationships among crops, among weed species, and among crops and weed species using molecular markers (Chan and Sun 1997; Garcia-Mas et al. 2000; Hongtrakul et al. 1997; Lima et al. 2002; McRoberts et al. 2005; Ranade et al. 1997; Ray and Roy 2009; Ulloa et al. 2003). Amplified Fragment Lneght Polymorphisms is a DNA fingerprinting technique based on the selective polymerase chain reaction (PCR) amplification of subsets of genomic restriction fragments (Vos et al. 1995). This technique can be used to generate hundreds of highly replicable markers from DNA of any organism without prior knowledge of sequence using a limited set of generic primers (Mueller and Wolfenbarger 1999; Vos et al. 1995). AFLPs are time and cost effective, and in terms of replicability and resolution, are superior or equal to allozymes, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and Single Sequence Repeats (SSRs) (Mueller and Wolfenbarger, 1999). AFLP markers have many applications including systematics, pathotyping, biotype genetics, DNA fingerprinting, and QTLS mapping (Arens et al. 1998; Gonzales et al. 1998; Heun et al. 1997; Huys et al. 1996; Janssen et al. 1997; Jin et al. 1998; Maughen et al. 1996; Voorips et al. 1997).

High levels of polymorphism were revealed in foxtail millet [*Setaria italica* (L.) P. Beauv.] and green foxtail millet [*Setaria viridis* (L.) P Beauv.] by AFLP markers, and since these millets were self-pollinating species, there was little variation within accessions, while among accessions variation was large (d'Ennequin et al. 2000). In a genetic diversity study of jointed goatgrass (*Aegilops cylindrica* Host.) accessions, both RAPD and AFLP markers detected limited genetic diversity. However, AFLPs separated almost all accessions and generated more scorable bands than RAPDs (Pester et al. 2003). Results of a study to evaluate genetic relationships between wild and domesticated cowpea (*Vigna Unguiculata* L.) accessions using AFLPs revealed wild accessions to be more diverse than domesticated cowpea accessions. It was also observed that cowpea accessions from areas expected to be centers of origin were found to be more diverse with gene flow among wild and domesticated types influencing genetic diversity, resulting in development of considerable biotypes of cowpea (Coulibaly et al. 2002). Results of cluster analysis carried out to study genetic relationships of various pigweed species grouped Palmer amaranth, a dioecious *Amaranthus* species, with spiny amaranth (*Amaranthus spinosus* L.), a monoecious *Amaranthus* species, suggesting possible hybrid formation between these two species (Wassom and Tranel 2005).

AFLPs are a robust procedure for detecting genetic diversity among closely related accessions (Pester et al. 2003). Therefore research was conducted to assess levels of genetic diversity in Palmer amaranth biotypes from North Carolina and Georgia using AFLPs and to characterize diversity among biotypes, geographical regions, glyphosate response, and gender.

Materials and Methods

Plant Material. Eight Palmer amaranth biotypes collected from North Carolina and Georgia were used for this study (Culpepper et al. 2008) (Table 1). Out of these, four biotypes were glyphosate susceptible (GS, one from North Carolina and three from Georgia) and four were glyphosate resistant (GR, one from North Carolina and three from Georgia). Seeds of Palmer amaranth were planted in excess in standard plastic 54-cm nursery trays[1] having six rectangular plastic pots each filled with commercial potting media[2] in the greenhouse. A week after emergence plants were fertilized[3] and seedlings thinned to one per pot. Twenty Palmer amaranth plants were maintained for each biotype. Approximately 2 wk after emeregence, four immature, unopened leaves were collected in 2.0 ml screw cap eppendorf[4] tubes containing two ceramic beads[5] and kept on ice until DNA extraction.

DNA extraction and AFLP analysis. DNA extraction was carried out using CTAB method (Afanador et al. 1993) with the modification that Fast prep® FP120[6] was used for tissue grinding. DNA was extracted from young leaves by grinding in Fast prep FP120 for 20 s along with 600 µl extraction buffer (2% CTAB, 1.4 M NaCl, 0.02M EDTA, 0.1M Tris-HCl pH 8.0 heated to 65°C + 1% β-mercaptoethanol). Eppendorfs were incubated in a water bath at 65°C for 30 minutes. After adding 600 µl chloroform:isoamyl alcohol (24:1), tubes were kept on a horizontal shaker[7] for 15 min and centrifuged[8] at 8000 rpm for 12 min. The aqueous layer was transferred into a new eppendorf and 600 µl isopropanol was added to it. Contents were mixed well and kept at -20°C for 10 min and then centrifuged at 13000 rpm for 7 min. The supernatant was discarded and the pellet washed in 70% cold (4°C) ethanol and dried in a vacuum dessicator for 12 min. The dried pellet was resuspended in 100 µl of TE, and 2 µl RNAase was added and tubes incubated in a water bath at 65°C for 10 min. The supernatant was transferred to clean tubes after centrifugation at 13000 rpm for 8 minutes. DNA was precipitated with 10 µl ammonium acetate and 300 µl cold (-20°C) ethanol. After mixing the contents very gently, tubes were kept at -20°C for 30 min. Tubes were again centrifuged at 13000 rpm for 10 min and the supernatant was discarded. The pellet was vacuum-dried for 12 min and resuspended in 100 µl TE. Tubes were centrifuged at 10000 rpm for 20 min and supernatant transferred to clean tubes. DNA was quantified using a fluorometer[9] and diluted to 20 ng/ µl and stored at 4 °C. AFLP reactions were performed following protocols of Milla et al. (2005) using fluorescently labeled [*Eco*RI + 3][10] and non-labeled [*Mse*I + 3][11] primers. Sample handling following amplification and polyacrylamide gel electrophoresis (PAGE) were carried out according to Milla et al. (2005). Master mix per sample for restriction digestion consisted of 1.6 µl water , 7.0 µl 5XR/L buffer+BSA , 0.6 µl *Eco*RI (12 U@20 U/µl) and 0.8 µl *Mse* I (12 U@20 U/µl). Ten microlitres of restriction digestion master mix was added to 25 µl of sample (20 ng/µl) and incubated at 37°C for 1 hr. After completion of digestion, 10 µl were removed from each sample and put in PCR plate already containing 2 µl loading buffer and run on 0.8% agarose gel. A smear in 100 to1200 bp region signified success of digestion. The digested template was stored at 4°C till ready for ligation. The master mix for adapter ligation per sample contained 1.2 µl 5XR/L buffer+BSA , 0.6 µl *Eco*RI adapter (@5pMo/ µl), 0.6 µl *Mse* I adapter (@5pMo/ µl), 0.6 µl ATP (10mM, pH 8.0) and 0.16 µl T4 DNA ligase (400 U/µl). Five µl ligation mix were added to 25 µl of restriction reaction and incubated at 37°C overnight. The mixture was diluted by addition of 220 µl of sd water**.** Pre-amplification master mixture per sample was prepared by mixing 2.0 µl of 10X PCR buffer , 9.96 µl sd water , 1.6 µl dNTP mixture (2.5mM each), 0.6 µl E01-A primer (@50 ng/ µl~8.3 µM), 0.6 µl M02-C primer ((@50 ng/

μl~8.3 μM) and 0.24 μl Taq polymerase(@5 U/μl). Fifteen μl of pre-amplification master mix was added to 5.0 μl aliquot of diluted R/L product (DNA template) in PP Plate resulting in 20 μl of PCR reaction. The PCR profile for pre- amplification for PTC-100 thermocyclers[12] is shown in Table 2. After completion of pre-amplification reaction, 5.0μl of mixture was pipetted and mixed with 5.0 μl low BFB loading buffer per sample and run on 0.8% agarose gel. A smear in 100 to1200 bp range signified success of pre-amplification. The remaining 15 μl of pre- amplification products were transferred to micro-dilution tubes and diluted 1:20 by addition of low TE (pH 8.0). The selective amplification master mix per sample contained 5.33 μl sd water, 2 μl of 10X PCR buffer, 1.6 μl dNTP mixture, 0.83 μl IRD-labeled E-primer (6 ng/ μl~1 μM), and 0.24 μl Taq polymerase(@5 U/μl). Ten μl of selective amplification master mix were dispensed into 5.0 μl of diluted pre-amplification DNA and 5.0 μl of M+3 primer (6 ng/ μl~1 μM) resulting in 20 μl of final reaction volume. The PCR profile for selective-amplification for PTC-100 thermocyclers is shown in Table 3. To 20 μl of selective amplification products, 10 μl of formamide loading buffer was added and samples were run on sequencing gels on LI-COR 4300 DNA analyzer[13].

Data analysis. To score amplified bands obtained using AFLP markers, AFLP-Quantar 1.0 software package[14] was used. The presence and absence of bands was treated as binary data where 0 and 1 denoted absence and presence of bands, respectively. Bands present at the same bp in different individuals were assumed to be the same allele. Binary data were used for calculating standard statistics for all primer combinations (PC). The statistics calculated were total number of bands, number of polymorphic bands, percentage of polymorphic bands, and size range of scored bands. Polymorphic information content (PIC) and marker index (MI) values were calculated for each PC so as to determine each primer combination's ability to detect polymorphisms. The mean PIC value for n loci was calculated as:

$$\mathrm{PIC} = \frac{\sum_{j-1}^{n}(1 - F_{aaj}^2 - F_{anj}^2)}{n}$$

where *j* is the *j*th locus, F_{aaj}^2 is the frequency of the amplified allele, and F_{anj}^2 is the frequency of the non-amplified allele (Geuna et al., 2003).The MI was calculated as MI = PIC x *n* x *ß*, where *n* is the total number of amplified fragments per primer, and *ß* is the proportion of polymorphic fragments (Varshney et al. 2007).

Individuals were grouped by biotype (Wayne, Johnston, Sumter, Macon, Emanuel, and Crisp), gender (male, female, unknown), geographical location (North Carolina and Georgia), or response to glyphosate (resistant and susceptible). Binary data were used to calculate Dice genetic similarity (GS) values (Dice 1945) for all pair-wise genotypic combinations using NTSYSpc v 2.2 software (Rohlf 2000). Similarity matrices thus obtained were used to calculate average GS values for each group in order to compare levels of diversity among the different groups of individuals. Genetic similarity values were also used to construct dendrograms using the neighbor-joining (NJ) clustering method(Saitou and Nei 1987). Using the Dcenter and Eigen options of NTSYSpc v 2.2 software, a principal coordinate (PCO) analysis was executed based on the distance matrix. The second and third principal coordinate scores were plotted. Furthermore, to partition molecular variation among and within groups, an analysis of molecular variance (AMOVA) was performed using Arlequin version 2.001[15] (Schneider et al., 2002)

Results and Discussion

AFLP Polymorphism. A total of 997 bands were generated using eight primer combinations. Out of those, 955 distinct, polymorphic bands ranging in size from 63 to 700 bp were scored. Standard statistics for all PCs are summarized in table 4. The number of polymorphic scored bands amplified per primer combination ranged from 98 to165, with an average of 119. PIC values ranged from 0.16 to 0.27, with an average of 0.20. The primer with the highest PIC and MI values was E43M51, indicating that this PC had the most discriminating power.

Genetic relationships. Average maximum and minimum genetic similarity values were 0.8605 and 0.0773, respectively. Average genetic similarity values when individuals were grouped by biotype are listed in Table 5. The biotype from Crisp County had the highest average within biotype genetic similarity value (0.5596) indicating that individuals within this biotype were least diverse and most closely related to one other. Sumter County biotype (GS) had the lowest within genetic similarity value (0.3569), implying that individuals within this biotype were the most diverse (Table 5). When comparisons were made among biotypes from different counties, it was observed that biotypes from Crisp (GS) and Emanuel (GR) counties were most closely related as evident from the highest among biotypes GS value of 0.4895 while biotypes from Sumter (GR) and Macon (GR) counties were least related with the lowest among biotypes GS of 0.2403.

Genetic similarities were also compared by grouping individuals by their response to glyphosate. As such, individuals were divided into two main groups; GR and S. GS values within R and S biotypes were 0.3439 and 0.3504, respectively while GS among GR and S biotypes was 0.3270 (Table 6). Genetic similarities did not vary much, indicating that biotypes either resistant or susceptible to glyphosate had almost similar levels of genetic diversity. It is generally assumed that a higher degree of genetic variability leads to better adaptation to prevailing environmental stresses. Therefore, glyphosate-resistant biotypes should be more diverse than susceptible biotypes since they are better adapted to withstand glyphosate applications. However, results from our study indicated that both GR and GS biotypes had similar levels of genetic variation. The major groups (GR and GS) were divided into subgroups based on geographical location forming four categories; resistant from North Carolina (RNC), susceptible from North Carolina (SNC), resistant from Georgia (RGA), and susceptible from Georgia (SGA) (Table 6). When comparing sub-groups based on geographical location, the GR biotype from North Carolina had the highest within biotype GS (0.4315) while the GR biotype group from Georgia had the lowest within biotype GS (0.3685). Among biotypes, both resistant and susceptible biotypes from Georgia were most similar based on the highest among biotypes GS value of 0.3504. Resistant biotypes from Georgia were least similar to susceptible biotypes from North Carolina as they shared a GS value of 0.2835 which was the lowest (Table 6). Overall, levels of genetic variability did not seem to be biased towards any of the groupings of individuals by response and then further regrouping by geographical location.

Another comparison within and among GS was made by grouping individuals as either male (M) or female (F) and further subgrouping them based on states as North Carolina males (MNC), North Carolina females (FNC), Georgia males (MGA), and Georgia females (FGA) (Table 7). GS values within males and females and among males and females varied little, indicating that neither group is more diverse that the other. The highest and the lowest within group GS were observed in females from Georgia (0.3863) and males from Georgia, respectively. The highest among group GS existed among males and females from North Carolina (0.3705) while the lowest among group GS was among males from North Carolina and females from Georgia. Overall, GS varied little when individuals were grouped by gender. Based on GS, grouping individuals by biotype provided the widest range in GS values and the best separation of individuals.

Cluster Analysis. Dice GS values were used to create a dissimilarity matrix which was then utilized to construct dendrograms using NJ method (Saitou and Nei 1987). Individuals clustered together mainly by biotype (Figure 1). Since every biotype was a representation of a specific geographical location, this might also be interpreted as grouping of individuals based on their geographical location. Overall, the dendrogram consisted of two major clusters. Cluster I consisted of Wayne (R) and Johnston (S) County biotypes from North Carolina along with both R and S biotypes from Sumter County in Georgia whereas cluster II consisted of individuals from Macon (R), Emanuel (R and S), and Crisp (S) counties in Georgia. Within cluster I there were three sub-clusters, A, B, and C. Sub-cluster A contained almost all of Johnston biotype individuals (seventeen) grouped with two Wayne County individuals and almost half of the individuals (twenty one) from the two R and S biotypes from Sumter County. Sub-cluster B consisted mostly of Wayne County biotype individuals. Sub-cluster C included all the remaining individuals from both R and S biotypes from Sumter County. In cluster II the presence of distinct sub-clusters was not as clearly evident, however all individuals from a biotype were clustered together with the exception of four individuals.

Two individuals from S Emanuel County clustered with Crisp County individuals, while two individuals from Crisp County clustered with S biotype from Emanuel County. Collectively, cluster analysis grouped together individuals from the same biotype except for Sumter County biotypes. Individuals from Sumter County biotypes fell with Wayne and Johnston County individuals instead of forming distinct clusters like other biotypes. Genetic similarities within these two biotypes from Sumter County were low as compared to other biotypes, indicating comparatively greater genetic diversity among individuals (Table 5). This might be responsible for all individuals not clustering together in these biotypes unlike other biotypes evaluated in this study.

An NJoin dendrogram was also constructed by averaging genetic similarities of individuals over biotypes (Figure 2). The dendrogram clustered biotypes into two major clusters just like the dendrogram in Figure 1. Cluster I was formed by Wayne, Johnston, and two Sumter County biotypes while cluster II was formed by Macon, Crisp, and two biotypes from Emanuel County (Figure 2). In this dendrogram, the two clusters were representative of biotypes from two major geographical locations (NC and GA). Biotypes from respective states clustered together with the exception of Sumter County biotypes which grouped with North Carolina biotypes instead of clustering with biotypes from Georgia. This might be due to higher levels of genetic variation present among individuals from these populations.

Principal Coordinate analysis. PCO was also used to evaluate genetic relationships among Palmer amaranth biotypes. The first two axes of the PCO accounted for 41% of the variation with the first and second eigenvalues explaining 35% and 6%, respectively. Results of PCO (Figure 3) corresponded well with those of the cluster analysis as it separated individuals into two distinct clusters like NJ dendrograms (Figures 1 and 2). Cluster I was formed by individuals from Wayne, Johnston and Sumter counties while cluster II consisted of biotypes from Macon, Emanuel, and Crisp counties having individuals tightly packed together (Figure 3). Cluster II individuals were not as closely packed together as cluster I and this cluster could be further divided into three groups. Group A consisted most of the individuals from Johnston County grouped with a couple of individuals from Sumter County biotypes (both R and S biotypes). Group C, which was not as closely packed as group A, contained most of the individuals from Wayne County mixed with some Sumter County individuals (both R and S biotypes) and two Johnston County individuals. Group B, which was the least closely packed of the three groups, grouped together the remaining individuals from both R and S Sumter County biotypes along with four individuals from Wayne and one Johnston County individual (Figure 3). Compact grouping of individuals from Macon, Emanuel and Crisp counties is reflective of higher within and among GS values for these biotypes (Figure 3 and table 5). Comparatively lower within and among GS values for biotypes from Wayne, Johnston and Sumter counties were responsible for their loose clustering (Figure 3 and table 5).

Analysis of molecular variance. Results of the AMOVA indicated that significant ($P < 0.0001$) differences existed among and within biotypes when individuals were analyzed by grouping them by biotype, by state, and by response to glyphosate (Tables 8, 9, and 10). However, among component of variation was not significant when individuals were grouped by gender, but within component of variation was significant (Table 11). Irrespective of grouping of individuals, the within component of variation was always significant (P <

0.0001) and accounted for a greater percentage of total variation (Tables 8,9,10, and 11). On grouping individuals by biotypes, among biotype variation accounted for 21.29% while within biotype component accounted for 78.71% of total variation (Table 8). When individuals were grouped by state, among and within components of variance accounted for 9.36% and 90.64% of variation, respectively (Table 9). Grouping by response to glyphosate partitioned total variation into 3.05% among and 96.95% within biotypes (Table 10). When individuals were grouped by gender, among component of variation was just 0.67% (not significant) while within component accounted for 99.33% of the total variation (Table 11). Based on these results, grouping of individuals by biotype provided the best separation of groups as it resulted in the highest (21.29%) among groupings component of variation.

AMOVA was also carried out by grouping individuals by biotype, by response to glyphosate and by gender and then nesting these groupings within states. Results of these analyses also indicated that irrespective of grouping, within biotypes component of variation was always significant ($P < 0.0001$) and accounted for a greater percentage of the total variation (Tables 12, 13, and 14). When biotypes were nested within states, variation among states accounted for 3.26 % of total variation which was not significant, while among and within biotypes components of variation were 19.49% and 77.26%, respectively and both were significant ($P < 0.0001$) (Table 12). Nesting response to glyphosate within states partitioned total variation as 6.46%, 5.91%, and 87.64% into among states, among biotypes, and within biotypes, respectively (Table 13). Among states variation did not account for a significant portion of total variation while among and within biotypes components were significant ($P < 0.0001$). On nesting gender within states, among states accounted for 9.02%

while among biotypes accounted for 0.74% of total variation and both were not significant (Table 14). However, within biotypes component accounted for 90.24% of total variation which was significant and greater than other two components. Overall, the highest contribution towards total variation (19.49%) by among groups nested within states component is obtained when individuals were grouped by biotype and nested within states (Table 12). The results of AMOVA were also supported by cluster analysis, which grouped individuals by biotype (Figure 1).

Collectively, AFLPs detected high levels of genetic diversity in the Palmer amaranth biotypes studied. The AMOVA results indicated that grouping by state and then biotype provides the best separation of groups. Cluster and PCO analyses results also supported these findings by grouping individuals based on biotype/geographical location. While there were significant differences in levels of diversity among groupings, the vast majority of genetic variation always resided within biotypes. These results highlighted the great degree of genetic variation present within Palmer amaranth biotypes that might be contributing to the competitive ability and success of this weed species under selection pressure from glyphosate. Furthermore, these results highlighted the importance of site specific weed management of Palmer amaranth biotypes as individuals belonging to a specific geographical location tend to cluster together.

Acknowledgements

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Sources of Materials

[1] Myers Industries Lawn & Garden Group,15200 Madison Road, P.O. Box 738, Middlefield, OH 44062-0738

[2] Fafard 4P potting mix, Conrad Fafard Inc. Agawam, MA.

[3] Scotts® Starter® Fertilizer, The Scotts Company LLC, Marysville, OH.

[4,5] Eppendorf AG, Barkhausenweg 1, 22339 Hamburg, Germany.

[6] Fast Prep® FP120, Qbiogene, Carlsbad, CA.

[7] Lab-line horizontal shaker, Fisher scientific, 2000 Park Lane Dr. Pittsburg, PA, 15275.

[8]Centirfuge 5424, Eppendorf AG, Barkhausenweg 1, 22339 Hamburg, Germany.

[9] Hoefer fluorometer, Hoefer Scientific Instruments, San Francisco, CA.

[10] Labeled EcoRI + 3 primers were purchased from LI-COR Inc., Lincoln, NE.

[11]All non-labeled primers and adapters were obtained from Sigma Genosys, The Woodlands, TX.

[12] BIO-RAD My Cycler™ Personal Thermal Cycler, Life Science Research, 2000 Alfred Nobel Drive, Hercules, CA 94547.

[13] LI-COR Biosciences ,4647 Superior St, Lincoln, NE 68504, USA.

[14]AFLP-Quantar 1.0 software package, Keygene Products B.V., Wageningen, Netherlands.

[15] Arlequin ver. 2.001, Univ. of Geneva, Geneva, Switzerland.

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Table 1. List of Palmer amaranth biotypes used for assessment of molecular diversity with AFLP markers.

County	State	Response to glyphosate
Wayne	North Carolina	Resistant
Johnston	North Carolina	Susceptible
Sumter	Georgia	Susceptible
Sumter	Georgia	Resistant
Macon	Georgia	Resistant
Emanuel	Georgia	Resistant
Emanuel	Georgia	Susceptible
Crisp	Georgia	Susceptible

Table 2. PCR profile for pre-amplification for thermocyclers.

28 cycles	94°C	15 seconds
	60°C	30 seconds
	72°C	60 sec,+1 sec/cycle extension
1 cycle	72°C	2 minutes
Hold	4°C	

Table 3. PCR profile for selective-amplification for thermocyclers.

3 cycles	94°C	10 seconds
	65°C	30 seconds,-0.7°C/cycle after cycle
	72°C	60 seconds
25 cycles	94°C	10 seconds
	56°C	30 seconds
	72°C	60 seconds, +1 second/cycle extension
1 cycle	72°C	2minutes
Hold	4°C	

Table 4. Standard statistics for AFLP primer combinations used to assess genetic diversity among Palmer amaranth biotypes.

PC	TB[1]	PB[2]	PPB[3]	SRSB[4]	PIC[5]	MI[6]
E38M60	103	98	95.14	63-692	0.22	21.44
E39M49	112	103	91.96	65-604	0.20	20.71
E39M53	87	81	93.10	71-615	0.16	12.59
E39M55	118	113	95.76	64-672	0.17	19.36
E40M59	125	120	96.00	81-700	0.19	23.67
E43M51	169	165	97.63	87-681	0.27	44.47
E44M49	155	150	96.77	74-675	0.18	27.57
E45M58	128	125	97.66	94-689	0.18	22.25
	997*	955	95.5**	63-700***	0.20**	24.01**

[1] TB: total number of bands (scored polymorphic + monomorphic bands)

[2] PB: number of polymorphic bands

[3] PPB: percentage of polymoprhic bands

[4]SRSB: size range of scored bands

[5]PIC: polymoprhic information content

[6]MI: marker index

* Total

** Average

*** Range

Table 5. Average genetic similarity values among and within Palmer amaranth biotypes collected from North Carolina and Georgia.

County	Wayne (R) (n*=20)	Johnston (S) (n=20)	Sumter (S) (n=20)	Sumter (R) (n=20)	Macon (R) (n=20)	Emanuel (R) (n=20)	Emanuel (S) (n=19)	Crisp (S) (n=20)
Wayne (R)	0.4315							
Johnston (S)	0.3179	0.4075						
Sumter (S)	0.3222	0.3232	0.3569					
Sumter (R)	0.2962	0.3132	0.3181	0.3637				
Macon (R)	0.2889	0.2648	0.2623	0.2403	0.5567			
Emanuel(R)	0.2962	0.2726	0.2829	0.2562	0.4425	0.5360		
Emanuel (S)	0.2703	0.2643	0.2642	0.2467	0.4004	0.4448	0.4626	
Crisp (S)	0.3188	0.2946	0.3010	0.2713	0.4399	0.4895	0.4761	0.5596

*n= number of individuals per biotype

Table 6. Average genetic similarity values among and within Palmer amaranth biotypes collected from North Carolina and Georgia by grouping them based on response to glyphosate.

Grouping	R[1]	S[2]		
R	0.3439			
S	0.3270	0.3504		
	RNC[3]	SNC[4]	RGA[5]	SGA[6]
RNC	0.4315			
SNC	0.3179	0.4075		
RGA	0.2937	0.2835	0.3685	
SGA	0.3042	0.2945	0.3504	0.3826

[1]Resistant to glyphosate (pooled over states)

[2]Susceptible to glyphosate (pooled over states)

[3]Resistant from North Carolina

[4]Susceptible from North Carolina

[5] Resistant from Georgia

[6] Susceptible from Georgia

Table 7. Average genetic similarity values among and within Palmer amaranth biotypes collected from North Carolina and Georgia by grouping them based on gender.

Grouping	M[1]	F[2]
M	0.329729	
F	0.333861	0.345512

	MNC[3]	MGA[4]	FNC[5]	FGA[6]
MNC	0.366221			
MGA	0.300629	0.347492		
FNC	0.370523	0.296812	0.363339	
FGA	0.291909	0.358424	0.286902	0.386291

[1]Male (pooled over states)

[2]Female (pooled over states)

[3] Male from North Carolina

[4] Male from Georgia

[5] Female from North Carolina

[6]Female from Georgia

Table 8. Analysis of molecular variance results when individuals were grouped by biotype.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P-value
Among biotypes	7	3434.67	20.81	21.29	<0.0001
Within biotypes	151	11623.50	76.98	78.71	<0.0001
Total	158	15058.16			

Table 9. Analysis of molecular variance results when individuals were grouped by state.

Source of variation	Df	Sum of squares	Variance components	Percentage of variation	P-value
Among biotypes	1	658.79	9.47	9.36	<0.0001
Within biotypes	157	14399.37	91.7	90.64	<0.0001
Total	158	15058.16			

Table 10. Analysis of molecular variance results when individuals were grouped by response to glyphosate.

Source of variation	Df	Sum of squares	Variance components	Percentage of variation	P-value
Among biotypes	1	328.45	2.95	3.05	<0.0001
Within biotypes	151	14729.72	93.82	96.95	<0.0001
Total	158	15058.16			

Table 11. Analysis of molecular variance results when individuals were grouped by gender.

Source of variation	Df	Sum of squares	Variance components	Percentage of variation	P-value
Among biotypes	2	246.21	0.64	0.67	0.045
Within biotypes	156	14811.96	94.95	99.33	<0.0001
Total	158	15058.16			

Table 12. Analysis of molecular variance results by nesting biotypes within states.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P-value
Among states	1	658.79	3.24	3.26	0.3724
Among biotypes within states	6	2775.87	19.42	19.49	<0.0001
Within biotypes	151	11623.50	76.98	77.26	<0.0001
Total	158	15058.16			

Table 13. Analysis of molecular variance results by nesting response to glyphosate within states.

Source of variation	Df	Sum of squares	Variance components	Percentage of variation	P-value
Among states	1	658.79	6.53	6.46	0.3480
Among biotypes within states	2	652.48	5.98	5.91	<0.0001
Within biotypes	155	13746.86	88.69	87.64	<0.0001
Total	158	15058.16			

Table 14. Analysis of molecular variance results by nesting gender within states.

Source of variation	Df	Sum of squares	Variance components	Percentage of variation	P-value
Among states	1	658.79	9.13	9.02	0.1026
Among biotypes within states	3	339.31	0.75	0.74	<0.0.264
Within biotypes	154	14060.06	91.29	90.24	<0.0001
Total	158	15058.16			

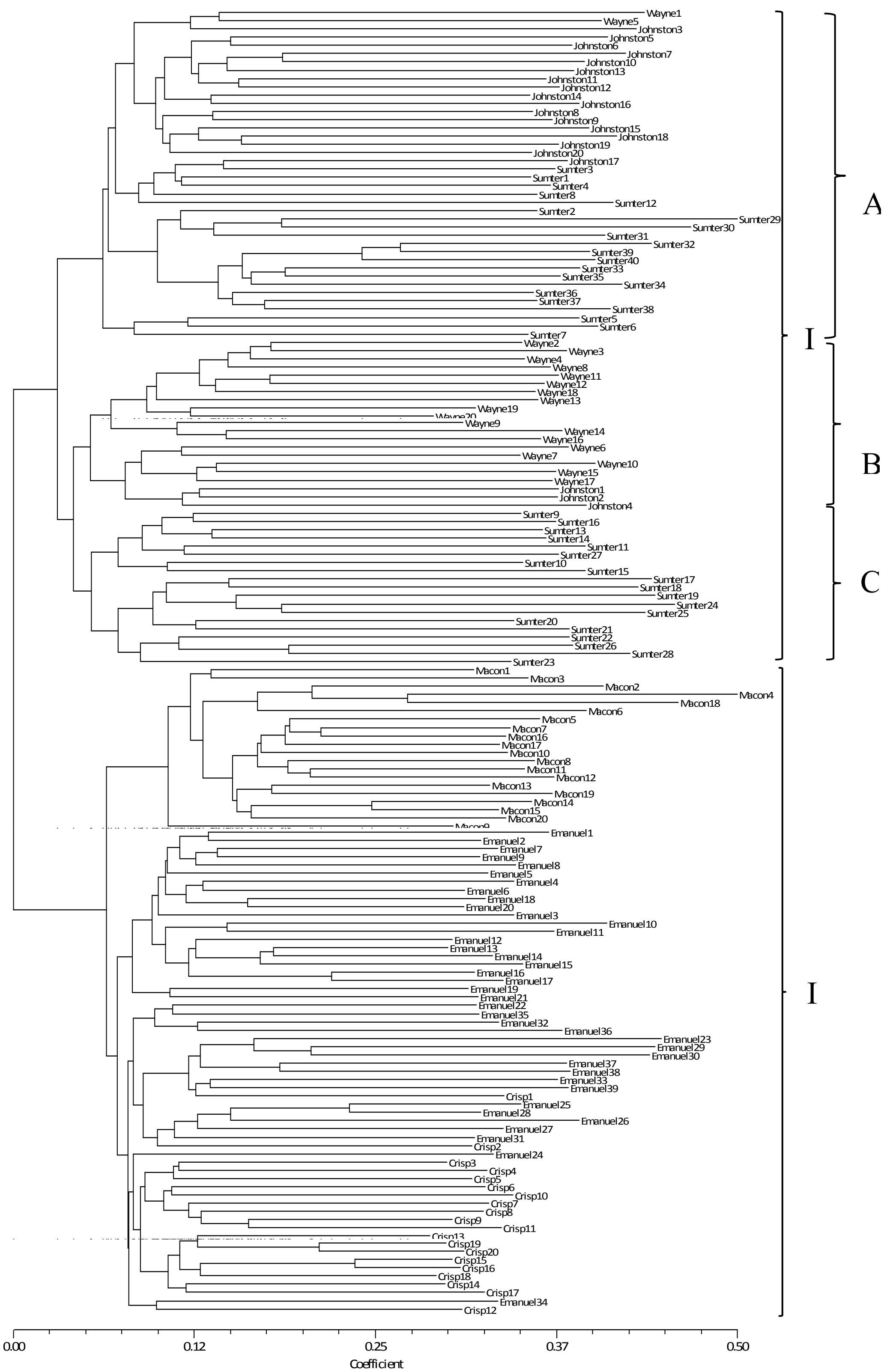

Figure 1. NJoin dendrogram of 159 individuals using eight AFLP markers.

