

Research Article

Interference of Selected Palmer Amaranth (*Amaranthus palmeri*) Biotypes in Soybean (*Glycine max*)

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Received 30 April 2012; Revised 16 July 2012; Accepted 20 July 2012

Academic Editor: Kassim Al-Khatib

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Palmer amaranth (*Amaranthus palmeri* S. Wats.) has become difficult to control in row crops due to selection for biotypes that are no longer controlled by acetolactate synthase inhibiting herbicides and/or glyphosate. Early season interference in soybean [*Glycine max* (L.) Merr.] for 40 days after emergence by three glyphosate-resistant (GR) and three glyphosate-susceptible (GS) Palmer amaranth biotypes from Georgia and North Carolina was compared in the greenhouse. A field experiment over 2 years compared season-long interference of these biotypes in soybean. The six Palmer amaranth biotypes reduced soybean height similarly in the greenhouse but did not affect soybean height in the field. Reduction in soybean fresh weight and dry weight in the greenhouse; and soybean yield in the field varied by Palmer amaranth biotypes. Soybean yield was reduced 21% by Palmer amaranth at the established field density of 0.37 plant m⁻². When Palmer amaranth biotypes were grouped by response to glyphosate, the GS group reduced fresh weight, dry weight, and yield of soybean more than the GR group. The results indicate a possible small competitive disadvantage associated with glyphosate resistance, but observed differences among biotypes might also be associated with characteristics within and among biotypes other than glyphosate resistance.

1. Introduction

Palmer amaranth is one of the most troublesome weeds of agronomic crops in the southeastern United States [1–3] because of its competitive ability, C₄ photosynthesis, higher water use efficiency, and rapid growth rate [4, 5]. This weed also possesses drought tolerance mechanisms which allow survival under limited water availability [6–8] and it adapts readily to shading [9]. Several biotypes of Palmer amaranth have evolved resistance to herbicides representing different modes of action, including 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors, mitotic inhibitors, acetolactate synthase (ALS) inhibitors, and photosynthetic inhibitors [10], which make it challenging to control in cropping systems.

Species, density, and time of emergence with respect to the crop determine the relative competitiveness of pigweed

species [11–14]. Interference of pigweed species including common waterhemp (*Amaranthus rudis* Sauer) [15–17], Palmer amaranth [11, 17, 18], and redroot pigweed (*Amaranthus retroflexus* L.) [14, 17, 19–22] has been evaluated in soybean. Soybean yield reduction as a result of interference increased from 17% to 68% with an increase in Palmer amaranth density from 0.33 to 10 plants m⁻¹ of row length [11]. Furthermore, in the same study, correlation between soybean yield reduction and Palmer amaranth density was linear up to two Palmer amaranth plants m⁻¹ of row, indicating that intraspecific interference between adjacent Palmer amaranth plants began at greater densities. Monks and Oliver [18] studied the competitive influence of common cocklebur (*Xanthium strumarium* L.), johnsongrass [*Sorghum helveticum* (L.) Pers.], Palmer amaranth, sicklepod [*Cassia obtusifolia* (L.) H. S. Irwin and Barneby], and tall morningglory [*Ipomea purpurea* (L.) Roth] on biomass and yield of two soybean

cultivars in Arkansas. Their results indicated a reduction in biomass of both soybean cultivars when growing within 50 cm of Palmer amaranth and reduction in soybean seed yield within a distance of 25 cm of Palmer amaranth. The distance of influence of Palmer amaranth was among the greatest for the weeds evaluated in this study. Among the three pigweed species (Palmer amaranth, redroot pigweed, and common waterhemp) interfering with soybean, Palmer amaranth accumulated the greatest biomass, followed by common waterhemp and then redroot pigweed [17]. Further, Palmer amaranth planted along with soybean at a density of 8 plants m^{-1} of row resulted in the greatest (79%) reduction in soybean yield, followed by common waterhemp (56%) and then redroot pigweed (38%).

Interference of Palmer amaranth has also been studied in other crops [23–29]. Palmer amaranth growing at a density of 0.9 plants m^{-2} resulted in up to 92% reduction in cotton (*Gossypium hirsutum* L.) lint yield [23]. 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 all densities [24]. 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 [25, 26]. There was a negative linear relationship between grain sorghum [*Sorghum bicolor* (L.) Moench] yield and density of Palmer amaranth. Increasing weed density decreased grain sorghum yield by reducing the numbers of grains produced in panicles [27]. Season-long Palmer amaranth interference in peanut (*Arachis hypogaea* L.) reduced peanut canopy diameter and one plant m^{-1} of row resulted in a predicted yield loss of up to 28% [28]. Yield reductions from 30 to 94% were reported in sweet potato [*Ipomoea batatas* (L.) Lam.] by Palmer amaranth densities ranging from 0.5 to 6.5 plants m^{-1} row [29].

