GLYPHOSATE-RESISTANT COMMON WATERHEMP CONFIRMED IN TEXAS

Ginger G. Light
Texas Tech University
Lubbock, TX
Maad Y. Mohammed
Borlaug Institute for International Agriculture – Texas A & M System
College Station, TX
Peter A. Dotray
Texas Tech University, Texas AgriLife Research and Extension
Lubbock, TX
James M. Chandler
Texas A & M University
College Station
Robert J. Wright
Texas Tech University and Texas AgriLife Research
Lubbock, TX

Abstract

Two independent common waterhemp populations exhibiting poor control by glyphosate were identified in Wharton County, Texas. The level of resistance in four putatively glyphosate-resistant common waterhemp biotypes was examined under greenhouse conditions. As a basis for comparison, confirmed glyphosate-resistant and susceptible common waterhemp populations were included in the analysis. The LD_{50} for the susceptible population (22.4 oz/A) was equivalent to the 1X labeled rate, while the putatively resistant lines exhibited a broad range of resistance from 3.5 to 58.5-fold greater than the susceptible biotype. The GR_{50} for the most resistant line was 2.5 fold greater than the susceptible biotype (8.1 oz/A). A second objective examined alternative postemergence herbicide options for glyphosate-resistant common waterhemp control. At College Station, Ignite® and Reflex® provided the most effective and consistent control. In Lubbock, greatest efficacy was observed following Staple® in two of four newly identified resistant lines. Development of specifically designed management strategies for individual producers will be essential in mitigating the spread of glyphosate-resistant common waterhemp in Texas.

Introduction

In 2008, 7.0 million acres of Roundup Ready cotton was grown in the U.S. (Anonymous 2009). During this same time period, glyphosate use has increased almost 10-fold. (NASS 1997 and NASS 2006). The popularity of Roundup Ready crops and intense management of weeds using glyphosate as the main control method has caused a shift in weed populations (Culpepper 2006 and Owen 2008) and created a selection pressure for glyphosate-resistant weeds. Currently, there are nine confirmed weed species in the U.S. and seven additional species in other countries resistant to glyphosate, including common waterhemp (*Amaranthus rudis* Sauer) (Heap 2009). Glyphosate-resistant common waterhemp has also been confirmed in Missouri, Illinois, Kansas, and Minnesota (Legleiter and Bradley 2008).

Glyphosate-resistant *Amaranthus* species are a recognized risk to U.S. agriculture. Estimates are that more than 3 million acres of crop land are now affected by glyphosate-resistant *Amaranthus* species (Heap 2009). This epidemic is particularly pertinent to agricultural regions that utilize intensive glyphosate management practices to control weedy pests. The food, horticulture and fiber industries in Texas alone generate $73 billion a year for the rural economy (TDA 2006). When a cotton producer in Wharton County failed to control common waterhemp in 2006 following multiple glyphosate applications, there was concern that a resistant biotype may have developed. A second independent location in the same county exhibited inconsistent glyphosate control of common waterhemp in 2008. The objectives of this research were to: 1) characterize the level of glyphosate resistance in the common waterhemp populations from each location; and 2) evaluate their potential for control with alternative postemergence herbicides.

Materials and Methods

Plant Material

Seed from thirty-two individual accession plants were collected on August 16, 2006 from Wharton County, Texas following glyphosate applications at either 44 or 88 oz/A when the plants were 4 to 6 inches tall. Individual seed heads were placed in paper bags and allowed to dry at ambient temperature. Seed were hand-threshed and placed at 4 °F for four months to reduce seed dormancy. Seed were stored at room temperature until used in these studies. A preliminary analysis of the selected accessions indicated that several were potentially resistant (data not shown). Two lines (15 and 19) were chosen to represent the resistance observed from the 2006 populations.

In 2008, a second independent Wharton County location was identified and sampled by collecting seed from five individual accessions. Each plant had received three sequential applications of glyphosate (30, 22, and 22 oz/A) on March 29, April 25, and May 15, respectively. Individual seed heads were harvested on July 2 and allowed to dry at ambient temperature. Seed were hand-threshed and allowed to remain at room temperature. Initial screening indicated that all five lines were potentially resistant (data not shown). Two lines (1b and 6c) were chosen to represent two distinct levels of resistance observed from this preliminary analysis.

Seed from a confirmed Missouri glyphosate-resistant common waterhemp population (Legleiter and Bradley 2008) was obtained to serve as a resistant control (hereafter referred to as MO). Seed from two susceptible lines was also included in the assessment of resistance. A confirmed susceptible line was obtained from the Belleville Research Center, Illinois (hereafter referred to as IL). This line served as the susceptible control against which LD_{50} and GR_{50} for resistant biotypes were compared. The second potentially susceptible line was collected from an uncultivated field in Wharton County, Texas and was processed in the same manner as lines 1b and 6c (hereafter referred to as TX-S).

