2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

WEED MANAGEMENT IN ROUNDUP READY FLEX COTTON IN ARIZONA

William B. McCloskey and Kwame O. Adu-Tutu
Department of Plant Sciences
University of Arizona
Tucson, AZ
T. Vint Hicks
Monsanto Company
Fountain Hills, AZ

Abstract

Experiments were conducted in 2002 and 2003 at the University of Arizona Maricopa Agricultural Center to evaluate the tolerance of RR Flex cotton to topical Roundup Weathermax (glyphosate) applications and to study weed management programs in RR Flex cotton. RR Flex cotton demonstrated excellent tolerance to glyphosate as measured by pollen viability and yield. The presence of the RR gene did not affect the yield of genotype pairs that were identical except for the presence or absence of the RR Flex genetic construct. In the weed management study, delaying the first topical glyphosate application resulted in larger, more difficult to control weeds and reduced cotton yield by allowing greater early season competition between weeds and cotton. The best weed control programs included early (1 to 2 leaf growth stage) topical applications at rates greater than 0.75 lb ae/A and a second Roundup application after the first post-planting irrigation (10 node growth stage). The data also suggested that there may be significant value in making a layby, directed-broadcast application that includes a residual herbicide such as Caparol (prometryn) at layby.

Introduction

The active ingredient of Roundup, glyphosate, inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) in the shikimate acid pathway of plants thereby inhibiting the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan causing the death of susceptible plants. The genetic construct (promoter and EPSP synthase gene) in current commercial Roundup Ready cotton varieties is not well expressed in male flower tissues (Pline et al., 2003). Exposure of Roundup Ready cotton to glyphosate can cause abnormal flowers with stunted filaments and anthers that do not release pollen. Lack of self-pollination and the resulting boll abortion can cause yield losses. To address this problem, Monsanto Company developed a new glyphosate resistant genetic construct consisting of a new promoter combined with the commercialized EPSP synthase gene joined to the current commercial promoter and EPSP synthase gene combination (i.e., a double gene construct with two different promoters). The experiments reported here evaluated several transformation events containing this genetic construct for tolerance to topical Roundup applications in 2002, evaluated the tolerance of the transformation event selected for commercialization (RR Flex cotton) in 2003, and studied weed management in RR Flex cotton in 2003.

Materials and Methods

Experiments were conducted in 2002 and 2003 at the University of Arizona Maricopa Agricultural Center near Maricopa, AZ. The 2002 tolerance trial used a randomized complete block design with 4 replicates and screened 5 different transformation events (labeled 161, 601, 9910, 9920 and 10371) along with the current Roundup Ready gene construct, labeled 1445. The field received a preplant-incorporated application of Prowl at 0.825 lb ai/A prior to planting and was cultivated during the season. Three glyphosate rates (0, 1.5 and 2.25 lb ae/A) were sprayed topically at the 3 leaf and 6, 10 and 14 node cotton growth stages on May 10, May 20, June 4 and June 19, 2002, respectively. After flowering began, visual flower sterility ratings were made on June 28 and July 24 using a 1 (fertile) to 5 (sterile) rating system. An unsprayed agronomic yield trial was also conducted in 2002 in which plants from lines 161, 601, 9910, 9920 and 10371 containing the new RR Flex genetic construct (+) were compared with other plants from the same genetic lines without the RR Flex genetic construct (-). The 2003 tolerance trial used a randomized complete block design with 4 replicates and the plot size was 4 rows (13.33 ft) by 40 ft long. The tolerance trial was “dry” planted on April 8, 2003. Treatments consisted of topically spraying Roundup Weathermax at 0.75, 1.5 or 3 lb ae/A mixed with ammonium sulfate (2% v/v) at various times as indicated in the data table. The 2003 weed management trial used a randomized complete block design with 5 replicates and the plot size was 4 rows (13.33 ft) by 40 ft long. The field received a preplant-incorporated application of Prowl at 0.412 lb ai/A but was not cultivated during the season. The experiment was “dry” planted on April 9, irrigated on April 15 and weeds emerged with the cotton on April 21, 2003. Roundup Weathermax mixed with ammonium sulfate (2% v/v) was applied topically and post-directed at 0.75, 1.125 or 1.5 lb ae/A in various treatments at various growth stages as indicated in the data tables. In all experiments, the cotton was grown using standard management practices and harvested using a spindle picker equipped with an automated weighing system.

