GLYPHOSATE RESISTANT PALMER AMARANTH IN ARIZONA

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Abstract

Seed was collected in July and August, 2012 from two populations of Palmer amaranth, *Amaranthus Palmerii*, in Buckeye and Glendale, AZ in western Maricopa County suspected of being resistant to glyphosate due to control failures. For comparison, seed was also collected from a known glyphosate susceptible Palmer amaranth population at the University of Arizona Maricopa Agricultural Center (MAC). Seeds of each biotype were planted in 4 inch pots, plants were grown to the 4 leaf growth stage in a greenhouse, sprayed with various rates of glyphosate and phytotoxicity was visually estimated to confirm glyphosate resistance. In the first experiment, the Buckeye biotype showed little response to slight stunting at glyphosate rates of 0.375, 0.75, 1.5 and 3.0 lb ae glyphosate/A. In contrast the Glendale and Maricopa biotypes were killed by rates of 0.375 and 0.75 lb ae glyphosate/A. In the second experiment, the phytotoxic response to increasing glyphosate rate from 0.0089 to 0.356 lb ae/A 14 days after treatment (DAT) was 3.5 to 9.9 for the Maricopa biotype and 0.25 to 9.9 for the Glendale biotype (0 to 10 scale with 10 representing death). In contrast, the phytotoxic response to increasing glyphosate rate from 2.67 to 9 lb ae/A 14 DAT was 3.5 to 6.6 for the Buckeye biotype. The Buckeye plants were severely stunted and chlorotic for a period of two weeks after treatment but eventually recovered, resumed growth and began flowering in the greenhouse. In conclusion, the Maricopa and Glendale biotypes collected in 2012 were susceptible to glyphosate but the Buckeye biotype was resistant to glyphosate. The management of glyphosate resistant Palmer amaranth in western Maricopa County will be a serious challenge to cotton producers locally while producers in the rest of the state are at risk of importing the resistance gene.

Introduction

Cotton producers and researchers in Arizona have been concerned about the spread of glyphosate resistant Palmer amaranth across the southeastern U.S. cotton-belt since the initial 2004 discovery of a resistant biotype in Georgia. However, neither this concern nor the educational efforts of University of Arizona (UA) Extension faculty reversed recent trends toward reduced amounts of cultivation for weed control, the reduced use of preplant-preemergence herbicides and increasing reliance on glyphosate for weed management in Arizona cotton. In July 2012, UA Extension faculty were called to evaluate a glyphosate-Palmer amaranth control failure in a Buckeye, AZ cotton field and subsequently in another field located in Glendale, AZ. The initial determination based on three field applications of glyphosate at 44 fl. oz./A (1.5 lb ae/A) was that the Buckeye Palmer amaranth population was likely resistant to glyphosate. Due to the large size of the plants and the severe injury resulting from high glyphosate rates, no determination could be made regarding Palmer amaranth population in the Glendale field. The objective of the experiments reported here were to test the hypothesis that the Buckeye and Glendale Palmer amaranth populations or biotypes were resistant to glyphosate herbicide.