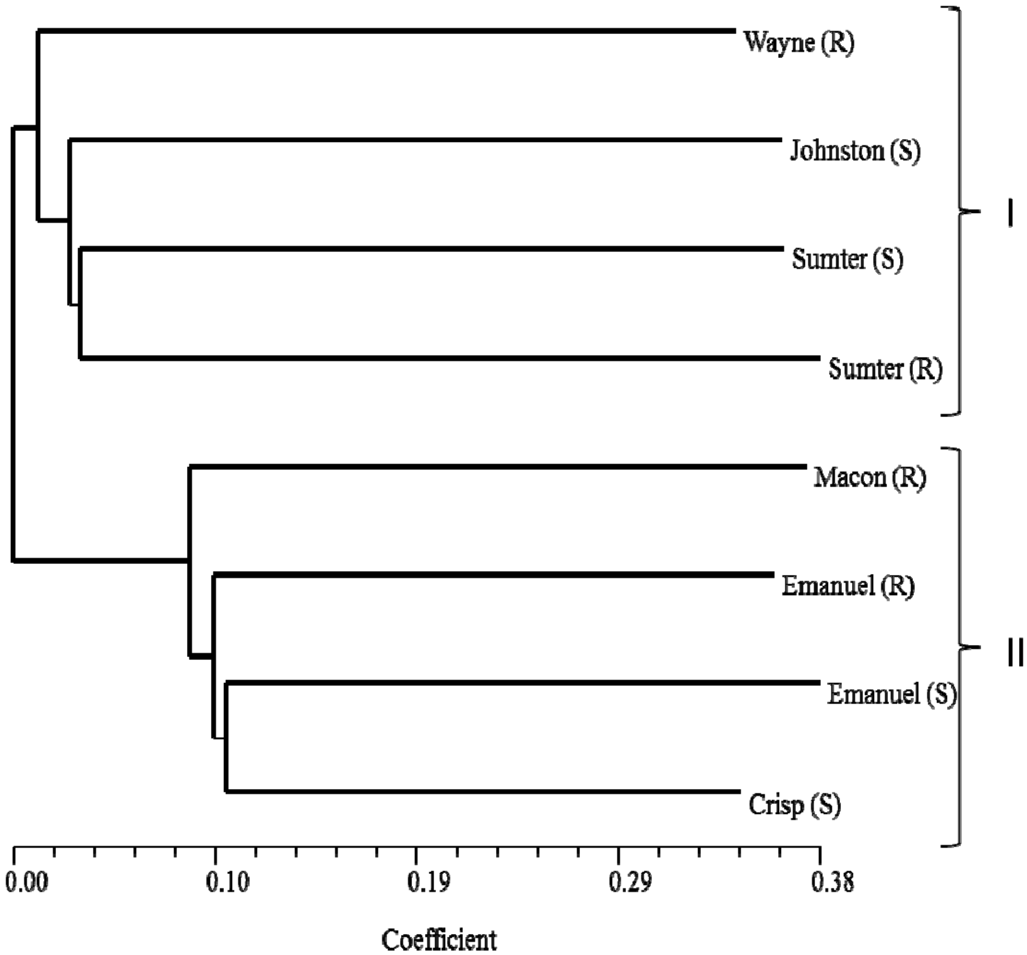

Figure 2. NJoin dendrogram of 159 individuals using eight AFLP markers pooled over biotypes.

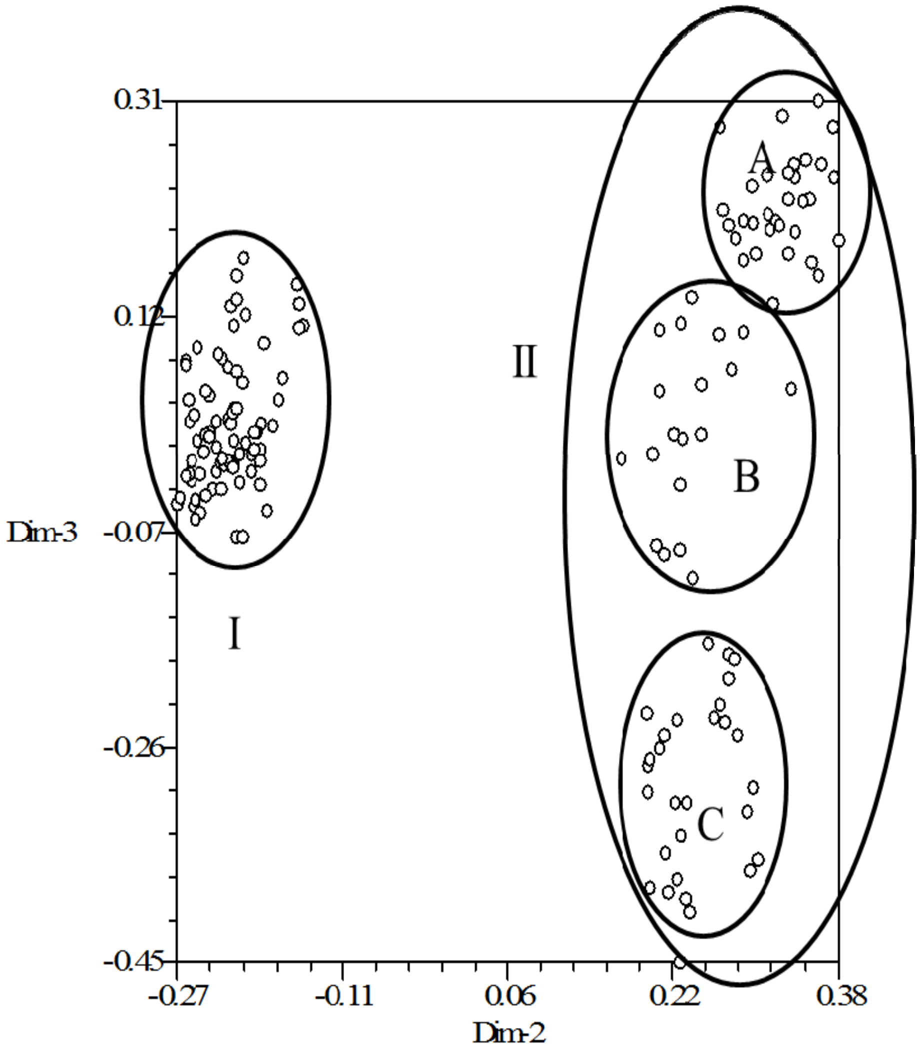

Figure 3: Principal Coordinate plot of 159 Palmer amaranth individuals for second and third eigenvalues estimated with 8 AFLP markers.

CHAPTER II

Elucidating the Inheritance of Evolved Resistance to Glyphosate in a Biotype of Palmer amaranth (*Amaranthus palmeri*) from North Carolina

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Abstract

Glyphosate-resistant corn, cotton and soybean are major crops in southern cropping systems. Widespread adoption of these crops replaced selective herbicides with glyphosate and intensified selection pressure on weeds for glyphosate resistance. Many biotypes of Palmer amaranth (*Amaranthus palmeri* S. Wats.), one of the most troublesome weeds in southern cropping systems, have developed resistance to glyphosate in recent years. Although appearance of glyphosate-resistant Palmer amaranth biotypes has been well documented, information associated with the mechanism of inheritance of resistance is limited. Research

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was conducted to study inheritance of glyphosate resistance in a Palmer amaranth biotype from North Carolina. I_{50} values for visible control of resistant (GR) and susceptible (GS) biotypes were 1735 and 51 g/ha, respectively. I_{50} values for F_1 progeny obtained from resistant female x susceptible male (RxS) and susceptible female x resistant male (SxR) biotypes were 1388 and 1159 g/ha, respectively. Dose response behavior of F_1 progeny indicated that resistance was not fully dominant over susceptibility. Furthermore, lack of significant differences between dose responses for reciprocal F_1 families suggested that genetic control of glyphosate resistance is governed by the nuclear genome. When looking at dose response results for BC_1F_1 families, chi-square values for many BC_1F_1 families conformed to monogenic inheritance. However, chi-square values for all BC_1F_1 families combined together were not consistent with a single gene hypothesis for both doses of glyphosate. These results indicated that inheritance of glyphosate resistance in studied biotype of Palmer amaranth from North Carolina was incompletely dominant, nuclear inherited, and might not be consistent with single gene mechanism of inheritance.

Nomenclature: Corn, *Zea mays* L.; cotton, *Gossypium* hirsutum L.; palmer amaranth, *Amaranthus palmeri* S. Wats.; soybean, *Glycine max* (L.) Merr.

Key words: Backcross families, F_1 families.

Abbreviations: GR, glyphosate resistant; GS, glyphosate susceptible; RxS, resistant female crossed with susceptible male; SxR, susceptible female crossed with resistant male.

Introduction

Glyphosate (*N*-[phosphonomethyl]glycine) has become the world's most widely used herbicide since its commercialization in 1974 because it is effective, economical, and comparatively safe to the environment (Dill et al. 2008; Duke and Powles 2008). Being non-selective, glyphosate is used to control a wide array of weed species including both grasses and broadleaf weeds (Powles 2008; Baylis 2000). When glyphosate-resistant crops (including canola (*Brassica napus* L.), corn, cotton, and soybean) were introduced beginning in 1996, glyphosate revolutionized agriculture by enabling growers to use this herbicide for weed control in standing crops (Dill et al. 2008). Glyphosate-resistant corn, cotton and soybean are major crops in southern cropping rotations. Widespread adoption of these crops replaced selective herbicides with glyphosate and intensified selection pressure on weeds. Currently 12 weed species with evolved resistance to glyphosate have been confirmed in the United States (Heap 2011). Palmer amaranth is among the most competitive weeds of southern cropping systems and populations of Palmer amaranth have developed resistance to glyphosate in recent years because of repeated applications of this herbicide (Culpepper et al. 2008; Heap 2011). Evolution of glyphosate resistance in weed species poses a great risk to incessant success of glyphosate-resistant crops (Powles 2008). Although the extent of glyphosate-resistant Palmer amaranth biotypes has been well documented (Culpepper et al. 2006, 2008; Norsworthy et al. 2008; Steckel et al. 2008), information is limited about the genetic control of resistance. Mode of inheritance, among other factors, is an important component affecting the evolution of resistance (Diggle and Neve 2001; Jasieniuk et al. 1994). The number of genes involved in governing resistance and their interactions influence not only the enrichment of resistance in a population, but also gene flow among populations (Gressel and Segal 1982; Maxwell et al. 1990; Roush et al. 1990).

Several studies have been conducted to determine herbicide resistance in weed species. In a population of wild oats (*Avena fatua* L.), resistance to Acetyl-CoA carboxylase (ACCase) inhibitors, including fenoxaprop-P, diclofop-methyl, and sethoxydim, was inherited as a single, partially dominant nuclear gene (Bruce et al. 1995). Similar results for inheritance of resistance to ACCase inhibitors were also reported in rigid ryegrass (*Lolium rigidum* Gaud.) (Tardif et al. 1996) and Italian ryegrass (*Lolium multiflorum* Lam.) (Betts et al. 1992). Inheritance of resistance to acetolactate synthase (ALS) inhibiting herbicide metsulfuron in a prickly lettuce (*Lactuca serriola* L.) biotype involved a single nuclear gene with incomplete dominance (Mallory-Smith et al. 1990). Similar results for genetic control of ALS resistance were observed in common sowthistle (*Sonchus oleraceus* L.) for the herbicides chlorsulfuron, flumetsulam, imazethapyr, imazapyr, and sulfometuron (Boutsalis and Powles 1995). Inheritance of resistance to dinitroaniline herbicides in green foxtail (*Setaria viridis* (L.) P. Beauv.) and goosegrass (*Eleusine indica* (L.) Gaertn.) was found to be controlled by a single nuclear recessive gene (Zeng and Baird 1997; Jasieniuk et al. 1994) while resistance to the bipyridilium herbicides diquat and paraquat was controlled by a single incompletely dominant nuclear gene (Purba et al. 1993).

The mechanism of inheritance of glyphosate resistance has been studied in several weed species. In a glyphosate-resistant population of rigid ryegrass from Australia, resistance was found to be an incompletely dominant trait encoded by a single nuclear gene (Lorraine-Colwill et al. 2001). In another study, dose responses of eight glyphosate-resistant populations of rigid ryegrass indicated that resistance was partially to fully dominant, within herbicide doses tested, and did not involve maternal inheritance (Wakelin and Preston 2006). Segregation of resistance in four out of five backcross populations in the same study was indicative of a single gene mechanism of inheritance. However, genetic control of glyphosate-resistance in a population of rigid ryegrass from California was reported to be incompletely dominant and multigenic involving at least two nuclear genes (Simarmata and Penner 2005). In glyphosate resistant horseweed (*Conyza canadensis* L.), resistance was found to be governed by a single nuclear incompletely dominant allele (Zelaya et al. 2004). Results of a glyphosate resistance inheritance study in goosegrass showed that resistance was inherited as a single, nuclear encoded incompletely dominant gene (Ng et al 2004).

Research on the inheritance of glyphosate resistance in Palmer amaranth has been limited. Only one study, looking at populations from Georgia (Gaines 2009) has been published to date. Resistance appeared to be a polygenic, additive, nuclear encoded, and a semi-dominant trait. However, probable apomixis occurring in the Palmer amaranth populations studied made it challenging to elucidate the mechanism of glyphosate inheritance. Therefore, the genetic control of evolved glyphosate resistance in this species needs to be further investigated. The present study was conducted with the objective to elucidate mechanism of inheritance of glyphosate resistance in a Palmer amaranth biotype from North Carolina.

Materials and Methods

Generation of F_1 families. Plants from a glyphosate-resistant (GR) biotype from Wayne county (I_{50} for visible control = 1770 g/ha) and a susceptible (GS) biotype from Johnston county (I_{50} for visible control = 89 g/ha) in North Carolina were used as parents (Whitaker et al. 2009). After collection from the field, seeds were kept at -20°C for a month. Seeds of Palmer amaranth were planted in excess in 10 cm square pots[1] containing commercial potting mix[2] in the greenhouse and were thinned to one plant per pot 8 d after emergence.

The greenhouse was maintained at 35 ± 5 °C and natural lighting was supplemented for 14 h each day with metal halide lighting[3] (400 µmol $m^{-2}s^{-1}$). Plants were kept enclosed in dialysis tubing[4] about 8 cm wide until they were ready to be paired for crosses to prevent any unintentional crossing. When plants began to form inflorescences, reciprocal crosses were made between GR and GS biotypes to generate 16 F_1 families. One plant each of the GR and GS biotypes were paired together and encased in dialysis tubing well before shedding of pollen occurred in order to prevent entry of foreign pollen. Fertilization was ensured by tapping the tubing twice every day. Single female plants were also enclosed in dialysis tubing as controls to check for efficacy of tubing in preventing entry of foreign pollen. Seeds from each female plant were harvested upon maturation and kept in separate envelopes to constitute individual F_1 families. As such, there were two sets of F_1 families: one originating from GR females (8 RxS families) and another from GS females (8 SxR families), making a total of 16 F_1 families. Germination tests on seeds from these families revealed a low germination percentage. Therefore, seeds were kept at -20°C for 50 d in order to break dormancy.

F_1 dose- response experiments. A dose response study for glyphosate was conducted on GS and GR biotypes and F_1 families. Fifteen seeds each for GS, GR and the 16 F_1 families were planted in 15-cm round pots containing commercial potting mix in the greenhouse. The potassium salt of glyphosate[5] was applied to Palmer amaranth at 3 to 4 leaf stage. Glyphosate was applied at 45, 90, 180, and 400 g ae/ha to the susceptible biotype, at 1000, 2000, 3000, and 4000 g ae/ha to the resistant biotype, and at 180, 400, 2000 and 3000 g ae/ha to the F_1 families. Glyphosate was applied in the greenhouse in 140 L/ha at 207 kPa using a CO_2-pressurized backpack sprayer with a flat-fan nozzle[6]. There were six replicate pots for each herbicide rate for GR and GS biotypes, and one pot for each of the 16 F_1 families (8 RxS and 8 SxR), thus families were used as replicates for each herbicide rate. Visible estimates of percent survival were taken 2 weeks after treatment and data from each group of maternal and paternal F_1 families were pooled.

Data Analysis for F_1 dose- response experiments. Percent survival rates were plotted against herbicide doses to develop a dose response curve using SIGMAPLOT 11.2[7]. I_{50} values for percent survival were calculated from the regression equation. Data were subjected to analysis of variance using GLM procedure in SAS[8] for the biotype by glyphosate rate factorial arrangement. Means were separated using Fisher's Protected LSD test at $p \leq 0.05$.

Generation of backcross families. Selected surviving males after 180 and 400 g ae/ha glyphosate application from each of the F_1 families were transplanted into round pots (10 cm diameter by 12 cm deep) and each paired with an GS female already in the pot in greenhouse. Both inflorescences were encased together in the same manner as outlined in the generation of F_1 families. Fertilization was ensured by tapping the tubing twice every day. Single female plants were also enclosed in dialysis tubing as controls. Seeds were harvested individually from each female and kept in separate envelopes forming 16 BC_1F_1 families. However, seed yield from one of the backcrosses was not enough and therefore was not included for further evaluation of glyphosate resistance. Seeds were kept at -20°C for 50 d to break dormancy.

Treating BC_1F_1 families with glyphosate. All fifteen BC_1F_1 families were assessed for resistance to glyphosate along with selected F_1 families (both RxS and RxS), GR, and GS biotypes as controls in the greenhouse. Seeds were planted in excess in 15-cm round pots and 10 d old seedlings were transplanted into rectangular nursery trays. About 50 seedlings were transplanted per tray and treated with 2000 and 3000 g ae/ha of glyphosate at 4 to 5 leaf stage. Glyphosate was applied as described previously. Numbers of surviving and dead individuals were recorded 3 wk after treatment and data from each group of maternal and paternal BC_1F_1 families were pooled.

Data Analysis for BC_1F_1 dose response experiments. Data for the observed number of surviving and dead plants were compared with predicted values by subjecting it to chi-square tests in order to understand if genetic control of glyphosate resistance in this biotype was governed by single or multiple genes. The proportion of surviving susceptible and F_1

individuals at each dose tested was used to calculate expected survival of BC_1F_1 families assuming monogenic inheritance (Tabashnik 1991). The equation used for calculating expected survival is as follows:

$Y_X = 0.05\ (W_{RS} + W_{SS})$ [1]

where,

Y_X = expected proportion alive

W_{RS} = proportion of individuals observed alive in the F_1 biotype (averaged over RxS and SxR families)

W_{SS} = proportion of individuals alive in GS biotype

Results and Discussion

Visible percent survival data indicated much less control of the glyphosate-resistant Palmer amaranth biotype as compared to susceptible (Figure 1). I_{50} values for R and S biotypes were 1735 and 51 g/ha, respectively. These values were similar to those obtained in earlier greenhouse studies involving the same biotypes of Palmer amaranth where I_{50}s for visual control were reported to be1769 and 89 g/ha for resistant and susceptible biotypes, respectively (Whitaker et al 2009). Variations in I_{50} values among experiments conducted at different times can occur possibly as a result of differences in seasonal conditions. Also, in an earlier study involving similar biotypes of Palmer amaranth, observations were recorded on visible control (Whitaker et al. 2009) while in present study data for percent survival were recorded.

I_{50} for combined RxS and SxR F_1 families were 1388 and 1159 g/ha, respectively. Both sets of F_1 families were treated with 180, 400, 2000 and 3000 g/ha of glyphosate (Table 1). Glyphosate rates common for GR parent biotype and F_1 families were 2000 and 3000g/ha while glyphosate rates common for GS parent biotypes and F_1 families were 180 and 400 g/ha. Therefore, comparisons between parent biotypes and both sets of F_1 families for percent survival were made at glyphosate rates that were common among them. Although, GR parents were not exposed to 180 and 400 g/ha of glyphosate, it was expected that all individuals would have survived at these rates because the I_{50} for this biotype was 1769 g/ha. Similarly, although GS parents were not exposed to 2000 and 3000g/ha of glyphosate, all individuals would have died at these rates given that the I_{50} for this biotype was 51 g/ha.

At any given dose, percent survival of F_1 progenies showed lower levels of resistance as compared to GR parent biotype and higher levels of resistance than GS parent biotype (Figure 1 and table 1). Response of both sets of maternal and paternal resistant F_1 families was closer to that of resistant parent (Figure 1). This was different from earlier observations where behavior of F_1 families was found to lean more towards the maternal parent (Gaines 2009). Dose response behavior of F_1 families indicated that resistance was not fully dominant over susceptibility. Also, values for percent survival of both sets of F_1 families at a given glyphosate dose were similar (Figure 1 and table 1). This lack of significant difference between dose responses of reciprocal F_1 families suggested that genetic control of glyphosate resistance is governed by the nuclear genome and there is no maternal or cytoplasmic inheritance involved. Inheritance of glyphosate resistance as an incompletely dominant trait controlled by nuclear genes has been reported in other weed species and other glyphosate-resistant populations of Palmer amaranth as well (Gaines 2009; Ng et al. 2004; Simarmata et al. 2005; Wakelin and Preston 2006; Zelaya et al. 2004).

To determine if genetic control of resistance involves single allele or multiple genes, the surviving males from sixteen F_1 families were backcrossed to susceptible females in individual crosses resulting in the formation of fifteen BC_1F_1 families. Plants were treated with 2000 and 3000 g/ha of glyphosate along with GS, GR, and F_1 controls. The number of surviving individuals from BC_1F_1 families was recorded 3 wk after treatment and compared with predicted values assuming monogenic inheritance. Because homogeneity chi-square was significant, data were pooled over backcross families. The chi-square probability values of combined BC_1F_1 families were not consistent with a single gene hypothesis at the herbicides rates tested (Tables 2 and 3). These results indicated that inheritance of glyphosate resistance may involve more than one gene in the biotype used in this study. Inheritance of glyphosate resistance has also been suggested to follow a polygenic additive pattern in biotypes of Palmer amaranth from Georgia (Gaines 2009). Probable involvement of one or more minor genes in conferring resistance to glyphosate at lower doses has also been reported earlier in a glyphosate-resistant rigid ryegrass population from Australia (Lorraine-Colwill et al. 2001). However, segregation of resistance in eleven out of fifteen individual backcross families tested at both doses in this study did conform to a monogenic inheritance hypothesis as evident from $P > 0.05$ (Tables 2 and 3). Since the majority of families followed monogenic inheritance of resistance, these results suggested that glyphosate resistance was inherited as a single gene in these families. Since the number of plants evaluated within each of the backcross families is not large enough (ranging from 25 to 52), interpretation of mechanism of resistance inheritance based on individual families is difficult. Moreover, earlier studies have shown that increased copy numbers of *EPSPS* gene confers resistance to glyphosate in Palmer amaranth and relative copy numbers of *EPSPS* gene in pseudo F_2 populations may range from one to greater than sum of copy numbers from both F_1 parents (Gaines et al. 2010). Furthermore, preliminary results of an experiment to study inheritance of *EPSPS* gene amplification in Palmer amaranth indicated a wide range (from 1to 80) in *EPSPS* gene copy number in the majority of the F_1 populations studied (Giacomini et al. 2011). Therefore, the *EPSPS* gene copy number present in the different backcross families evaluated in the present study could be highly variable and might be responsible for the inconsistent segregation of resistance among backcross families. Since *EPSPS* gene copy number in the GR and GS parents, in the F_1 parents used to generate backcross families, and in the BC_1F_1 individuals themselves is not known at present, interpretation about the number of genes involved in glyphosate inheritance is challenging. It would be informative to know the copy numbers of the *EPSPS* gene in the parental biotypes and their subsequent progenies. This information could aid in providing better interpretation of results.

Collectively, these data suggest that glyphosate resistance in the studied biotype of Palmer amaranth is nuclear inherited with no maternal or cytoplasmic inheritance involved. Resistance is incompletely dominant and is not consistent with a single gene mechanism of inheritance when data are pooled over backcross families. However, many individual backcross families did follow monogenic inheritance. Possible inconsistency in the inheritance of the copy number of *EPSPS* gene in the biotype studied might be responsible for the variable results about the number of genes involved in inheritance of glyphosate resistance in the biotype of Palmer amaranth used in this experiment.

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Sources of Materials

[1]ITML green standard pots, Myers Industries Lawn & Garden Group,15200 Madison Road, P.O. Box 738, Middlefield, OH 44062.

[2]Fafard 4P potting mix, Conrad Fafard Inc. Agawam, MA 01001.

[3]Hubbell Lighting, Inc.,701 Millennium Blvd, Greenville, SC 29607.

[4]Carolina Biological Supply Company, PO Box 6010, Burlington, NC 27216.

[5]Roundup Weathermax, Monsanto Company, St. Louis, Mo 63167.

[6] 8002 Spray nozzles. Spraying Systems Company, Wheaton, IL 60189.

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

[8]Statistical Analysis Systems®, version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

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Table 1: Percent survival of parent Palmer amaranth biotypes (GR and GS) and F_1 families (RxS and SxR) after treatment with glyphosate.

	Herbicide rate (g/ha)			
Biotype	180	400	2000	3000
	%			
Resistant (GR)	-	-	44 a	32 a
Susceptible (GS)	9.5 b	2.9 c	-	-
F1 Res female (RxS)	86 a	81 a	22 b	10 b
F1 Sus female (SxR)	83 a	68 a	22 b	4 b

Table 2. Chi-square analysis of segregation for glyphosate resistance at 2000 g/ha in BC_1F_1 families assuming monogenic inheritance.

	Observed			Expected			
Backcross family	Alive	Dead	Total	Alive	Dead	χ^2	p-value
BC_1F_1 SxR1	8	38	46	5.98	40.02	0.376	0.460
BC_1F_1 SxR2	7	45	52	6.76	45.24	0.921	0.663
BC_1F_1 SxR3	12	38	50	6.5	43.5	0.021	0.114
BC_1F_1 SxR4	20	32	52	6.76	45.24	0.000	<0.0001
BC_1F_1 SxR5	3	49	52	6.76	45.24	0.121	0.272
BC_1F_1 SxR6	3	37	40	5.2	34.8	0.301	0.417
BC_1F_1 SxR7	7	23	30	3.9	26.1	0.092	0.239
BC_1F_1 RxS1	19	33	52	6.76	45.24	0.000	<0.0001
BC_1F_1 RxS2	15	37	52	6.76	45.24	0.000	<0.0001
BC_1F_1 RxS3	4	48	52	6.76	45.24	0.255	0.386
BC_1F_1 RxS4	33	19	52	6.76	45.24	0.000	<0.0001
BC_1F_1 RxS5	6	46	52	6.76	45.24	0.754	0.615
BC_1F_1 RxS6	6	46	52	6.76	45.24	0.754	0.615
BC_1F_1 RxS7	7	45	52	6.76	45.24	0.921	0.663
BC_1F_1 RxS8	4	23	27	3.51	23.49	0.779	0.623
Total	154	559	713	92.69	620.31	0.000	<0.0001
Homogeneity						5.296	0.979

Behavior of parental controls

S Parent	0	52	52
R Parent	39	13	52
F1 SxR	11	36	47
F1 RxS	15	37	52

Table 3. Chi-square analysis of segregation for glyphosate resistance at 3000 g/ha in BC_1F_1 families assuming monogenic inheritance.

	Observed			Expected			
Backcross family	Alive	Dead	Total	Alive	Dead	χ^2	p-value
BC_1F_1 SxR1	1	38	39	3.12	35.88	0.211	0.354
BC_1F_1 SxR2	2	50	52	4.16	47.84	0.270	0.396
BC_1F_1 SxR3	3	47	50	4	46	0.602	0.562
BC_1F_1 SxR4	4	48	52	4.16	47.84	0.935	0.666
BC_1F_1 SxR5	0	52	52	4.16	47.84	0.033	0.145
BC_1F_1 SxR6	2	41	43	3.44	39.56	0.418	0.482
BC_1F_1 SxR7	1	32	33	2.64	30.36	0.293	0.411
BC_1F_1 RxS1	21	31	52	4.16	47.84	0.000	<0.0001
BC_1F_1 RxS2	11	41	52	4.16	47.84	0.000	<0.05
BC_1F_1 RxS3	2	50	52	4.16	47.84	0.270	0.396
BC_1F_1 RxS4	24	28	52	4.16	47.84	0.000	<0.0001
BC_1F_1 RxS5	8	44	52	4.16	47.84	0.050	0.176
BC_1F_1 RxS6	9	43	52	4.16	47.84	0.013	0.092
BC_1F_1 RxS7	14	36	50	4	46	0.000	<0.0001
BC_1F_1 RxS8	1	24	25	2	23	0.461	0.503
Total	103	605	708	56.64	651.36	0.000	<0.0001
Homogeneity						3.556	0.941

Behavior of parental controls

S Parent	0	52	52
R Parent	35	17	52
F1 SxR	10	40	50
F1 RxS	6	44	50

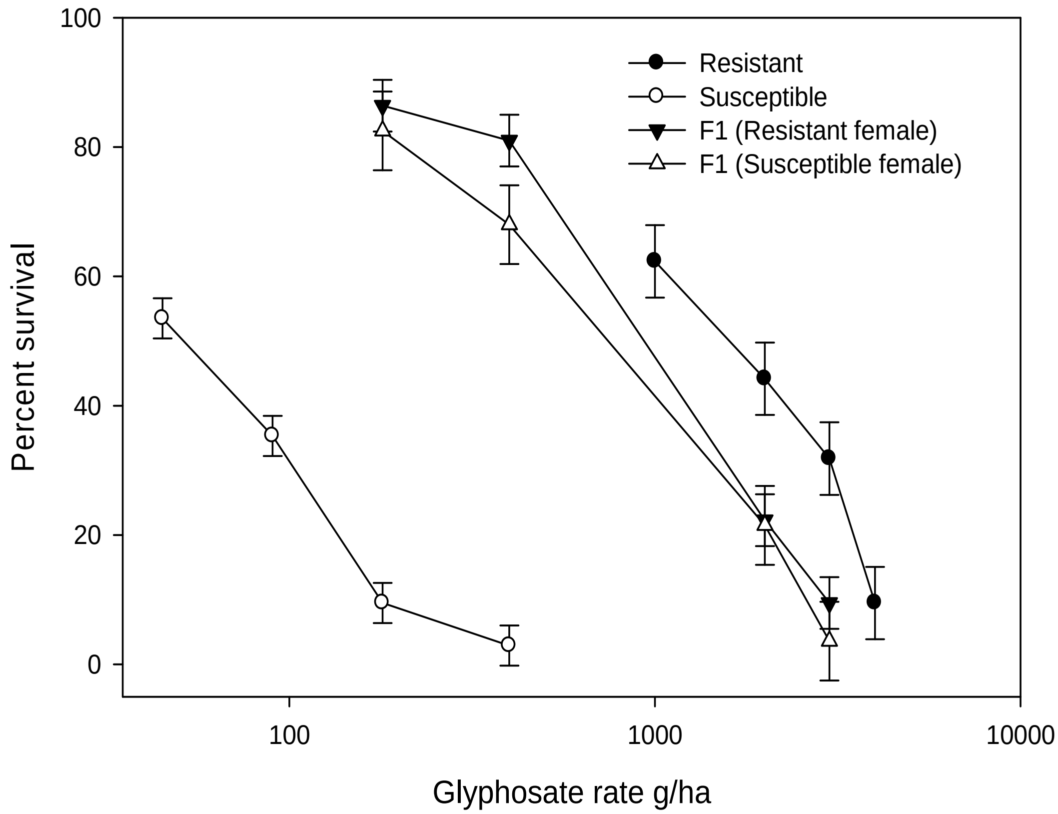

Figure 1. Glyphosate dose responses of resistant, susceptible, and F_1 (maternal GR and GS) biotypes of Palmer amaranth. Points represent mean percent survival ± SE.

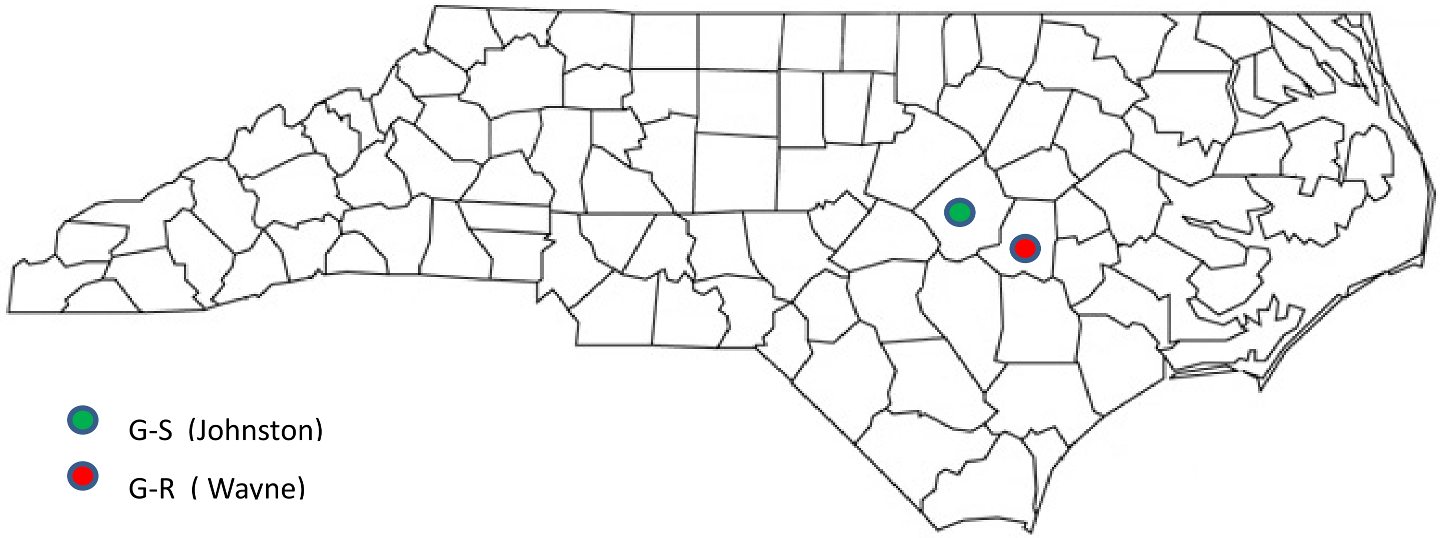

Figure 2. North Carolina counties from where biotypes used in the study were collected

CHAPTER III

Recovery of Selected Palmer amaranth (*Amaranthus palmeri*) Biotypes from Drought Stress

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Abstract

Palmer amaranth is an economically important weed in crop production systems in North Carolina due to its competitive ability and presence of resistance to herbicides representing different modes of action. In some cases, herbicide-resistant weed biotypes carry a fitness penalty compared with non-resistant wild types. A greenhouse experiment was conducted to determine recovery of fifteen Palmer amaranth biotypes collected across North Carolina following exposure to 0, 1, 3, 5, 7, and 9 days of soil moisture stress beginning 14 days after seeding emergence (DAE). Five biotypes each expressing confirmed glyphosate resistance

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(GR), acetolactate synthase (ALS) resistance (ALSR), and susceptibility to both ALS inhibiting herbicides and glyphosate (ALSS/GS) were compared. Variation in recovery, based on percent reduction in height or dry weight relative to a non-stressed control, was noted for the fifteen Palmer amaranth biotypes 24 and 30 DAE. When biotypes were grouped as GR, ALSR and ALSS/GS (5 biotypes in each group), the interaction of biotype group by duration of moisture stress was not significant for height. However, percent reduction in height for the GR group was less compared with ALSS/GS and ALSR groups 24 DAE. By 30 DAE, the GR group maintained an advantage over the ALSS/GS group but reduction in height did not differ for GR and ALSR groups. Dry weight of both ALSR and GR groups was affected less by moisture stress than the ALSS/GS group. Stress for 7 or 9 d affected height and dry weight while stress for 5 or fewer days did not, and this relationship was noted for differences in leaf stomatal conductance and photosynthesis for one example biotype. Collectively, these data suggest that herbicide resistance and recovery from stress may be related, although a larger pool of biotypes is needed to clearly define this relationship.

