Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2012, Article ID 176108, 7 pages
doi:10.1155/2012/176108

Research Article

Inheritance of Evolved Glyphosate Resistance in a North Carolina Palmer Amaranth (*Amaranthus palmeri*) Biotype

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Received 27 June 2012; Accepted 31 July 2012

Academic Editor: Kent Burkey

Inheritance of glyphosate resistance in a Palmer amaranth biotype from North Carolina was studied. Glyphosate rates for 50% survival of glyphosate-resistant (GR) and glyphosate-susceptible (GS) biotypes were 1288 and 58 g ha^{-1}, respectively. These values for F1 progenies obtained from reciprocal crosses (GR × GS and GS × GR) were 794 and 501 g ha^{-1}, respectively. Dose response of F1 progenies indicated that resistance was not fully dominant over susceptibility. Lack of significant differences between dose responses for reciprocal F1 families suggested that genetic control of glyphosate resistance was governed by nuclear genome. Analysis of F1 backcross (BC1F1) families showed that 10 and 8 BC1F1 families out of 15 fitted monogenic inheritance at 2000 and 3000 g ha^{-1} glyphosate, respectively. These results indicate that inheritance of glyphosate resistance in this biotype is incompletely dominant, nuclear inherited, and might not be consistent with a single gene mechanism of inheritance. Relative 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) copy number varied from 22 to 63 across 10 individuals from resistant biotype. This suggested that variable *EPSPS* copy number in the parents might be influential in determining if inheritance of glyphosate resistance is monogenic or polygenic in this biotype.

1. Introduction

Glyphosate 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 [1, 2]. Being nonselective, glyphosate is used to control a wide array of weed species including both grasses and broadleaf weeds [3, 4]. When glyphosate-resistant (GR) crops {including canola (*Brassica napus* L.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* (L.) Merr.)} were commercialized beginning from 1996 to 1998, glyphosate revolutionized production of these crops by enabling growers to use this herbicide for weed control in standing crops [1]. With widespread adoption of glyphosate-resistant corn, cotton, and soybean, glyphosate replaced many previously used selective herbicides and intensified pressure of glyphosate on weeds.

Currently, glyphosate resistance has been confirmed in 13 weed species in the United States, including Palmer amaranth (*Amaranthus palmeri* S. Wats.) [5]. Palmer amaranth is among the most competitive weeds of southern cropping systems [6] and populations of Palmer amaranth have evolved resistance to glyphosate in recent years because of repeated applications of this herbicide [7]. Evolution of glyphosate resistance in weed species poses a great risk to the continued success of GR crops [4]. Although the extent of GR Palmer amaranth biotypes has been well documented [8–10], information is limited about the genetic control of resistance in this species. Mode of inheritance, among other factors, is an important component affecting the evolution

of resistance [11, 12]. 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 [13–15].

Resistance to herbicides has been studied in many weed species. In most studies, resistance is the result of a single gene [16]; however, resistance as the result of more than one gene has been reported [17, 18]. Resistance in all situations except target site resistance to the triazine herbicides is encoded on the nuclear genome [16]. Target site triazine resistance is encoded on the chloroplastic genome [19], accounting for its cytoplasmic inheritance. Typically, resistance is partially dominant over susceptibility [16]; however, both completely dominant and, less commonly, recessive inheritance patterns of resistance have also been reported. Herbicide resistance was found to be inherited as a single, partially dominant, nuclear trait in common sow thistle (*Sonchus oleraceus* L.), Italian ryegrass (*Lolium multiflorum* Lam.), prickly lettuce (*Lactuca serriola* L.), rigid ryegrass (*Lolium rigidum* Gaud.), and wild oats (*Avena fatua* L.) [20–24]. 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 [12, 25].