Materials and Methods

Palmer amaranth seed were collected from mature female plants in the Buckeye and Glendale fields discussed above. Seed was also collected from a known glyphosate susceptible Palmer Amaranth population growing at the University of Arizona Maricopa Agricultural Center (MAC) in order to facilitate comparison of susceptible and putative glyphosate resistant biotypes in the greenhouse. Seed (several per pot) were planted in an artificial soil mix in 4 inch pots that were put in a greenhouse. After emergence, plants were thinned, fertilized and irrigated as needed. Palmer amaranth plants were grown to the 4 (mostly) to 6 true leaf growth stage for experiments. The first experiment was planted on September 27, 2012 and sprayed on October 18, 2012. The second experiment with more plants was planted on October 11, 2012 and sprayed on November 1, 2012. For treatment, the pots were removed from the greenhouse and sprayed using a three nozzle boom (XR8001VS nozzles spaced 20 inches apart) and CO_2 pressurized backpack sprayer calibrated to deliver 9.95 GPA at 25 PSI and 2.5 MPH. The formulation of glyphosate used was Aquamaster (4 lb ae glyphosate/gallon) because it did not contain surfactant. A nonionic surfactant, Activator 90, was added to all spray solutions at 0.5% v/v. Pots were returned to the greenhouse and plant response to glyphosate was monitored for 1 to 5 weeks after spraying. Phytotoxicity was visually estimated using a 10 point scale with 0 indicating no effect and 10 indicating death. In the second experiment, the number of Palmer amaranth main-stem nodes and plant height were also measured at 14 and 33 DAT (days after treatment). The data were subjected to analysis of variance and mean separation as indicated on the data tables.

Results and Discussion

The initial resistance assay was conducted using the Buckeye, Glendale and Maricopa biotypes and glyphosate rates from 0.1875 lb ae/A to 3 lb ae/A (Table 1). Not all biotypes received every treatment as indicated in the tables because seed dormancy and lack of emergence reduced the number of plants available for the experiment. The Buckeye biotype exhibited few glyphosate injury symptoms although there were occasional plants that showed minor injury. In contrast, the Glendale and Maricopa biotypes were severely injured by glyphosate and appeared to be equally susceptible to the herbicide (Table 1).

Table 1. Response of three Palmer amaranth biotypes to glyphosate in a greenhouse experiment initiated on September 27, 2012. Phytotoxicity was visually estimated 7 DAT; 0 indicates no injury, 10 indicates plant death. Data are means ± standard deviation (n=number of plants/replicates). The data were transformed prior to analysis of variance using the arcsine square root percent transformation; means followed by the same letter within a column do not significantly differ (P = 0.05) according the Student-Newman-Keuls test.

Glyphosate Rate	Phytotoxicity 7 DAT		
	Buckeye	Glendale	Maricopa
(lb ae/A)	*(0-10)*	*(0-10)*	*(0-10)*
untreated	0 ± 0 (7) *b*	0 ± 0 (3) b	0 ± 0 (4) b
0.1875	0 ± 0 (6) a	--	9.3 ± 1.2 (3) a
0.375	0 ± 2.2 (7) a	8.0 ± 1.0 (3) a	9.5 ± 0.6 (4) a
0.75	0.1 ± 3.8 (7) a	9.3 ± 0.5 (4) a	8.3 ± 1.5 (3) a
1.125	0 ± 2.2 (7) a	--	--
1.5	0 ± 0 (7) a	8.3 ± 1.3 (4) a	--
2.25	0 ± 0 (6) a	9.3 ± 0.6 (3) a	--
3.0	0 ± 3.1 (7) a	--	--

A second experiment was conducted to determine the response of the Glendale and Maricopa biotypes to low rates of glyphosate (0.01, 0.05, 0.1, 0.2 and 0.4 kg ae/ha) and the response of the Buckeye biotype to high rates of glyphosate (2.52, 3.36, 5.04, 6.7, 10.1 kg ae/ha). In addition to visually estimating phytotoxicity, plant height and number of main-stem nodes were measured. For clarity, the Glendale and Maricopa data (Table 2a & 2b) are reported separately from the Buckeye data (Table 3). The Buckeye plants were not treated with low glyphosate rates because of the lack of response to glyphosate shown in the first experiment.

The results of the second experiment confirmed that the Glendale and Maricopa Palmer amaranth populations were susceptible to glyphosate. The normal field dose of glyphosate is 0.75 lb ae/A and in this experiment glyphosate rates of ½, ¼ and less severely injured the Glendale and Maricopa Palmer amaranth biotypes (Table 2a). It is not likely that these plants would have survived in normal field situations outside the greenhouse. The plant height and main-stem node data showed similar trends; rates of 0.09 lb ae/A (0.1 kg ae/ha) and greater stopped growth in height and node production (Table 2b). Treated plants turned chlorotic in the first week after being sprayed with glyphosate while the untreated plants remained green and healthy albeit somewhat etiolated due to the low light levels in the greenhouse and shorter days of the fall and winter. There did not appear to be any noticeable difference in susceptibility to glyphosate between the Maricopa and Glendale Palmer amaranth biotypes.