Determination of LD_{50} and GR_{50} Values

To determine the level of heritable glyphosate resistance, seeds were planted at a depth of 1/4" in 20" by 11" flats containing 520 in^3 of potting soil and watered to initiate germination. Germination rates varied from 5 to 95% depending on whether or not the seed had received cold pre-treatment (data not shown). Plants were grown in contained greenhouse facilities at both Lubbock and College Station, Texas during early spring. Once the plants reached the 2-leaf stage, they were hand-transplanted to 5 to 6" pots with 2 plants per pot containing 27.5 in^3 of potting soil. Once the plants reached 3 to 5" in height, glyphosate was applied.
Glyphosate (MON 0139) was applied using a stationary greenhouse sprayer equipped with TurboTee 110015 spray tips calibrated to deliver 12.5 gal/A. Glyphosate plus non-ionic surfactant (MON 59162) at 0.25% v/v was used for each treatment to alleviate phytotoxicity which was likely to occur at high rates if commercially available glyphosate was used for this study. Glyphosate rates for susceptible populations were 0, 1/32, 1/16, 1/8, 1/4, 1/2, 1, and 2X with 1X = 22 oz/A. Glyphosate rates for resistant populations were 0, 1/2, 1, 2, 4, 6, 8, 12, and 16X with 1X = 22 oz/A. Each experiment unit was replicated four times for a total of eight plants per rate in a completely randomized design. The trial was conducted at two locations: Lubbock and College Station, Texas. Throughout the trial, surface drip irrigation was applied to maximize healthy growth.

At 28 DAT, the numbers of surviving common waterhemp were counted at each glyphosate rate. A survivor was defined as possessing any green leaf tissue, regardless of the physical injury. Additionally, above ground biomass was removed for each rate and bulked. The biomass was allowed to dry inside paper bags for 14 d prior to obtaining dry weights. The lethal dose needed to kill 50 percent of the population (LD_{50}) and the dose required to cause a 50% reduction in growth (GR_{50}) were determined using PROC PROBIT in SAS, Version 9.2. Confidence intervals (95%) were calculated and used to assess rate effects on the accessions studied.

Control of Resistant Lines with Alternative Postemergence Herbicides

To examine the control by alternative herbicides, resistant common waterhemp lines (1b, 6c, 15, 19, and MO) were planted as previously described. The following postemergence herbicides were applied as previously described to 3 to 5" tall plants: Staple® 3.2 EC at 2.5 oz/A, Pursuit® 2 AS at 4 oz/A, Permit® 75 WG at 1.33 oz/A, Reflex® 2 SL at 1.5 pt/A, Aatrex® 4 L at 2 pt/A, MSMA® 6 L at 2.67 pt/A, Ignite® 280 SL at 29 oz/A, and Direx® 4 L at 1.2 qt/A. Non-ionic surfactant at 0.25% v/v was added to Staple.

At 28 DAT, common waterhemp visual injury symptoms were evaluated on a 10 point scale with 0 = no symptoms and 10 = complete control. The experimental design was a three-way factorial with complete randomization and four replications of each herbicide for all putative resistant lines (1b, 6c, 15, 19, and MO). Susceptible lines were not included because they could be adequately controlled by glyphosate at recommended label rates. The experiments were conducted at two locations: Lubbock and College Station, Texas. Data were subjected to the analysis of variance using the PROC GLM procedure of SAS, Version 9.2. Due to significant interactions, data was not pooled across treatments, locations or biotypes.

Results and Discussion

LD_{50} and GR_{50} Determinations

The lethal dose response curves illustrate three distinct groups: susceptible, low resistance, and high resistance (Figure 1). The LD_{50} values ranged from 1.0 to 59.7X the labeled rate (22 oz/A) of glyphosate (Table 1). The susceptible genotypes had similar glyphosate LD_{50} values of 1.0X (22.4 oz/A) and 1.6X (35 oz/A) for IL and TX-S, respectively. The dose response increase for the TX-S population compared to the IL susceptible population may be indicative of the initial phase of resistance development due to pollen flow.