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

Key words: Moisture stress, herbicide resistance, acetolactate synthase, glyphosate.

Abbreviations: GR, glyphosate resistance; ALSR, acetolactate synthase resistance; ALSS, acetolactate synthase susceptible, GS, glyphosate susceptible; DAE, days after emergence.

Introduction

There are a number of ways weeds can interact with crops and subsequently affect crop yield (Aldrich 1984; Harper 1977). Weeds can affect crop growth by allelopathy, by serving as hosts for insects or plant diseases, and by competing with crops for light, water, carbon dioxide, oxygen, and soil nutrients. The final impact on crop yield is affected by weed density, species, distribution, and duration of interference.

Soil moisture is one of the most important environmental factors in crop-weed interactions (Bleasdale 1960; Ogg et al 1994; Riffle 1990; Zimdahl 2004). The ability of crops and weeds to extract water from soil and their response to moisture stress is a key factor in determining the outcome of crop-weed interference under drought (Patterson 1995; Wiese and VanDiver 1970). Interference of common cocklebur (*Xanthium strumarium* L.) with soybean [*Glycine max* (L.) Merr.] reduced soybean yield by 29% and 12% under well-watered and drought-stress conditions, respectively (Mortensen and Coble 1989). The reduction in interference was attributed to differential responses of both soybean and cocklebur to moisture stress. Ability to absorb water from soil under limited water availability, water use efficiency (WUE), and transpiration vary among crop and weed species (Aldrich 1984; Geddes et al. 1979; Patterson 1986; Patterson and Flint 1983). For example, WUE of genotypes of vegetable amaranth (*Amaranthus tricolor* L., *Amaranthus blitum* L., and *Amaranthus cruentus* L.) was not affected in response to drought stress. However, stress significantly reduced total plant dry mass and leaf area per unit root dry mass and increased root dry mass ratio differently in genotypes (Liu and Stüzel 2004). Under limited water conditions, plants respond differently and show a wide range of drought tolerance mechanisms both in terms of morphological and physiological aspects (Blum 1996). In another experiment involving the same genotypes of vegetable amaranth, significant variation was found to exist among genotypes for transpiration and stomatal conductance which was positively correlated with relative decrease in dry weight across four genotypes (Liu and Stüzel 2002).

Critical period for crop-weed interference is influenced by soil moisture availability. Differential effects of drought stress on crop and weed growth is responsible for the influence of moisture availability on the length of critical period (Patterson 1995). While competing with natural populations of common ragweed (*Ambrosia artemisifolia* L.), the critical period for soybean was 2 wk in a dry year but 4 wk in a wet year (Coble et al. 1981). Similar results showing shorter critical period for soybeans under dry conditions than under adequate soil moisture availability when competing with natural weed populations were also reported by Jackson et al. (1985). At the same time, there are also reports of comparatively longer critical period under moisture stress than under sufficient moisture conditions. The critical period for soybean in competition with giant foxtail (*Setaria faberi* Herrm.) was 10 to15 d when abundant soil moisture was available while it was 25 d during a dry year (Harrison et al. 1985).

Palmer amaranth is an economically important weed to manage in crop production systems in southern United States (Webster 2004, 2005; Webster and Coble 1997) due to its competitive ability, C_4 photosynthesis, higher water use efficiency and growth rate, and presence of resistance to herbicides representing different modes of action, including glycines, dinitroanilines, herbicides that inhibit acetolactate synthase and photosynthetic inhibitors (Black et al. 1969; Heap 2011; Horak and Loughin 2000). Growth analysis of Palmer amaranth, common waterhemp (*Amaranthus rudis* Sauer), redroot pigweed (*Amaranthus retroflexus* L.) and tumble pigweed (*Amaranthus albus* L.) showed that Palmer amaranth was the tallest, possessed the largest volume and greatest amount of branching, and produced the greatest leaf area, dry matter, specific leaf area, leaf area ratio, relative growth rate, and net assimilation rate among the four species (Horak and Loughin 2000). Plants which produce more dry matter per unit of water used tend to be more prolific than those which have higher water requirements under moisture stress conditions. Water requirement of C_4 plants is much lower as compared to C_3 plants for fixing one gram of CO_2, Radosevich et al. 1997). In a study aimed to access water relations, photosynthesis, and growth of soybean and seven weed species under controlled environment conditions in a phytotron, it was observed that smooth pigweed (*Amaranthus hybridus* L.), a C_4 plant, had the greatest net photosynthetic rates, net assimilation rates, and water use efficiency based on whole plant as well as on a single leaf basis (Patterson and Flint 1983). Based on these results, one would assume that all C_4 plants are always better equipped to acclimatize to moisture stress than C_3 plants. But there can possibly be some exceptions based on individual physiological responses of species to imposed drought stress. The effect of long-term drought stress on leaf gas exchange and fluorescence parameters in C_3 and C_4 plants was studied in a field experiment (Hura et al 2007). The results of this study suggested that field bean (*Vicia faba* L.), a C_3 plant and maize, a C_4 plant (*Zea mays* L.) responded with better acclimatization responses to water deficit by activating a photoprotective mechanism (which may be related to synthesis of phenolic compounds) and by effectively scattering excessive excitation energy by its partial transfer to PS I and thus avoided injury to PS II. With triticale (C_3) (*x Triticosecale* Wittmack) and amaranth (C_4) (*Amaranthus cruentus* L.) there were changes observed in red fluorescence and a decrease in maximum quantum yield of PS II which could have been due to serious photoinhibitory injuries because of lack of transfer of excitation energy from PS II to PS I (Hura et al. 2007).

Previous research suggests that in some cases herbicide-resistant weed biotypes have a fitness penalty compared with non-resistant wild types. Ability of a particular genotype to produce viable offspring relative to other genotypes in a population is known as relative fitness of that biotype (Preston et al. 2009). In a population, alleles that carry a larger fitness penalty are less frequent than those with a smaller fitness penalty associated with them. It is generally assumed that herbicide resistance alleles are infrequent in populations in the absence of selection pressure exerted by herbicide possibly because of the fitness penalty they might have (Jasieniuk et al. 1996; Preston and Powels 2002). Pederson et al. (2007), in an attempt to study ecological fitness of a glyphosate-resistant population of rigid ryegrass (*Lolium rigidum* Gaudin), selected susceptible individuals from within a glyphosate-resistant population and compared them with resistant individuals from the same population. Their results indicated that resistant plants produced seeds which were larger in size but fewer in number compared to susceptible plants. In other experiments, segregating F_2 populations of rigid ryegrass generated by crossing resistant and susceptible plants were assessed for change in the frequency of resistant individuals over time in the field. After 4 yr, the proportion of glyphosate-resistant plants in the population decreased (Preston and Wakelin 2008; Wakelin and Preston 2006). Fitness costs of maternally inherited triazine-resistance were studied in populations of smooth pigweed. Results from studies with triazine resistant smooth pigweed indicated that resistance reduced several components of fitness, including early seedling emergence, early growth, mid-season leaf number, and total above ground biomass, but differences varied among years and populations (Jordan 1996 and 1999). These results suggest that fitness is plastic and it depends strongly on environmental factors strongly (Cousens and Mortimer 1995; Jordan 1996 and 1999).

Comparing vegetative and reproductive characteristics of biotypes expressing various levels of herbicide resistance from different regions can provide information useful in predicting changes in populations and response to management. The ability of Palmer amaranth biotypes expressing ALSR or GR during early season growth where interference with crops is critical has not been reported. Therefore, a greenhouse experiment was conducted to determine recovery of Palmer amaranth populations [five populations each expressing confirmed glyphosate resistance (GR), acetolactate synthase resistance (ALSR), and susceptibility to both ALS inhibiting herbicides and glyphosate (ALSS/GS)] were compared under various durations of imposed soil moisture stress 14 to 24 DAE.

Materials and Methods

Seed of Palmer amaranth collected from across North Carolina (Whitaker et al. 2007) (Figure 1) were planted in excess in round plastic pots (10-cm dia by 12-cm deep) containing commercial potting mix[1] making sure that all pots contained the same weight of potting mix. Eight DAE, seedlings were thinned to one per pot and at 10 DAE, plants were fertilized[2] for optimum growth. Plants were watered daily manually to ensure that each pot received the same volume of water. Greenhouse was maintained at 35 ± 5 °C and natural lighting was supplemented for 14 h each day with metal halide lighting[3] (400 μmol $m^{-2}s^{-1}$). Beginning 14 DAE, plants were not watered in order to induce soil moisture stress for 0, 1, 3, 5, 7, or 9 d after which soil was brought back to full saturation 24 DAE and maintained at a moisture status to ensure optimum growth for the remainder of the experiment. Plant height (cm) was recorded 14, 16, 18, 20, 22, and 24 DAE corresponding to 0, 1, 3, 5, 7, and 9 days of moisture stress regime. Plant height and above-ground fresh weight and dry weight were recorded 30 DAE. The experimental design was randomized complete block with ten replications and the experiment was repeated. Percent reduction in height, fresh weight, and dry weight were calculated relative to the non-stressed control. Data were subjected to analysis of variance[5] testing main effects and interactions of biotypes and moisture stress treatment. However, two analyses were performed with variation in biotype designation. In one analysis, the 15 biotypes, irrespective of herbicide resistance expression, were subjected to ANOVA[4] to test main effects and interactions associated with moisture stress treatment. In a second analysis, biotypes were grouped based on expression of herbicide resistance (GR, ALSR, and ALSS/GS) and tested for main effects and interactions with moisture stress treatment. Means of significant main effects and interactions were separated using Fisher's Protected LSD test at $p \leq 0.05$.

Photosynthetic assimilation (umol CO_2 $m^{-2}s^{-1}$) and stomatal conductance (mol H_2O $m^{-2}s^{-1}$) were determined[5] in another experiment to document stress for one susceptible population at 14, 16, 18, 20, 22, and 24 DAE as well as 25 DAE after bringing plants back to saturation. The fourth fully expanded leaf from the top of each plant was used for measurements which were made between 10.00 and 14.00 hrs EST in the greenhouse using a portable photosynthesis system[6] and a leaf chamber fluorometer equipped with Version 6.2 software. Each measurement was taken over a 30 s period of time. There were five replicate plants per treatment and six measurements were taken per plant and the experiment was repeated.

It is important to understand treatment structure which is shown in Figure 1. When plant heights were recorded at 14 DAE, plants were not under moisture stress. When observations were taken at 16 DAE, 1 day of moisture stress regime was complete and treated plants were brought back to full saturation after plant heights were recorded. However, plants receiving 3, 5, 7, and 9 days of moisture stress treatments were still under stress at this stage. Similarly, when observations were recorded at 18 DAE, 3 day moisture stress regime was over and treated plants were fully saturated after recording plant height, while plants undergoing 5, 7, and 9 days of stress treatment were still under moisture stress. The longest duration of moisture stress treatment was 9 days which was achieved at 24 DAE. Therefore, by 24 DAE, treatments were complete and all plants were brought back to full saturation soil moisture after observations were recorded. Once a particular stress regime was complete, plants were watered to ensure optimum growth.

Results and Discussion

Main effects of biotype and period of moisture stress and the interaction of these factors were significant for percent reduction in height, fresh weight and dry weight at 24 and 30 DAE (Table 1). These results were expected given degree of phenotypic variation often observed among Palmer amaranth biotypes (Klingaman et al., 1994). However, the primary objective of this experiment was to determine the relationship between herbicide resistance expression and recovery from soil moisture stress. When biotypes were segregated into three groups as GR, ALSR and ALSS/GS groupings, the interaction of biotype group by duration of moisture stress was not significant for percent reduction in height, fresh weight and dry weight (Table 2). However, main effect of biotype group was significant for percent reduction in height (24 and 30 DAE) and dry weight but was not significant for percent fresh weight reduction. Main effect of duration of moisture stress was also significant for percent reduction in height, fresh weight and dry weight 30 DAE (Table 2). At 24 DAE, the percent reduction in height for GR group (11.7%) was less than reduction in height for ALSS/GS (13.6%) and ALSR (14.5%) groups (Table 3). By 30 DAE, the GR group maintained an advantage over the ALSS/GS group but reduction in height did not differ for GR and ALSR groups (Table 3). While differences in reduction in fresh weight were not observed when comparing biotype groups, dry weight of both ALSR and GR groups were affected less by moisture stress than the ALSS/GS group (Table 3). Percent dry weight reduction for GR and

ALSR groups was 16.8% and 17.5%, respectively, while this value for ALSS/GS group was 20.2% (Table 3). These results suggest possible relationship between herbicide resistance and ability to recover from moisture stress. In contrast to a possible fitness penalty when considering evolved GR or ALSR, these data suggest a possible fitness advantage in addition to herbicide resistance.

When pooled over biotype groups based on resistance characteristics, moisture stress for 1 or 3 d did not reduce Palmer amaranth height at 24 DAE (Table 4). Moisture stress for five or more days reduced height of Palmer amaranth, with greater reduction in height as the duration of stress increased from 5 to 9 d (Table 4). However, by 30 DAE, moisture stress for up to 5 d did not cause a reduction in plant height while stress for 7 and 9 d reduced plant height by 11.5% and 18.5%, respectively (Table 4). Moisture stress for up to 5d did not reduce fresh weight at 30 DAE, but there was reduction in fresh weight by 7 and 9 days of stress. Fresh weight was reduced 15.6% and 26.5% as a result of moisture stress for 7 and 9 days, respectively (Table 4). There was reduction in dry weight irrespective of biotype group when plants were subjected to moisture stress for 5 or more days. Reductions in dry weight of 16%, 21%, and 33% was observed for moisture stress periods of 5, 7, and 9 d, respectively (Table 4).

Wilting of Palmer amaranth leaves was observed 5 d after stress was initiated. There was no reduction in photosynthesis (μmol CO_2 $m^{-2}s^{-1}$) and stomatal conductance (mol H_2O $m^{-2}s^{-1}$) of a susceptible Palmer amaranth biotype for up to 3 d of moisture stress (Tables 5 and 6).

However, moisture stress for 5, 7, and 9 d reduced photosynthesis and stomatal conductance (Tables 5 and 6). Plants resumed normal photosynthesis and stomatal activity 1d after bringing soil back to full saturation (Table 6). Actual values of photosynthetic assimilation and stomatal conductance at 20, 22, 24, and 25 DAE are listed in Table 6. Visually, plant wilting was noticed 5 days after moisture stress was induced corresponding with 20 DAE.

Collectively, these data suggest that herbicide resistance and recovery from soil moisture stress may be related to some degree. These data also suggested a possible advantage for plants expressing herbicide resistance in that they have greater ability to recover from drought stress. However, the number of biotypes in each group was limited to five in this study. A more comprehensive screening of a higher number of biotypes with extended moisture stress past 9 d would be more informative in defining relationships of herbicide resistance and ability to recover from drought stress. Additionally, while the impact of a longer duration of stress would have been more informative, this experiment was conducted during the first 30 d when weeds, especially Palmer amaranth, have the greatest potential to impact growth, development, and yield of crops.

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Sources of Materials

[1]Fafard 4P potting mix, Conrad Fafard Inc., Agawam, MA 01001.

[2]Scotts® Starter® Fertilizer, The Scotts Company LLC, Marysville, OH 43041.

[3]Hubbell Lighting, Inc.,701 Millennium Blvd, Greenville, SC 29607.

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

[5]LI-6400 Instruction Manual, Version 6 v6.1, LI-COR Bioseceinces, P.O. Box 4425 Lincoln, Nebraska USA, 68504.

[6]LI-6400 Portable Photosynthesis System, LI-COR Bioseceinces, P.O. Box 4425 Lincoln, Nebraska USA, 68504.

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Table 1. P > F for percent reduction in height, fresh and dry weight of 15 Palmer amaranth biotypes.

	Height		Fresh weight	Dry weight
	DAE		DAE	DAE
Source of variation	24	30	30	30
Biotype	<0.0001	<0.0001	<0.0001	<0.0001
Moisture stress	<0.0001	<0.0001	<0.0001	<0.0001
Biotype x stress	0.0113	0.0374	<0.0001	<0.0001
Coefficient of variation (%)	95.3	114.7	95.1	92.3

Table 2. P > F for percent reduction in height, fresh and dry weight of Palmer amaranth when biotypes were grouped as susceptible (ALSS/GS), glyphosate resistant (GR), and acetolactate synthase resistant (ALSR).[a]

	Height		Fresh weight	Dry weight
	DAE		DAE	DAE
Source of variation	24	30	30	30
Biotype	0.0006	0.0193	0.5019	0.0092
Moisture stress	<0.0001	<0.0001	<0.0001	<0.0001
Biotype x stress	0.3562	0.7132	0.1098	0.4797
Coefficient of variation (%)	98.0	116.6	99.5	97.2

[a] Data pooled over two runs

Table 3. Percent reduction in height, fresh and dry weight of Palmer amaranth when biotypes were grouped as susceptible (ALSS/GS), glyphosate resistant (GR), and acetolactate synthase resistant (ALSR) pooled over periods of moisture stress.[a]

	Height		Fresh weight	Dry weight
	DAE		DAE	DAE
Biotype grouping	24	30	30	30
	% Reduction			
ALSS/GS	13.6 a	10.6 a	13.6 a	20.2 a
GR	11.7 b	8.8 b	12.9 a	16.8 b
ALSR	14.5 a	10.0 ab	13.7 a	17.5 b

[a] Means within a parameter followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p \leq 0.05$. Data are pooled over two experiments and durations of moisture stress.

Table 4. Percent reduction in height, fresh and dry weight of Palmer amaranth pooled over 3 groups of biotypes when biotypes were grouped as susceptible (ALSS/GS), glyphosate resistant (GR), and acetolactate synthase resistant (ALSR).[a]

	Height		Fresh weight	Dry weight
	DAE		DAE	DAE
Duration of moisture stress	24	30	30	30
	% Reduction			
0	7.8 d	6.7 c	8.8 c	10.9 d
1	7.3 d	7.5 c	8.9 c	11.5 d
3	7.4 d	6.3 c	8.7 c	12.9 d
5	10.5 c	8.1 c	9.7 c	16.2 c
7	15.6 b	11.5 b	15.6 b	21.4 b
9	31.0 a	18.5 a	26.5 a	32.6 a

[a] Means within a parameter followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p \leq 0.05$. Data are pooled over two experiments.

Table 5. P > F for influence of moisture stress on photosynthesis and stomatal conductance of an ALSS/GS Palmer amaranth biotype.

Days after stress initiation	Photosynthesis $\mu mol\ CO_2\ m^{-2}s^{-1}$	Stomatal conductance $mol\ H_2O\ m^{-2}s^{-1}$
	p-value	
14	0.1991	0.6476
16	0.1777	0.1051
18	0.2886	0.0931
20	0.0109	0.0026
22	0.0039	0.0003
24	<0.0001	<0.0001
25 (saturated)	0.3968	0.1573

[a] Data are pooled over two experiments.

Table 6. Influence of moisture stress on photosynthesis and stomatal conductance of an ALSS/GS Palmer amaranth biotype.[a]

	Photosynthesis				Stomatal conductance			
	DAE				DAE			
Days after stress initiation	20	22	24	25[b]	20	22	24	25[b]
	——— $\mu mol\ CO_2\ m^{-2}s^{-1}$ ———				——— $mol\ H_2O\ m^{-2}s^{-1}$ ———			
0	27.3 a	22.3 b	23.0 b	31.8 a	0.16 a	0.11 a	0.13 a	0.18 a
1	27.8 a	26.1 ab	27.3 b	28.6 a	0.16 a	0.12 a	0.15 a	0.17 a
3	29.9 a	29.6 ab	23.1 b	30.2 a	0.19 a	0.14 a	0.16 a	0.17 a
5	21.7 b	32.5 a	28.6 b	26.8 a	0.10 b	0.14 a	0.13 a	0.17 a
7	21.6 b	12.2 c	35.0 a	29.4 a	0.11 b	0.04 b	0.17 a	0.22 a
9	20.6 b	17.5 c	7.8 c	33.0 a	0.11 b	0.06 b	0.02 b	0.21 a

[a]Means within a parameter followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p \leq 0.05$. Data are pooled over two experiments.

[b]Saturated soil.

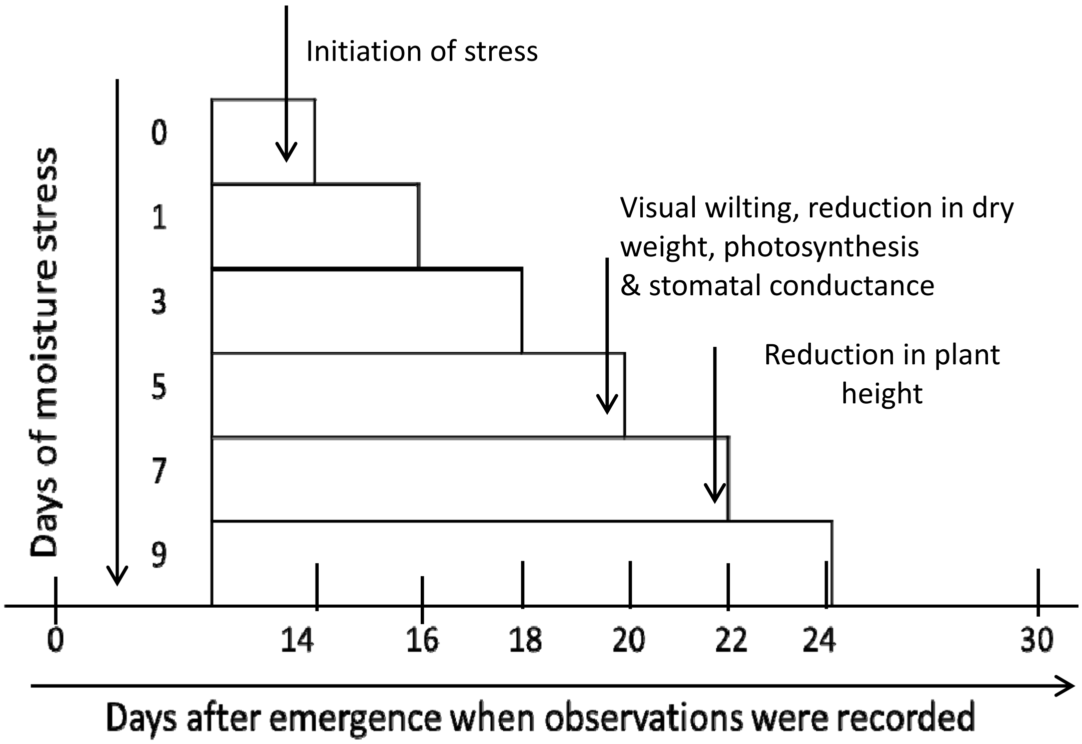

Figure 1. Moisture stress regimes relative to days after emergence.

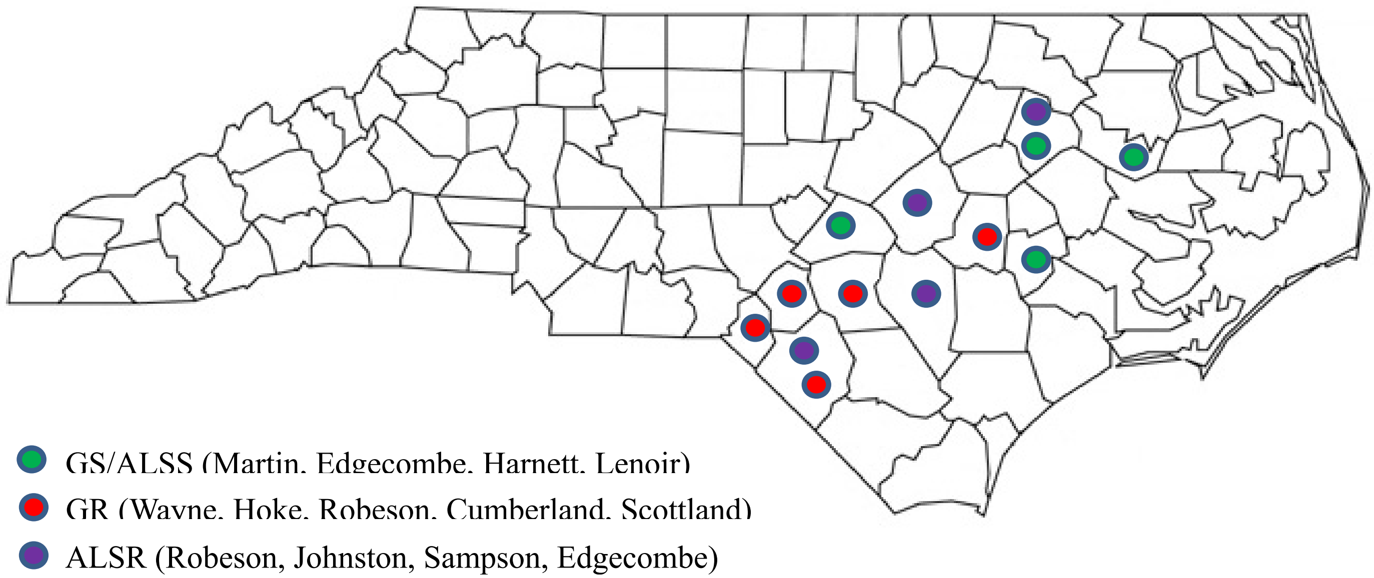

Figure 2. North Carolina counties from where populations used in the study were collected.

CHAPTER IV

Relative Interference of Eight Palmer amaranth (*Amaranthus palmeri*) Biotypes with Selected Crops

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Abstract

Palmer amaranth has become difficult to control in southern row crops due to selection for biotypes that are no longer controlled by acetolactate synthase-inhibiting herbicides and/or glyphosate. Previous research suggests that biotypes of individual weed species can interfere differently with crops. Determining the relative difference in interference by glyphosate-resistant (GR) biotypes and glyphosate-susceptible (GS) biotypes is of interest in order to evaluate whether or not there is a fitness penalty for glyphosate resistance in GR weed

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biotypes compared with GS biotypes. Early season interference with corn, cotton, peanut, snap bean, and soybean by eight Palmer amaranth biotypes (4 GR and 4 GS) collected across Georgia and North Carolina was compared in the greenhouse. Interference of seven Palmer amaranth biotypes (4 GR and 3 GS) with soybean was compared in the field. Interactions among crops and Palmer amaranth biotypes did not occur with respect to percent reduction in height, fresh weight and dry weight during early season interference of growth. When pooled over crops, the percent reduction for fresh weight was 26% in presence of GS biotypes and 20% in presence of GR biotypes. Results from field study revealed that several Palmer amaranth biotypes grew more quickly and competed more effectively with soybean. Differences in soybean yield were noted when comparing interference of biotypes. Increase in soybean yield was noted as the distance from interfering Palmer amaranth increased. Given the relatively small number of Palmer amaranth biotypes compared in this study, differences noted among biotypes might be associated with characteristics within biotypes other than glyphosate resistance.

Nomenclature: Corn, *Zea mays* L.; cotton, *Gossypium* hirsutum L.; palmer amaranth, *Amaranthus palmeri* S. Wats.; peanut, *Arachis hypogea* L.; snap bean, *Phaseolus vulgaris* L.; soybean, *Glycine max* (L.) Merr.

Key words: Interference, fitness, glyphosate, herbicide, resistant, susceptible.

Abbreviations: GR, glyphosate-resistant; GS, glyphosate-susceptible.

Introduction

Palmer amaranth (*Amaranthus palmeri* S.Wats.) is among the most troublesome weeds of agronomic crops in southern cropping systems including corn, cotton, and soybean (Webster 2004, 2005; Webster and Coble 1997). It is difficult to manage this weed due to its competitive ability, C_4 photosynthesis, higher water use efficiency and growth rate, and presence of resistance to herbicides representing different modes of action, including 5-enolpyruvylshikimate-3-phosphate synthase inhibitors, mitotic inhibitors, acetolactate synthase inhibitors, and photosynthetic inhibitors (Black et al. 1969; Heap 2011; Horak and Loughin 2000). Growth analysis of Palmer amaranth, common waterhemp (*Amaranthus rudis* Sauer), redroot pigweed (*Amaranthus retroflexus* L.), and tumble pigweed (*Amaranthus albus* L.) showed that Palmer amaranth was the tallest, possessed the largest volume and branching, and produced the greatest leaf area, dry matter, specific leaf area, leaf area ratio, relative growth rate, and net assimilation rate among the four species (Horak and Loughin 2000). Many biotypess of this weed have developed confirmed resistance to glyphosate in the southern United States, making it further difficult to manage (Culpepper et al. 2006, 2008; Norsworthy et al. 2008; Steckel et al. 2008).

Competition with Palmer amaranth at a density of 10 plants/m^2 reduced soybean yield up to 68%, while reduction of up to 92% in cotton lint yield was reported at 0.9 plants/m^2 (Klingaman and Oliver 1994; Rowland et al 1999). Palmer amaranth reduced corn leaf area index (LAI) and corn grain yield from 11% to 91% as density increased from 0.5 to 8 plants/ m^2 (Massinga et al. 2001; Massinga and Curie 2002). While a linear decrease in cotton yield was observed from 13% to 54% with an increase in Palmer amaranth density from 1 to 10 plants/ m^2, volume and biomass of Palmer amaranth remained unaffected by intraspecific competition at any of these densities (Morgan et al. 2001). There was a negative linear relationship between grain sorghum [*Sorghum biclor* (L.) Moench] yield and density of Palmer amaranth. Increasing weed density decreased grain sorghum yield by reducing the numbers of grains produced in panicles (Moore et al. 2004). Season-long Palmer amaranth interference in peanut reduced peanut canopy diameter, and one plant/m of row resulted in a predicted yield loss of up to 28% (Burke et al. 2007). Yield reductions from 30 to 94% were reported in sweetpotato [*Ipomoea batatas* (L.) Lam.] by Palmer amaranth densities ranging from 0.5 to 6.5 plants/m row (Meyers et al. 2010).

Previous research has suggested herbicide-resistant weed biotypes sometimes have a fitness penalty compared with non-resistant wild types. For example, reduced photosynthesis led to 10 to 75% reduction in biomass and/or seed production for many triazine-resistant weed species (Holt 1996; Jasieniuk et al. 1996; Warwick 1991). Pederson et al. (2007) studied the ecological fitness of a glyphosate resistant population of rigid ryegrass (*Lolium rigidum* Gaudin) by selecting susceptible individuals from a glyphosate-resistant population. Their results indicated that resistant plants produced seeds which were larger in size but fewer in number compared to susceptible plants. In other experiments, segregating F_2 populations of rigid ryegrass generated by crossing resistant and susceptible plants were assessed for change in the frequency of resistant individuals over time in the field. After 4 yr, the proportion of glyphosate resistant plants in the population decreased (Preston and Wakelin 2008; Wakelin and Preston 2006). Fitness costs of maternally inherited triazine resistance were studied in populations of smooth pigweed (*Amaranthus hybridus* L.). Results indicated that resistance reduced several components of fitness, including early seedling emergence, early growth, mid-season leaf number, and total above-ground biomass, but differences varied among years and populations (Jordan 1996, 1999).

Understanding of fitness costs involved with herbicide resistance can be exploited for formulating better resistance management strategies and can be a useful tool for studying ecological and evolutionary aspects of herbicides resistance (Jordan 1999). Therefore, research was conducted with the objective to compare early season interference of growth of major row crops of North Carolina by selected GR and GS biotypes of Palmer amaranth. Research was also conducted to compare the differences in growth of crops by grouping Palmer amaranth biotypes as GR and GS and to evaluate any fitness costs that might be involved with glyphosate resistance. Field experiments were conducted to study differences in growth and competitiveness of Palmer amaranth biotypes and their effect on soybean yield following season-long interference.