Table 2a. Response of the Glendale and Maricopa Palmer amaranth biotypes to glyphosate in a greenhouse experiment initiated on October 11, 2012. Phytotoxicity (0 indicates no injury, 10 indicates plant death) was visually estimated 14 and 25 DAT. Data are means ± standard deviation of four replicates; means followed by the same letter within a column do not significantly differ (P = 0.05) according the Student-Newman-Keuls test. An arcsine square root percent transformation was used to transform the data prior to analysis of variance.

Glyphosate Rate	Phytotoxicity 14 DAT		Phytotoxicity 25 DAT	
	Glendale	Maricopa	Glendale	Maricopa
lb ae/A	*(0-10)*	*(0-10)*	*(0-10)*	*(0-10)*
untreated	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c
0.009	0.25 ± 0.5 c	3.5 ± 2.3 b	0 ± 0 c	4.2 ± 2 b
0.045	5.7 ± 3 b	6.9 ± 0.8 a	7.8 ± 1.7 b	8.8 ± 0.5 a
0.09	8.6 ± 1.7 ab	8.4 ± 1.6 a	9.1 ± 1 ab	8.6 ± 0.2 a
0.18	7.4 ± 2 ab	7.5 ± 0.9 a	9.0 ± 0.7 ab	9.1 ± 0.6 a
0.36	9.9 ± 0.06 a	9.9 ± 0.06 a	10 ± 0 a	10 ± 0 a

Table 2b. Plant height and number of nodes for the Glendale and Maricopa Palmer amaranth biotypes 14 days after treatment (DAT) with glyphosate in a greenhouse experiment initiated on October 11, 2012. Data are means ± standard deviation of four replicates; means followed by the same letter within a column do not significantly differ (P = 0.05) according the Student-Newman-Keuls test. The data were transformed prior to analysis of variance as indicated in the footnotes.

Glyphosate Rate	Height		Main-stem nodes	
	Glendale[1]	Maricopa[2]	Glendale[1]	Maricopa[1]
(lb ae/A)	*(cm)*	*(cm)*	*(n)*	*(n)*
untreated	49.3 ± 0.2 a	43.7 ± 0.1 a	16.9 ± 0.4 a	17.2 ± 0.2 a
0.009	33.0 ± 0.2 b	24.6 ± 0.2 b	16.2 ± 0.3 a	16.7 ± 0.6 a
0.045	9.4 ± 0.3 c	8.4 ± 0.1 c	5.7 ± 0.7 b	3.7 ± 0.1 b
0.09	5.1 ± 1.2 c	6.3 ± 0.05 c	3.3 ± 1 b	5.2 ± 0.2 b
0.18	7.2 ± 0.8 c	5.5 ± 0.07 c	5.6 ± 0.4 b	4.7 ± 0.3 b
0.36	3.6 ± 0.9 c	5.6 ± 0.1 c	1.8 ± 0.6 b	0.6 ± 0.4 c

[1]Square Root(n+0.5); [2]Log(n+1)