The mode of inheritance of glyphosate resistance has been studied in a few weed species. In rigid ryegrass [26, 27], horseweed (*Conyza canadensis* L.) [28], and goosegrass [29], resistance was inherited as a single dominant or partially dominant nuclear gene with no influence from maternal effects. Other studies have pointed to more complex mechanisms of inheritance. For example, 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 [17]. Inheritance of glyphosate resistance has also been suggested to follow a polygenic additive pattern in biotypes of Palmer amaranth from Georgia [30]. Probable involvement of one or more minor genes in conferring resistance to glyphosate at lower doses has also been reported in a GR rigid ryegrass population from Australia [26]. Furthermore, earlier studies on glyphosate resistance and the inheritance of the *EPSPS* gene in Palmer amaranth have shown that increased copy numbers of this gene confer resistance to glyphosate [31]. Preliminary results of an experiment carried out by Giacomini et al. [32] indicated a wide range, from 1 to 80, in *EPSPS* copy number in the majority of the F1 populations studied indicating that inheritance of those additional copies from parents to progeny can be highly unpredictable. Research on the inheritance of evolved glyphosate resistance in Palmer amaranth has been somewhat limited. The present study was conducted with the objective to further investigate the mechanism of inheritance of evolved glyphosate resistance in a Palmer amaranth biotype from North Carolina.

2. Materials and Methods

2.1. Generation of F1 Families. Seeds from a GR biotype from Wayne county (glyphosate rate for 50% visible control = 1770 g ha^{-1}) and a GS biotype from Johnston county (glyphosate rate for 50% visible control = 89 g ha^{-1}) in North Carolina [33] were subjected to two cycles of recurrent selection with glyphosate at 840 g ha^{-1} in the greenhouse in order to obtain the individuals used as parents for all experiments. Glyphosate rates required for 50% visible control of GR and GS biotypes in recurrent generations were similar (data not shown) suggesting that the biotypes used for this study were homozygous for the glyphosate resistance trait. After collection, seeds were kept at −20°C for a month and subsequently planted in excess in 10-cm square pots containing commercial potting mix (Fafard 4P potting mix, Conrad Fafard Inc., Agawam, MA 01001) in the greenhouse. Seedlings were thinned to one plant per pot 8 days after emergence. The greenhouse was maintained at 35 ± 5°C and natural lighting was supplemented for 14 h each day with metal halide lighting (Hubbell Lighting, Inc.,701 Millennium Blvd, Greenville, SC 29607) delivering

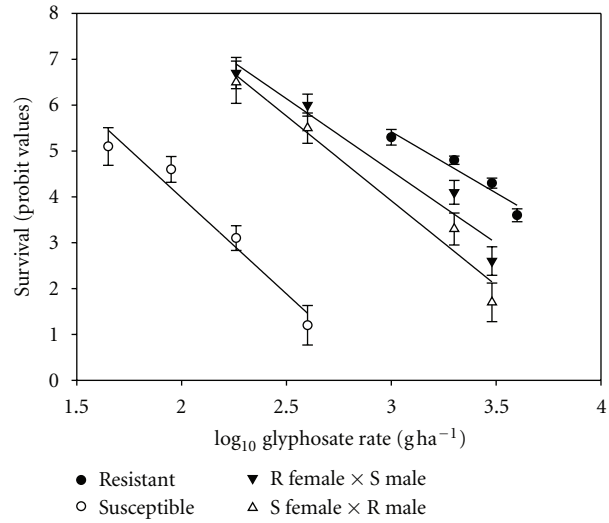

Figure 1: Glyphosate dose responses of glyphosate-resistant (GR), glyphosate-susceptible (GS), F1 R × S (GR female × GS male), and F1 S × R (GS female × GR male) Palmer amaranth populations. Points are mean values ± S.E. Best fit curves for these respective populations are: $y = 13.4 - 2.7x$, $P = 0.0390$, $r^2 = 0.92$; $y = 12.4 - 4.2x$, $P = 0.0221$, $r^2 = 0.96$; $y = 14 - 3.1x$, $P = 0.0246$, $r^2 = 0.95$; and $y = 15 - 3.7x$, $P = 0.0170$, $r^2 = 0.97$.

Table 1: Percent survival of parent Palmer amaranth biotypes (GR and GS) and F1 families (R × S and S × R) after treatment with glyphosate*.