Materials and Methods

Greenhouse Study. Seeds from eight Palmer amaranth biotypes (Table 1), along with corn[1], cotton[2], peanut (Mozingo et al. 2000), soybean[3], and snap bean[4], were planted in 15-cm round plastic pots[5] containing commercial potting soil[6] in two parallel rows 2.5 cm apart. Out of eight Palmer amaranth biotypes, four biotypes were GS (one from North Carolina and three from Georgia) and four were GR (one from North Carolina and three from Georgia) (Culpepper et al. 2008) (Figures 3 and 4). Approximately six crops seeds and 25 Palmer amaranth seeds were planted in each pot and seedlings were eventually thinned to one crop and one Palmer amaranth plant per pot 10 days after emergence (DAE). Plants were fertilized[7] every ten days to ensure optimum plant growth with 25 ml/pot of a 4.6 g/L fertilizer solution. Greenhouse was maintained at 35 ± 5 °C, and natural illumination was supplemented for 14 h each day with metal halide lighting[8] (400 $\mu mol\ m^{-2}s^{-1}$). The experimental design was a randomized complete block with treatments replicated ten times and the experiment was conducted twice.

Height of the Palmer amaranth and crop plants (cm) was determined every 5 d beginning 1 wk after pots were thinned, corresponding to 15, 20, 25, 30, 35, and 40 DAE. Plant height measurements were taken from the soil surface to the base of the last fully opened leaf for all crops. At 40 DAE, Palmer amaranth and crop plants were severed at the soil surface and fresh and dry weight determined. Number of corn leaves and nodes per soybean plant were also recorded at harvest.

Data for plant height and fresh and dry weight were subjected to analysis of variance considering the factorial treatment structure including six levels of crop (no crop, corn, cotton, peanut, snap bean, soybean) and nine levels of Palmer amaranth biotypes (no Palmer amaranth and eight biotypes from North Carolina and Georgia). Analysis of variance was also carried out by grouping biotypes based on expression of glyphosate resistance (GR and GS) and tested for main effects of crop and biotype and interactions of crop by weed biotype. Means of significant main effects and interactions were separated using Fisher's Protected LSD test at $p \leq 0.05$.

Field Study. The experiment was conducted in conventionally planted soybean[3] during 2008 and 2009 at the Cunningham Research Station near Kinston,NC on a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) in rows spaced 91 cm apart. Plot size was one row by 6 m, with 91-cm row spacing. Two border rows were included between experimental units. In separate plots, approximately 10 seeds of each biotype (Table 1) were planted 3 cm apart adjacent to the crop row immediately after planting soybean. Both GR and GS biotypes were thinned to one plant per plot (one per row) about 20 DAE. At 35 DAE, Palmer amaranth plants were covered with large plastic bags and potassium salt of glyphosate[9] at 1.1 kg ae/ha were applied over the entire test area to control all other plants. Hand removal of weeds was performed during entire duration of experiment. Height of Palmer amaranth was recorded at 30, 60, 90, and 120 DAE. Palmer amaranth and soybean plants were harvested manually with clippers at soybean maturity. Data were recorded for Palmer amaranth fresh weight and soybean yield at 0 to 30, 31 to 60, 61 to 90, 91 to 120 and 121 to 150 cm on either side of Palmer amaranth. Soybean plot length corresponded to a density designed to reduce soybean yield by 11.2% based on computations from HADSS® (Herbicide Application Decision Support System[10]). Ten soybean plants on each side of Palmer amaranth plant were considered the weed/crop density required to reduce soybean yield by 11.2%. Data for Palmer amaranth height and weight and soybean yield were subjected to analysis of variance. Means of significant main effects and interactions were separated using Fisher's Protected LSD test at $p \leq 0.05$.

Results and Discussion

Greenhouse study. The interaction of crop by biotype for percent reduction in plant height was not significant for either crop or Palmer amaranth biotype (Table 2). However, the main effect of percent reduction in crop height was significant for both crop and Palmer amaranth biotype. The main effect of biotype was significant for only Palmer amaranth biotype at 15, 20, 25, and 30 DAE (Table 2). These results indicated that as expected there were differences in heights of crops studied in this experiment at all DAE. However, differences in the height of Palmer amaranth biotypes existed only at 15, 20, 25, and 30 DAE.

The interaction of crop by Palmer amaranth biotype was not significant for crop and Palmer amaranth fresh weight and dry weight reduction (Table 3). The main effects of crop and biotype were significant for percent fresh and dry weight reduction for crop. While the main effect of crop was significant for Palmer amaranth fresh and dry weight reduction, the main effect of biotype was significant for only percent dry weight reduction of Palmer amaranth biotype. Lack of an interaction of crop by biotype for crop fresh weight and dry weight reductions suggested that interference from Palmer amaranth biotypes is similar for corn, cotton, peanut, soybean, and snap bean (Table 3). Lack of an interaction of crop by biotype for Palmer amaranth fresh weight and dry weight reduction indicated that crops interfered with Palmer amaranth biotypes similarly. The main effect of biotype for soybean nodes/plant and number of corn leaves/plant at 40 DAE was not significant indicating that early season interference from Palmer amaranth did not affect number of soybean nodes/plant and number of corn leaves/plant (Table 4).

When data were pooled over Palmer amaranth biotypes, the lowest reductions for plant height, fresh weight and dry weight were observed for corn and snap bean indicating that these crops were most competitive or least affected by interference from Palmer amaranth biotypes (Table 5). Cotton and peanut had the greatest and similar percent fresh weight and dry weight reduction as compared to other crops pointing towards their less competitive nature. When pooled over crops, interference from GR biotypes from Emanuel, Macon, Sumter and Wayne counties resulted in lower fresh and dry weight reduction as compared to other biotypes (Table 6). However, the effect of GR biotype from Wayne County was similar to that of GS from Crisp County in terms of fresh and dry weight reduction. The GS biotypes from Crisp, Emanuel Johnston, and Sumter counties appeared to be more competitive and reduced fresh and dry weight of crops more as compared to GR biotypes.

The GR biotype of Palmer amaranth from Macon County had the highest dry weight reduction when data were pooled over crops (Table 7). The percent reduction in dry weight was the lowest for GR biotype from Emanuel County but was similar to all the GS biotypes and GR biotypes from Wayne County. When data were pooled over Palmer amaranth biotypes, peanut was least competitive resulting in 20 and 21% reduction in fresh and dry weight of biotypes, respectively (Table 8). Corn and snap bean were the most competitive among all crops resulting in the greatest fresh and dry weight reduction of Palmer amaranth biotypes.

One important question of interest was whether or not there was a fitness penalty for glyphosate resistance in GR weed biotypes compared with GS biotypes. Analysis of variance results when Palmer amaranth biotypes were grouped as either GR or GS indicated that main effect of crop was significant for percent reduction in height, fresh weight, and dry weight for both crop and Palmer amaranth biotype 40 DAE (Tables 9 and 10). The main effect of Palmer amaranth biotype was significant for reduction in fresh weight and dry weight for crop, but was not significant for Palmer amaranth biotype (Table 10). The interaction of crop by biotype was not significant for fresh weight and dry weight reduction for both crop and Palmer amaranth biotype.

The percent reduction in crop height, fresh weight and dry weight (pooled over crops), was lower when in competition with GR biotypes than GS biotypes of Palmer amaranth (Table 11). The reduction in crop height was 8 and 12% when facing interference from GR and GS biotypes of Palmer amaranth, respectively. Percent fresh weight reduction of crops was 20% in presence of GR biotypes while it was 26% in presence of GS biotypes (Table 11). The reduction in crop dry weight was 19% and 25% in presence of GR and GS biotypes, respectively. Although results from these experiments indicated a possible advantage of GS Palmar amaranth biotypes in terms of competitive ability with crops, these results could also be associated with natural variability in biotypes selected for these experiments. Including a higher number of both GR and GS biotypes, representing an even large geographical distribution, could help in better understanding of involvement of resistance trait towards fitness.

Field study. The main effect of year and distance from the weed and the interaction of year by biotype were significant for soybean yield (Table 12). Soybean yield was greater in 2009 than in 2008 for all biotypes except for biotype from Crisp County (Table 13). While GR biotypes from Emanuel and Sumter counties were least competitive in 2009, based on soybean yield, they were not so in 2008 (Table 13). The lowest soybean yield of 1130 kg/ha was observed due to interference by Wayne county biotype (GR), and yield loss due to this biotype was similar to yield loss resulting from interference by GR biotypes from Emanuel and Macon counties and GS biotypes form Johnston county in 2008 (Table 13). Significant effect of distance from Palmer amaranth was reflected in increasing yield of soybean as distance from Palmer amaranth increased (Table 14). The lowest soybean yield of 1220 kg/ha was observed at a distance of 0 to 30 cm from Palmer amaranth while the greatest yield of 1930 kg/ha was obtained at 150 cm.

The main effect of year, biotype, and evaluation interval (30, 60, 90, and 120 DAE) was significant for Palmer amaranth height (Table 15). Interactions of interval by year and interval by biotype were also found to be significant. The GS biotype from Crisp County was taller as compared to other biotypes at 90 and 120 DAE (Figure 3). Overall, GS biotypes from Crisp, Emanuel, and Johnston counties were taller than GR biotypes from Emanuel, Macon, and Sumter counties throughout, while the height of GR biotype from Wayne county was intermediate between these two groups when grown along with soybean for entire season in the field (Figure 3). Similar to results obtained with early season interference in the greenhouse (Figure 4), GS biotypes from Crisp, Emanuel, Johnston, and Sumter counties were taller than GR biotypes from Emanuel, Macon, Sumter, and Wayne counties (Figure 3). These results indicated that Palmer amaranth biotypes that were comparatively taller at younger stages of growth in the greenhouse maintained that competitive advantage in height throughput entire season.

The main effect of biotype and interaction of biotype and year were significant for Palmer amaranth fresh weight following full-season interference with soybean (Table 16). The Emanuel County biotype (GS) had the greatest fresh weight (615 g/plant) and all other biotypes had lower and similar fresh weights in 2008 (Table 17). However, in 2009, Crisp county biotype (GS) had the greatest fresh weight, and fresh weight the other biotypes was less and similar. These data revealed that several Palmer amaranth biotypes grew more quickly and competed more effectively with soybean. Differences in soybean yield were noted when comparing interference of biotypes. As the distance from Palmer amaranth plants decreased, there was a significant decrease in soybean yield. Collectively, results from these experiments indicate that interference can vary among Palmer amaranth biotypes for major crops grown in North Carolina. Although data suggest that there may be a fitness penalty due to the GR trait, a larger pool of biotypes is needed to conclusively define a fitness cost to glyphosate resistance in Palmer amaranth.

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Sources of Materials

[1]Corn hybrid 31G71. Pioneer Hi-Bred, Johnston, IA 50131.

[2]Cotton cultivar DP 424 BGRR. Monsanto Company, St. Louis, MO 63167.

[3]Soybean cultivar AG6301. Monsanto Company, St. Louis, MO 63167.

[4]Snap bean cultivar Kentucky Wonder, Wyatt-Quarles Seed Company, Garner NC 27529.

[5]ITML green standard pots, Myers Industries Lawn & Garden Group, Middlefield, OH 44062.

[6]Fafard 4P potting mix, Conrad Fafard Inc. Agawam, MA 0.1001.

[7]Scotts® Starter® Fertilizer, The Scotts Company LLC, Marysville, OH 43041.

[8]Hubbell Lighting, Inc., Greenville, SC 29607.

[9]Roundup Weathermax, Monsanto Company, St. Louis, Mo 63167.

[10]HADSS® [Herbicide Application Decision Support System], Ag Renaissance Software, LLC, Raleigh, NC 27695.

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Table 1. List of Palmer amaranth biotypes used for greenhouse and field experiment.

County	State	Response to glyphosate
Wayne	North Carolina	Resistant
Johnston	North Carolina	Susceptible
Sumter	Georgia	Susceptible
Sumter	Georgia	Resistant
Macon	Georgia	Resistant
Emanuel	Georgia	Resistant
Emanuel	Georgia	Susceptible
Crisp	Georgia	Susceptible

Table 2. P > F for percent reduction in crop and Palmer amaranth height at all days after emergence.[a]

	Days after emergence											
	Crop height						Palmer amaranth biotype height					
Source of variation	15	20	25	30	35	40	15	20	25	30	35	40
Crop	0.0137	0.0217	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Palmer Biotype	0.3416	0.0919	0.2118	0.1161	0.2695	0.0550	0.0015	<0.0001	0.0003	0.0269	0.2329	0.5457
Crop x Palmer Biotype	0.6886	0.2543	0.6306	0.2257	0.6165	0.8698	0.6311	0.9919	0.8792	0.8065	0.9151	0.2129
Coefficient of variation (%)	159	166	185	178	175	134	250	209	179	164	155	61

[a] Data are pooled over experiments.

Table 3. P > F for percent reduction in fresh weight and dry weight of crop and Palmer amaranth 40 days after emergence[a].

Source of variation	Crop		Palmer amaranth	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Crop	<0.0001	<0.0001	<0.0001	<0.0001
Palmer biotype	0.0044	0.0403	0.4616	0.0110
Crop x Palmer biotype	0.8063	0.8509	0.0563	0.2065
Coefficient of variation (%)	91	98	43	47

[a] Data are pooled over experiments.

Table 4. P > F for soybean nodes per plant and corn leaf tips per plant 40 days after emergence.

Source of variation	Soybean nodes/plant	Corn leaves /plant
Palmer biotype	0.7549	0.6239
Coefficient of variation (%)	13	5

Table 5. Percent reduction in crop height, fresh weight, and dry weight 40 days after emergence[a].

Crop	Height	Fresh wt.	Dry wt.
		%	
Cotton	12	35	34
Corn	5	11	12
Peanut	15	32	32
Snap bean	6	10	9
Soybean	11	26	23
LSD ($p \leq 0.05$)	4	5	5

[a] Data are pooled over Palmer amaranth biotypes and experiments.

Table 6. Percent reduction in crop height fresh weight and dry weight as influenced by Palmer amaranth biotypes 40 days after emergence[a].

Biotype	Height	Fresh wt.	Dry wt.
		%	
Wayne (GR)	11	19	19
Johnston (GS)	10	26	26
Sumter (GS)	12	25	25
Sumter (GR)	7	20	19
Macon (GR)	10	22	22
Emanuel (GR)	10	18	18
Emanuel (GS)	12	28	25
Crisp (GS)	7	24	23
LSD ($p \leq 0.05$)	4	6	6

[a] Data are pooled over crops and experiments.

Table 7. Percent reduction in Palmer amaranth dry weight 40 days after emergence[a].

Biotype	Dry wt.
Wayne (GR)	53
Johnston (GS)	54
Sumter (GS)	50
Sumter (GR)	55
Macon (GR)	62
Emanuel (GR)	48
Emanuel (GS)	54
Crisp (GS)	53
LSD ($p \leq 0.05$)	7

[a] Data are pooled over crops and experiments.

Table 8. Percent reduction in Palmer amaranth fresh weight and dry weight as influenced by crops 40 days after emergence[a].

Biotype	Fresh wt.	Dry wt.
	%	
Cotton	34	34
Corn	88	85
Peanut	20	21
Snap bean	86	83
Soybean	46	46
LSD ($p \leq 0.05$)	5	6

[a] Data are pooled over Palmer amaranth biotypes and experiments.

Table 9. P > F for percent reduction in crop and Palmer amaranth height at all days after emergence when biotypes were grouped as glyphosate resistant (GR) and susceptible (GS)[a].

	Days after emergence											
	Crop height						Palmer amaranth biotype height					
Source of variation	15	20	25	30	35	40	15	20	25	30	35	40
Crop	0.0134	0.0225	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Palmer Biotype	0.6236	0.5941	0.1241	0.5934	0.7873	0.2632	0.7620	0.2614	0.1241	0.2521	0.6169	0.1980
Crop x Palmer Biotype	0.2738	0.7811	0.0913	0.7686	0.6406	0.7250	0.5595	0.7174	0.0913	0.1575	0.1648	0.6573
Coefficient of variation (%)	159	167	180	179	175	134	252	211	179	163	154	61

[a] Data are pooled over experiments.

Table 10. P > F for percent reduction in fresh weight and dry weight of crop and Palmer amaranth 40 days after emergence when biotypes were grouped as glyphosate resistant (GR) and susceptible (GS)[a].

	Crop		Palmer amaranth biotype group	
Source of variation	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Crop	<0.0001	<0.0001	<0.0001	<0.0001
Palmer biotype group	<0.0001	0.0009	0.2993	0.3140
Crop x Palmer biotype group	0.3339	0.3582	0.1058	0.3263
Coefficient of variation (%)	90	97	43	48

[a] Data are pooled over experiments.

Table 11. Percent reduction in crop height, fresh weight and dry weight as influenced by Palmer amaranth biotypes 40 days after emergence when biotypes were grouped as glyphosate resistant (GR) and susceptible (GS)[a].

Biotype	Height	Fresh wt.	Dry wt.
		%	
Resistant (GR)	8	20	19
Susceptible (GS)	12	26	25
LSD ($p \leq 0.05$)	2	3	3

[a] Data are pooled over crops and experiments.

Table 12. P > F for soybean yield following full season interference by Palmer amaranth biotypes.

Source of variation	Yield
Year	<0.0001
Biotype	0.5716
Year x biotype	0.0009
Distance	<0.0001
Year x distance	0.3139
Biotype x distance	0.4638
Year x biotype x distance	0.9734
Coefficient of variation (%)	35.7

Table 13. Soybean yield as influenced by full season interference by Palmer amaranth biotypes.

State	County	Resistance	Soybean yield 2008	Soybean yield 2009
			kg/ha	
North Carolina	Wayne	Yes	1130	1930
North Carolina	Johnston	No	1370	1900
Georgia	Sumter	Yes	1390	2070
Georgia	Macon	Yes	1210	2000
Georgia	Emanuel	Yes	1180	2260
Georgia	Emanuel	No	1440	1730
Georgia	Crisp	No	1440	1680
LSD ($p \leq 0.05$)			260	

Table 14. Soybean yield as influenced by distance from Palmer amaranth.

Distance from Palmer	Yield
Cm	kg/ha
0 to 30	1220
30 to 60	1470
60 to 90	1610
90 to 120	1750
120 to150	1930
LSD ($p \leq 0.05$)	157

*Data pooled over biotypes and years

Table 15. P > F for Palmer amaranth height following full-season interference by soybean.

Source of variation	Yield
Year	0.0001
Biotype	<0.0001
Year x Biotype	0.6543
Interval	<0.0001
Year x Interval	<0.0001
Biotype x Interval	<0.0001
Year x Biotype x Interval	0.7003
Coefficient of variation (%)	17

Table 16. Analysis of variance (P > F) for Palmer amaranth fresh weight following full season interference by soybean.

Source of variation	Yield
Year	0.2296
Biotype	<0.0001
Year x biotype	0.0021
Coefficient of variation (%)	17

Table 17. Palmer amaranth fresh weight following full season interference by soybean.

State	County	Resistance	Fresh wt. 2008	Fresh wt. 2009
			g/plant	
North Carolina	Wayne	Yes	252	863
North Carolina	Johnston	No	212	487
Georgia	Sumter	Yes	155	524
Georgia	Macon	Yes	140	234
Georgia	Emanuel	Yes	278	772
Georgia	Emanuel	No	615	1131
Georgia	Crisp	No	340	2509
LSD ($p \leq 0.05$)			286	1065
			697	

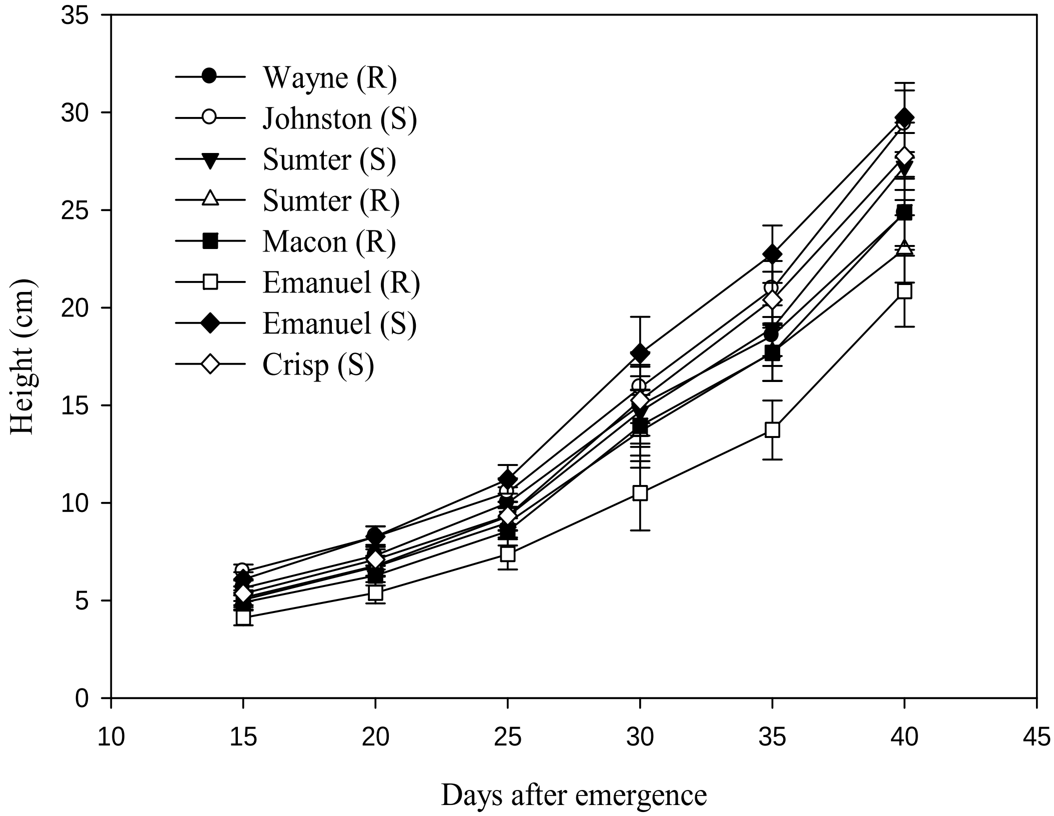

Figure 1. Height of Palmer amaranth biotypes at various intervals following early season interference by soybean in the greenhouse. Data points are mean heights ± SE.

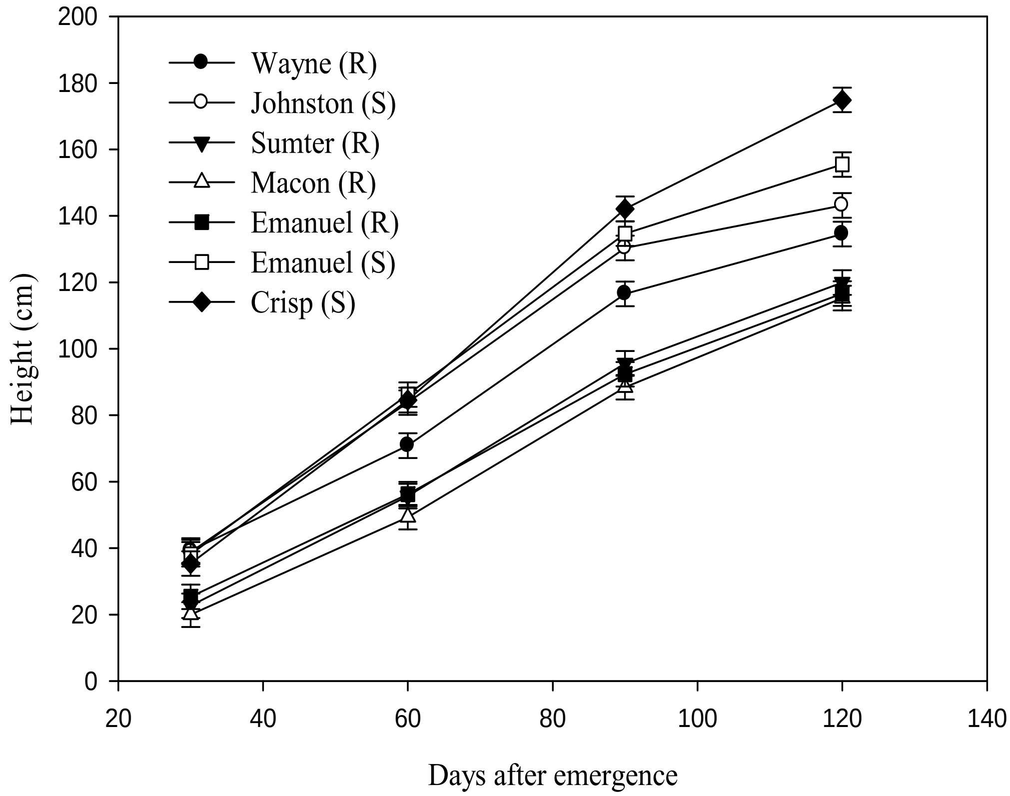

Figure 2. Height of Palmer amaranth biotypes at various intervals following full season interference by soybean in the field. Data points are mean heights ± SE.

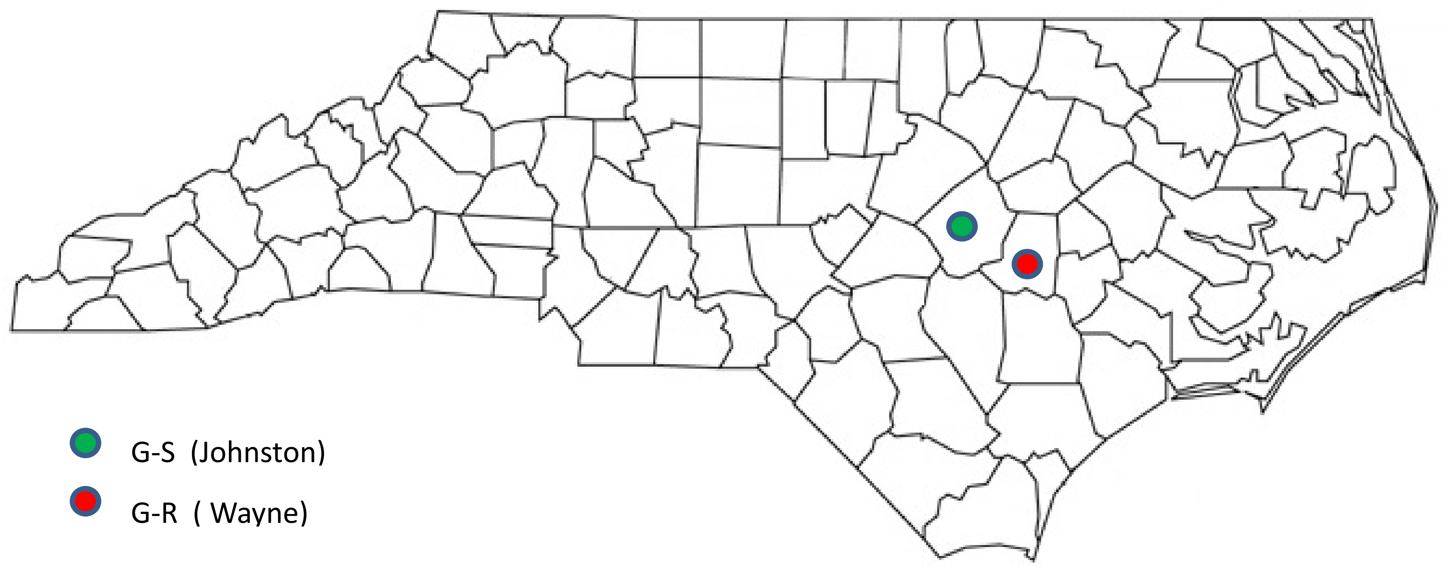

Figure 3. North Carolina counties from where biotypes used in the study were collected

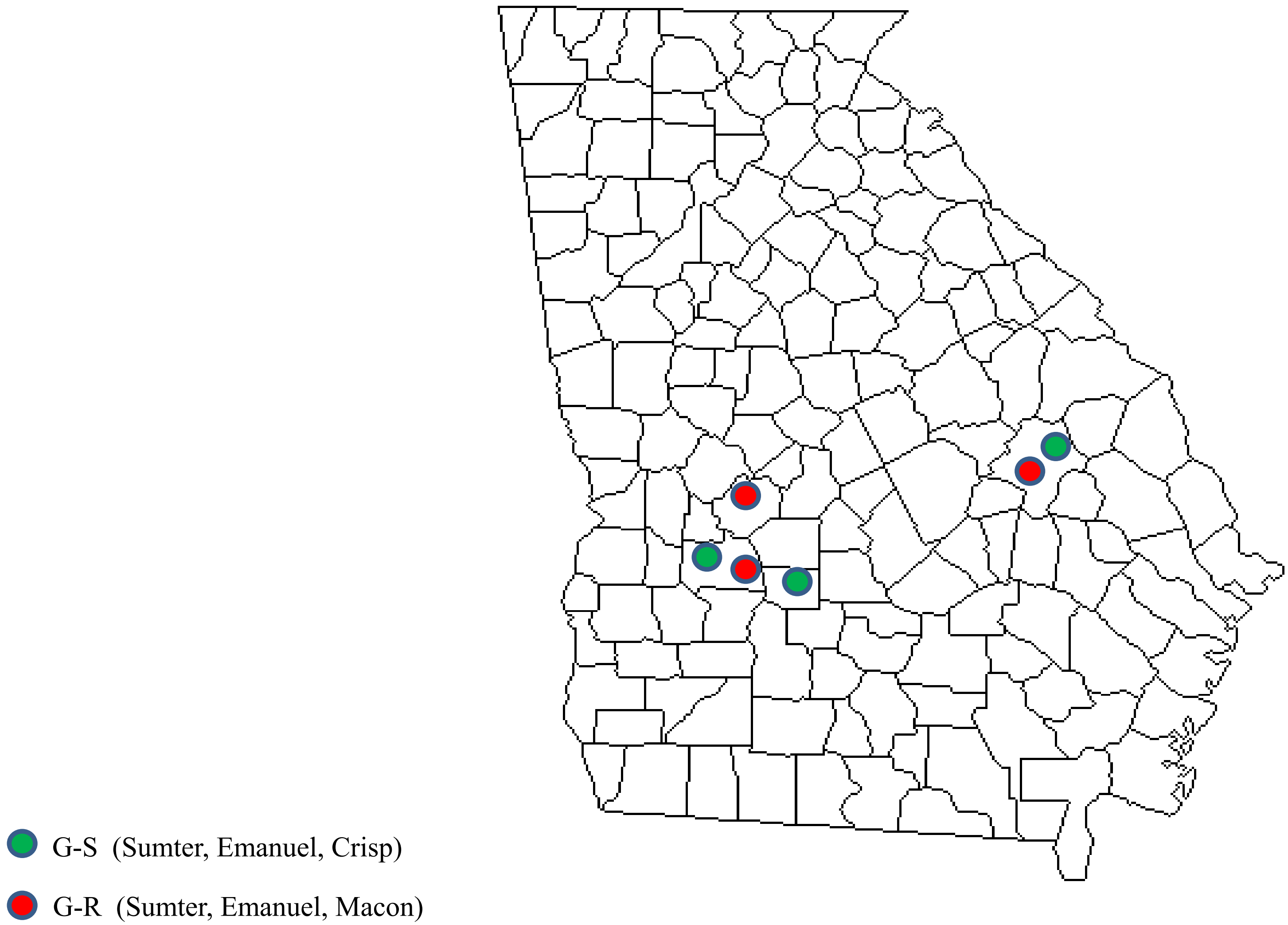

Figure 4. Georgia counties from where biotypes used in the study were collected

CHAPTER V

Efficacy of Selected Herbicides on Eight Palmer amaranth (*Amaranthus palmeri*) Biotypes

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Abstract

Considerable variation in phenotypic and genotypic characteristics has been observed for Palmer amaranth biotypes. Research was conducted to determine if differences existed among 15 biotypes for control by herbicides with different modes of action (MOA). In greenhouse experiments, no difference in Palmer amaranth control by atrazine, flumioxazin, or fomesafen applied preemergence (PRE) was noted while control by pendimethalin, pyrithiobac sodium, and *S*-metolachlor applied PRE was affected by biotype. Palmer

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amaranth control by dicamba, glufosinate, glyphosate, lactofen, and trifloxysulfuron applied postemergence (POST) differed among Palmer amaranth biotypes while control by paraquat was not affected. When glyphosate resistant (GR) and glyphosate susceptible (GS) biotypes were pooled and compared within an herbicide, in some instances herbicide efficacy varied due to presence or absence of glyphosate resistance. Generally, control was greater for the GR biotypes compared with the GS biotypes with POST herbicides. When pretreated with glyphosate, variation in control by herbicides with different MOAs was also noted. These data suggest that Palmer amaranth response to PRE and POST herbicides may vary depending upon biotype and that in some instances presence or absence of glyphosate resistance may be a factor. However, differences in efficacy observed across biotypes may have been associated with characteristics within a biotype other than glyphosate resistance.

Nomencalture: Atrazine; dicamba; flumioxazin; fomesafen; glufosinate; glyphosate; lactofen; paraquat; pendimethalin; pyrithiobac sodium; *S*-metolachlor; trifloxusulfuron; Palmer amaranth (*Amaranthus palmeri* S. Wats.).

Key words: herbicide efficacy; glyphosate resistance.

Abbreviations used: ALS, acetolactate synthase; DAT, days after treatment; EPSPS, 5-enolpyruvylshikimate-3-phosphate; GS, glyphosate susceptible biotype; GR, glyphosate resistant biotype; MOA, mode of action; PRE, premeergence; POST postemergence; PPO, protoporphyrinogen oxyidase; preplant incorporated, PPI.