Results and Discussion

The flower sterility ratings on June 28, 2002 showed that there were no differences in pollen shed within genetic lines between the three glyphosate rates (unsprayed, 1.5 and 2.25 lb ae/A) except for line 1445 where the unsprayed flowers shed pollen while those sprayed with 1.5 and 2.25 lb ae/A released almost no pollen (Table 1). Similarly, the flower sterility ratings for lines 1445 and 161 on July 24, 2002 showed that pollen shed was reduced by topical glyphosate applications but pollen shed by the other lines (601, 9910, 9920 and 10371) was unaffected by topical glyphosate applications (Table 1). The yield data from the 2002 tolerance trial indicated that within a genetic line, there were no differences in yield between the unsprayed and glyphosate sprayed treatments except for line 1445 where all three treatments (i.e., unsprayed, 1.5 lb ae/A and 2.25 lb ae/A) were significantly different (Table 2). There were significant yield differences between genetic lines treated at the same glyphosate rate (Table 2). The 2002 agronomic yield trial with pairs of genotypes that differed only in the presence or absence of RR Flex cotton genetic construct (+ or -) showed that insertion of the genetic construct did not affect yield (i.e., none of the yield differences were statistically significant). The yields of the 601+ and 601- genotype pair were 2296 and 2067 lb/A of seed cotton, respectively; the yield of the 9910+ and 9910- genotype pair were 2100 and 2061 lb/A of seed cotton, respectively; and the yield of the 9920+ and 9920- genotype pair were 2155 and 2144 lb/A of seed cotton, respectively.

The transformation event selected for commercialization as RR Flex cotton was treated topically with a number of different glyphosate rates at various application timings designed to test tolerance during flowering and boll development in the 2003 tolerance trial. Following each application date, plots were visually inspected for symptoms of chlorosis and necrosis but such symptoms were never found in response to the topical glyphosate applications (Table 3). All treatments received topical glyphosate applications at the 1 to 2 true leaf, 4 to 5 true leaf, and 8 node growth stages but the total lb ae/A applied and the amount applied at each application time were different (Table 4). Treatments 1 to 5 received 4 topical Roundup Weathermax applications with the last application made at various rates and times in the season during the first part of the primary fruiting cycle (i.e., at 9 node, first flower, or 17 days after first flower [DAFF]). There were no significant yield differences between these treatments or compared to the untreated control (Table 4). Treatments 6, 7 and 8 all received a total of 5 topical Roundup Weathermax applications with the first four applications made at the 1 to 2 true leaf, 4 to 5 true leaf, 8 node, and first flower growth stages followed by a 3 lb ae/A application at either 31 DAFF, 5NAWF, and 20% cracked boll. There were no significant yield differences between these treatments or compared to the untreated control (Table 4). These data show that RR Flex cotton has excellent reproductive tolerance to glyphosate and will provide growers with greater application and rate flexibility than the current commercial RR cotton varieties.