Biotype	Herbicide rate (g/ha)			
	180	400	2000	3000
	%			
GR Parent	—	—	44a	32a
GS Parent	10b	3c	—	—
F1 R × S	86a	81a	22b	10b
F1 S × R	83a	68a	22b	4b

*Abbreviations used: GR: glyphosate-resistant; GS: glyphosate-susceptible; R × S and S × R: reciprocal crosses where, the first alphabet denotes female parent. Means within a glyphosate rate followed by the same letter are not significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

Table 2: Chi-square analysis of segregation for glyphosate resistance at 2000 g/ha in BC1F1 families assuming monogenic and two gene additive inheritance*.

Backcross family	Observed			One gene model Expected		χ^2	P value	Two gene additive model Expected		χ^2	P value
	Alive	Dead	Total	Alive	Dead			Alive	Dead		
BC1F1 S × R1	8	38	46	6.01	39.99	0.759	0.384	9.01	36.99	0.142	0.707
BC1F1 S × R2	7	45	52	6.79	45.21	0.007	0.932	10.19	41.81	1.241	0.265
BC1F1 S × R3	12	38	50	6.53	43.47	5.267	0.022	9.80	40.20	0.616	0.432
BC1F1 S × R4	20	32	52	6.79	45.21	29.539	<0.0001	10.19	41.81	11.750	0.001
BC1F1 S × R5	3	49	52	6.79	45.21	2.436	0.119	10.19	41.81	6.308	0.012
BC1F1 S × R6	3	37	40	5.22	34.77	1.090	0.296	7.84	32.16	3.713	0.054
BC1F1 S × R7	7	23	30	3.92	26.08	2.787	0.095	5.88	24.12	0.266	0.606
BC1F1 R × S1	19	33	52	6.79	45.21	25.235	<0.0001	10.19	41.81	9.477	0.002
BC1F1 R × S2	15	37	52	6.79	45.21	11.407	0.001	10.19	41.81	2.825	0.093
BC1F1 R × S3	4	48	52	6.79	45.21	1.321	0.250	10.19	41.81	4.675	0.031
BC1F1 R × S4	33	19	52	6.79	45.21	116.308	<0.0001	10.19	41.81	63.516	<0.0001
BC1F1 R × S5	6	46	52	6.79	45.21	0.106	0.744	10.19	41.81	2.142	0.143
BC1F1 R × S6	6	46	52	6.79	45.21	0.106	0.744	10.19	41.81	2.142	0.143
BC1F1 R × S7	7	45	52	6.79	45.21	0.007	0.932	10.19	41.81	1.241	0.265
BC1F1 R × S8	4	23	27	3.53	23.47	0.073	0.787	5.29	21.71	0.391	0.532
Total						196.449	<0.0001			110.446	<0.0001
Pooled	154	559	713	93.14	619.86	45.750	<0.0001	139.70	573.30	1.819	0.177
Homogeneity						150.699	<0.0001			108.627	<0.0001
Response of parental controls											
GR Parent	39	13	52								
GS Parent	0	52	52								
F1 R × S	15	37	52								
F1 S × R	11	36	47								

*Abbreviations used: GR: glyphosate-resistant; GS: glyphosate-susceptible; R × S and S × R: reciprocal crosses where, the first alphabet denotes female parent; BC1F1: backcross family; F1: first filial.

400 μmol m^{-2} s^{-1}. Palmer amaranth is dioecious, therefore female and male plants were selected and paired for crosses. Plants were enclosed in dialysis tubing (Carolina Biological Supply Co., P.O. Box 6010, Burlington, NC 27216) to prevent any unintentional crossing until they were ready to be paired for crosses. When plants began to form inflorescences, reciprocal crosses were set up between GR and GS individuals to generate 16 F1 families. There were two sets of F1 families: one originating from GR females (8 R × S families) and another from GS females (8 S × R families). One plant each of the GR and GS biotypes were paired together and encased in dialysis tubing well before pollen shed in order to prevent entry of foreign pollen. Pollination was ensured by tapping the tubing twice every day. Single female plants were also enclosed in dialysis tubing as controls to check for the efficacy of the tubing in preventing entry of foreign pollen. Seeds from each female plant were harvested upon maturation, kept in separate envelopes to constitute individual F1 families, and stored at −20°C for 50 days to break seed dormancy.