Introduction

Palmer amaranth has become difficult to control in southern row crops due to development of resistant biotypes that are no longer controlled by glyphosate (Culpepper et al. 2006, 2008c; Norsworthy et al. 2008; Steckel et al. 2008) and several other herbicides with MOAs different from glyphosate (Heap 2011). This weed is extremely competitive and can cause significant reduction in crop yield (Klingaman and Oliver 1994; Massinga et al. 2001; Massinga and Curie 2002; Rowland et al. 1999). Timing of application is critical with herbicides other than glyphosate used to control Palmer amaranth (Norsworthy et al. 2008). Weed management strategies designed to control herbicide-resistant Palmer amaranth include rotation to herbicide chemistry with a MOA different from the herbicide in question (Bond et al. 2006; Culpepper et al. 2008a 2008b) and incorporation of non-chemical weed control techniques. More than 90% control of Palmer amaranth was observed with acifluorfen, lactofen, and acifluorfen plus bentazon in peanut (*Arachis hypogea* L.) (Grichar 1997). Pendimethalin and trifluralin applied preplant incorporated (PPI) have been reported effective in controlling early season Palmer amaranth in cotton (*Gossypium hirsutum* L.) (Keeling et al. 1991). POST application of glyphosate and pyrithiobac resulted in variable Palmer amaranth control in cotton (Dotray et al. 1996; Scott et al. 2002). When evaluated 21 days after treatment (DAT) acifluorfen and lactofen controlled Palmer amaranth 84% and 87%, respectively (Mayo et al. 1995).

Differences in weed biotype response to herbicides have been reported previously (Bond et al. 2006; Li et al. 2004; Padzolt et al. 2002). Palmer amaranth control ranged from 20 to

94% 21 DAT with pyrithiobac, depending on the accession (Bond et al. 2006). Differential response was observed among common waterhemp (*Amaranthus rudis* L.) and tall waterhemp [*A. tuberculatus* (Moq.) Sauer] accessions for visible control by atrazine, glyphosate, and imazethapyr. However, there was no difference in response of accessions to fomesafen (Li et al. 2004).

It is suspected that differences in weed response to herbicides might be influenced by biotype, and determining the relative difference in controlling GR biotypes and GS biotypes with herbicides other than glyphosate is of interest. The objective of this research was to compare efficacy of PPI, PRE, and POST herbicides with MOAs different than glyphosate on eight palmer amaranth biotypes collected from Georgia and North Carolina.

Materials and Methods

Preemergence herbicides. Sterilized loamy sand soil was placed in nursery flats[1] 25 cm x 51cm in the greenhouse. Seeds from eight Palmer amaranth biotypes collected from North Carolina and Georgia (Figures 1 and 2) were planted shallowly in separate rows for each biotype. Out of these, four biotypes were GS (one from North Carolina and three from Georgia) and four were GR (one from North Carolina and three from Georgia) (Culpepper et al. 2008). Herbicides (Table 1) representing different MOA, including atrazine[2], flumioxazin[3], fomesafen[4], pendimethalin[5], pyrithiobac sodium[6], and *S*-metolachlor[7], were applied PRE to the soil surface prior to activation by 0.25 cm of water from sprinkler irrigation. Each herbicide was applied at 0.25 and 0.75 times the manufacturer's recommended rate for field applications of each herbicide. Atrazine (560 and 1680 g ai/ha), flumioxazin (18 and 53 g ai/ha), fomesafen (70 and 210 g ae/ha), pendimethalin (416 and 1247 g ai/ha), pyrithiobac sodium (15 and 44 g ai/ha), and *S*-metolachlor (425 and 1275 g ai/ha) were applied in 229 L/ha aqueous solution at 207 kPa pressure using a single even spray flat-fan nozzle[8] in an enclosed spray chamber. Plants were fertilized[9] every 10d to ensure optimum plant growth. The greenhouse was maintained at 35 ± 5 °C and natural lighting was supplemented for 14 h daily with metal halide lighting[10] (400 µmol $m^{-2}s^{-1}$). Plants were irrigated daily using overhead sprinkler system for 7 minutes four times daily. The experimental design was a split-plot with herbicides and herbicide rates serving as whole plot units and Palmer amaranth biotypes serving as subplot units. Treatments were replicated four times and the experiment was conducted twice.

Postemergence herbicides. Seeds from the eight Palmer amaranth biotypes were planted in excess in10 cm square pots[11] in a commercial soil medium[12] and thinned to four plants per pot prior to POST herbicide application (Table 1). Atrazine[2] (560 and 1680 g/ha), dicamba[13] (70 and 210 g ae/ha), potassium salt of glyphosate[14] (210 and 630 g ae/ha), glufosinate[15] (118 and 353 g ae/ha), lactofen[16] (70 and 210 g ai/ha), paraquat[17] (35 and 105 g ai/ha), and trifloxysulfuron[18] (1.25 and 3.75 g ai/ha) were applied POST to Palmer amaranth 10 to 12 cm in height at 4 to 5 leaf stage. The application rates were equivalent to 0.25 and 0.75 times the manufacturer's suggested use rate. Non-ionic surfactant[19] was included with dicamba, lactofen, paraquat, and trifloxusulfuron. Crop oil concentrate[20] was included with atrazine. No adjuvant was included with glyphosate and glufosinate. Palmer amaranth biotypes, fertilization schedule and irrigation schedules, and greenhouse conditions were similar as described for PRE experiment. The experimental design was a split plot with herbicide and herbicide rate combinations serving as whole plot units and Palmer amaranth biotypes serving as sub-plot units. The experiment was conducted twice and treatments were replicated four times.

In the same experiment a separate set of GR biotypes were pretreated with field recommended rate of glyphosate (840 g ae/ha) to check for any segregation that might be occurring in the biotypes for resistance to glyphosate. A week after glyphosate treatment POST herbicides were applied at the same rates as described previously. The experimental design was randomized complete block with four replications and the experiment was repeated.

Measurements and statistical analyses. Visible estimates of percent weed control were recorded 28 DAT in the PRE experiment and 21 DAT in the POST experiment using a scale of 0 to 100, where 0 = no control and 100 = plant death (Frans et al. 1986). Fresh weight of above-ground tissue was also determined immediately after making the visible estimates in the POST experiment which was used to calculate percent reduction in fresh weight for data analysis. Data for visible control and fresh weight were subjected to analysis of variance appropriate for the factorial treatment arrangement (herbicide by herbicide rate by Palmer amaranth biotype) in the PRE and POST experiments. Data in the POST experiment for GR biotypes were also analyzed to compare control following pre-treatment with glyphosate versus no pre-treatment of glyphosate. Plants within each biotype surviving glyphosate were assumed to be GR. In a final analysis, data for the GR and glyphosate susceptible GS biotypes were pooled into their respective groups to compare efficacy within herbicides. A non-treated control was included in both experiments. Means of significant main effects and interactions were separated using Fisher's Protect LSD test at $p \leq 0.05$.

Results and Discussion

Preemergence herbicides. The interaction of herbicide rate by Palmer amaranth biotype was significant for visible control by pendimethalin, pyrithiobac sodium, and *S*-metolachlor but not for atrazine, flumioxazin, and fomesafen (Table 2). Control by atrazine, flumioxazin, and fomesafen was not affected by main effects of herbicide rate and biotype or the interaction of these treatment factors because these herbicides controlled Palmer amaranth biotypes 100% at both the application rates (data not presented in tables). The main effect of biotype was significant for pendimethalin, pyrithiobac sodium, and *S*-metolachlor. The main effect of herbicide rate was not significant for pyrithiobac sodium but it was significant for both pendimethalin and *S*-metolachlor. The interaction of herbicide rate and biotype was caused by variation in control within rates among biotypes. Visible control by pendimethalin ranged from 63 to 96% with a significant increase in control as the rate of herbicide increased for all biotypes of Palmer amaranth (Table 3). The GS biotypes from Emanuel and Crisp counties were controlled more effectively by pendimethalin than GS and GR biotypes from other counties when applied at 0.25 times the manufacturer's field rate. When applied at the higher rate, control of the GR biotype from Emanuel County was lower than control of all other biotypes. Palmer amaranth control by pyrithiobac sodium ranged from 50 to 69%.

With respect to control by *S*-metolachlor, applying the highest rate controlled all Palmer amaranth biotypes completely. When comparing rates of *S*-metolachlor for each biotype, increasing the rate increased control of all biotypes.

Results for analysis of variance when biotypes were grouped as either GR or GS indicated that the main effect of herbicide rate was significant for pendimethalin and *S*-metolachlor but the main effect of biotype was significant only for penidmethalin (Table 4). The interaction of herbicide rate by Palmer amaranth biotype was not significant for any of the herbicides used in the study. When pooled over biotypes, visible control increased from 69 to 91% when rate of pendimethalin increased from 0.25 to 0.75 times the manufacturer's field recommended rate while it increased from 84 to 100% with an increase in rate of *S*-metolachlor from 0.25x to 0.75x. When data were pooled over herbicide rates for pedimethalin visible control of GS biotypes was greater (82%) as compared with GR biotypes (77%) (Table 5).

Postemergence herbicides. The interaction of herbicide rate by biotype was significant for atrazine and lactofen for visible control and for atrazine and glyphosate for Palmer amaranth fresh weight reduction (Table 6). Control by paraquat was complete regardless of biotype or herbicide rate. The main effect of herbicide rate for visible control was significant for atrazine, dicamba, glufosinate, glyphosate, lactofen, and trifloxysulfuron and this effect was also significant for percent fresh weight reduction for these herbicides except trifloxysulfuron. The main effect of Palmer amaranth biotype was significant for atrazine, dicamba, glufosinate, glyphosate, and trifloxysulfuron for both control and fresh weight reduction.

When pooled over biotypes, visible control of Palmer amaranth by dicamba, glufosinate, glyphosate, and trifloxysulfuron increased by 16, 24, 16, and 18%, respectively when the rate was increased from 0.25 to 0.75 times the recommended field rate (Table 7). When comparing Palmer amaranth fresh weight, there was and increase of 33, 37, 25, and 6% in fresh weight reduction with dicamba, glufosinate, lactofen, and trifloxysulfuron, respectively when the rate was increased from 0.25 to 0.75 times the recommended field rate.

Palmer amaranth visible control by dicamba, glufosinate, glyphosate, and trifloxysulfuron ranged from 74 to 95%, 73 to 89%, 26 to 93%, and 27 to 63%, respectively (Table 8). When pooled over herbicide rates, percent fresh weight reduction of Palmer amaranth biotypes with dicamba, glufosinate, and trifloxysulfuron ranged from 42 to 77%, 56 to 87%, and 7 to 39%, respectively (Table 9). When comparing visible control of Palmer amaranth biotypes within herbicide, the control was similar at 0.75 times rate of atrazine and lactofen for all biotypes while there were differences in control at 0.25 times rate (Table 10). At 0.25 times manufacturer's field rate, GS biotypes from Johnston and Sumter counties were controlled less with atrazine as compared to other biotypes. Among all the biotypes of Palmer amaranth, GS biotype from Sumter County was controlled the least at 0.25 times rate of lactofen. Percent fresh weight reduction was the lowest for GS biotype from Johnston County at 0.25 times the manufacturer's field rate of atrazine and this reduction was similar for all Palmer amaranth biotypes at 0.75 times rate. Percent fresh weight reduction for Palmer amaranth biotypes ranged from 8 to 91% for glyphosate.

One of the objectives of this research was to determine if differences in control of GS and GR biotypes differ when comparing control by herbicides other than glyphosate. When biotypes were grouped by confirmed resistance to glyphosate (GR and GS), the main effect of rate was significant for atrazine, dicamba, glufosinate, glyphosate, lactofen, and trifloxysulfuron for visible control (Table 11). This effect was also significant for percent fresh weight reduction for all herbicides except trifloxysulfuron. These results suggested that increasing rate of these herbicides from 0.25 to 0.75 times the manufacturer's field rate increased control and fresh weight reduction of Palmer amaranth biotypes. The main effect of biotype was significant for atrazine, dicamba, and glyphosate for visible control and fresh weight reduction of Palmer amaranth biotypes. This suggested that response of GR and GS biotypes of Palmer amaranth was variable for atrazine dicamba, and glyphosate. Interaction of rate by biotype was significant for atrazine, dicamba, and glufosinate for visible control and for only atrazine for fresh weight reduction.

When pooled over herbicide rates, visible control and fresh weight reduction were higher for GR biotypes than GS biotypes of Palmer amaranth when treated with dicamba (Table 12). As expected, the control and fresh weight reduction of GS biotypes of Palmer amaranth was higher (74 and 80%) as compared to GR biotypes (36 and 20%) with glyphosate application. Visible control by atrazine, glufosinate, and lactofen and fresh weight reduction by atrazine at 0.75 times manufacturer's field rate was similar for GR and GS biotypes. However, there were differences in visible control and fresh weight reduction of GR and GS biotypes at 0.25 times rate. With the exception of visible control by glufosinate, control by atrazine and lactofen and fresh weight reduction by atrazine at 0.25 times rate was higher for GR biotypes as compared to GS biotypes (Table 13). Visible control was similar for both GR and GS biotypes with glufosinate at 0.25 times the manufacturer's field rate.

For one part of this experiment GR biotypes were pretreated with glyphosate and then after a week POST herbicides were applied. Analysis of variance results for these indicated that interaction of rate by biotype for visible control was significant for lactofen and this interaction was significant for dicamba for fresh weight reduction (Table 14). Main effect of herbicide rate was significant for atrazine, dicamba, and glufosinate for both visible control and fresh weigh reduction. Main effect of biotype was significant for atrazine, dicamba, lactofen, and trifloxysulfuron for visible control and for atrazine and lactofen for fresh weight reduction. When pooled over GR biotypes visible control and fresh weight reduction were higher when atrazine, dicamba, and glufosinate were applied at 0.75 times manufacturer's filed recommended rate (Table 15). There were differences in control and fresh weigh reduction of GR biotypes when data were pooled over rates (Table 16). Visible control ranged from 69 to 92%, 74 to 90%, and 38 to 66% for atrazine, dicamba, and trifloxysulfuron, respectively. Percent fresh weight reduction was the lowest for GR biotype from Johnston County with atrazine and this value was the least and similar for GR biotypes from Johnston and Sumter counties with lactofen. Visible control of GR biotypes form Wayne and Johnston counties was the lowest and similar at 0.25 times manufacturer's recommended rate of lactofen (Table 17). Among all the GR biotypes of Palmer amaranth the greatest fresh weight reduction was observed for Emanuel county biotype when treated with dicamba at 0.75 times manufacturer's field recommended rate.

Results from these experiments indicate that differences in control can vary among biotypes for PRE and POST herbicides. On grouping biotypes by confirmed resistance to glyphosate, GR biotypes were controlled less by pendimethalin (PRE) and more by atrazine, glufosinate, and lactofen (POST) as compared to GS biotypes of Palmer amaranth. Realistically, these differences were minor and probably not biologically significant. Although these data suggest that resistance to glyphosate may also contribute to effectiveness of herbicides with other MOAs, the pool of biotypes (four GS versus four GR biotypes) was limited. Differences in efficacy observed across biotypes may have been associated with characteristics within a biotype other than glyphosate resistance.

Acknowledgments

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Sources of Materials

[1]Nursery flats, Myers Industries Lawn and Garned group, 15200 Madison Road, Middlefield, OH 44062.

[2]AAtrex herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[3]Valor SX herbicide®. Valent USA Corp., Valent USA Corporation, Walnut Creek, CA 94596.

[4]Reflex herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[5] Prowl H2O herbicide® . BASF Corp., Research Triangle Park, NC 27709.

[6]Staple herbicide®. Bayer Crop Science, Research Triangle Park, NC 27709.

Dual Magnum herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[8]TeeJet TP8003E even-fan spray nozzles, Spraying Systems Co., Wheaton, IL 60189.

[9]Scotts® Starter® Fertilizer, The Scotts Company LLC, Marysville, OH 43041.

[10]Hubbell Lighting, Inc.,701 Millennium Blvd, Greenville, SC 29607.

[11]ITML green standard pots, Myers Industries Lawn & Garden Group,15200 Madison Road, P.O. Box 738, Middlefield, OH 44062.

[12]Fafard 4P potting mix, Conrad Fafard Inc. Agawam, MA 01001.

[13]Clarity herbicide®. BASF Corp., Research Triangle Park, NC 27709.

[14]Roundup Weather Max®. Monsanto Co., St. Louis, MO 63198.

[15]Ignite herbicide®. Bayer Crop Science, Research Triangle Park, NC 27709.

[16]Cobra 2EC herbicide®. Valent USA Corp., Valent USA Corporation, Walnut Creek, CA 94596.

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

[18]Envoke herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[19]Induce® nonionic surfactant. Proprietary blend of alkyl aryl polyoxyalkane ethers, free fatty acids, and dimothyl polysiloxane, 90%. Helena Chemical Company, Collierville, TN 38107.

[20]Agri-Dex® spray adjuvant. Proprietary blend of alkyl aryl polyoxyalkane ethers, free fatty acids, and dimothyl polysiloxane, 90%. Helena Chemical Company, Collierville, TN 38107.

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Table 1. Common name, trade name, family, and MOA of herbicides used in PRE and POST experiments[a].

Herbicide	Trade Name	Family	Mode of action
Preemergence			
Atrazine	Aatrex	Triazine	Photosystem II inhibitor
Flumioxazin	Valor SX	N-phenylphthalimide	PPO inhibitor
Fomesafen,	Reflex	Diphenylether	PPO inhibitor
Pendimethalin	Prowl H2O	Dinitroaniline	Root growth inhibitor
Pyrithiobac sodium	Staple LX	Pyrimidinyl(thio)benzoate	ALS inhibitor
S-metolachlor	Dual II Magnum	Chloroacetamide	Shoot growth inhibitor
Postemergence			
Atrazine	Aatrex	Triazine	Photosystem II inhibitor
Dicamba	Clarity	Benzoic acid	Growth regulator
Glyphosate	Roundup Weathermax	Glycine	EPSPS inhibitor
Glufosinate	Ignite	Phosphonic acid	Glutamine synthetase inhibitor
Lactofen	Cobra	Diphenylether	PPO inhibitor
Paraquat	Gramaxone Inteon	Bipyrilidium	Photosystem I inhibitor
Trifloxysulfuron	Envoke	Sulfonylurea	ALS inhibitor

Table 1 continued

[a]Abbreviations: ALS, acetolactate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate; PPO, protoporphyrinogen oxidase.

Table 2. P > F for visible control of Palmer amaranth biotypes as influenced by PRE herbicides 4 weeks after treatment[a].

Source of variation	Pendimethalin	Pyrithiobac- sodium	S-metolachlor
Rate	< 0.0001	0.4932	0.0005
Biotype	<0.0001	< 0.0001	< 0.0001
Rate x Biotype	<0.0001	0.0017	< 0.0001
Coefficient of variation (%)	10	11	8

[a] Data are pooled over experiments.

Table 3. Palmer amaranth visible control as influenced by the interaction of PRE herbicide rate and biotypes 4 weeks after treatment[a].

	Pendimethalin		Pyrithiobac sodium		*S*-metolachlor	
Biotype	0.25x	0.75x	0.25x	0.75x	0.25x	0.75x
	%					
Wayne (GR)	68	94	64	66	79	100
Johnston (GS)	68	96	65	59	78	100
Sumter (GS)	66	91	56	64	81	100
Sumter (GR)	63	87	61	64	80	100
Macon (GR)	70	95	59	64	88	100
Emanuel (GR)	66	76	66	65	87	100
Emanuel (GS)	76	95	69	65	94	100
Crisp (GS)	76	91	57	50	86	100
LSD ($P \leq 0.05$)	6.0		5.0		5.0	

[a] Data are pooled over experiments.

Table 4. P > F for visible control of Palmer amaranth biotypes as influenced by PRE herbicides 4 weeks after treatment when biotypes were grouped as glyphosate resistant (GR) and glyphosate susceptible (GS)[a].

Source of variation	Pendimethalin	S-metolachlor
Rate	< 0.0001	0.0005
Biotype	0.0059	0.6651
Rate x Biotype	0.9491	0.6651
Coefficient of variation (%)	12	9

[a] Data are pooled over experiments.

Table 5. Palmer amaranth visible control as influenced by herbicide rates 4 weeks after treatment when biotypes were grouped as glyphosate resistant (GR) and glyphosate susceptible (GS)[a].

Biotype	Pendimethalin	*S*-metolachlor
0.25 x	69	84
0.75 x	91	100
P > F	<0.0001	0.0005

[a] Data are pooled over Palmer amaranth biotypes and experiments.

Table 6. Palmer amaranth visible control as influenced by pendimethalin and biotypes 4 weeks after treatment when biotypes were grouped as glyphosate resistant (GR) and glyphosate susceptible (GS)[a].

Biotype	Pendimethalin
Resistant(GR)	77
Susceptible (GS)	82
P > F	0.0059

[a] Data are pooled over herbicide rates and experiments.

Table 7. P > F for visible control and percent reduction in fresh weight of Palmer amaranth as influenced by POST herbicides 21 days after treatment[a].

Source of variation	Atrazine	Dicamba	Glufosinate	Glyphosate	Lactofen	Trifloxysulfuron
Visible control	p-value					
Rate	<0.0001	<0.0001	<0.0001	0.0020	<0.0001	<0.0001
Biotype	<0.0001	<0.0001	0.0230	<0.0001	0.4915	<0.0001
Rate x Biotype	<0.0001	0.2353	0.0682	0.5391	0.0010	0.3151
Coefficient of variation (%)	12	14	21	52	13	44
Fresh weight reduction						
Rate	<0.0001	<0.0001	<0.0001	0.0087	<0.0001	0.0575
Biotype	<0.0001	<0.0001	0.0008	<0.0001	0.6419	<0.0001
Rate x Biotype	<0.0001	0.2881	0.0901	0.0483	0.8712	0.9313
Coefficient of variation (%)	12	37	28	42	33	83

[a] Data are pooled over experiments.

Table 8. Palmer amaranth visible control and fresh weight reduction as influenced by POST herbicide rate 21 days after treatment[a].

Herbicide Rate	Dicamba	Glufosinate	Glyphosate	Lactofen	Trifolxysulfuron
	%				
Visible control					
0.25 x	75	68	47	-	31
0.75 x	91	92	63	-	49
LSD (P ≤0.05)	4	6	10	-	*
Fresh weight reduction					
0.25 x	38	53	-	67	19
0.75 x	71	90	-	92	25
LSD (P ≤0.05)	7	7	-	*	ns

[a] Data are pooled over Palmer amaranth biotypes and experiments.

Table 9. Palmer amaranth visible control as influenced by biotype and POST herbicides 21 days after treatment[a].

Biotype	Dicamba	Glufosinate	Glyphosate	Trifolxysulfuron
	%			
Wayne (GR)	78	86	32	32
Johnston (GS)	77	75	53	29
Sumter (GS)	74	76	90	27
Sumter (GR)	80	76	58	36
Macon (GR)	91	73	26	63
Emanuel (GR)	95	89	31	42
Emanuel (GS)	83	88	61	48
Crisp (GS)	85	78	93	45
LSD ($P \leq 0.05$)	8	12	20	13

[a] Data are pooled over herbicide rates and experiments.

Table 10. Palmer amaranth percent fresh weight reduction as influenced by biotype and POST herbicides 21 days after treatment[a].

Biotype	Dicamba	Glufosinate	Trifolxysulfuron
		%	
Wayne (GR)	50	64	11
Johnston (GS)	42	56	7
Sumter (GS)	44	74	29
Sumter (GR)	43	67	18
Macon (GR)	77	87	39
Emanuel (GR)	68	79	20
Emanuel (GS)	59	66	35
Crisp (GS)	53	80	17
LSD ($P \leq 0.05$)	14	14	13

[a] Data are pooled over herbicide rates and experiments.

Table 11. Palmer amaranth control and percent fresh weight reduction as influenced by the interaction of POST herbicide rate and biotypes 21 days after treatment[a].

	Visible control				Fresh weight reduction			
	Atrazine		Lactofen		Atrazine		Glyphosate	
Biotype	0.25 x	0.75 x	0.25 x	0.75 x	0.25x	0.75 x	0.25x	0.75 x
	%							
Wayne (GR)	96	100	84	99	95	100	13	16
Johnston (GS)	69	98	83	98	47	95	82	87
Sumter (GS)	69	100	73	97	72	100	52	91
Sumter (GR)	97	100	94	97	96	100	13	32
Macon (GR)	100	100	96	97	100	100	23	32
Emanuel (GR)	98	100	94	95	96	100	18	8
Emanuel (GS)	87	100	97	99	86	100	69	86
Crisp (GS)	98	100	90	98	98	100	88	85
LSD (P ≤ 0.05)	11		9		11		21	

[a] Data are pooled over experiments.

Table 12. P > F for visible control and percent fresh weight reduction of Palmer amaranth biotypes as influenced by POST herbicides 3 weeks after treatment when biotypes were grouped as glyphosate resistant (GR) and glyphosate susceptible (GS)[a].

Source of variation	Atrazine	Dicamba	Glufosinate	Glyphosate	Lactofen	Trifloxysulfuron
	p-value					
Visible control						
Rate	< 0.0001	< 0.0001	< 0.0001	0.0052	< 0.0001	< 0.0001
Biotype	0.0001	0.0096	0.9798	< 0.0001	0.1190	0.1072
Rate x Biotype	0.0002	0.3945	0.0225	0.5737	0.0444	0.2511
Coefficient of variation (%)	13	16	21	58	11	51
Fresh weight reduction						
Rate	< 0.0001	< 0.0001	< 0.0001	0.0117	0.0001	0.0906
Biotype	< 0.0001	0.0031	0.5838	< 0.0001	0.9736	0.9853
Rate x Biotype	0.0002	0.5606	0.4367	0.2270	0.1788	0.4738
Coefficient of variation (%)	16	40	30	44	24	93

Table 13. Palmer amaranth control and percent fresh weight reduction as influenced by POST herbicides 21 days after treatment when biotypes were grouped as glyphosate resistant (GR) and glyphosate susceptible (GS)[a].

Herbicide Rate	Dicamba	Glyphosate
	%	
Visible control		
Resistant (GR)	86	36
Susceptible (GS)	80	74
P > F	0.0096	< 0.0001
Fresh weight reduction		
Resistant (GR)	61	20
Susceptible (GS)	49	80
P > F	0.0031	< 0.0001

[a] Data are pooled over herbicide rates and experiments.

Table 14. Palmer amaranth control and percent fresh weight reduction as influenced by the interaction of herbicide rate and biotype grouping 21 days after treatment when biotypes were grouped as glyphosate resistant (GR) and glyphosate susceptible (GS)[a].

	Visible control						Fresh weight reduction	
	Atrazine		Glufosinate		Lactofen		Atrazine	
Biotype group	0.25 x	0.75 x	0.25 x	0.75 x	0.25 x	0.75 x	0.25 x	0.75 x
	%							
Resistant (GR)	98	100	73	89	92	97	97	100
Susceptible (GS)	81	99	66	96	86	98	76	99
LSD ($P \leq 0.05$)	6		9		5		7	

[a] Data are pooled over experiments.

Table 15. P > F for visible control and percent fresh weight reduction of Palmer amaranth as influenced by POST herbicides 21 days after treatment when glyphosate resistant (GR) biotypes were pretreated with glyphosate[a].

Source of variation	Atrazine	Dicamba	Glufosinate	Glyphosate	Lactofen	Trifloxysulfuron
Visible control	p-value					
Rate	<0.0001	0.0013	0.0227	0.5166	0.1457	0.8902
Biotype	0.0007	0.0010	0.5954	0.4156	<0.0001	0.0292
Rate x Biotype	0.0749	0.0547	0.7668	0.3077	0.0086	0.3772
Coefficient of variation (%)	19	14	51	52	25	49
Fresh weight reduction						
Rate	<0.0001	0.0078	<0.0001	0.1400	0.3718	0.2734
Biotype	0.0013	0.1623	0.2070	0.9673	0.0027	0.0628
Rate x Biotype	0.0620	0.0276	0.0955	0.5431	0.0839	0.3860
Coefficient of variation (%)	35	62	75	173	47	107

[a] Data are pooled over experiments.

Table 16. Palmer amaranth control and fresh weight reduction as influenced by POST herbicide rate 21 days after treatment when glyphosate resistant (GR) biotypes were pretreated with glyphosate[a].

Herbicide Rate	Atrazine	Dicamba	Glufosinate
		%	
Visible control			
0.25 x	69	78	49
0.75 x	93	88	66
P > F	< 0.0001	0.0013	0.0227
Fresh weight reduction			
0.25 x	53	41	20
0.75 x	88	64	60
P > F	< 0.0001	0.0078	<0.0001

[a] Data are pooled over experiments and Palmer amaranth biotypes.

Table 17. Palmer amaranth control and fresh weight as influenced by biotype and POST herbicide 21 days after treatment when glyphosate resistant (GR) biotypes were pretreated with glyphosate[a].

	Visible control			Fresh weight reduction	
Biotype	Atrazine	Dicamba	Trifloxysulfuron	Atrazine	Lactofen
	%				
Wayne (GR)	69	79	38	51	51
Sumter (GR)	79	90	51	71	80
Macon (GR)	92	87	66	88	75
Emanuel (GR)	85	74	53	73	46
LSD ($P \leq 0.05$)	11	8	18	18	21

[a] Data are pooled over herbicide rates and experiments.

Table 18. Palmer amaranth control and fresh weight as influenced by the interaction of herbicide rate and biotype 21 days after treatment when glyphosate resistant (GR) biotypes were pretreated with glyphosate[a].

	Visible control		Fresh weight reduction	
	Lactofen		Dicamba	
Biotype group	0.25 x	0.75 x	0.25 x	0.75 x
	—— % ——			
Wayne (GR)	48	64	47	49
Emanuel (GR)	94	73	41	89
Macon (GR)	74	89	62	54
Sumter (GR)	54	71	16	64
LSD ($P \leq 0.05$)	—— 18 ——		—— 33 ——	

[a] Data are pooled over experiments.

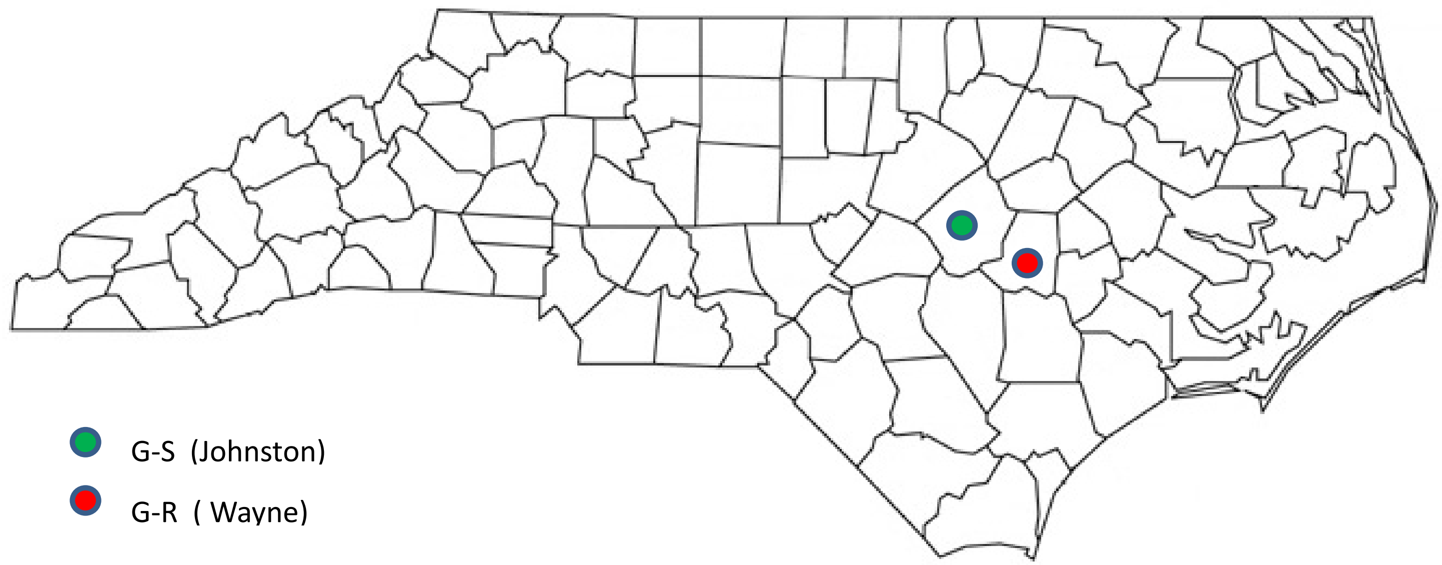
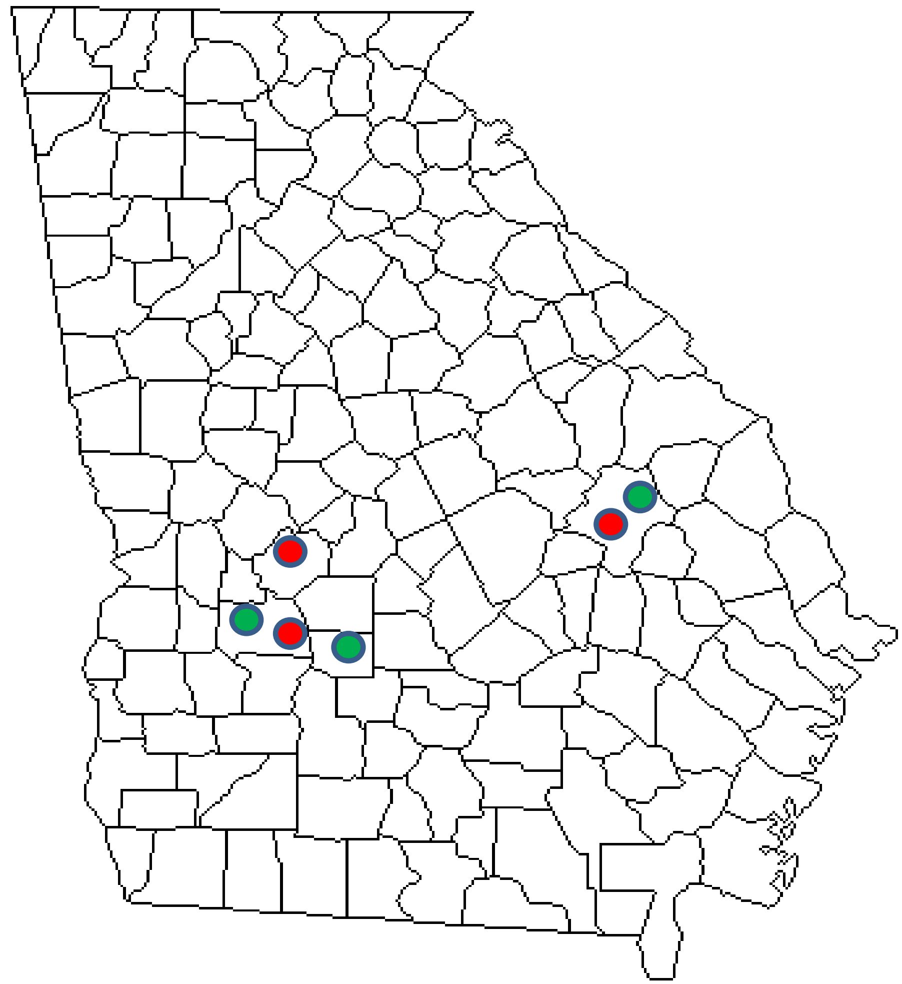


Figure 1. North Carolina counties from where biotypes used in the study were collected



G-S (Sumter, Emanuel, Crisp)

G-R (Sumter, Emanuel, Macon)

Figure 2. Georgia counties from where biotypes used in the study were collected

CHAPTER VI

Multiple Resistance to Acetolactate Synthase and Acetyl Co-A Carboxylase Inhibitors in North Carolina Italian Ryegrass (*Lolium perenne* ssp. *multiflorum*)

Aman Chandi, Alan C. York, David L. Jordan, and Josh B. Beam*

Abstract

Diclofop-resistant Italian ryegrass is widespread in southwestern North Carolina, and growers have resorted to using acetolactate synthase (ALS) inhibitors such as mesosulfuron and pyroxsulam to control this weed in wheat. In the spring of 2007, mesosulfuron failed to control Italian ryegrass in several fields. Seed were collected from six fields in two counties and greenhouse studies were conducted to determine response to mesosulfuron and the acetyl-CoA carboxylase (ACCase) inhibitors diclofop and pinoxaden. All populations were resistant to diclofop and cross resistant to pinoxaden. Five of the six populations displayed multiple resistance to the ACCase inhibitors and the ALS inhibitor. An additional study with two biotypes confirmed cross resistance to the ALS inhibitors imazamox, mesosulfuron, and pyroxsulam. Resistance to mesosulfuron was heritable.