Many biotypes of Palmer amaranth have developed confirmed resistance to glyphosate in the southern United States, making it difficult to manage [30–33]. Herbicide-resistant weed biotypes sometimes have a fitness penalty compared with nonresistant wild types [34–45]. Several components of fitness of maternally inherited triazine-resistant smooth pigweed (*Amaranthus hybridus* L.) were reduced, including early seedling emergence, early growth, mid-season leaf number, and total above-ground biomass, but differences varied among years and populations [36, 37]. Evolved resistance in Powell's amaranth (*Amaranthus powellii* S. Wats.) to ALS-inhibiting herbicides resulted in thinner roots and stems and reduced leaf area, resulting in a 67% reduction in above-ground vegetative mass and a 30% reduction in seed biomass [38]. A mutant of blackgrass (*Alopecurus myosuroides* Huds.) resistant to herbicides that inhibit acetyl coenzyme A carboxylase (ACCase) when grown in competition with wheat (*Triticum aestivum* L.) under limited water supply had a 6%, 42%, and 26% reduction in height, vegetative, and reproductive biomass, respectively, as compared to the wild biotype [39]. The proportion of resistant individuals in segregating F_2 populations of rigid ryegrass (*Lolium rigidum* Gaud.) decreased as compared to susceptible individuals

over a period of 4 years [40, 41]. Baucom and Mauricio [42] reported a high fitness cost of glyphosate resistance in tall morningglory. Glyphosate-resistant genotypes produced fewer seeds as compared to susceptible genotypes in the absence of selection pressure from glyphosate.

Determining relative differences in interference of GR and GS Palmer amaranth biotypes in soybean could be of benefit to evaluate possible competitive disadvantage associated with GR trait. Therefore, greenhouse experiment was conducted to compare early season interference by selected GR and GS biotypes of Palmer amaranth grown with soybean. A field experiment was conducted to study the effect of season-long interference by these biotypes on soybean.

2. Materials and Methods

2.1. Greenhouse Experiment. Seeds from six Palmer amaranth biotypes [31] collected from fields in Georgia and North Carolina during the fall of 2005 were grown along with Roundup Ready soybean cultivar AG6301 (Monsanto Company, St. Louis, MO 63167, USA) in 15 cm round plastic pots containing commercial potting soil (Fafard 4P potting mix, Conrad Fafard Inc. Agawam, MA 01001). Three Palmer amaranth biotypes were GR (one from North Carolina and two from Georgia) and three were GS (one from North Carolina and two from Georgia) (Figure 1). Approximately six soybean seeds and 25 Palmer amaranth seeds were planted in two parallel rows spaced 2.5 cm apart in each pot. Seedlings were thinned to one soybean and one Palmer amaranth plant pot^{-1} 10 days after emergence (DAE). Controls included a single soybean or Palmer amaranth plant pot^{-1} . Plants were fertilized (Scotts Starter Fertilizer, The Scotts Company LLC, Marysville, OH 43041, USA) with 25 mL of a 4.6 g L^{-1} fertilizer solution per pot every 10 days to ensure optimum plant growth. Pots were spaced sufficiently enough to avoid shading from adjacent pots during the entire duration of experiment. Plants were irrigated daily using an overhead sprinkler system. The greenhouse was maintained at $35 \pm 5^\circ C$, and natural illumination was supplemented for 14 hours each day with metal halide lighting ($400 \mu mol m^{-2} s^{-1}$) (Hubbell Lighting, Inc., Greenville, SC 29607). The experimental design was a randomized complete block with treatments replicated 10 times and the experiment was repeated.

Height of the Palmer amaranth and soybean was determined every 5 days beginning 1 week after pots were thinned, corresponding to 15, 20, 25, 30, 35, and 40 DAE. Plant height was measured from the soil surface to the base of the upper most fully expanded leaf for both soybean and Palmer amaranth plants. At 40 DAE, Palmer amaranth and soybean plants were severed at the soil surface to determine shoot fresh weight and dry weight. The samples were dried in paper bags in oven at $60^\circ C$ for 72 hours for dry weight measurements.