2.2. F1 Dose Response Experiments. A dose response study for glyphosate was conducted on GS and GR biotypes and F1 families. Fifteen seeds each for GS, GR and the 16 F1 families were planted in 15-cm round pots containing commercial potting mix in the greenhouse. The potassium salt of glyphosate (Roundup Weathermax, Monsanto Company, St. Louis, Mo 63167) was applied to Palmer amaranth plants at the 3- to 4-leaf stage. Glyphosate was applied at 45, 90, 180, and 400 g ha^{-1} to the susceptible biotype, at 1000, 2000, 3000, and 4000 g ha^{-1} to the resistant biotype, and at 180, 400, 2000, and 3000 g ha^{-1} to the F1 families. Glyphosate was applied in 140 L ha^{-1} at 207 kPa using a CO_2-pressurized backpack sprayer with a flat-fan nozzle (8002 Spray nozzles, Spraying Systems Company, Wheaton, IL 60189). There were six replicate pots for each herbicide rate for the GR and GS biotypes, and one pot for each of the 16 F1 families. Thus, the different families were used as replicates for each herbicide rate. Visible estimates of percent survival were taken 2 wk after treatment. Data from each group of maternal and paternal F1 families were pooled for analysis.

2.3. Data Analysis for F1 Dose Response Experiments. Probit values for percent survival rates were plotted versus $\log_{10}$ herbicide doses to develop a dose response curve using SIGMAPLOT 11.2 (SigmaPlot, version 11.0, Systat Software, Inc., 1735 Technology Drive, Suite 430, San Jose, CA, 95110). The values for 50% percent survival were calculated

Table 3: Chi-square analysis of segregation for glyphosate resistance at 3000 g/ha in BC1F1 families assuming monogenic and two gene additive inheritance*.

Backcross family	Observed			One gene model Expected		χ^2	P value	Two gene additive model Expected		χ^2	P value
	Alive	Dead	Total	Alive	Dead			Alive	Dead		
BC1F1 S × R1	1	38	39	3.12	35.88	1.566	0.211	4.68	34.32	3.288	0.070
BC1F1 S × R2	2	50	52	4.16	47.84	1.219	0.270	6.24	45.76	3.274	0.070
BC1F1 S × R3	3	47	50	4	46	0.272	0.602	6	44	1.705	0.192
BC1F1 S × R4	4	48	52	4.16	47.84	0.007	0.935	6.24	45.76	0.914	0.339
BC1F1 S × R5	0	52	52	4.16	47.84	4.522	0.033	6.24	45.76	7.091	0.008
BC1F1 S × R6	2	41	43	3.44	39.56	0.655	0.418	5.16	37.84	2.199	0.138
BC1F1 S × R7	1	32	33	2.64	30.36	1.107	0.293	3.96	29.04	2.514	0.113
BC1F1 R × S1	21	31	52	4.16	47.84	74.097	<0.0001	6.24	45.76	39.674	<0.0001
BC1F1 R × S2	11	41	52	4.16	47.84	12.224	<0.0001	6.24	45.76	4.126	0.042
BC1F1 R × S3	2	50	52	4.16	47.84	1.219	0.270	6.24	45.76	3.274	0.070
BC1F1 R × S4	24	28	52	4.16	47.84	102.849	<0.0001	6.24	45.76	57.441	<0.0001
BC1F1 R × S5	8	44	52	4.16	47.84	3.853	0.050	6.24	45.76	0.564	0.453
BC1F1 R × S6	9	43	52	4.16	47.84	6.121	0.013	6.24	45.76	1.387	0.239
BC1F1 R × S7	14	36	50	4	46	27.174	<0.0001	6	44	12.121	<0.0001
BC1F1 R × S8	1	24	25	2	23	0.543	0.461	3	22	1.515	0.218
Total						237.429	<0.0001			141.087	<0.0001
Pooled	103	605	708	56.64	651.36	41.245	<0.0001	84.96	623.04	4.353	0.037
Homogeneity						196.184	<0.0001			136.734	<0.0001
Response of parental controls											
GR Parent	35	17	52								
GS Parent	0	52	52								
F1 R × S	6	44	50								
F1 S × R	10	40	50								

*Abbreviations used: R: glyphosate-resistant; S: glyphosate-susceptible; R × S and S × R: reciprocal crosses where, the first alphabet denotes female parent; BC1F1: backcross family; F1: first filial.

from the regression equations. Data for percent survival for glyphosate rates common across F1 populations and GR and GS parents were subjected to ANOVA using the GLM procedure in SAS (Statistical Analysis Systems, version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513). Means were separated using Fisher's Protected LSD test at $P \leq 0.05$.