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Nomenclature: diclofop; imazamox; mesosulfuron; pinoxaden; pyroxsulam; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot.; wheat, *Triticum aestivum* L.

Key words: Herbicide resistance, selection pressure, chlorsulfuron.

Introdution

Italian ryegrass is one of the most troublesome weeds of winter wheat (*Triticum aestivum* L.) in the Southeast, Mid-South, and Pacific Northwest regions of the United States (Bridges 1992). This winter annual grass competes with wheat for nutrients and light, reduces wheat tillering, causes lodging of wheat, interferes with harvesting, and contaminates harvested grain (Appleby et al. 1976; Ball et al. 1995; Hashem et al. 1998; Justice et al. 1994; Liebl and Worsham 1987). Liebl and Worsham (1987) reported wheat yield losses of 4.2% for every 10 Italian ryegrass plants per m^2. Dense populations of this weed are common, and wheat yield reductions of 50% or greater are often recorded in uncontrolled populations (Appleby et al. 1976; Bailey and Wilson 2003; Brewster et al. 1977; Crooks et al. 2003, 2004; Justice et al. 1994). In addition to yield loss, the presence of Italian ryegrass seed in wheat grain can lead to a substantial price dockage (Fast et al. 2009).

Italian ryegrass is a common weed across all of North Carolina, but traditionally the greatest problems with this weed have been in the western half of the state. Irrigation capacity is very limited in this region, and many of the soils typically do not produce economically sustainable corn (*Zea mays* L.) yields. A common production system has been wheat followed by double-cropped soybean [*Glycine max* (L.) Mer.] for several years continuously. Prior to 1980, North Carolina growers had no herbicide options for Italian ryegrass in wheat. Dense infestations were common, and in many cases growers were forced to abandon wheat crops or forgo wheat production because of severe infestations. Diclofop, an herbicide that inhibits ACCase (EC.6.4.1.2) in sensitive plants (HRAC 2011), was commercialized in 1980 (US EPA 2000). Diclofop applied POST was highly effective on Italian ryegrass (Barnes et al. 2001; Brewster et al. 1977; Griffin 1985; Justice et al. 1994; Khodayari et al. 1983; Shaw and Wesley 1991), and growers used it routinely. Unfortunately, excessive reliance on diclofop led to selection of resistant biotypes. Diclofop-resistant Italian ryegrass was first reported in the United States in Oregon in 1987 (Stanger and Appleby 1989) and North Carolina in 1990 (Heap 2011). It is now present in at least 10 states in the United States and is also found in Chile, France, Italy, and the United Kingdom (Heap 2011). Other *Lolium* species with resistance to diclofop include rigid ryegrass (*Lolium rigidum*) in Australia, Chile, France, Greece, Iran, Israel, Saudi Arabia, South Africa, Spain, and Tunisia, perennial ryegrass (*Lolium perenne*) in Chile, and Persian darnell (*Lolium persicum*) in Montana (Heap 2011).

After selection for diclofop resistance, wheat growers in North Carolina had few options to control Italian ryegrass. They generally resorted to the commercial mixture of clorsulfuron plus metsulfuron, two sulfonylurea herbicides that inhibit ALS (EC.4.1.3.18) (HRAC 2011). The label for this mixture, registered in 1986 (PANNA 2011), claims only suppression of Italian ryegrass (Anonymous 2011d). Italian ryegrass control by chlorsulfuron plus metsulfuron has been inconsistent and often inadequate (Bailey and Wilson 2003; Bailey et al. 2003; King and Hagood 2003). Averaged across numerous trials in North Carolina, late-season Italian ryegrass control by chlorsulfuron plus metsulfuron has averaged about 50% (A. C. York, unpublished data).

Mesosulfuron, an ALS-inhibiting herbicide (HRAC 2011), was registered for use on wheat in March, 2004 (US EPA 2004). Mesofulfuron, applied POST alone or in combination with iodosulfuron, was very effective on both diclofop-resistant and -susceptible Italian ryegrass (Bailey and Wilson 2003; Bailey et al. 2003; Crooks et al. 2003, 2004; King and Hagood 2003). North Carolina growers began using mesosulfuron extensively for Italian ryegrass control beginning in the 2004/05 wheat crop.

In the spring of 2007, mesosulfuron failed to control Italian ryegrass in several fields in southwestern North Carolina, the region of the state where diclofop-resistant Italian ryegrass is most common. Italian ryegrass resistant to mesosulfuron had previously been found in Arkansas, and populations with multiple resistance to diclofop and chlorsulfuron or triasulfuron had been confirmed in Arkansas and Idaho (Heap 2011). The objective of our study was to confirm resistance to the ALS inhibitors mesosulfuron, pyroxsulam, and imazamox in the North Carolina biotypes, and to determine if the biotypes exhibited multiple resistance to these ALS inhibitors and the ACCase inhibitors diclofop and pinoxaden.

Materials and Methods

Methods common to all experiements. Seeds from Italian ryegrass suspected of being resistant to mesosulfuron were collected from multiple plants and bulked during the spring of 2007 from four wheat fields in Union County and two wheat fields in Lincoln County in the southwestern Piedmont region of North Carolina (Table 1). Each of these fields had been treated with mesosulfuron earlier in the season and Italian ryegrass control was poor. Seeds were also collected from an Italian ryegrass population in Edgecombe County in the central Coastal Plain region of eastern North Carolina that is believed to have never been exposed to any of the herbicides evaluated in this experiment.

Seeds were planted in 10-cm square plastic pots containing commercial potting media[1]. Seedlings were thinned to six plants per pot shortly after emergence. Plants were fertilized four times at weekly intervals beginning 1 wk after emergence with 25 ml/pot of a 4.6 g/L fertilizer solution[2]. Greenhouse temperature was maintained at 29/21 C day/night ± 3 C. Natural light was supplemented for a 12-h photoperiod by metal halide lamps providing an additional 400 μmol $m^{-2} s^{-1}$ photosynthetic photon flux at soil level. Herbicides were applied to two-tiller Italian ryegrass 8 to 10 cm in height in a spray volume of 229 L/ha at 207 kPa pressure using a single even-spray flat-fan nozzle[3] in an enclosed spray chamber. No adjuvant was included with diclofop (Jordan 2010). Adjuvants were included with other herbicides based upon the manufacturers' recommendations. A blend of methylated rape seed oil and ethoxylated alcohols[4] at 0.7 L/ha was included with pinoxaden (Anonymous 2011a). A nonionic surfactant[5] at 0.25% (v/v) was included with imazamox (Anonomous

2011c). A crop oil concentrate[6] at 1.0% (v/v) was included with pyroxsulam (Anonymous 2011g). A methylated seed oil[7] at 1.75 L/ha was included with mesosulfuron (Anonymous 2011f).

Plants were returned immediately to the greenhouse after herbicide application. Ryegrass control was estimated visually 3 wk after herbicide application using a scale of 0 to 100, where 0 = no control and 100 = plant death (Frans et al. 1986). Fresh weight of live shoot tissue (green tissue) was recorded following the visual estimates of control. Percent reduction in shoot fresh weight was calculated relative to the non-treated control for each biotype.

The experimental design of each experiment was a randomized complete block with treatments replicated three (experiments 1 and 2) or four (experiment 3) times, and each experiment was repeated. Data for percent weed control and percent fresh weight reduction were pooled over runs of the experiment and subjected to analysis of variance. In order to describe dose response of ryegrass biotypes, Gompertz [$y = a*\exp(-\exp(-(x-x_0)/b))$] logarithmic [$y = y_0 + a \ln(x- x_0)$], logistic [$y = 93.8/(1+ (x/ x_0)^b)$], polynomial [$y = y_0+ax$], and sigmoidal [$y = a/(1+\exp(-(x- x_0)/b))$] three-parameter regression equations were used in SigmaPlot 11.0[8]. I_{50} values for herbicides were constructed using regression equations.

In experiment 2, I_{50} values for weed control and percent fresh weight reduction of putative resistant biotypes L1 and U3 were divided by the I_{50} values of susceptible biotype E to calculate R/S ratios, where R = I_{50} of resistant biotype and S = I_{50} of susceptible biotype. This ratio gave an idea about the level of resistance in the suspected resistant population as compared to the susceptible control used in the study.

In experiment 3, in order confirm if resistance to mesosulfuron was heritable, data for percent survival, percent weed control, and percent fresh weight reduction for the F_2 generation from the L1 and U3 biotypes and the F_1 generation of the E biotype were subjected to ANOVA using the GLM procedure in SAS[6]. Main effects of biotype and dose and interaction of dose and biotypes were separated using Fisher's protected LSD at $p \leq 0.05$.

Experiment 1. This experiment included the E biotype and the six putative resistant biotypes from Lincoln and Union counties (Table 1). Diclofop[10], mesosulfuron[11], and pinoxaden[12] were applied to each biotype at 0.25, 0.5, 1, 4, 16, and 32 times the manufacturers' suggested use rates, where 1X = 840 g ai/ha of diclofop, 15 g ai/ha of mesosulfuron, and 62.5 g ai/ha of pinoxaden (Anonymous 2011a, 2011f; Jordan 2010). A non-treated check was included for each biotype.

Experiment 2. This experiment included biotypes E, L1, and U3 (Table 1). Diclofop, mesosulfuron, pinoxaden, imazamox ammonium salt[13], and pyroxsulam[14] were applied to each biotype at 0.05, 0.1, 0.25, 0.5, 1, 3, 6, 12, 24, 48, and 96 times the manufacturers' suggested use rates, where 1X = 840 g/ha of diclofop, 15 g/ha of mesosulfuron, 62.5 g/ha of pinoxaden, 52 g ae/ha of imazamox, and 16 g ai/ha of pyroxsulam (Anonymous 2011a, 2011c, 2011f, 2011g; Jordan 2010). A non-treated check was included for each biotype.

Experiment 3. Seedlings of the L1 and U3 biotypes were treated with mesosulfuron at 150 g/ha (10 times manufacturer's suggested use rate). Surviving seedlings were transplanted into 30-cm (dia) plastic pots containing the potting media and grown to maturity for seed collection. Seed of the F2 generation from the L1 and U3 biotypes and the F1 generation of the E biotype were planted in 10-cm square pots and grown as previously described. These seedlings were treated with mesosulfuron at 0, 15, 60, and 240 g/ha.

Results and Discussion

Experiment 1. I_{50} values could not be calculated for response of some biotypes to one or more of the herbicides due to the limited herbicide rate ranges evaluated (Tables 2 and 3). From the regression curves of both visible control and shoot fresh weight reduction, however, it is obvious that all biotypes from Lincoln and Union counties were resistant to diclofop (Figures 1A and 2A). Differences in response to diclofop among the biotypes were noted. Biotypes L1, L2, U2, and U3 were more resistant to diclofop than biotypes U1 and U4. Diclofop at 840 g/ha, the manufacturer's suggested field use rate, controlled biotype E 97% and reduced fresh weight 81%. Biotypes L1, L2, U2, and U3 were controlled 2 to 9% and shoot fresh weight was reduced 12 to 41 % compared with 23 to 35% control and 41 to 57 % shoot fresh weight reduction of biotypes U1 and U4.

The I_{50} for visible control of biotype E by pinoxaden was 37 g/ha (Table 2) while the I_{50} for shoot fresh weight reduction was less than 15.6 g/ha, the lowest rate tested (Table 3). All biotypes from Lincoln and Union counties were resistant to pinoxaden. Less resistance was noted with biotype U1 than with the other Lincoln and Union biotypes, but visible control and shoot fresh weight reduction of U1 by the manufacturer's suggested field use rate of 62.5

g/ha was less than 30% (Figures 1B and 2B). Biotypes L1, L2, U2, U3, and U4 were highly resistant to pinoxaden. Pinoxaden at 2000 g/ha, 32 times the suggested field use rate, provided less than 50% visible control and shoot fresh weight reduction of biotypes L1, L2, U2 U3, and U4.

I_{50} values for mesosulfuron could not be calculated for the E biotype or the U1 biotype as both visible control and shoot fresh weight reduction were greater than 50% with all rates evaluated (Figures 1C and 2C). Mesosulfuron at the manufacturer's suggested field use rate of 15 g/ha controlled these biotypes and reduced fresh weights greater than 95%. The inadequate control of biotype U1 observed in the field in the spring of 2007 was apparently due to causes other than resistance. In contrast, mesosulfuron at 15 g/ha controlled the other Lincoln and Union biotypes 30% or less and reduced shoot fresh weight 50% or less.

Experiment 2. The I_{50} for visible control and shoot fresh weight reduction of biotype E by diclofop was 218 and 251 g/ha, respectively (Tables 4 and 5). Diclofop at 840 g/ha, the manufacturer's suggested field use rate, controlled Italian ryegrass and reduced fresh weight approximately 90 and 85 %, respectively (Figures 3A and 4A). Similar to results in experiment 1, the L1 and U3 biotypes were shown to be highly resistant to diclofop, with R/S ranging from 89 to 177 (Tables 4 and 5). The E biotype was highly susceptible to pinoxaden, and I_{50} values for pinoxaden applied to the E biotype could not be determined. Visible control and shoot fresh weight reduction of the E biotype were at least 95% by pinoxaden at 3.125 g/ha, the lowest rate tested and only 5% of the manufacturer's suggested field use rate. Without an I_{50} for biotype E, R/S for biotypes L1 and U3 treated with pinoxaden could not be determined. Pinoxaden was more effective on biotypes L1 and U3 in experiment 2 (Figures 3B and 4B) than in experiment 1 (Figures 1B and 2B). However, the two experiments clearly indicate cross resistance in these biotypes to both ACCase-inhibiting herbicides.

Cross resistance among the three ALS-inhibiting herbicides was also demonstrated. Additionally, multiple resistance to the ALS-inhibiting herbicides and the ACCase-inhibiting herbicides was observed. Compared to biotype E, R/S values for visible control and shoot fresh weight reduction ranged from 5 to 7 for imazamox, 12 to 22 for mesosulfuron, and 19 to 20 for pyroxsulam applied to biotype L1 (Tables 4 and 5). For the U3 biotype, *R/S* values for visible control and shoot fresh weight reduction ranged from 12 to 18 for imazamox, 64 to 65 for mesosulfuron, and 24 to 29 for pyroxsulam.

Experiment 3. All rates of mesosulfuron controlled the E biotype completely (Table 6). All F2 plants of the L1 biotype survived mesosulfuron at the field use rate of 15 g/ha and shoot fresh weight was reduced only 8%. Ninety-five percent of the L1 plants survived mesosulfuron at 240 g/ha, and shoot fresh weight was reduced only 45%. Ninety-eight percent of the F2 plants of the U3 biotype survived mesosulfuron applied at 60 g/ha, and 88% survived mesosulfuron applied at 240 g/ha. Visible control and shoot fresh weight reduction by mesosulfuron at 240 g/ha applied to the U3 biotype were only 25 and 29%, respectively. The F2 plants of biotypes L1 and U3 were from parents treated with mesosulfuron at 150 g/ha. Results of this experiment demonstrate that resistance to mesosulfuron in these biotypes is a heritable trait.

Detailed herbicide use records on the fields from which the Lincoln and Union biotypes were collected in 2007 were unavailable. However, the growers noted that each field had been treated with mesosulfuron at least twice since the spring of 2005. Each field had also been treated for several years prior to 2005 with clorsulfuron plus metsulfuron. The growers also mentioned a long history of diclofop use during the 1980's through the mid 1990's. Pinoxaden, pyroxsulam, and imazamox had never been applied to any of the fields.

These experiments demonstrate cross resistance to diclofop and pinoxaden in Italian ryegrass in North Carolina. Diclofop, an aryloxyphenoxy propionate, and pinoxaden, a phenylpyrazolin, are chemically dissimilar yet both inhibit ACCase (Burton et al. 1989; Hofer et al. 2006). Resistance to diclofop or other aryloxyphenoxy propionates does not necessarily infer cross resistance to pinoxaden (Ellis et al. 2010; Owen and Powles 2009; Rauch et al. 2010). However, cross resistance to aryloxyphenoxy propionates and pinoxaden has previously been reported in several weedy grasses, including Italian ryegrass, wild oat (*Avena fatua*), hood canarygrass (*Phalaris paradoxa*), littleseed canarygrass (*Phalaris minor*), rigid ryegrass (*Lolium rigidum*), and blackgrass (*Alopecurus myosuroides*) (Chhokar and Sharma 2008; Collavo et al. 2011; Petit et al. 2010; Rauch et al. 2010; Uludag et al. 2008; Yu et al. 2007). Resistance to pinoxaden was often observed before the herbicide was commercialized (Kuk et al. 2008; Yu et al. 2010). The fields from which the Lincoln and Union biotypes were collected had never received pinoxaden. Resistance to pinoxaden in these populations was likely selected for by widespread use of diclofop (Kuk et al. 2008; Owens and Powles 2009; Petit et al. 2010; Rauch et al. 2010).

Resistance to ALS inhibitors is widespread in *Lolium* spp. (Heap 2011). Kuk and Burgos (2007) reported the first mesosulfuron-resistant population of Italian ryegrass in the United States. That population, from Arkansas, was cross resistant to chlorsulfuron, sulfometuron, and imazamox, but it was not resistant to diclofop or pinoxaden. The next report of a mesosulfuron-resistant Italian ryegrass population was in Texas (Ellis et al. 2008). This population also was not resistant to diclofop or pinoxaden. More recently, Italian ryegrass populations with cross resistance to mesosulfuron, imazamox, and pyroxsulam and multiple resistance to diclofop were reported in Arkansas (Salas et al. 2010). Our experiments demonstrate that Italian ryegrass populations with cross resistance to mesosulfuron, imazamox, and pyroxsulam and multiple resistance to diclofop and pinoxaden also occur in North Carolina.

Multiple resistance to ACCase and ALS inhibitors in Italian ryegrass presents a serious challenge to wheat growers. Except for the commercial mixture of flufenacet plus metribuzin (Anonymous 2011b), all herbicides currently available for Italian ryegrass control in North Carolina are either ACCase or ALS inhibitors. Metribuzin could be applied early POST to wheat, but the label excludes North Carolina and most other southeastern states (Anonymous 2011e). Italian ryegrass control by metribuzin is inconsistent (Kinfe and Peeper 1991; Shaw and Wesley 1991), and wheat injury is sometimes unacceptable (Grey and Bridges 2003; Shaw and Wesley 1991). Flufenacet plus metribuzin applied PRE or early POST can control Italian ryegrass well (Grey and Bridges 2003; Ritter and Menbere 2002). The rate of metribuzin in this commercially formulated mixture is low, and except for metribuzin-sensitive cultivars (Grey and Bridges 2003), wheat tolerance is acceptable. In numerous trials in North Carolina, wheat tolerance of flufenacet plus metribuzin has been good but Italian ryegrass control has been inconsistent due to the requirement for timely rainfall to activate the herbicide prior to weed emergence (A. C. York, unpublished data). Late-season Italian ryegrass control ranged from 15 to 100%, and averaged only 67%. In 50% of the trials, late-season control was less than 80%. Growers need a new mode of action to control Italian ryegrass in wheat.

Sources of Materials

[1]Fafard 4P Mix, Conrad Fafard, Inc., Agawam, MA 01001-0790.

[2]Peters Professional Water Soluble 20-20-20 Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH 43041.

[3]TeeJet TP8003E even-fan spray nozzles, Spraying Systems Co., Wheaton, IL 60189.

[4]Adigor Adjuvant, Syngenta Crop Protection Canada, Inc, Guelph, Ontario, Canada N1G 4Z3.

[5]Induce nonionic surfactant, Helena Chemical Co. Collierville, TN 38017.

[6]Agri-Dex crop oil concentrate, Helena Chemical Co. Collierville, TN 38017.

[7]Premium MSO Methylated Spray Oil, Helena Chemical Co. Collierville, TN 38017.

[8] SYSTAT Software Inc. (SSI), San Jose, CA, USA 95110.

[9]Hoelon herbicide, Bayer CropScience, Research Triangle Park, NC 27709.

[10]Statistical Analysis Systems, version 9.2, SAS Institute, Cary, NC 27513.

[11]Osprey herbicide, Bayer CropScience, Research Triangle Park, NC 27709.

[12]Axial herbicide, Syngenta Crop Protection Inc, Greensboro, NC 27419-8300.

[13]Beyond herbicide, BASF Corp., Research Triangle Park, NC 27709.

[14]PowerFlex herbicide, Dow AgroSciences LLC, Indianapolis, IN 46268.

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Table 1. Italian ryegrass biotype designations and Global Positioning Systems (GPS) coordinates of collection sites.

Biotype designation	North Carolina County	GPS Coordinates	
		North	West
E	Edgecombe	35.9905	-77.7622
L1	Lincoln	35.5375	-81.3394
L2	Lincoln	35.5266	-81.3217
U1	Union	35.1761	-80.2981
U2	Union	34.8393	-80.3643
U3	Union	35.0919	-80.4871
U4	Union	35.1300	-80.3600

Table 2. Visible control of Italian ryegrass by diclofop, pinoxaden, and mesosulfuron. Experiment 1.

Bio-type	Herbicide	Regression equation	R^2	I_{50} [a]	SE
				g/ha	
L1	Diclofop	y = 65.7*exp (-exp (-(x-5521.5)/6017.7))	0.94	13,284	6.8
L1	Pinoxaden	y = 0.09+0.001x	0.83	> 2000 [b]	2.3
L1	Mesosulfuron	y = 83.1*exp (-exp (-(x-46.3)/35.6))	0.96	70.3	9.0
L2	Diclofop	y = 52.3*exp (-exp (-(x-14235.6)/11217.1))	0.99	>26,880	1.4
L2	Pinoxaden	y = −2.2+0.014x	0.91	> 2000	3.4
L2	Mesosulfuron	y = 96.4*exp (-exp (-(x-19.4)/21.5))	0.96	28.4	8.8
U1	Diclofop	y = 77.5 *exp (-exp (-(x- 1034.2)/1071.8))	0.95	1916	8.5
U1	Pinoxaden	y = 93.8 *exp (-exp (-(x- 107.9)/112.6))	0.97	160	7.8
U1	Mesosulfuron	y = 99.5*exp (-exp (-(x-1.7)/4.2))	0.99	< 3.75	1.8
U2	Diclofop	y = 7.1 + 0.002x	0.91	21,450	8.8
U2	Pinoxaden	y = 0.23+0.003x	0.90	> 2000	0.72
U2	Mesosulfuron	y = 82.9*exp (-exp (-(x-30.7)/55.1))	0.92	68.3	9.9
U3	Diclofop	y = 7.0 + 0.001x	0.81	> 26,880	6.0
U3	Pinoxaden	y = 0.92+0.006x	0.84	> 2000	1.9
U3	Mesosulfuron	y = 85.5*exp (-exp (-(x-14.3)/18.5))	0.93	26.0	9.8
U4	Diclofop	y = 82.4*exp (-exp (-(x-534.3)/1886.8))	0.93	1846	7.8
U4	Pinoxaden	y = 0.14+0.001x	0.74	> 2000	0.5
U4	Mesosulfuron	y = 95.0*exp (-exp (-(x-20.6)/40.0))	0.91	38.0	11.8
E	Diclofop	y = 98.2/(1+exp (-(x-180.3)/159.0))	0.98	< 210	2.7
E	Pinoxaden	y = 97.4*exp (-exp (-(x-27.1)/23.1))	0.99	37	2.9
E	Mesosulfuron	y = 99.9*exp (-exp (-(x-1.0)/3.4))	0.99	< 3.75	0.1

Table 2 continued

[a]I_{50} values are herbicide concentration that controlled the Italian ryegrass 50%. Manufacturers' suggested use rates are 840,15, and 62.5 g/ha for diclofop, mesosulfuron, and pinoxaden, respectively.

[b]Values cannot be estimated based on the dose range used.

Table 3. Shoot fresh weight reduction of Italian ryegrass by diclofop, pinoxaden, and mesosulfuron. Experiment 1.

Biotype	Herbicide	Regression equation	R^2	I_{50}[a]	SE
				g/ha	
L1	Diclofop	y = 71.9*exp (-exp (-(x-2188.9)/4508.4))	0.90	6742	9.0
L1	Pinoxaden	y =4.1+0.006x	0.62	> 2000[b]	3.3
L1	Mesosulfuron	y = 84.1*exp (-exp (-(x-33.7)/38.7))	0.93	59.0	10.5
L2	Diclofop	y = 59.1*exp (-exp (-(x-5091.0)/8818.1))	0.92	20,873	5.9
L2	Pinoxaden	y = 59.7/(1+exp (-(x-1119.8)/479.4))	0.93	1906	5.3
L2	Mesosulfuron	y = 94.2*exp (-exp (-(x-101.1)/26.9))	0.98	24.0	4.8
U1	Diclofop	y = 79.2*exp (-exp (-(x-418.9)/990.9))	0.97	1189	5.1
U1	Pinoxaden	y = 94.7/(1+exp (-(x-92.4)/59.3))	0.96	99	7.7
U1	Mesosulfuron	y = 98.8/(1+exp (-(x-1.1)/3.6))	0.98	<3.75	2.1
U2	Diclofop	y = 40.6+0.0003x	0.10	> 26,880	8.5
U2	Pinoxaden	y = 20.8/(1+exp (-(x-15.2)/0.8))	0.30	>2000	4.7
U2	Mesosulfuron	y = 58.6*exp (-exp (-(x-3.0)/9.9))	0.60	21.2	13.8
U3	Diclofop	$y = 52.8/(1+ (x/885.5)^{-0.9})$	0.96	21,095	3.7
U3	Pinoxaden	$y = 51.4/(1+ (x/571.2)^{-1.1})$	0.99	> 2000	1.8
U3	Mesosulfuron	y = 89.1*exp (-exp (-(x-7.0)/14.4))	0.95	15.0	7.5
U4	Diclofop	$y = 93.8/(1+ (x/255.3)^{-0.43})$	0.75	346	9.3
U4	Pinoxaden	y = 5.96+0.006x	0.34	> 2000	6.1
U4	Mesosulfuron	y = 95.1*exp (-exp (-(x-11.5)/35.9))	0.91	27.4	10.6
E	Diclofop	$y = 99.9/(1+ (x/73.2)^{-1.1})$	0.99	< 210	1.1
E	Pinoxaden	y = 99.5/(1+exp (-(x-6.9)/32.3))	0.99	< 15.6	1.6
E	Mesosulfuron	y = 99.8/(1+exp (-(x-0.8)/3.7))	0.99	<3.75	0.4

[a]I_{50} values are herbicide concentration reduced fresh weight of Italian ryegrass 50%. Manufacturers' suggested use rates are 840,15, and 62.5 g/ha for diclofop, mesosulfuron, and pinoxaden, respectively. [b]Values cannot be estimated based on the dose range used.

Table 4. Visible control of Italian ryegrass by diclofop, imazamox, mesosulfuron, pinoxaden, and pyroxsulam. Experiment 2.

Biotype	Herbicide	Regression equation	R^2	I_{50} [a]	SE	R/S [b]
				g/ha		
L1	Diclofop	y = 77.6*exp (-exp (-(x-21567.5)/20748.2))	0.96	38601.0	5.3	177
L1	Imazamox	y = 95.0*exp (-exp (-(x-51.5)/69.4))	0.98	82.0	6.0	7
L1	Mesosulfuron	y = 86.3*exp (-exp (-(x-15.7)/21.1))	0.93	28.5	11.1	22
L1	Pinoxaden	y = 83.9*exp (-exp (-(x-7.2)/9.5))	0.88	13.5	12.3	—
L1	Pyroxsulam	y = 93.0*exp (-exp (-(x-15.2)/35.0))	0.93	32.0	10.1	20
U3	Diclofop	y = 79.7*exp (-exp (-(x-8188.3)/14753.9))	0.93	19416.0	7.2	89
U3	Imazamox	y = 91.1*exp (-exp (-(x-128.9)/174.7))	0.98	218.0	6.0	18
U3	Mesosulfuron	y = 86.4*exp (-exp (-(x-40.3)/71.4))	0.89	83.2	12.0	64
U3	Pinoxaden	y = 93.3*exp (-exp (-(x-4.4)/7.4))	0.93	7.8	8.8	—
U3	Pyroxsulam	y = 79.4*exp (-exp (-(x-21.7)/21.1))	0.99	38.0	10.0	24
E	Diclofop	y = 94.4*exp (-exp (-(x-132.6)/197.8))	0.98	218.0	6.1	—
E	Imazamox	y = 99.1*exp (-exp (-(x-7.1)/13.0))	0.99	12.0	1.8	—
E	Mesosulfuron	y = 98.9*exp (-exp (-(x-0.64)/1.6))	0.99	1.3	1.8	—
E	Pinoxaden	y = 99.2*exp (-exp (-(x-1.2)/0.62))	0.99	< 3.1 [c]	2.0	—
E	Pyroxsulam	y = 98.5*exp (-exp (-(x-0.24)/3.42))	0.97	1.6	3.7	—

Table 4 continued

[a]I_{50} values are herbicide concentration that controlled the Italian ryegrass 50%. Manufacturers' suggested use rates are 840, 52, 15, 62.5, and 16 g/ha for diclofop, imazamox, mesosulfuron, pinoxaden, and pyroxsulam, respectively.

[b]R/S ratios were calculated based on I_{50} values of putative resistant biotypes L1 and U3 relative to susceptible biotype E.

[c]Values cannot be estimated based on the dose range used.

Table 5. Shoot fresh weight reduction of Italian ryegrass by diclofop, imazamox, mesosulfuron, pinoxaden, and pyroxsulam. Experiment 2.