Data for percent reduction in plant height and percent reduction in fresh and dry weight relative to controls without interference were subjected to ANOVA using Proc. GLM (Statistical Analysis Systems, version 9.1, SAS Institute Inc.,

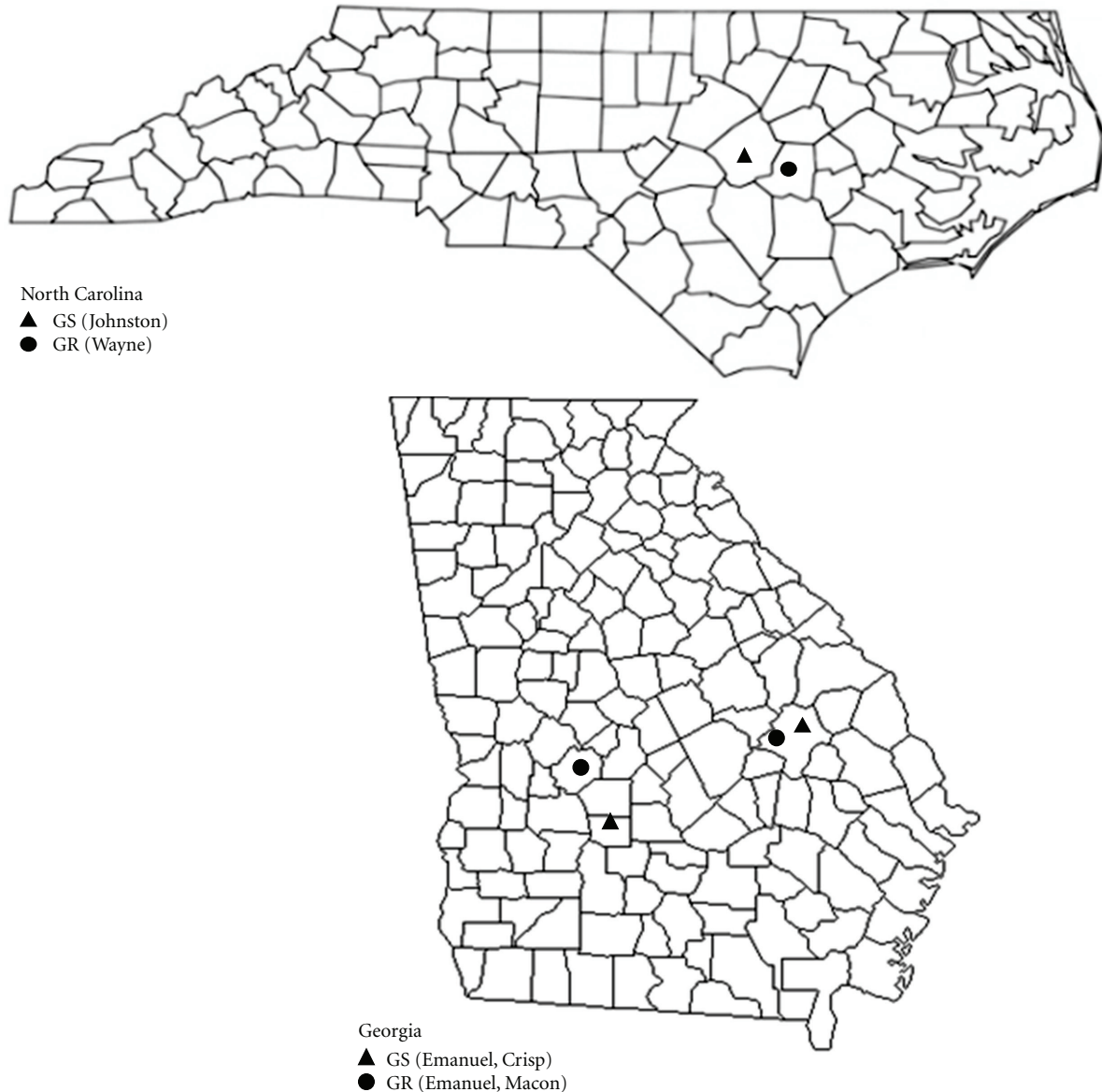


FIGURE 1: Locations of North Carolina and Georgia biotypes used in the study.

SAS Campus Drive, Cary, NC 2751, USA). Due to lack of interaction data were pooled over the two runs. In a separate analysis, data were grouped for biotypes expressing resistance or susceptibility to glyphosate (GR biotype group and GS biotype group) and subjected to ANOVA. Means of significant effects were separated using Fisher's Protected LSD test.