2.4. Generation of Backcross Families. Sixteen selected surviving males after 400 g ha^{-1} glyphosate application from each of the F1 families were transplanted into round pots (10 cm diameter by 12 cm deep) and each paired with a female from the original GS biotype already in the pot in the greenhouse. Both inflorescences were encased together in the same manner as outlined in the generation of F1 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 BC1F1 families. However, seed yield from one of the backcrosses was low and therefore this family was not included for further evaluation of glyphosate resistance. Seeds were stored at −20°C for 50 days to break dormancy.

2.5. Treating BC1F1 Families with Glyphosate. All fifteen BC1F1 families were assessed for resistance to glyphosate along with F1 families (both R × S and S × R), GR, and GS biotypes as controls in the greenhouse. Seeds were planted in excess in 15-cm round pots and 10 day old seedlings were transplanted into nursery trays. About 50 seedlings were transplanted per tray and each treated with 2000 and 3000 g ha^{-1} of glyphosate at the 4- to 5-leaf stage. Glyphosate was applied as described previously. Numbers of surviving and dead individuals were recorded 3 wk after treatment.

2.6. Data Analysis for BC1F1 Dose-response Experiments. Data for the observed number of surviving and dead plants were compared with predicted values by subjecting data 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 F1 individuals at each dose tested was used to calculate expected survival of BC1F1 families assuming monogenic inheritance [34]. The following equation was used for calculating expected survival:

$$\mathrm{YX} = 0.5\ (\mathrm{WRS} + \mathrm{WSS}), \quad (1)$$

where, YX = expected proportion alive, WRS = proportion of individuals observed alive in the F1 biotype (averaged over R × S and S × R families), WSS = proportion of individuals alive in GS biotype.

A similar calculation was used to test for fit to a two gene additive model. A homogeneity test was conducted in order to test whether data could be pooled over backcross families.

2.7. EPSPS Gene Copy Number Determination. Copy number of the *EPSPS* gene was determined in 2 and 10 plants of the original GS and GR biotypes, respectively, in order to elucidate whether or not gene copy number played a role in the inheritance of resistance in derived F1 and BC1F1 populations. The following procedure is a modification of the quantitative real-time polymerase chain reaction (qPCR) method followed by Gaines et al. [31]. Genomic DNA was extracted from fresh tissue using DNEasy Plant Mini Kits (Qiagen, 27220 Turnberry Lane, Suite 200, Valencia, CA 91355) and checked for quality by gel electrophoresis. Amount and purity of the samples was determined with a ND-1000 Nanodrop instrument (Thermo Scientific, 28W092 Commercial Avenue, Barrington, IL 60010). The DNA was diluted to a 2-ng/μL concentration in highly purified 18 mΩ water. Using PerfeCTa SYBR Green Supermix with ROX (Quanta Biosciences, 202 Perry Parkway, Gaithersburg, MD 20877), the SYBR Green Supermix, upstream and downstream primers, and water were combined in a 1.5-mL tube to create a SYBR Green master mix. The genomic DNA templates (10 ng) were run with each primer set in triplicate in 12.5-μL reaction volumes using the SYBR Green master mix on a Polymerase Chain Reaction (PCR) plate. The plate was covered by Microseal "B" film (Bio-Rad, 2000 Alfred Nobel Drive, Hercules, CA 94547), which is optically clear for real-time PCR detection, and fluorescence data were captured in real time during each amplification cycle. The ABI Prism 7000 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA 94547) was run with the following thermoprofile: 15 min at 95 C, 40 cycles of 95 C for 30 sec, and 60 C for 1 min, and finally a melt-curve analysis to check for primer-dimers. No-template controls, consisting of 10 μL of Master Mix and 2.5 μL of water, served as the negative controls for this procedure. No primer-dimers and no amplification products were seen in the melt-curve analysis and the controls, respectively. The melting peaks for both primer sets were 81C.