Biotype	Herbicide	Regression equation	R^2	I_{50}[a]	SE	R/S[b]
				g/ha		
L1	Diclofop	y = 74.0*exp (-exp (-(x-21633.2)/32925.6))	0.96	42144.0	4.8	168
L1	Imazamox	y = 96.5*exp (-exp (-(x-61.4)/57.6))	0.98	86.0	6.0	5
L1	Mesosulfuron	y = 88.9*exp (-exp (-(x-11.8)/21.7))	0.93	23.7	10.4	12
L1	Pinoxaden	y = 97.2*exp (-exp (-(x-91.8)/405.4))	0.75	74.0	15.0	—
L1	Pyroxsulam	y = 91.1*exp (-exp (-(x-27.4)/54.2))	0.96	55.0	7.1	19
U3	Diclofop	y = 98.5*exp (-exp (-(x-25763.2)/32925.6))	0.92	38933.0	7.4	155
U3	Imazamox	y = 78.2*exp (-exp (-(x-128.4)/112.6))	0.94	219.0	9.8	12
U3	Mesosulfuron	y = 82.6*exp (-exp (-(x-59.6)/92.4))	0.93	123.3	9.9	65
U3	Pinoxaden	y = 95.2*exp (-exp (-(x-3.1)/24.9))	0.93	14.0	7.6	—
U3	Pyroxsulam	y = 75.0*exp (-exp (-(x-40.9)/50.2))	0.94	85.0	8.9	29
E	Diclofop	y = 88.0*exp (-exp (-(x-194.3)/100.0))	0.93	251.0	10.5	—
E	Imazamox	y = 98.9*exp (-exp (-(x-16.6)/6.2))	0.99	19.0	2.8	—
E	Mesosulfuron	y = 98.5*exp (-exp (-(x-1.5)/1.1))	0.99	1.9	2.8	—
E	Pinoxaden	y = 94.5 + 0.81 ln(x-1.96)	0.32	< 3.1[c]	3.6	—
E	Pyroxsulam	y = 97.9*exp (-exp (-(x-2.0)/2.3))	0.97	2.9	6.0	—

[a]I_{50} values are herbicide concentration that reduced fresh weight of Italian ryegrass 50%. Manufacturers' suggested use

Table 5 continued

rates are 840, 52, 15, 62.5, and 16 g/ha for diclofop, imazamox, mesosulfuron, pinoxaden, and pyroxsulam, respectively.

[b]R/S ratios were calculated based on I_{50} values of putative resistant biotypes L1 and U3 relative to susceptible biotype E.

[c]Values cannot be estimated based on the dose range used.

Table 6. Survival, visible control, and shoot fresh weight reduction of the E biotype and F2 generation of L1 and U3 biotypes of Italian ryegrass following mesosulfuron application. Experiment 3.[a]

	Survival			Visible control			Shoot fresh weight reduction		
	Mesosulfuron (g/ha)			Mesosulfuron (g/ha)			Mesosulfuron (g/ha)		
Biotype	15	60	240	15	60	240	15	60	240
					%				
E	0	0	0	100	100	100	100	100	100
L1	100	98	95	9	17	41	8	22	45
U3	98	98	88	0	2	25	1	6	29
LSD		8			11			12	

[a]The F2 generation of biotypes L1 and U3 was from parents surviving mesosulfuron applied

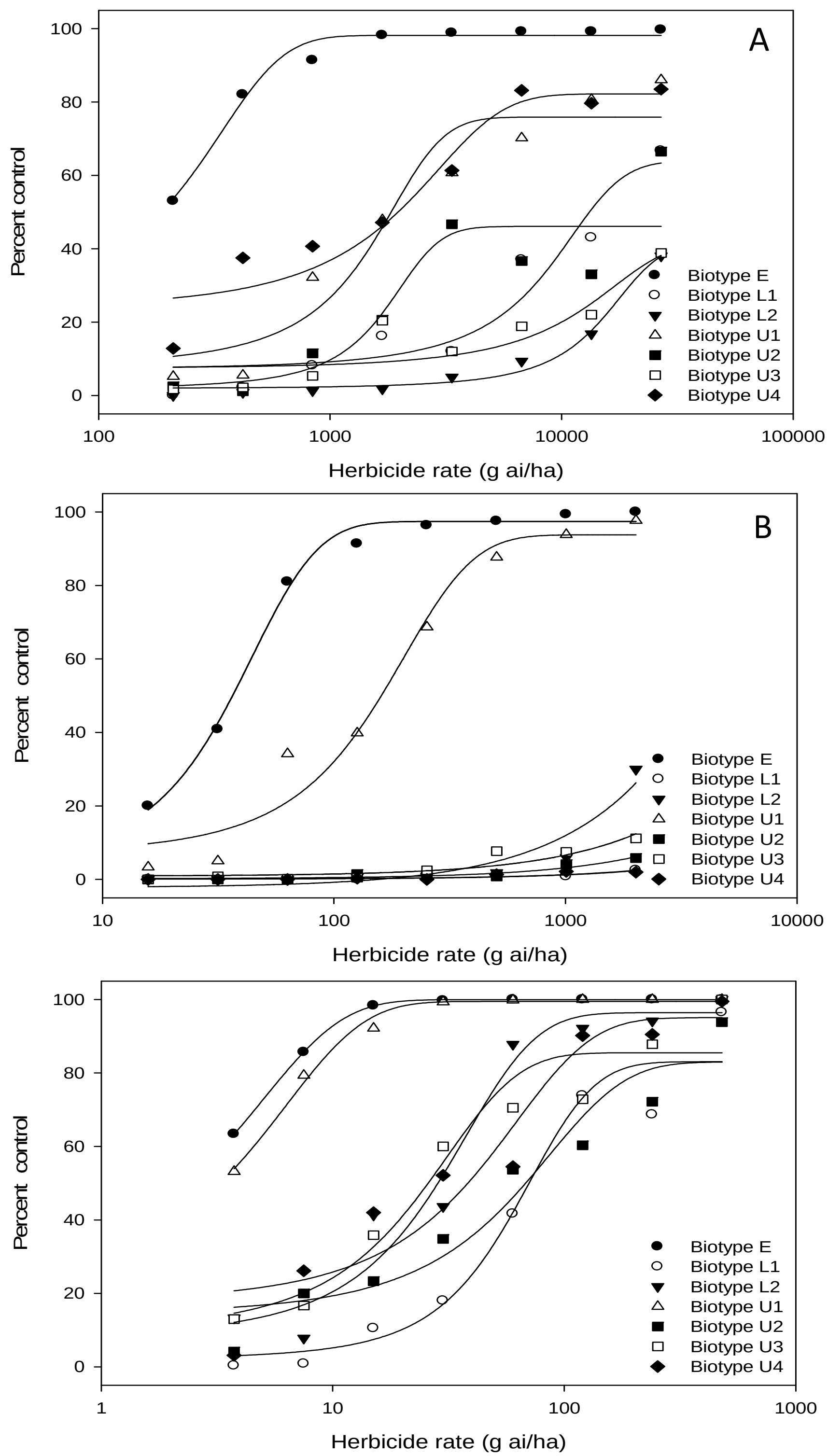

Figure 1. Visible control of seven Italian ryegrass biotypes by diclofop (A), pinoxaden (B), and mesosulfuron (C). Experiment 1.

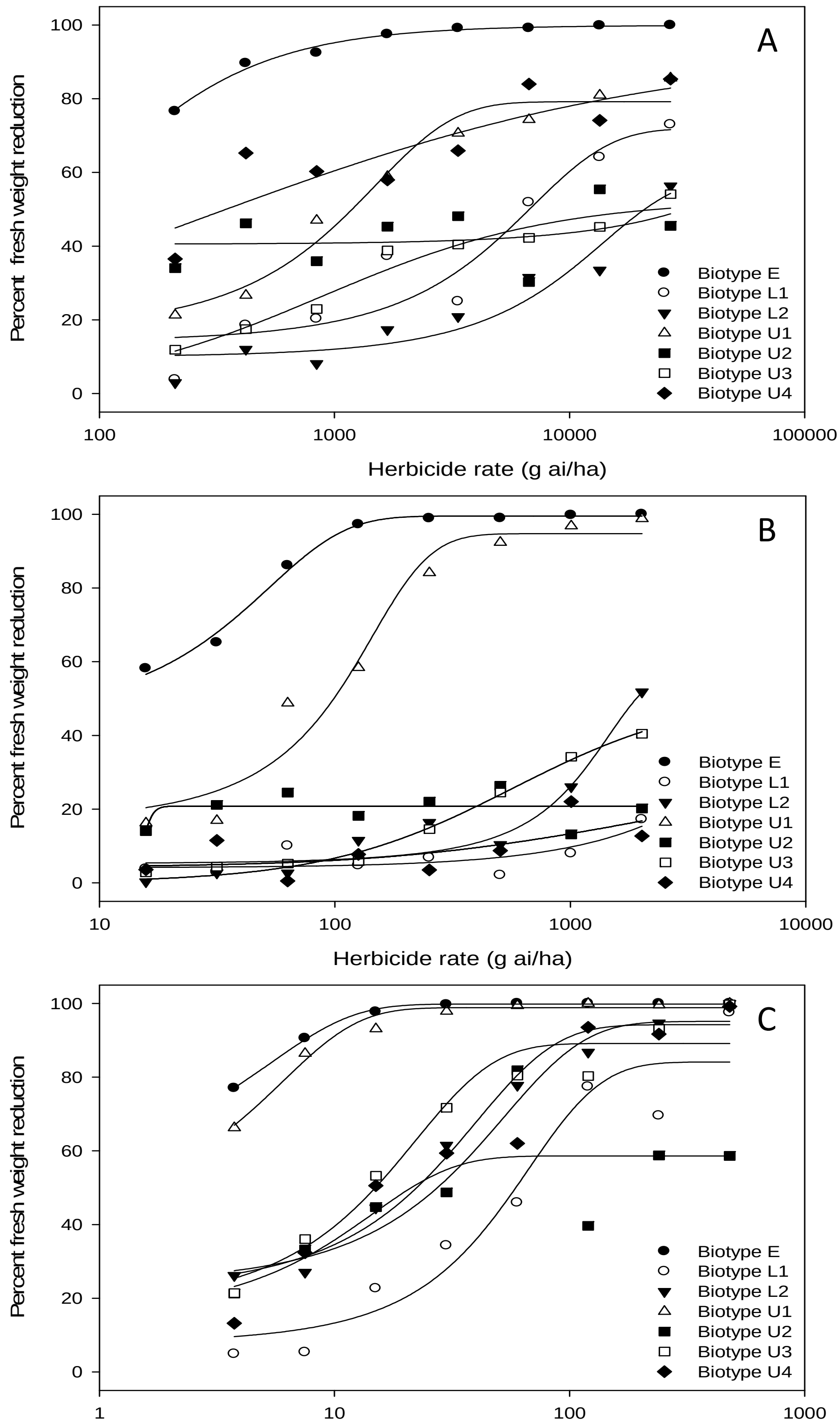

Figure 2. Shoot fresh weight reduction of seven Italian ryegrass biotypes by diclofop (A), pinoxaden (B), and mesosulfuron (C). Experiment 1.

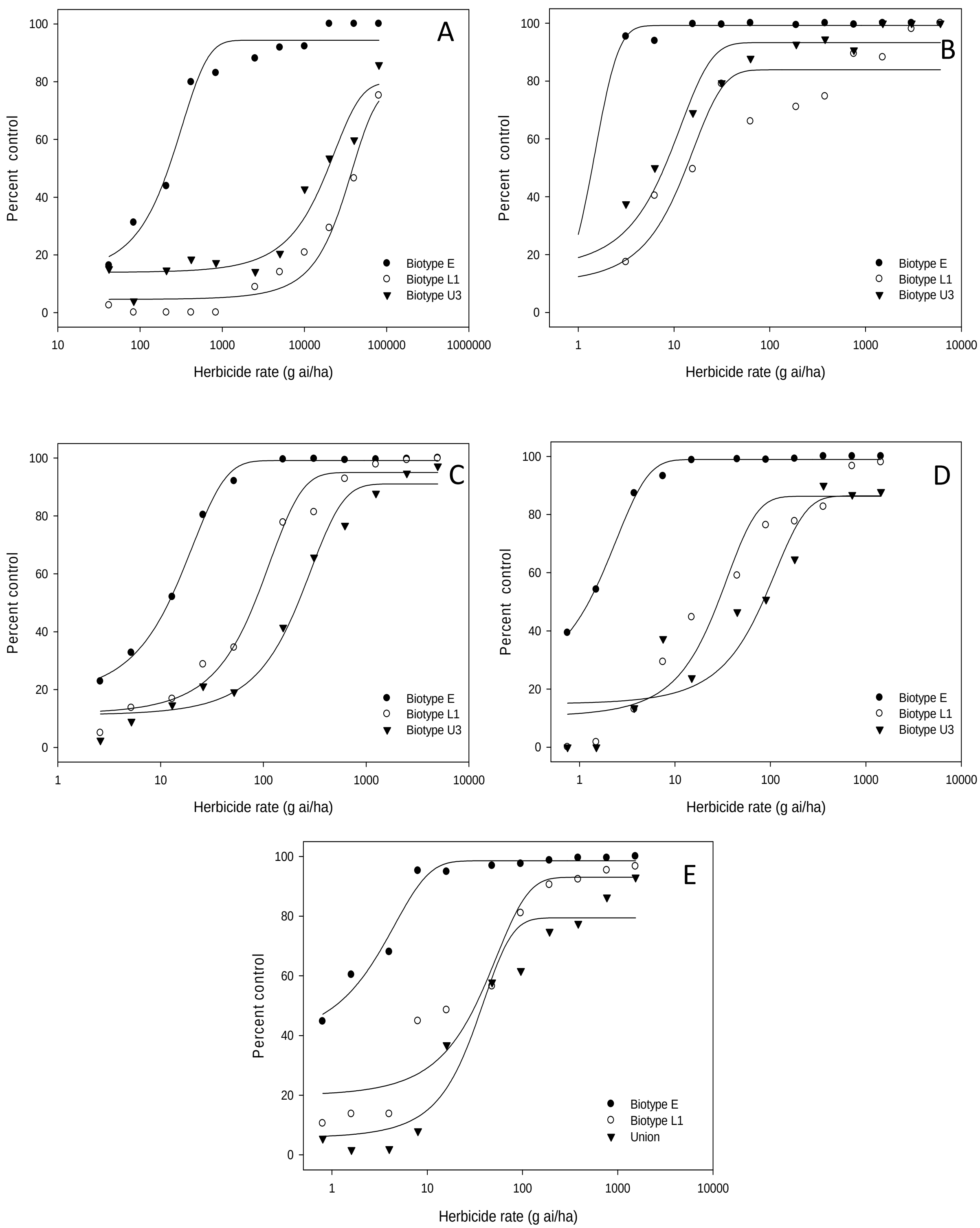

Figure 3. Visible control of Italian ryegrass by diclofop (A), pinoxaden (B), imazamox (C), mesosulfuron (D), and pyroxsulam (E). Experiment 2.

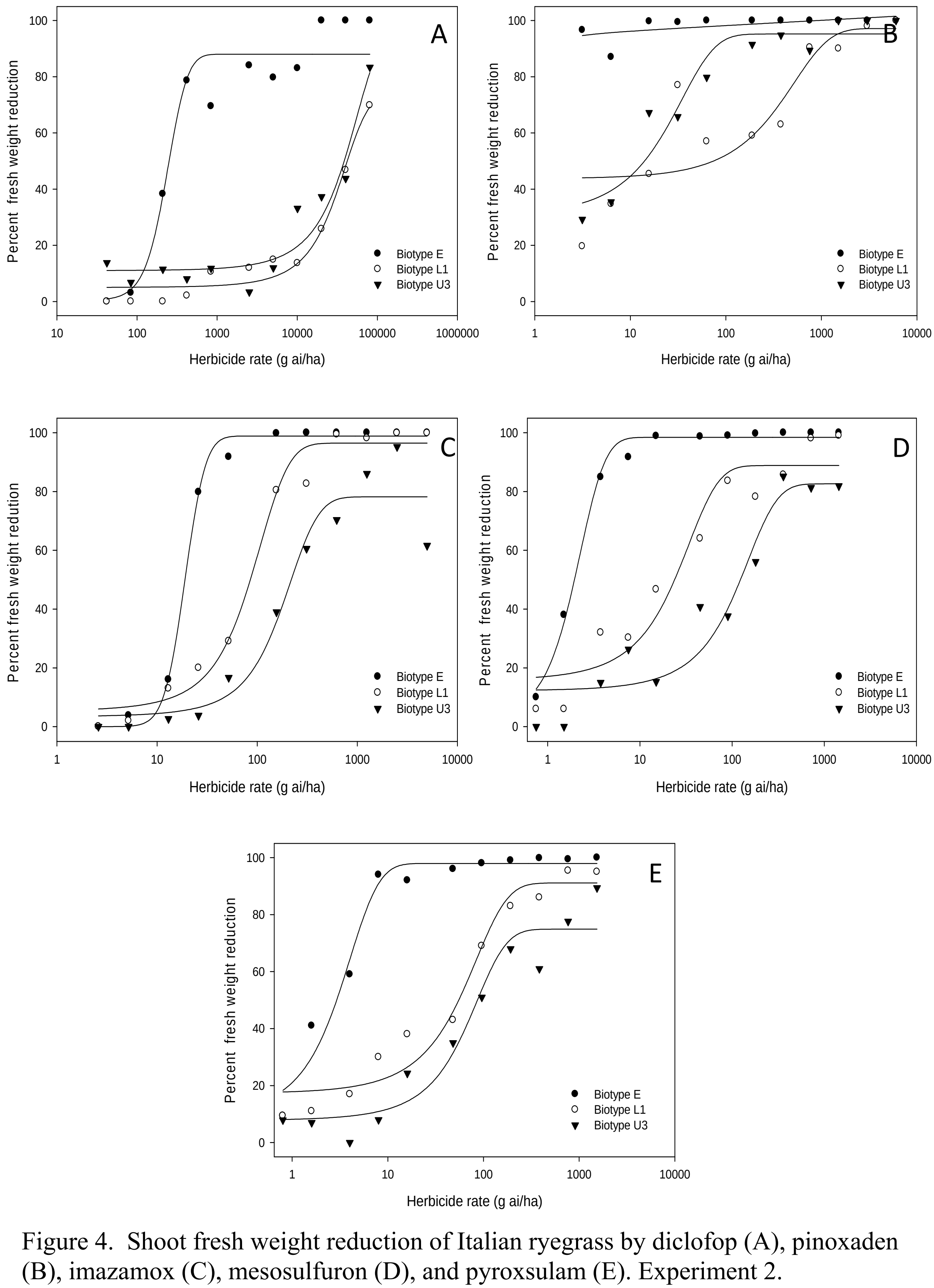

Figure 4. Shoot fresh weight reduction of Italian ryegrass by diclofop (A), pinoxaden (B), imazamox (C), mesosulfuron (D), and pyroxsulam (E). Experiment 2.

CHAPTER VII

Confirmation and Management of Common Ragweed Resistant to Diclosulam

Aman Chandi, David L. Jordan, Alan C. York, and Bridget R. Lassiter*

Abstract

Selection for biotypes of common ragweed expressing resistance to acetolactate synthase (ALS)-inhibiting herbicides has increased in North Carolina and surrounding states. Research was conducted in North Carolina to confirm common ragweed resistance to the ALS-inhibiting herbicide diclosulam in a field with suspected resistance and to compare herbicide programs in that field designed to control ALS-resistant common ragweed in corn, cotton, peanut, and soybean. In greenhouse experiments, I_{50} values following postemergence application of diclosulam for mortality of plants, visual estimates of percent control, and percent reduction in plant fresh weight were 557 to 653 fold higher for the suspected ALS-resistant biotype compared with a suspected ALS-susceptible biotype. Herbicides with modes of action different from ALS-inhibiting herbicides, including: atrazine, dicamba, and glyphosate in corn; fomesafen, glyphosate, MSMA, and prometryn in cotton; bentazon, flumioxazin, and lactofen in peanut; and flumioxazin, glyphosate, and lactofen in soybean

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controlled common ragweed more effectively in many instances than programs relying on cloransulam (soybean), diclosulam (peanut), thifensulfuron (corn), and trifloxysulfuron (cotton) that typically control common ragweed populations not resistant to ALS-inhibiting herbicides. Applying tank-mix or sequential applications of herbicides with different modes of action were effective in controlling ALS-resistant common ragweed in all crops.

Nomenclature: Atrazine; cloransulam; dicamba; diclosulam; flumioxazin; fomesafen; glyphosate; lactofen, nicosulfuron; prometryn, trifloxysulfuron, common ragweed, *Ambrosia artemisiifolia* L.; corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.; peanut, *Arachis hypogaea* L.; soybean, *Glycine max* (L.) Merr.

Key Words: herbicide resistance, weed management.

Introduction

Development of herbicide-resistant weed biotypes has increased considerably over the past decade in many regions of the United States (Heap 2010). Among all herbicide modes of action, the greatest number of species with resistant biotypes has been observed with ALS-inhibiting herbicides (Heap 2010). Herbicides that inhibit the ALS enzyme are used in most agronomic crops, including corn, cotton, peanut, and soybean, in the United States. Popularity and effectiveness of this mode of action in these crops have lead to tremendous selection pressure in many fields and the subsequent development of cross resistance (Tranel and Wright 2002).

Common ragweed is an important weed in agronomic crops in the southern region of the United States (Webster 2009). Use of the ALS-inhibiting herbicide diclosulam has been important in managing this weed and minimizing interference with peanut yield (Bailey et al. 1999; Everman et al. 2006; Price et al. 2002). However, in recent years, biotypes of common ragweed resistant to ALS-inhibiting herbicides have been reported in the United States (Heap 2010). Resistance to ALS-inhibiting herbicides, combined with documentation of common ragweed resistance to glyphosate (Heap 2010), has increased concern over ability of practitioners to effectively manage these and other weed species in southern row crops.

Rotating herbicides with different modes of action, applying herbicides with different modes of action sequentially or in mixtures, and implementing non-chemical control methods where feasible are important tactics in managing herbicide-resistant biotypes in corn (Gunsolus 2008), cotton (Burgos et al. 2006), peanut (Jordan et al. 2007), and soybean (Loux et al. 2010). Preventing resistance from evolving and detecting resistant populations as early as possible also are critical in weed management systems, especially for ALS-inhibiting herbicides that are prone to having resistant populations develop.

Defining herbicide programs that control susceptible and ALS-resistant common ragweed is important in developing a comprehensive weed management strategy in southern row crops. Therefore, research was conducted in North Carolina to confirm putative ALS resistance in a population of common ragweed and develop herbicide programs to control a mixture of ALS- resistant and -susceptible populations in corn, cotton, peanut, and soybean.

Materials and Methods

Confirmation of ALS resistance in common ragweed. During 2005, common ragweed plants escaping applications of diclosulam applied PRE at 26 g ai/ha followed by imazapic applied POST at 70 g ai/ha were observed in a series of weed management experiments in a research field at the Upper Coastal Plain Experiment Station located near Rocky Mount, NC (Lassiter et al. 2007). This field was used routinely in previous years by weed scientists to evaluate performance of a wide range of herbicides, including ALS-inhibiting herbicides. Soil was a Goldsboro loamy sand (fine-loamy, siliceous, thermic Aquic Paleudalts) with humic matter (Mehlich 1984) ranging from 1.6 to 2.1% and pH ranging from 5.8 to 6.2. In 2006, 2007, and 2008, experiments were conducted to determine distribution and frequency of common ragweed expressing possible resistance to ALS-inhibiting herbicides in this field. Common ragweed response to ALS-inhibiting herbicides was observed in tilled fallow areas in plots 2 m by 6 m in randomly selected sites in the field. New sites were selected each year. Total size of the field was 2.2 ha.

Treatments consisted of diclosulam[1] at 26 and 130 g/ha applied to common ragweed 15 to 20 cm in height. These rates of diclosulam represent the manufacturer's suggested use rate (Anonymous 2010) and five times the suggested use rate. A non-treated control was also included. Diclosulam plus nonionic surfactant[2] at 0.25% (v/v) was applied in 145 L/ha aqueous solution using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles.[3] Clethodim[4] at 140 g ai/ha plus crop oil concentrate[5] at 1.0% (v/v) was applied POST as needed, separate from diclosulam, to minimize interference of annual grasses with common ragweed.

The experimental design was a randomized complete block and treatments were replicated 12, 10, and 6 times in 2006, 2007, and 2008, respectively. The number of plants surviving herbicide application was recorded 4 weeks after diclosulam application and was subjected to analysis of variance using the GLM procedure of SAS.[6] Means were separated using Fisher's Protected LSD test at $p \leq 0.05$.

In 2007, seed was collected from common ragweed plants surviving diclosulam at 130 g/ha. Similarly, seed from a common ragweed biotype suspected to be ALS-susceptible was collected in Raleigh, NC in 2007. Plants for the suspected ALS-susceptible biotype were found in a non-agricultural area that most likely had not been exposed to ALS-inhibiting herbicides. Approximately 10 seeds for each biotype were planted in the greenhouse in pots (10 cm by 10 cm) containing a commercial potting mixture.[7] Plants were thinned to one plant per pot two weeks after seeding and fertilized using a water soluble nutrient solution.[8] When common ragweed had 4 to 6 leaves, diclosulam at 26, 104, 208, 416, 832, and 1664 g/ha was applied to the putative ALS-resistant biotype. Diclosulam at 0.4, 0.8, 1.6, 3.2, 6.4, and 26 g/ha was applied to the suspected ALS-susceptible plants. Nonionic surfactant[2] at 0.25% (v/v) was included with diclosulam.

The experimental design was a randomized complete block with treatments replicated 10 times, and the experiment was repeated. The number of surviving plants was recorded 3 weeks after application of diclosulam. Visual estimates of percent control were recorded at this time using a scale of 0 to 100% where 0 = no control and 100 = complete control. Foliar chlorosis, necrosis, and plant stunting were used when making the visual estimates. Plants were then severed at the soil surface and shoot fresh weight determined. Percent reduction in shoot fresh weight relative to the non-treated control was calculated. Data for plant mortality, visual estimates of common ragweed control, and percent reduction in common ragweed weight were subjected to analysis of variance using the GLM procedure of SAS[6]. Polynomial curve fitting was used to construct I_{50} values for suspected ALS-susceptible and ALS-resistant biotypes.

Management programs for ALS-resistant common ragweed. The experiment was conducted from 2007 through 2010 in the previously described field. Plot size was eight rows (2007 and 2010) or four rows (2008 and 2009) spaced 91 cm apart by 20 m (2007) or 8 m (2008, 2009, and 2010) in conventionally prepared raised seedbeds. In-row crop populations were 8, 12, 15, and 28 plants/m for corn, cotton, peanut, and soybean, respectively. The glyphosate-resistant corn hybrid was 31G71[9]. Cotton cultivar was DP 424

BGRR[10] in 2007 and 2008 and DP 0920 B2RF[11] in 2009 and 2010. The soybean cultivar was AG6301[12]. The peanut cultivar was VA 98R (Mozingo et al. 2000). Except for weed control, production practices for each crop were standard for the region.

Herbicide programs in each crop consisted of two "base" herbicides (an ALS-inhibiting herbicide that is normally effective on ALS-susceptible common ragweed and a non-ALS-inhibiting herbicide normally effective on common ragweed) and three "complement" herbicide options within each base herbicide program (Table 1). The ALS-inhibiting base herbicides included thifensulfuron[13] (4.5 g ai/ha) applied EPOST to corn, trifloxysulfuron[14] (5.0 g ai/ha) applied MPOST (mid POST) to cotton, diclosulam[1] (26 g/ha) applied PRE to peanut, and clorsansulam[15] (18 g ai/ha) applied EPOST to soybean. Non-ALS-inhibiting base herbicides included atrazine[16] (2240 g ai/ha) applied MPOST to corn, fomesafen[17] (280 g ai/ha) applied PRE to cotton, flumioxazin[18] (71 g ai/ha) applied PRE to peanut and soybean. Complement herbicide options in corn included no herbicide, dicamba[19] (280 g ai/ha) applied MPOST, and glyphosate[20] (840 g ae/ha) applied MPOST. In cotton, complement herbicide options were no herbicide, sequential applications of glyphosate[20] (840 g/ha) applied MPOST followed by the same rate LPOST (late POST), and prometryn[21] (1120 g ai/ha) plus MSMA[22] (2240 g ai/ha) applied POST-directed. Complement herbicide options for peanut included no herbicide, paraquat[23] (140 g ai/ha) plus bentazon[24] (280 g ai/ha) applied EPOST followed by bentazon[24] (1120 g/ha) applied LPOST, and imazapic[25] (70 g ai/ha) plus lactofen[26] (280 g ai/ha) applied MPOST. Complement herbicide options in soybean included no herbicide, lactofen[26] (280 g/ha) applied MPOST, and sequential applications of glyphosate (840 g/ha) applied MPOST followed by the same rate LPOST. *S*-metolachlor[27] at 1.7 kg ai/ha was applied PRE on the entire test area for cotton, peanut, and soybean, including the non-treated control. Nicosulfuron[28] was applied EPOST over the entire test area for corn. *S*-metolachlor and nicosulfuron were used to control annual grasses and small-seeded broadleaf weeds. Neither of these herbicides completely controls common ragweed (Mekki and Leroux 1994). Nicosulfuron was applied three days prior to application of thifensulfuron or atrazine. Clethodim (140 g/ha) was applied EPOST and/or LPOST to control annual grasses in cotton, peanut, and soybean except when glyphosate was applied as a complementary herbicide treatment. Clethodim was also applied to non-treated controls. Atrazine and thifensulfuron were applied MPOST when corn was 25 cm tall with three to four leaves. Dicamba and glyphosate were applied LPOST when corn was 70 cm tall. Trifloxysulfuron and glyphosate were applied when cotton had four to five leaves and was 12 to 20 cm tall. The second application of glyphosate occurred approximately 14 days later when cotton was 30 to 40 cm tall. Prometryn plus MSMA was applied when cotton was 50 to 60cm tall using a hooded sprayer. Paraquat and bentazon were applied EPOST when peanut canopy was 8 to 10 cm wide. Imazapic and lactofen or bentazon were applied MPOST when peanut canopy was 25 to 30 cm wide. Cloransulam-methyl was applied to soybean with two trifoliates and was 8 to 10 cm tall while lactofen and the first of two glyphosate applications occurred when soybean was 15 to 20 inches tall. The second application of glyphosate was made 14 to 21 days later.

Herbicides were applied in 145 L/ha aqueous solution using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles[3]. Nonionic surfactant[2] was included with lactofen (soybean), prometryn plus MSMA, thifensulfuron, and trifloxysulfuron at 0.25% (v/v) or 0.125% (v/v) with paraquat plus bentazon. Crop oil concentrate[5] at 1.0% (v/v) was included with atrazine, bentazon, nicosulfuron, and imazapic plus lactofen. No adjuvant was included with dicamba or glyphosate.

Visual estimates of percent common ragweed control were recorded 8 weeks after planting (WAP) in all experiments using a scale of 0 to 100%, where 0 = no control and 100 = complete control (Frans et al. 1986). The number of common ragweed plants in the center two rows (1.8 m width) of each plot was recorded 8 WAP and converted to the number of plants/m^2. Visual estimates of percent control of large crabgrass [*Digitaria sanguinalis* (L.) Scop.] in corn in all years and control of common lambsquarters (*Chenopodium album* L.) in cotton during 2008 and 2009. Pitted morningglory (*Ipomoea lacunosa* L.) during 2007 and 2009 and purple nutsedge (*Cyperus purpurea* L.) during 2008 and 2010 were recorded in peanut. Each crop was mechanically harvested to determine yield. Corn and soybean yields were adjusted to 15.5% and 13% moisture, respectively. Cotton yield is reported as seedcotton yield. Peanut pods were dug and vines inverted based on pod mesocarp color (Williams and Drexler 1981). Peanut was harvested 4 to 7 d after digging and vine inversion and final yield was adjusted to 8% moisture.

The experimental design for each crop was a randomized complete block with treatments replicated four times (2007) or five times (2008, 2009, and 2010). Data for visual estimates of percent control of common ragweed, common lambsquarters, large crabgrass, pitted morningglory, and purple nutsedge; common ragweed density, and crop yield were subjected to analysis of variance appropriate for the factorial arrangement of treatments for a two (base herbicides) by three (complement herbicides) treatment arrangement. The non-treated control was excluded from this analysis. Data for visual estimates of percent weed control were arcsine square root transformed before analysis. Means of significant main effects and interactions were separated using Fisher's Protected LSD test at $p \leq 0.05$.

Results and Discussion

Confirmation of ALS resistance in common ragweed. Common ragweed densities in the non-treated control varied by year, ranging from 104 to 429 plants/m^2 (Table 1). Some common ragweed each year survived diclosulam applied at one or five times the manufacturer's suggested use rate. There was no difference in the number of surviving plants when comparing the two diclosulam rates (Table 1). Averaged over diclosulam rates, the percentage of the population surviving the herbicide was 5.5, 1.4, and 18.8% in 2006, 2007, and 2008, respectively, or almost 9% when averaged over years. Variation in response across years most likely reflected differences in populations in the field, random selection of sites each year, and rainfall patterns that may have influenced common ragweed emergence prior to application of diclosulam. Although common ragweed was present across the entire field, the population density varied and it is suspected that selection pressure for ALS-resistant biotypes most likely varied across the field based on previous cropping systems and/or herbicide program. Previous research (Everman et al. 2006; Lancaster et al. 2007) reported that diclosulam applied POST at the manufacturer's suggested use rate of 26 g/ha controls ALS-susceptible common ragweed completely.