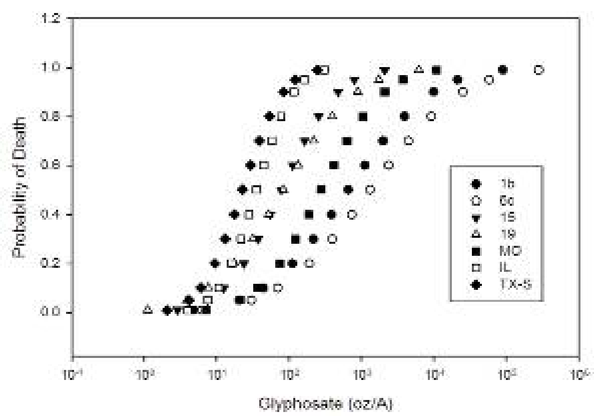

Figure 1. PROBIT analysis dose response curves to predict the lethal glyphosate dose needed to kill 50% of the common waterhemp when treated at 3 to 5" tall.

Table 1. LD_{50} and GR_{50} rates for glyphosate control and growth reduction of susceptible and resistant biotypes of common waterhemp.

Biotype	Source Location	LD_{50} rate[a]	GR_{50} rate[a]
1b	Wharton County, TX	29.7	1.4
6c	Wharton County, TX	59.7	1.0
15	Wharton County, TX	3.5	0.4
19	Wharton County, TX	3.7	0.2
MO	Missouri	12.6	1.4
IL	Illinois	1.0	0.4
TX-S	Wharton County, TX	1.6	0.3

[a]1X = 22 oz/A

The LD_{50} values for lines 1b, 6c, 15, 19 and MO were more diverse but clustered in two groups with no overlap of 95% fiducial limits. A low level of resistance (<4X) was observed for lines 15 (77 oz/A) and 19 (82 oz/A), while a high level of resistance (>12X) was observed for lines MO (277 oz/A), 1b (654 oz/A) and 6c (1313 oz/A). The resistance observed in the MO biotype was in agreement with published resistance levels of 9 and 19-fold compared to the susceptible biotype (Legleiter and Bradley 2008). Variable common waterhemp control has been reported in previous studies (Smith and Hallett 2006), and the proportion of common waterhemp most sensitive to glyphosate control is decreasing (Volenberg et al. 2007).

The growth reduction response curves illustrate separation of response into two distinct categories (Figure 2). The GR_{50} values ranged from 0.2 to 1.4X the labeled rate of glyphosate (Table 1). Less than a 0.5X rate was required to reduce growth by 50% in lines TX-S, IL, 15, and 19. In contrast, lines 1b, 6c, and MO required a 1 to 1.4X rate of glyphosate to reduce growth by 50%. These values reflect a three-fold increased in the rate required to reduce growth compared to the susceptible lines. In spite of the growth reduction, all resistant lines flowered and produced seed when 12 h day length induced early floral initiation.

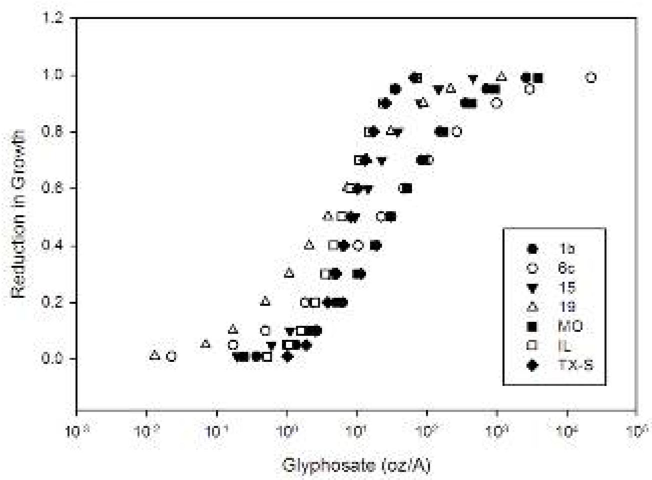

Figure 2. PROBIT analysis dose response curves to predict the glyphosate dose required to cause a 50% reduction in growth of common waterhemp when treated at 3 to 5" tall.

Alternative Postemergence Herbicide Control

Significant interactions were observed among locations, treatments, and biotypes in this portion of the study. At College Station, the most effective treatments were Ignite® and Reflex® (Table 2). Ignite provided the most effective and consistent control across biotypes, while Reflex® acceptably controlled all populations except the MO population. This result was expected since Missouri common waterhemp expresses multiple resistance to PPO-inhibitors (Legleiter and Bradley 2008). Poor control of lines 1b and 6c by Staple® at College Station may be related to poor inhibition of the ALS target site under high temperatures experienced in the contained greenhouse facility (Light et al. 1999).

Table 2. Visual injury evaluations of five glyphosate-resistant common waterhemp populations 28 DAT with various postemergence herbicides in College Station, TX[a-c].