2.2. Field Experiment. The experiment was conducted in conventionally planted (row to row distance = 91 cm) Roundup Ready soybean cultivar AG6301 during 2008 and 2009 at the Cunningham Research Station near Kinston, NC on a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiodults). Plot size was one row by 6 m, and two border rows were included between experimental units. Approximately 10 seeds of each Palmer amaranth biotype

were planted 3 cm apart and 4 cm to the side of soybean row immediately after planting soybean in the middle of the plot. 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 (Roundup Weathermax, Monsanto Company, St. Louis, MO 63167, USA) at $1.1 \text{ kg ae ha}^{-1}$ was applied over the entire test area to control other weeds. For the remainder of the season, weeds other than the one desired Palmer amaranth per plot were removed by hand. A control was included without Palmer amaranth. The experimental design was a randomized complete block with eight replications.

Soybean height was recorded 30, 60, 90, and 120 DAE at a distance of 30, 60, 90, and 120 cm on either side of Palmer amaranth within the soybean row. Mature soybean plants were harvested manually in row sections of 0 to 30,

31 to 60, 61 to 90, 91 to 120, and 121 to 150 cm on either side of Palmer amaranth. Soybean plants were threshed using stationary thresher. Observations on soybean were converted to percent reduction in height and yield relative to the control in absence of Palmer amaranth.

Data for percent reduction in soybean height and yield were subjected to ANOVA as explained earlier when considering Palmer amaranth biotypes individually. In a separate analysis, data were grouped for biotypes expressing resistance or susceptibility to glyphosate (GR and GS biotype groups) and subjected to ANOVA. Means of significant main effects and interactions were separated using Fisher's Protected LSD test. Due to lack of interaction data were pooled over the two runs. Percent reduction in soybean yield was linearly regressed against distance from Palmer amaranth using Sigmaplot 12.0 (Systat Software Inc. 1735 Technology Drive, Suite 430, San Jose, CA 95110, USA). The regression expression used was $y = ax + b$, where, y = percent reduction in yield, x = distance from Palmer amaranth plant, and a and b are constants.

3. Results and Discussion

3.1. Greenhouse Experiment. Differences among individual Palmer amaranth biotypes were not observed for soybean height reduction at any of the six times that height was recorded (data not shown). Averaged over biotypes, soybean height was reduced 10, 10, 8, 11, 10, and 5% at 15, 20, 25, 30, 35, and 40 DAE (data not shown). Differences among individual Palmer amaranth biotypes were observed for soybean fresh weight ($P \leq 0.05$) and dry weight ($P \leq 0.10$) reduction. Both fresh weight and dry weight reduction was similar with the Emanuel (GR), Macon, (GR), Crisp (GS), Emanuel (GS), and Johnston (GS) biotypes (Table 1). The Wayne (GR) biotype reduced fresh and dry weight less than the Macon (GR), Crisp (GS), and Emanuel (GS) biotypes.

When Palmer amaranth biotypes were grouped with respect to response to glyphosate, no differences in soybean height reduction were noted between the two groups (data not shown). However, differences among Palmer amaranth biotype groups were observed for soybean fresh weight ($P \leq 0.05$) and dry weight ($P \leq 0.10$) reduction. The GS biotype group reduced fresh weight and dry weight of soybean more than the GR biotype group (Table 1). Soybean fresh weight was reduced 31% and 23% as result of interference from the GS and GR Palmer amaranth biotype groups, respectively. Interference from GS and GR biotype groups reduced soybean dry weight by 27% and 21%, respectively.

3.2. Field Experiment. Interference of Palmer amaranth biotypes and biotype groups did not affect soybean height at 30, 60, 90, or 120 DAE and at distance of 30, 60, 90, or 120 cm from Palmer amaranth (data not shown). These results were unexpected given the competitive ability of Palmer amaranth [1–9, 11, 17, 18]. Chivinge and Schweppenhauser [46] reported that competition with smooth pigweed reduced branching, shoot dry weight, leaf area index, number of pods per plant, and grain yield of soybean, but plant height,

TABLE 1: Percent reduction in soybean fresh weight and dry weight at harvest (40 days after emergence) caused by early season interference by Palmer amaranth in greenhouse experiment.^{a,b}

Palmer amaranth biotype	Fresh weight reduction	Dry weight reduction
	%	
Individual Palmer amaranth biotypes ^c		
Emanuel (GR)	24ab	23ab
Macon (GR)	31a	28a
Wayne (GR)	13b	11b
Crisp (GS)	31a	26a
Emanuel (GS)	37a	31a
Johnston (GS)	26ab	24ab
Palmer amaranth biotypes grouped by response to glyphosate ^d		
GR group	23z	21z
GS group	31y	27y

^aData are pooled over runs of the experiment. Abbreviations: GR, glyphosate resistant; GS, glyphosate susceptible.