Primer efficiency curves were created for each primer set by using a 1/10x dilution series of genomic DNA from a resistant plant. The *EPSPS* primers EPSF1 (5′-ATG-TTGGACGCTCTCAGAACTCTTGGT-3′) × EPSR8 (5′-TGAATTTCCTCCAGCAACGGCAA-3′) (195-bp product) had an efficiency of 95.16% and the *ALS* primers ALSF2 (5′-GCTGCTGAAGGCTACGCT-3′) × ALSR2 (5′-GCG-GGACTGAGTCAAGAAGTG-3′) (118-bp product) had an efficiency of 95.62%. These efficiencies are very similar and thus directly comparable in later calculations.

Threshold cycles (Ct) were calculated by the ABI Prism 7000 program, and relative copy number was determined by using a modified version of the $2^{-\Delta\Delta Ct}$ method from [35]. The *ALS* gene was used as a reference gene present in the genome at a copy number of one. Quantification of *EPSPS* was calculated by finding ΔCt = (Ct, *ALS*-Ct, *EPSPS*) and calculating 2ΔCt to get a relative *EPSPS* copy number count.

3. Results and Discussion

Percent survival data indicated much reduced control of the GR Palmer amaranth biotype compared with the GS biotype (Figure 1). Glyphosate rate for 50% percent survival of GR and GS biotypes were 1288 and 58 g ha^{-1}, respectively. Whitaker et al. [33] using these same biotypes of Palmer amaranth reported the values for 50% percent visible control to be 1769 and 89 g ha^{-1} for GR and GS biotypes, respectively. Differences in values between their results and those observed in our study most likely reflect differences in methodology (i.e., percent survival versus percent visible control).

The values for 50% percent survival for combined GR × GS and GS × GR F1 families were 794 and 501 g ha^{-1}, respectively. Both sets of F1 families were treated with 180, 400, 2000, and 3000 g ha^{-1} of glyphosate (Table 1). Glyphosate rates common for the GR parent biotype and F1 families were 2000 and 3000 g ha^{-1}, while glyphosate rates common for the GS parent biotype and F1 families were 180 and 400 g ha^{-1}. Therefore, comparisons between parent biotypes and both sets of F1 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^{-1} of glyphosate, it was expected that all individuals would have survived at these rates because the glyphosate rate for 50% survival for this biotype was 1288 g ha^{-1}. Similarly, although GS parents were not exposed to 2000 and 3000 g ha^{-1} of glyphosate, all individuals would have died at these rates given that the rate for 50% survival for this biotype was 58 g ha^{-1}. At any given dose, percent survival of F1 progenies showed lower levels of resistance as compared to the GR parent biotype and higher levels of resistance than the GS parent biotype (Figure 1 and Table 1). Response of both sets of maternal and paternal resistant F1 families was closer to that of the resistant parent (Figure 1). Dose-response behavior of F1 families indicated that resistance was not fully dominant over susceptibility. Inheritance of glyphosate resistance as an incompletely dominant trait controlled by nuclear genes has been reported in other weed species [17, 27–29]. Values for percent survival of both sets of F1 families at a given glyphosate dose were not significantly different from each other (Figure 1 and Table 1), indicating that genetic control of glyphosate resistance is governed by the nuclear genome and that there is no maternal or cytoplasmic inheritance involved. These results are also similar to those of Gaines [30], who reported that resistance in GR Palmer amaranth was due to an incompletely dominant, nuclear-inherited gene.

To determine if genetic control of resistance involves single or multiple genes, BC1F1 families were developed and treated with glyphosate at 2000 and 3000 g ha^{-1}. The homogeneity chi-square was significant ($P < 0.0001$); therefore, data could not be pooled over backcross families.