In the greenhouse, the I_{50} value for surviving plants of the putatively resistant biotype grown from seed collected from plants surviving diclosulam at 130 g/ha in the field was 613 g/ha (Figure 1). In contrast, the I_{50} value for the suspected ALS-susceptible biotype was 1.1 g/ha (Figure 1). The I_{50} value for the visual estimates of control were 1.1 g/ha for ALS-susceptible and 713 g/ha for ALS-resistant (Figure 1). Fresh weight I_{50} values for ALS-resistant and ALS-susceptible biotypes were 784 g/ha and 1.2 g/ha, respectively (Figure 1). These data reveal a 557 to 653 fold higher difference in susceptibility of the two biotypes to diclosulam as measured by plant mortality, visual estimates of percent control, and percent reduction in fresh weight. These data confirm common ragweed resistance to diclosulam in this particular field. Other research (Patzoldt et al. 2001) has confirmed ALS-resistance in common ragweed populations at a resistant/susceptible ratio of 5,000 for cloransulam-methyl.

Management programs for ALS resistant common ragweed. The interaction of year by base herbicide by complement herbicide was significant for common ragweed control in all crops (Table 3). When common ragweed control was analyzed for each year, the interaction of base herbicide by complement herbicide was significant for control in corn during all years and for control in cotton during 2007, 2008, and 2009 (Table 4). The main effect of complement herbicide was significant for common ragweed control in 2010 for cotton (Table 4). The interaction of base herbicide by complement herbicide was significant for common ragweed control in peanut in 2008 and 2010; the main effect of complement herbicide was significant in all years. The interaction of base herbicide by complement herbicide was significant for control in soybean in 2008. Although main effects and interactions for these treatment factors were not significant in 2009 in soybean, the main effect of complement herbicide was significant in 2010 (Table 4).

The interaction of year by base herbicide by complement herbicide was significant for common ragweed density in corn and soybean Table 4). Common ragweed population was affected by the interaction of base herbicide by complement herbicide in corn in 2007 and 2008 but was not affected by either treatment factor or their interaction in 2010 (Table 5). The interaction of base herbicide by complement herbicide as well as year by base herbicide was significant for common ragweed population in cotton (Table 3). In peanut, the main effect of complement herbicide was also significant (Table 3). With soybean, the interaction of base herbicide by complement herbicide was significant for common ragweed population in 2008; population was not affected by these treatment factors in 2009 or 2010 (Table 5).

Common ragweed control was higher in corn when thifensulfuron was followed by either dicamba or glyphosate or when atrazine was applied alone or followed by complementary herbicides in all years (Table 6). Likewise, when complement herbicides were applied following trifloxysulfuron or when fomesafen was applied alone or with the complementary herbicides prometryn plus MSMA or glyphosate, common ragweed control in cotton exceeded that of trifloxysulfuron alone in 2007, 2008, and 2009 (Table 4). Common ragweed control in 2010 was higher when glyphosate was applied compared with no complement herbicide or prometryn plus MSMA regardless of base herbicide. Control by prometryn plus MSMA exceeded control when complement herbicides were not applied (Tables 6 and 7). Even though ALS-resistant common ragweed is present in this field, diclosulam was more effective than the alternative base herbicide flumioxazin for peanut in 2008 and 2010 (Table 6). While flumioxazin controls common ragweed, the rate required to avoid significant peanut injury is considered low for many weed species (Anonymous 2010b). Additionally, common ragweed that is not resistant to ALS-inhibiting herbicides is considered very susceptible to diclosulam. In experiments evaluating reduced herbicide programs in peanut with rates at or below the manufacturer's suggested use rate, diclosulam either PRE or POST consistently controlled common ragweed when applied at rates lower than 26 g/ha. The frequency of ALS-resistant common ragweed was relatively low in this field (9% on average, Table 1), and therefore the more effective control of ALS-susceptible common ragweed by diclosulam explains the difference between the two base herbicides. In contrast to results with peanut, flumioxazin alone was more effective than cloransulam-methyl in 2008 (Table 6). In 2009, common ragweed control exceeded 95% regardless of herbicide program (data nor shown). In 2010, common ragweed control lactofen or glyphosate controlled common ragweed the same and more effectively than not applying a complement herbicide (Table 7).

Common ragweed density differed in 1 of 3 years in corn when comparing individual herbicide programs with non-treated corn (Table 6). In 2008, common ragweed density following thifensulfuron alone was similar to the non-treated control while thifensulfuron followed by the dicamba or glyphosate or atrazine alone or followed complement herbicides had no surviving common ragweed plants. In 2009, all herbicide programs were effective in eliminating common ragweed compared with non-treated corn. Common ragweed density was similar regardless of herbicide program in 2010 and did not differ from non-treated corn.

Common ragweed density in cotton decreased when herbicides were applied when pooled over years (Table 6). Applying fomesafen alone or followed by complement herbicides as well as trifloxysulfuron followed by two applications of glyphosate was more effective in reducing common ragweed populations than trifloxysulfuron alone or prometryn plus MSMA.

Common ragweed population was lower when complement herbicides were applied regardless of base herbicide (Table 7). In soybean, common ragweed density was similar among all herbicide programs in 2009 and 2010, and only in 2009 did density in the non-treated control exceed that of soybean treated with herbicide (Table 4). In 2008, common ragweed density following cloransulam-methyl alone was similar to the non-treated control. Applying complement herbicides following cloransulam-methyl or flumioxazin alone or followed by lactofen or glyphosate decreased population of common ragweed compared with cloransulam-methyl alone or non-treated soybean.

Several other weeds were present in uniform and high enough populations to evaluate. Large crabgrass control in corn and purple nutsedge control in peanut were affected by the interaction of year and complement herbicide and the main affect of base herbicide (Table 8). Common lambsquarters control in cotton was affected by the interaction of year by base herbicide by complement herbicide while pitted morningglory control in peanut was affected by interactions of year by base herbicide and year by complement herbicide (Table 8). Purple nutsedge control was affected by the main effect of base herbicide the interaction of year by complement herbicide (Table 8).

When pooled over years and complement herbicides, large crabgrass control in corn was higher when atrazine was applied compared with thifensulfuron (Table 9). Large crabgrass control in 2008 and 2010 was higher when glyphosate was applied compared with no complement herbicide or dicamba. In 2009, control was nearly complete regardless of complement herbicide.

Pitted morningglory control in peanut was similar in 2007 regardless of base or complement herbicides (Table 9). However, in 2009 flumioxazin was less effective than diclosulam regardless of complement herbicide. Paraquat plus bentazon and imazapic plus lactofen were equally effective in controlling pitted morningglory regardless of base herbicide program (Table 9). As expected, purple nutsedge control by diclosulam exceeded that by flumioxazin and neither of these herbicides controlled this weed completely (Table 9). Although no differences in control were noted in 2007 when comparing complement herbicides, paraquat plus bentazon and imazapic plus lactofen controlled purple nutsedge similarly in 2009.

Common lambsquarters control in cotton varied slightly when comparing years (Table 10). In 2008, control by trifloxysulfuron followed by complement herbicides or fomesafen alone or followed by complement herbicides exceeded that of trifloxysulfuron alone. Trifloxysulfuron alone or followed by prometryn plus MSMA was similar while control by trifloxysulfuron followed by glyphosate exceeded that of trifloxysulfuron alone. No differences in common lambsquarters control were noted when comparing among complement herbicides when fomesafen was the base herbicide.

The interaction of year, base herbicide, and complement herbicide was not significant for yield of any crop (Table 3). The interaction of base herbicide and complement herbicide was significant for corn yield. The interaction of year by complement herbicide was significant for cotton, peanut, and soybean yield.

When pooled over years, corn yield ranged from 4410 kg/ha to 6590 kg/ha when herbicides were applied compared with 3820 kg/ha for non-treated corn (Table 11). Yield was highest when thifensulfuron was followed by glyphosate. Yield following thifensulfuron alone or followed by dicamba was similar as was yield when atrazine was applied alone or followed by atrazine or glyphosate. Applying atrazine and glyphosate increased yield over thifensulfuron alone.

In absence of herbicides, cotton yield was 590 kg/ha or less when comparing across years (Table 12). In cotton, yield following fomesafen was higher than when trifloxysulfuron was applied in 2 of 4 years. Yield was also higher in 2 of 4 years when complement herbicides were applied, and in two years applying glyphosate increased yield over prometryn plus MSMA or no complement herbicide irrespective of base herbicide.

Peanut and soybean yields were not affected by the main effect of base herbicides (Table 3). Applying imazapic plus lactofen increased yield in 2 of 4 years when compared to not applying complement herbicides (Table 12). Yield following paraquat plus bentazon was higher in only one of 4 years compared with not including a complement herbicide. In soybean, no difference in yield among complement herbicides was noted in 2009. However, yield in 2008 applying glyphosate increased yield over the no-complement treatment. However, in 2010 applying glyphosate increased yield over the other complement herbicide treatments.

Collectively, results from these experiments confirm resistance of common ragweed to the ALS-inhibiting herbicide diclosulam. Results from field trials demonstrate the value of using herbicides that control common ragweed with a mode of action different from ALS inhibition in minimizing interference of common ragweed with corn, cotton, peanut, and soybean. However, these data also indicate that herbicide alternatives to ALS-inhibiting herbicides may be less effective in controlling the entire population of resistant and susceptible common ragweed in a particular field. For example, while diclosulam did not control ALS-resistant common ragweed, control of the overall common ragweed population was greater than the non-ALS-inhibiting alternative flumioxazin. However, extended selection pressure with diclosulam only most likely would render diclosulam less effective than flumioxazin in controlling common ragweed.

Acknowledgments

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Sources of Materials

[1]Strongarm herbicide®. Dow AgroSciences, Indianapolis, IN 46268.

[2]Induce® nonionic surfactant. Proprietary blend of alkyl aryl polyoxyalkane ethers, free fatty acids, and dimethyl polysiloxane, 90%. Helena Chemical Company, Collierville, TN 38107.

[3]8002 Spray nozzles. Spraying Systems Company, Wheaton, IL 60189-7900.

[4]Select 2EC herbicide®. Valent USA Corporation, Walnut Creek, CA 94596.

[5]Agri-Dex® spray adjuvant. Proprietary blend of alkyl aryl polyoxyalkane ethers, free fatty acids, and dimethyl polysiloxane, 90%. Helena Chemical Company, Collierville, TN 38107.

[6]SAS Institute. 2006. GLM Procedure. 100 SAS Campus Drive, Cary, NC 27513-2414.

[7]Metro Mix 200®, Scotts-Sierra Horticultural Products Company, Marysville, OH 43044.

[8]Peters Professional® Water Soluble 20-20-20 Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH 43044.

[9]Corn hybrid 31G71. Monsanto Company, St. Louis, MO 63167.

[10]Cotton cultivar DP 424 BGRR. Monsanto Company, St. Louis, MO 63167.

[11]Cotton cultivar DP 0920 B2RF. Monsanto Company, St. Louis, MO 63167.

[12]Soybean cultivar AG6301. Monsanto Company, St. Louis, MO 63167.

[13]Harmony G herbicide®. E. I. du Pont de Nemours and Co., Wilmington, DE 19898.

[14]Envoke herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[15]First Rate herbicide®. Dow AgroSciences, Indianapolis, IN 46268.

[16]Attrex herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[17]Reflex herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[18]Valor SX herbicide®. Valent USA Corporation, Walnut Creek, CA 94596.

[19]Clarity herbicide®. BASF Corp., Research Triangle Park, NC 27709.

[20]Roundup Weather Max®. Monsanto Co., St. Louis, MO 63198.

[21]Caparol herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[22]MSMA Plus herbicide®. Drexel Chemical Co., Memphis, TN 38113.

[23]Gramoxone Inteon herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[24]Basagran herbicide®. BASF Corporation, Research Triangle Park, NC 27709.

[25]Cadre herbicide®. BASF Corporation, Research Triangle Park, NC 27709.

[26]Cobra 2EC herbicide®. Valent USA Corp., Valent USA Corporation, Walnut Creek, CA 94596.

[27]Dual Magnum herbicide®. Syngenta Crop Protection, Greensboro, NC 27419.

[28]Accent herbicide®. E. I. du Pont de Nemours and Co., Wilmington, DE 19898.

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Anonymous. 2010b. Valor SX herbicide label. Valent USA Corp., Walnut Creek, CA.

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Table 1. Herbicide programs evaluated in corn, cotton, soybean, and peanut.

	Base herbicides[a]		Complementary herbicides
Crop	PRE	EPOST[b]	applied MPOST or LPOST[c]
Corn	—	Thifensufuron	—
	—	Thifensufuron	Dicamba
	—	Thifensufuron	Glyphosate
	—	Atrazine	—
	—	Atrazine	Dicamba
	—	Atrazine	Glyphosate
Cotton	—	Trifloxysulfuron	—
	—	Trifloxysulfuron	Prometryn plus MSMA
	—	Trifloxysulfuron	Glyphosate then glyphosate
	Fomesafen	—	—
	Fomesafen	—	Prometryn plus MSMA
	Fomesafen	—	Glyphosate then glyphosate
Peanut	Diclosulam	—	—
	Diclosulam	Paraquat plus bentazon	Bentazon
	Diclosulam	—	Imazapic plus lactofen

Table 1 continued

	Flumioxazin	—	—
	Flumioxazin	Paraquat plus bentazon	Bentazon
	Flumioxazin	—	Imazapic plus lactofen
Soybean	—	Cloransulam-methyl	—
	—	Cloransulam-methyl	Lactofen
	—	Cloransulam-methyl	Glyphosate then glyphosate
	Flumioxazin	—	—
	Flumioxazin	—	Lactofen
	Flumioxazin	—	Glyphosate then glyphosate

[a]Rates of PRE and EPOST herbicides included the following: atrazine (2240 g/ha), cloransulam (18 g/ha), diclosulam (26 g/ha), flumioxazin (71 g/ha), fomesafen (280 g/ha), glyphosate (840 g/ha), paraquat plus bentazon (140 + 280 g/ha), thifensulfuron (4.5 g/ha), and trifloxysulfuron (5 g/ha).

[b]Abbreviation: EPOST, early postemergence.

[c]Rates of herbicides applied MPOST or LPOST included the following: bentazon (1220 g/ha), dicamba (280 g/ha), glyphosate (840 g/ha), imazapic plus lactofen (70 + 280 g/ha), lactofen (280 g/ha), and prometryn plus MSMA (1120 + 2240 g/ha).

Table 2. Number of common ragweed plants surviving diclosulam applied POST.[a]

Herbicide	Rate	2006	2007	2008
	g/ha	—— No./m^2. ——		
Non-treated	-	429 a	104 a	165 a
Diclosulam	26	27 b	2 b	46 b
Diclosulam	130	21 b	1 b	16 b

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

Table. 3. P > F for common ragweed control, common ragweed density, and crop yield as influenced by year, base herbicides, and complement herbicides.

Treatment factor	Corn	Cotton	Peanut	Soybean
Common ragweed control				
Year	<0.0001	<0.0001	<0.0001	<0.0001
Base herbicide (Base)	<0.0001	<0.0001	<0.0001	0.0004
Complement (Comp)	<0.0001	<0.0001	<0.0001	<0.0001
Base*Comp	<0.0001	<0.0001	<0.0001	0.0002
Year*Base	<0.0001	<0.0001	<0.0001	0.0176
Year*Comp	<0.0001	<0.0001	<0.0001	<0.0001
Year*Base*Comp	<0.0001	0.0004	0.0497	0.0375
Coefficient of variation (%)	8.2	12.8	14.5	12.6
Common ragweed density				
Year	0.0003	<0.0001	0.0405	0.0047
Base	0.0006	0.0002	0.8671	0.0259
Comp	0.0191	0.0028	0.0016	0.0003
Base*Comp	0.0060	0.0056	0.9640	0.3138
Year*Base	0.2304	0.0012	0.8507	0.1218
Year*Comp	0.0060	0.0998	0.1864	0.0236
Year*Base*Comp	0.0467	0.1073	0.9952	0.0107

Table 3 continued

Coefficient of variation (%)	175.5	273.9	243.7	185.4
Crop yield				
Year	<0.0001	<0.0001	0.0072	<0.0001
Base	0.7988	0.0014	0.4823	0.2417
Comp	0.0002	<0.0001	0.0001	<0.0001
Base*Comp	0.0123	0.3021	0.1427	0.1590
Year*Base	0.1438	0.0552	0.9742	0.0984
Year*Comp	0.3869	<0.0001	0.0179	0.0126
Year*Base*Comp	0.4822	0.1955	0.2335	0.5182
Coefficient of variation (%)	23.5	27.1	23.7	20.6

Table 4. P > F for common ragweed control in corn, cotton, peanut, and soybean as influenced by base herbicide and complement herbicide for each year.

Source	2007	2008	2009	2010
Corn				
Base herbicide (Base)	-	<0.0001	0.0179	0.0122
Complement herbicide (Comp)	-	<0.0001	0.0061	0.0007
Base*Comp	-	<0.0001	0.0061	0.0312
Coefficient of variation (%)	-	7.2	5.8	10.8
Cotton				
Base	<0.0001	<0.0001	0.0375	0.5094
Comp	0.0011	<0.0001	0.0177	<0.0001
Base*Comp	0.0023	<0.0001	0.0177	0.1021
Coefficient of variation (%)	14.3	15.0	2.0	18.5
Peanut				
Base	0.0731	0.0225	0.5690	<0.0001
Comp	<0.0001	<0.0001	0.0011	<0.0001
Base*Comp	0.2116	0.0162	0.3710	<0.0001
Coefficient of variation (%)	9.5	6.8	23.2	11.3

Table 4 continued

Soybean				
Base	-	<0.0001	0.7854	0.1433
Comp	-	<0.0001	0.0886	<0.0001
Base*Comp	-	<0.0001	0.9269	0.1192
Coefficient of variation (%)		7.2	1.3	23.3

Table 5. P > F for common ragweed population in corn and soybean as influenced by base herbicide and complement herbicide for each year.

Source	2007	2008	2009
Corn			
Base herbicide (Base)	0.0001	0.0177	0.1872
Complement herbicide (Comp)	<0.0001	0.0060	0.6227
Base*Comp	<0.0001	0.0060	0.9920
Coefficient of variation (%)	111.5	211.9	165.8
Soybean			
Base	0.0007	0.5497	0.4973
Comp	<0.0001	0.0563	0.2616
Base*Comp	0.0002	0.6951	0.5425
Coefficient of variation (%)	106.4	300.0	170.1

Table 6. Influence of base herbicides and complement herbicides on visual estimates of common ragweed control and density.[a]

		Common ragweed response								
		Visual control				Plant density				
Base herbicide	Complement herbicide	2007	2008	2009	2010	2007	2008	2009	2010	Pooled
		%				No./m^2				
Corn										
Thifensulfuron	None	-	24 b	84 b	68 b	-	3 a	0 b	1 a	-
Thifensulfuron	Dicamba	-	100 a	100 a	99 a	-	0 b	0 b	2 a	-
Thifensulfuron	Glyphosate	-	100 a	100 a	99 a	-	0 b	0 b	1 a	-
Atrazine	None	-	100 a	100 a	93 a	-	0 b	0 b	0 a	-
Atrazine	Dicamba	-	100 a	100 a	94 a	-	0 b	0 b	1 a	-
Atrazine	Glyphosate	-	100 a	100 a	100 a	-	0 b	0 b	0 a	-
Non-treated control		-	-	-	-	-	4 a	5 a	1 a	-
Cotton										
Trifloxysulfuron	None	40 c	32 c	95 b	-	-	-	-	-	5 b
Trifloxysulfuron	Prometryn plus	66 b	78 b	100 a	-	-	-	-	-	2 c

Table 6 continued

	MSMA									
Trifloxysulfuron	Glyphosate[b]	100 a	100 a	100 a	-	-	-	-	-	0 d
Fomesafen	None	99 a	93 ab	100 a	-	-	-	-	-	0 d
Fomesafen	Prometryn plus MSMA	100 a	97 ab	100 a	-	-	-	-	-	0 d
Fomesafen	Glyphosate[b]	100 a	100 a	100 a	-	-	-	-	-	0 d
Non-treated control		-	-	-	-	-	-	-	-	13 a
Peanut										
Diclosulam	None	-	82 b	-	83 abc	2 b	0 b	0 b	3 ab	-
Diclosulam	Paraquat plus bentazon	-	100 a	-	95 a	0 b	0 b	0 b	0 b	-
Diclosulam	Imazapic plus lactofen	-	100 a	-	93 ab	0 b	0 b	0 b	0 b	-
Flumioxazin	None	-	66 c	-	15 d	2 b	0 b	0 b	2 ab	-
Flumioxazin	Paraquat plus bentazon	-	99 a	-	82 bc	0 b	0 b	0 b	0 b	-

Table 6 continued

Flumioxazin	Imazapic plus lactofen	-	100 a	-	79 c	1 b	0 b	0 b	0 b	-
Non-treated control		-	-	-	-	45 a	3 a	3 a	4 a	-
Soybean										
Cloransulam-methyl	None	-	40 d	-	-	-	2 a	0 b	1 a	-
Cloransulam-methyl	Lactofen	-	91 b	-	-	-	0 b	0 b	1 a	-
Cloransulam-methyl	Glyphosate[b]	-	100 a	-	-	-	0 b	0 b	0 a	-
Flumioxazin	None	-	80 c	-	-	-	0 b	0 b	1 a	-
Flumioxazin	Lactofen	-	100 a	-	-	-	0 b	0 b	0 a	-
Flumioxazin	Glyphosate[b]	-	100 a	-	-	-	0 b	0 b	0 a	-
Non-treated control		-	-	-	-	-	3 a	2 a	1 a	-

[a]Means within a year and crop followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$.

[b]Sequential applications 14 to 21 days apart.

Table 7. Influence of complement herbicides on visual estimates of common ragweed control in cotton and peanut and common ragweed density in peanut.[a]

Complement herbicides	Common ragweed control 2007	2008	2009	2010	Common ragweed density Pooled
	%				No./m^2
Cotton					
None	-	-	-	27 c	-
Prometryn plus MSMA	-	-	-	81 b	-
Glyphosate then glyphosate	-	-	-	100 a	-
Peanut					
None	67 c	-	63 b	-	2 a
Paraquat plus bentazon	96 a	-	94 a	-	0 b
Imazapic plus lactofen	88 b	-	100 a	-	0 b
Non-treated control	-	-	-	-	14
Soybean					
None	-	-	-	38 b	-

Table 7 continued

Lactofen	-	-	-	92 a	-
Glyphosate then glyphosate	-	-	-	99 a	-

[a]Means within a year or when pooled over years followed by the same letter are not significantly different according to Fisher's Protected LSD test at $p < 0.05$. Data are pooled over levels of base herbicide for common ragweed control in cotton and peanut. Data for common ragweed density are pooled over years and base herbicides.

Table. 8. P > F for large crabgrass control in corn, common lambsquarters control in cotton, and purple nutsedge and pitted morningglory control in peanut as influenced by year, base herbicide, and complement herbicide.[a]

	Control			
	Corn	Cotton	Peanut	
Treatment factor	Large crabgrass	Common lambsquarters	Purple nutsedge	Pitted morningglory
Year	<0.0001	0.6143	0.0037	<0.0001
Base herbicide (Base)	0.0086	0.0023	0.0059	0.0011
Complement (Comp)	<0.0001	<0.0001	0.0002	0.0002
Base*Comp	0.0909	0.0007	0.5015	0.5146
Year*Base	0.0947	0.1605	0.1615	0.0061
Year*Comp	0.0005	0.0395	0.0280	0.0004
Year*Base*Comp	0.3361	0.0408	0.5443	0.6090
Coefficient of variation (%)	32.9	18.4	36.5	26.4

[a]Data for large crabgrass control in corn are from 2008, 2009, and 2010. Data for common lambsquarters control in cotton are from 2008 and 2009. Data for pitted morningglory control in peanut are from 2007 and 2008. Data for purple nutsedge control in peanut are from 2008 and 2010.

Table 9. Influence of base herbicide and complement herbicides on corn, cotton, peanut, and soybean yield.[a]

Treatment factor[b]	2007	2008	2009	2010	Pooled
	%				
Large crabgrass control in corn					
Base herbicides					
Thifensulfuron	-	-	-	-	58 b
Atrazine	-	-	-	-	70 a
Complement herbicides					
None	-	21 b	91 a	53 b	-
Dicamba	-	32 b	85 a	38 b	-
Glyphosate	-	87 a	98 a	75 a	-
Pitted morningglory control in peanut					
Base herbicides					
Diclosulam	100 a	-	82 a	-	-
Flumioxazin	96 a	-	45 b	-	-

Table 9 continued

Complement herbicides					
None	97 a	-	27 b	-	-
Paraquat plus bentazon	98 a	-	81 a	-	-
Imazapic plus lactofen	98 a	-	84 a	-	-
Purple nutsedge control in peanut					
Base herbicides					
Diclosulam	-	-	-	-	75 a
Flumioxazin	-	-	-	-	55 b
Complement herbicides					
None	-	74 a	-	17 b	-
Paraquat plus bentazon	-	89 a	-	75 a	-
Imazapic plus lactofen	-	88 a	-	60 a	-

[a]Means within a year, crop, and treatment factor followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$. Data for each treatment factor are pooled over levels of the other treatment factor. Data for the non-treated control were not included in the analysis of variance to allow consideration of the treatment structure.

Table 9 continued

[b]Base herbicides included acetolactate synthase (ALS)-inhibiting herbicides or non-ALS inhibiting herbicides applied either PRE or early POST.

Complement herbicides were applied POST following each level of base herbicide.

Table 10. Influence of base herbicide and complement herbicides on common lambsquarters control in cotton.[a]

Base herbicide	Complement herbicide	2008	2009
		%	
Trifloxysulfuron	None	32 b	70 b
Trifloxysulfuron	Prometryn plus MSMA	100 a	86 ab
Trifloxysulfuron	Glyphosate then glyphosate	100 a	100 a
Fomesafen	None	92 a	87 ab
Fomesafen	Prometryn plus MSMA	99 a	92 a
Fomesafen	Glyphosate then glyphosate	100 a	100 a

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

Table 11. Influence of base herbicide and complement herbicides on corn yield.[a]

Base herbicide	Complement herbicide	Yield
		kg/ha
Thifensulfuron	None	4410 c
Thifensulfuron	Dicamba	4700 bc
Thifensulfuron	Glyphosate	6590 a
Atrazine	None	5050 bc
Atrazine	Dicamba	5050 bc
Atrazine	Glyphosate	5420 b
Non-treated control		3820

[a]Means followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$. Data are pooled over years. Data for the non-treated control were not included in the analysis of variance to allow consideration of the treatment structure.

Table 12. Influence of base herbicide and complement herbicides on corn, cotton, peanut, and soybean yield.[a]

Treatment factor[b]	2007	2008	2009	2010
	kg/ha			
Cotton				
Base herbicides				
Trifloxysulfuron	1110 b	1270 b	2640 a	1610 a
Fomesafen	1650 a	1840 a	2570 a	1830 a
Complement herbicides				
None	680 c	1250 b	2370 b	90 c
Prometryn plus MSMA	1180 b	1580 ab	2640 ab	1980 b
Glyphosate then glyphosate	2280 a	1850 a	2800 a	3010 a
Non-treated control	0	590	0	0
Peanut				
Base herbicides				
Diclosulam	3400 a	4070 a	3390 a	3390 a
Flumioxazin	3360 a	3900 a	3360 a	3190 a

Table 11 continued

Complement herbicides				
None	2890 b	3980 a	3060 a	2100 b
Paraquat plus bentazon	3550 ab	4020 a	3510 a	3940 a
Imazapic plus lactofen	3710 a	3970 a	3550 a	3830 a
Non-treated control	1150	2270	2060	1150
Soybean				
Base herbicides				
Cloransulam-methyl	-	3030 a	1560 a	1710 a
Flumioxazin	-	3360 a	1740 a	1540 a
Complement herbicides				
None	-	2970 b	1510 a	1170 b
Lactofen	-	3150 ab	1700 a	1390 b
Glyphosate then glyphosate	-	3460 a	1740 a	2310 a
Non-treated control	-	2070	1430	900

[a]Means within a year, crop, and treatment factor followed by the same letter are not significantly different according to Fisher's Protected LSD at $p \leq 0.05$. Data for each

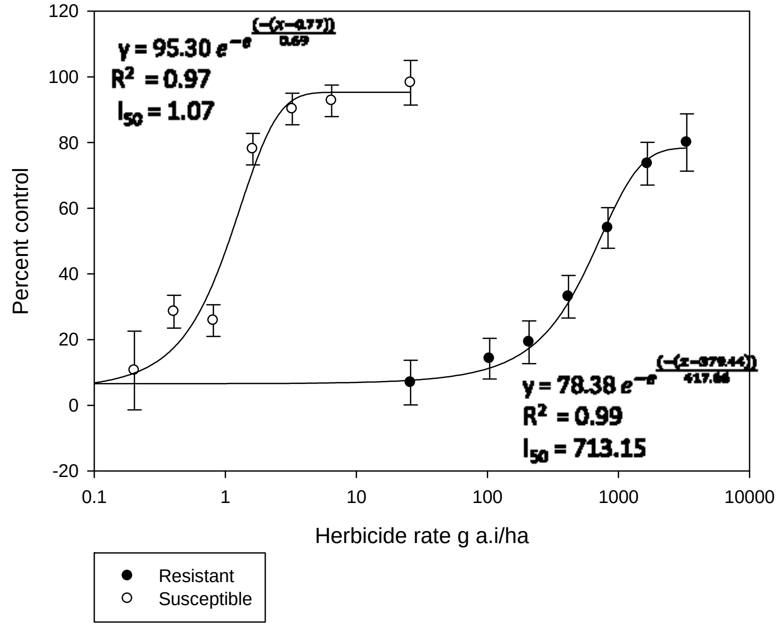
Table 11 continued

treatment factor are pooled over levels of the other treatment factor. Data for the non-treated control were not included in the analysis of variance to allow consideration of the treatment structure.

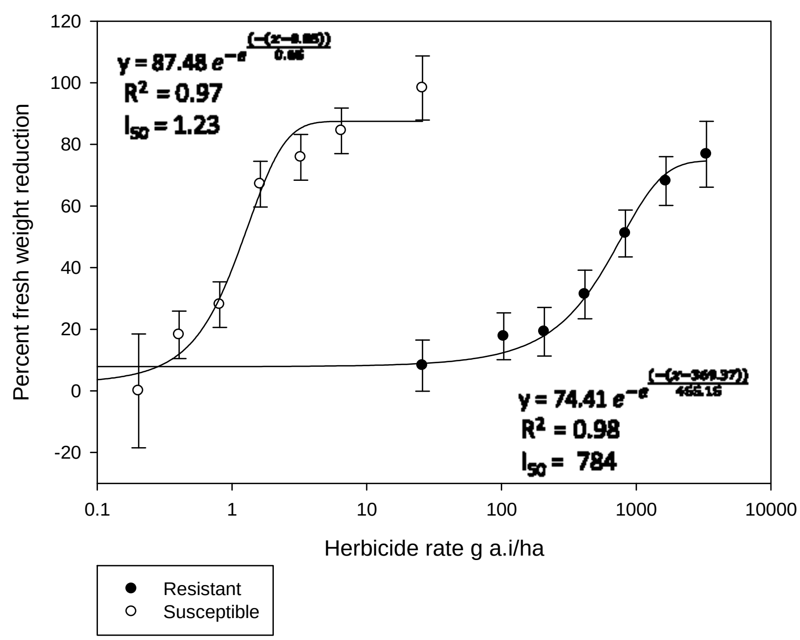
[b]Base herbicides included acetolactate synthase (ALS)-inhibiting herbicides or non-ALS inhibiting herbicides applied either PRE or early POST. Complement herbicides were applied POST following each level of base herbicide.

Figure1. Comparison of mortality of plants, percent visual control, and percent reduction in fresh weight for suspected diclosulam-susceptible and diclosulam-resistant biotypes.

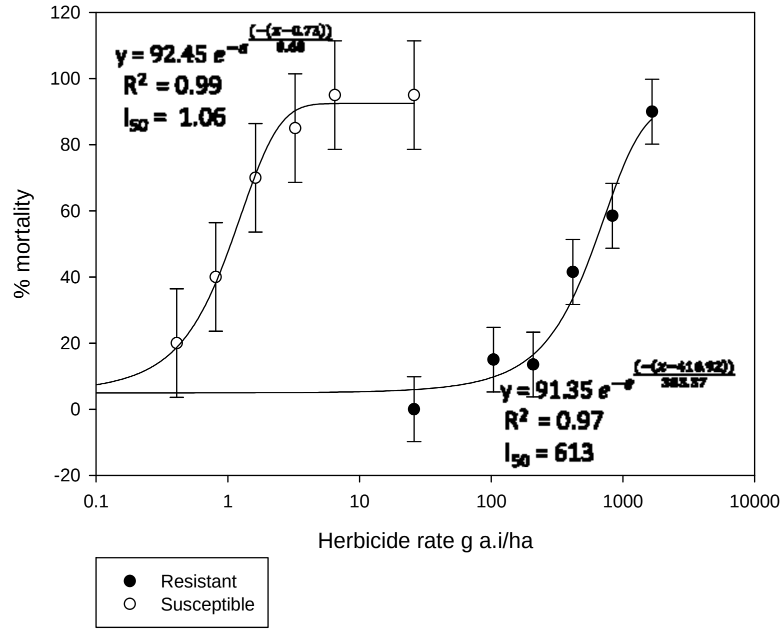
Percent weed control



Percent fresh weight reduction



% mortality



y = 92.45 e
R² = 0.99
I50 = 1.06
y = 91.35 e
R² = 0.97
I50 = 613
% mortality
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
Herbicide rate g a.i/ha
Resistant
Susceptible