^bMeans within a parameter and analysis followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$ for fresh weight reduction and $P \leq 0.10$ for dry weight reduction.

^cConsists of six Palmer amaranth biotypes.

^dConsists of a group of three glyphosate-resistant (GR) and a group of three glyphosate-susceptible (GS) Palmer amaranth biotypes.

number of seeds per pod, and 1000-seed weight were not affected. The main effects of year, biotype, distance, and their interactions were not significant for percent reduction in soybean height when Palmer amaranth biotypes were considered individually or as biotype groups at 30, 60, 90, and 120 DAE and at distance of 30, 60, 90, and 120 cm from Palmer amaranth (data not shown). When Palmer amaranth biotypes were considered individually, the main effects of year, biotype, and distance from Palmer amaranth were significant for percent reduction in soybean yield (Table 2). However, the interactions of these factors were not significant. Similar results were obtained when Palmer amaranth biotypes were grouped based on response to glyphosate (GR and GS biotype groups).

There were differences in soybean yield reduction as a result of interference from individual Palmer amaranth biotypes averaged over years and five 30 cm distance increments from Palmer amaranth (Table 3). Interference from all GS biotypes reduced soybean yield similarly. Among GR biotypes, interference from the Emanuel biotype reduced soybean yield more than Macon and Wayne biotypes. Soybean yield reduction by the GR Emanuel biotype was similar to GS biotypes. When biotype groups were compared, interference from the GS biotype group reduced soybean yield more than the GR biotype group. The GS biotype group reduced soybean yield 23% compared with 19% reduction by the GR biotype group.

A significant effect of distance from Palmer amaranth was reflected in increasing yield of soybean as distance from Palmer amaranth increased (Figure 2). The greatest yield reduction, 34%, was noted at 15 cm from Palmer amaranth

TABLE 2: $P > F$ for percent reduction in soybean yield caused by season-long interference by Palmer amaranth in field experiment.^a

Source of variation	Individual Palmer amaranth biotypes ^b	Palmer amaranth biotypes grouped by response to glyphosate ^c
$P > F$ value		
Year	<0.0001	<0.0001
Biotype	0.0013	0.0010
Year × biotype	0.9135	0.4459
Distance	<0.0001	<0.0001
Year × distance	0.1296	0.1457
Biotype × distance	0.1980	0.8564
Year × biotype × distance	0.9150	0.6201
Coefficient of variation (%)	90.4	96.3

^aData are pooled over runs of the experiment.

^bConsists of six Palmer amaranth biotypes.

^cConsists of a group of three glyphosate-resistant (GR) and a group of three glyphosate-susceptible (GS) Palmer amaranth biotypes.

TABLE 3: Percent reduction in soybean yield caused by season-long interference by Palmer amaranth in field experiment.^{a,b}

Palmer amaranth biotype	Yield reduction
	%
Individual Palmer amaranth biotypes ^c	
Emanuel (GR)	24a
Macon (GR)	17b
Wayne (GR)	16b
Crisp (GS)	24a
Emanuel (GS)	22a
Johnston (GS)	24a
Palmer amaranth biotypes grouped by response to glyphosate ^d	
GR	19z
GS	23y

^aData are pooled over five 30 cm distance increments from Palmer amaranth and runs (years) of the experiment. Abbreviations: GR, glyphosate resistant; GS, glyphosate susceptible.

^bMeans within a parameter and analysis followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

^cConsists of six Palmer amaranth biotypes.

^dConsists of a group of three glyphosate-resistant and a group of three glyphosate-susceptible Palmer amaranth biotypes.

while yield was reduced 28%, 22%, 16%, and 11% at 45 cm, 75 cm, 105 cm, and 135 cm from Palmer amaranth, respectively. Soybean yield and biomass reduction when growing within a distance of 25 cm and 50 cm of Palmer amaranth, respectively, were reported by Monks and Oliver [18].