Thus, tests on individual families were considered (Tables 2 and 3). Segregation of resistance in 10 out of 15 individual backcross families tested at 2000 g ha^{-1} glyphosate and 8 out of 15 individual backcross families tested at 3000 g ha^{-1} glyphosate conformed to a monogenic inheritance (Tables 2 and 3). When tested against a 2-gene additive model, the homogeneity test was also significant ($P < 0.0001$). When individual families were considered, 10 out of 15 families fitted the model at 2000 g ha^{-1} and 3000 g ha^{-1} glyphosate. The inheritance analysis suggested that the monogenic model fitted the data better than the two-gene additive model.

While in the majority of the BC1F1 families inheritance of glyphosate resistance was consistent with a single gene hypothesis, this was not the case in a few families. An examination of the data showed that there was an excess of resistant individuals in these families (Tables 2 and 3). Moreover, in three families (BC1F1 S × R4, BC1F1 R × S1, and BC1F1 R × S4) the excess of resistant individuals at both glyphosate rates used was large. This suggests some other form of inheritance is occurring in these families. In an earlier study on the inheritance of glyphosate resistance in Palmer amaranth biotypes from Georgia, an overabundance of resistant progeny was found in some but not all of the families evaluated [30]. Further studies on the inheritance of *EPSPS* gene in these populations showed that inheritance of additional copies of the gene from parents to progeny was highly unpredictable [31, 32]. Because the copy number determination technique was not available to us at the time dose response experiments were performed, the *EPSPS* gene copy number in the F1 parents and the BC1F1 individuals could not be determined. This information could have aided in providing a better interpretation of the results. However, plants from the GR and GS biotypes used to generate our F1 and BC1F1 families were analyzed for copy number. While the GS parent possessed only one copy of *EPSPS* relative to *als*, the number of copies in the GR parent ranged from 22 to 63 (Figure 2). These results confirm that increased *EPSPS* copy number is closely associated with glyphosate resistance in the resistant Palmer amaranth biotype used in this study. Moreover, *EPSPS* copy number in this biotype is highly variable. Given that the inheritance of glyphosate resistance in individuals with increased number of copies of *EPSPS* has been demonstrated to be unpredictable in previous studies [30, 32], it is likely that this is also the cause for the variability in the inheritance of glyphosate resistance observed in this study.

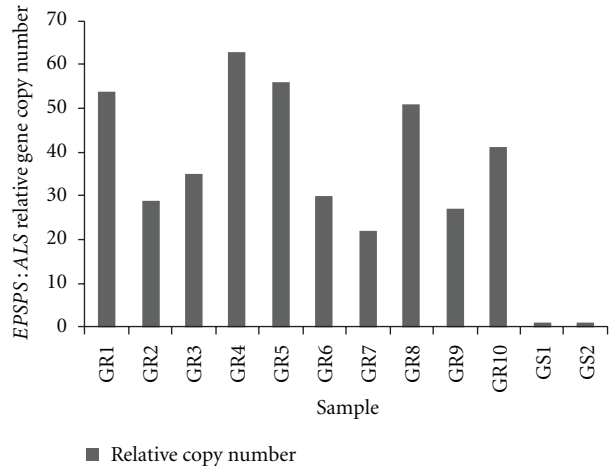

Figure 2: *EPSPS* gene copy number relative to *ALS* from 10 glyphosate-resistant (GR) and 2 glyphosate-susceptible (GS) plants.

4. Conclusions

Collectively, these data suggest that glyphosate resistance in the studied biotype of Palmer amaranth is incompletely dominant, and nuclear-inherited with no maternal or cytoplasmic effects involved. While resistance is consistent with a single gene mechanism of inheritance for many backcross families, a number of individual backcross families seem to follow polygenic inheritance. Differences in copy number of the *EPSPS* gene in the resistant biotype of Palmer amaranth studied and the unpredictable behavior in the inheritance of these copies might be responsible for the variable results among families regarding the number of genes involved in inheritance of glyphosate resistance. Further analysis looking at *EPSPS* copy numbers in each generation of GR Palmer amaranth progenies might help elucidate these issues.

Acknowledgments

Monsanto Company and Syngenta Crop Protection provided partial funding. Appreciation is expressed to C. Foresman and Dr. V. Shivrain for discussions relative to research and to R. Seagroves, P. Eure, and J. Hinton for technical assistance.

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