The Buckeye Palmer amaranth biotype was injured by the high rates of glyphosate used in the second experiment as shown by phytotoxicity 14 and 33 DAT (Table 3). The plants turned chlorotic to various degrees and growth stopped for a period of about 2 weeks as shown by the height and node measurements collected 14 DAT (Table 3). As the experiment progressed, it became increasingly difficult to maintain the untreated plants due to their very large size relative to the small 4 inch pots. After a severe desiccation event, the untreated plants were discarded. The much smaller treated plants were not desiccated and slowly began to recover. This was reflected in the increase in main-stem node production and increases in plant height (Table 3). In addition to producing new shoot growth, many plants began to produce inflorescences and many of the male plants were shedding pollen at the time the experiment was terminated (data not shown). An interesting feature of the recovery was the very large amount of plant to plant variation in the height and node measurements as shown by the large standard deviations of these measures (Table 3). While the mechanism of glyphosate resistance in the Buckeye Palmer amaranth population is currently unknown, this variation would be consistent with plant to plant variation in the number of gene copies for

EPSPS (5-enolpyruylshikimate-3-phosphate synthase). The resultant plant to plant variation in EPSPS enzyme levels in the plants could result in differential responses at the same glyphosate rate (Gains et al., 2011). Gene amplification and increases in plant EPSPS enzyme levels was shown to confer glyphosate resistance in Palmer amaranth populations from Georgia and other southeastern states (Gains et al., 2010) and variation in injury due to variation in EPSPS copy number (Gains et al., 2011). The glyphosate resistance trait in the Buckeye Palmer amaranth population appears to be heritable since the progeny germinated from seed collected from female plants in Buckeye were resistant to glyphosate in the greenhouse.

Table 3. Phytotoxicity, height and number of main-stem nodes 14 and 33 DAT with glyphosate for the Buckeye Palmer amaranth biotype. Data are means ± standard deviations of four replicates; means followed by the same letter within a column do not significantly differ (P = 0.05) a according the Student-Newman-Keuls test.

Glyphosate Rate	Phytotoxicity		Height		Main-stem nodes	
	14 DAT	33 DAT	14 DAT	33 DAT	14 DAT	33 DAT
(lb ae/A)	*(0-10)*	*(0-10)*	*(cm)*	*(cm)*	*(n)*	*(n)*
Untreated	0 ± 0 c	--	33 ± 8 a	--	16.8 ± 1.7 a	--
2.67	6.2 ± 3.2 a	4.5 ± 3.4 a	11 ± 7 b	19 ± 14 a	5.3 ± 3.9 b	10 ± 6 a
3	5.4 ± 4.1 ab	5 ± 5 a	10 ± 5 b	22 ± 18 a	7.5 ± 3.7 b	8 ± 6 a
4.5	5.3 ± 4.0 ab	3.8 ± 4.1 a	13 ± 5 b	28 ± 14 a	7.3 ± 3.9 b	14 ± 10 a
6	3.5 ± 2.1 ab	2.8 ± 0.9 a	14 ± 10 b	35 ± 13 a	9.0 ± 2.9 b	18 ± 5 a
9	6.6 ± 0.6 a	4.4 ± 2.0 a	9 ± 1 b	17 ± 7 a	6.0 ± 1.4 b	17 ± 5 a

Summary

Glyphosate-resistant Palmer amaranth was discovered in Buckeye, AZ in July 2012 after three 1.5 lb ae/A glyphosate applications failed to control Palmer amaranth plants. Seed were collected from mature female plants in the Buckeye field and used to conduct greenhouse assays in fall 2012. Four-leaf Palmer amaranth plants survived high rates of glyphosate from 2X to 6X (3 to 9 lb ae/A) the normal field rate of 0.75 lb ae/A. Growth of treated plants was inhibited for about 2 weeks after spraying but height and number of main-stem node data showed the plants recovered and resumed growth. Most of these plants began producing inflorescences and male plants were shedding pollen when the experiment was terminated. The presence of glyphosate resistant Palmer amaranth in central Arizona presents cotton growers with a serious challenge. Growers must adopt rigorous sanitation practices to stop the spread of glyphosate resistance through seed and pollen movement. Furthermore, growers need to diversify their weed control tactics, especially in the use of preplant, preemergence herbicides, and move away from glyphosate only programs to avoid selecting for glyphosate-resistant weeds.

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