Palmer amaranth density established in this experiment was 0.37 Palmer amaranth plants m^{-2} or 0.33 Palmer amaranth plants m^{-1} of row length (calculated based on effective harvested plot size of 3 m by 0.91 m). Soybean yield loss corresponding to this density was 22% (averaged over five 30 cm distance increments from Palmer amaranth). Soybean

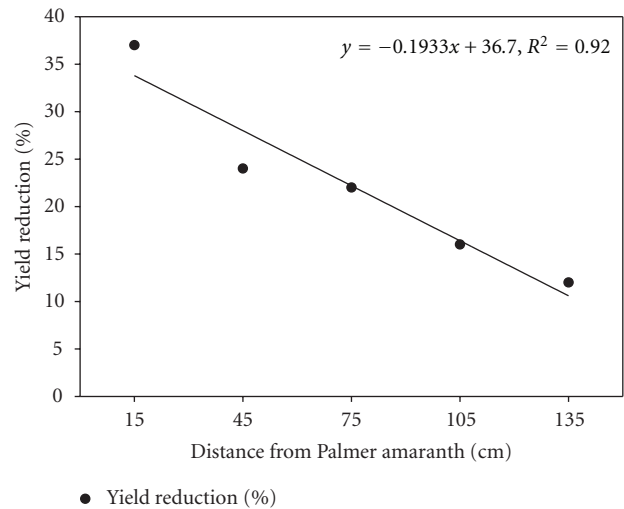


FIGURE 2: Percent reduction in soybean yield as influenced by distance from Palmer amaranth in the field experiment.

yield loss of 17% at a Palmer amaranth density of 0.33 m^{-1} of row was reported by Klingaman and Oliver [11]. Herbicide Application Decision Support System for field crops (WebHADSS version 2004.0.3) predicted soybean yield loss of 11.2% at density of 0.37 Palmer amaranth plants m^{-2} .

Results from both the greenhouse and field studies indicate a possible small competitive disadvantage associated with the glyphosate resistance trait in the biotypes of Palmer amaranth examined. A fitness penalty associated with glyphosate resistance [40–42, 45] as well as other herbicides [36–39] has been reported previously. A fitness penalty associated with GR Palmer amaranth has not been reported. On the other hand, the observed differences may be due to reasons unrelated to glyphosate resistance. A wide range of phenotypic variation has been reported in Palmer amaranth accessions [47–50]. Genetic variability in Palmer amaranth biotypes used in this study was assessed in another experiment using Amplified Fragment Length Polymorphisms [51]. Pair-wise genetic similarity values were found to be relatively low, averaging 0.34. The variation among GR and GS biotype groups used in this study was also found to be less than the overall genetic variability present within all the individual biotypes. It is possible that a high degree of phenotypic and genetic variability present among and within Palmer amaranth biotypes used in the study was responsible for the observed differences in interference.

4. Conclusions

Collectively, results from these experiments indicate that interference in soybean can vary among Palmer amaranth biotypes. Although data suggest that there may be a small competitive disadvantage due to the GR trait, a larger pool of biotypes is needed to conclusively define a fitness cost to glyphosate resistance in Palmer amaranth. The observed differences in interference may have been associated with

inherent diversity existing within and among Palmer amaranth biotypes.

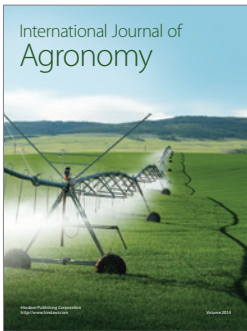
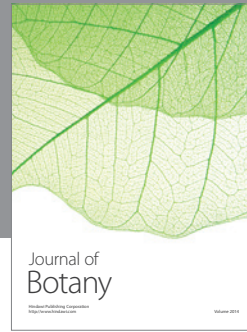
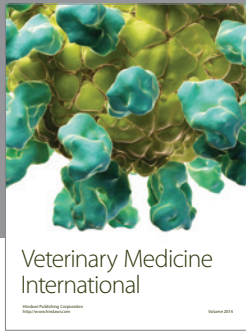
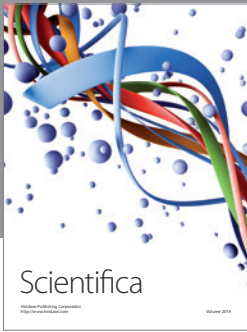
Acknowledgments

Syngenta Crop Protection and Monsanto Company provided partial funding for this research. Appreciation is expressed to Rick Seagroves, Jamie Hinton, Peter Eure, and Gurinderbir Chahal for technical assistance and to Navinderpal Singh and Chuck Foresman for providing advice on this project.

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