Treatment	Rate	Biotype					LSD
	(oz/a)	1b	6c	15	19	MO	
Untreated		0 E a	0 E a	0 C a	0 F a	0 C a	0
Staple	2.5	3 CD ab	2.8 D ab	2 BC b	1.5 DEF b	5.3 AB a	3.1
Pursuit	4.0	3 CD a	3.5 CD a	2.3 BC a	3 DE a	3.5 B a	2.4
Permit	1.33	2.3 DE ab	0.3 E b	1.5 BC b	0.8 EF b	4.3 B a	2.3
Reflex	24	7 AB ab	9.8 A a	6.8 A b	8.3 AB ab	3.5 B c	2.9
Aatrex	32	6.3 AB a	4.5 BC ab	2 BC b	6 BC a	6.8 AB a	3.1
MSMA	43	4.8 BC b	5.3 B b	4.5 AB bc	3.8 CD c	6.3 AB a	1.0
Ignite	29	8.3 A a	9.3 A a	6.8 A a	8.8 A a	8.3 A a	4.0
Direx	38	7 AB ab	4 C b	5.5 A ab	6 BC ab	8.3 A a	3.5
LSD		2.3	1.1	3.2	2.7	3.3	

[a]Scale from 0 to 10 with 10 indicating complete necrosis.
[b]Means within a column followed by the same uppercase letter are not significantly different at P=0.05, according to Fisher's LSD test.
[c]Means within a row followed by the same lowercase letter are not significantly different at P=0.05, according to Fisher's LSD test.

In Lubbock, effective control was not obtained by any treatment for lines 15, 19 and MO (Table 3). Staple® provided effective control of lines 1b and 6c, while Reflex® provided adequate control. In contrast to College Station, Ignite® did not provide effective control in Lubbock. Ignite® provides greatest efficacy in high humidity environments which may not have been achieved at this location (Coetzer et al. 2001).

Table 3. Visual injury evaluations of five glyphosate-resistant common waterhemp populations 28 DAT with various postemergence herbicides in Lubbock, TX[a-c].

Treatment	Rate	Biotype					
	(oz/A)	1b	6c	15	19	MO	LSD
Untreated		0 C a	0 C a	0 C a	0 D a	0 C a	0
Staple	2.5	6.5 A ab	8.8 A a	2.8 AB c	2.5 BC c	3.8 A bc	2.9
Pursuit	4.0	3.3 B b	6 AB a	2.3 AB b	1.8 CD b	2.8 AB b	2.0
Permit	1.33	3 B a	3.5 BC a	1.8 B a	1.3 CD a	1.5 BC a	2.3
Reflex	24	6 A a	7 AB a	1.8 B b	1.8 CD b	1.5 BC b	3.4
Aatrex	32	2.3 BC b	8.8 A a	1.5 BC b	2 BC b	3.3 AB b	2.1
MSMA	43	3 B b	5.8 AB a	3.5 A b	4.5 A ab	3 AB b	1.6
Ignite	29	2 BC ab	5 B a	2 AB ab	1.8 CD b	1.5 BC b	3.1
Direx	38	1.8 BC a	5 B a	3.5 A a	3.8 AB a	3.3 AB a	3.7
LSD		2.46	3.7	1.63	1.94	1.93	

[a]Scale from 0 to 10 with 10 indicating complete necrosis.
[b]Means within a column followed by the same uppercase letter are not significantly different at *P*=0.05, according to Fisher's LSD test.
[c]Means within a row followed by the same lowercase letter are not significantly different at *P*=0.05, according to Fisher's LSD test.

Lines 15, 19 and MO were not acceptably controlled by any of the ALS-inhibiting herbicides at either location. The MO line has confirmed resistance to ALS herbicides (Legleiter and Bradley 2008), and control of lines 15, 19, and MO was similar with all the ALS-inhibiting herbicides. Coupled with a field history of ALS chemistry use at the site of collection for lines 15 and 19, the likelihood exists that lines 15 and 19 are also ALS-resistant.

Summary

Texas has documented its first case of glyphosate-resistance with the confirmation of several biotypes of common waterhemp. Long term management strategies are crucial to mitigating the presence of glyphosate-resistant common waterhemp in Texas. In addition to crop rotation and cultural control, mechanical and chemical control may be necessary to combat glyphosate-resistant common waterhemp. The use of cultivation alone in a cropping system reduced the number of common waterhemp in the soil seed bank by 93% over a four-year period (Buhler et al. 2001). Continued use or reintroduction of preemergence herbicides into a weed management strategy may also be essential in controlling glyphosate-resistant common waterhemp. Preemergence herbicides with varying modes of action such as acetachlor, *S*- metolachlor, dimethenamid, sulfentrazone, chlorimuron, and pendimethalin provide effective control of *Amaranthus* species (Nolte and Young 2002; and Steckel et al. 2002). Finally, rotating crop technologies may be an important strategy in mitigating the increase in glyphosate-resistant common waterhemp.

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