In the 2003 RR Flex cotton weed management study, the first topical Roundup Weathermax (glyphosate) applications were made at either the 1.5 true leaf, 4 true leaf, or 10 node cotton growth stages at one of three rates, 0.75 lb ae/A, 1.125 lb ae/A or 1.5 lb ae/A. Ivyleaf morningglory control following topical applications at the 1.5 and 4 true leaf growth stages of cotton increased as the rate of glyphosate increased (Table 5). As expected, the size of the weeds (ivyleaf morningglory and Palmer amaranth) increased as the first topical glyphosate application was delayed and resulted in reduced morningglory control compared to plots treated earlier as shown by weed control evaluations on May 22 and June 26 (Table 5). The superior weed control in plots treated earlier in the season was in part due to a second early season glyphosate application in plots treated at the 1.5 and 4 leaf growth stages, however, cumulative morningglory weed control ratings on July 30 showed that even after all treatments had received multiple glyphosate applications, delaying the first topical application still reduced morningglory control (large IPOHE, Table 6). Despite the large size of Palmer amaranth at the 10 node topical application, control was still good (88 to 100 % control) in all treatments due to the sensitivity of this species to glyphosate (data not shown). Delaying the initial topical application until the 10 node growth stage after the first post-planting irrigation resulted in significant cotton-weed competition that stunted the cotton as indicated by the June 26 visual evaluation (Table 5). These height reductions were not as significant later in the season on July 16 after herbicide applications reduced weed competition (ANOVA for cotton height on July 16 was P=0.0722).

The yield data from the RR Flex cotton weed management study indicated that all treatment regimes provided substantial control of Palmer amaranth and ivyleaf morningglory and at least partially protected cotton lint yield with the least effective treatment yielding 76.6% of most effective treatment (Table 6). All treatments regimes completely eliminated Palmer amaranth from the plots. Annual morningglory species are the most troublesome weeds in Arizona cotton fields and the representative species in this study was ivyleaf morningglory. The percent control of large IPOHE plants indicated the degree of control of plants that either emerged with the cotton, shortly afterwards, or after the first post-planting irrigation. Comparison of treatments 2, 4, 5, 6, and 7 indicated that delaying the first topical Roundup Weathermax application decreased the control of IPOHE on July 30 and earlier as discussed above (Table 5) and appeared to be associated with a trend of decreasing yield (Table 6). Control of the small morningglory that emerged mid-season or shortly before layby was more variable and was poor in treatments 8 and 10 because the post-direct treatments were band applications and because the layby applications did not include Caparol which provided residual control and increased burn-down activity (Table 6). A better measure of the ability of late-emerging morningglory to compete with cotton was the percent IPOHE canopy cover on 13 October 2003. The percent canopy cover measure was a visual estimate of the percentage of the area of the cotton canopy that had morningglory tendrils and leaves at the top of the cotton canopy. Treatments 2, 4, 5, 6, 7, 11 and 12 all provided sufficient

control of later-emerging morningglory plants to keep the cotton canopy free of significant morningglory foliage and minimized competition of this weed with cotton. The data suggest that the best weed control programs with RR Flex cotton should include early topical applications at rates greater than 0.75 lb ae/A of glyphosate, a second application after the first post-planting irrigation and that there may be significant value in making a layby, directed-broadcast application that includes a residual herbicide such as Caparol (Table 6).

References

Pline, W.A., K.L. Edmisten, J.W. Wilcut, R. Wells, and J. Thomas. 2003. Glyphosate induced reductions in pollen viability and seed set in glyphosate-resistant cotton and attempted remediation by gibberellic acid (GA3). Weed Sci. 51:19-27.

Table 1. Effect of four topical glyphosate applications on flower sterility at the Maricopa Agricultural Center, Maricopa, AZ. Visual flower sterility ratings were made using a 1 to 5 scale with 1 indicating fertile flowers with pollen shed and 5 indicating infertile flowers with no pollen shed. Six flowers were rated in each plot. Values are means of four replications; means within a row (i.e., the same genetic line) from the same date followed by the same letter (a or b) are not significantly different at P=0.05 according to the Ryan-Einot-Gabriel-Welsch multiple range test. Means within a column on the same date followed by the same letter (x or y) are not significantly different at P=0.05 according to the Ryan-Einot-Gabriel-Welsch multiple range test.

	June 28, 2002			July 24, 2002		
Genetic line	**0**	**1.5**	**2.25**	**0**	**1.5**	**2.25**
	(lb ae/A)	*(lb ae/A)*	*(lb ae/A)*	*(lb ae/A)*	*(lb ae/A)*	*(lb ae/A)*
161	1.4 a	1.5 a	1.4 a y	1.2 b	2.1 ab	2.5 a xy
601	1.5 a	1.1 a	1.4 a y	1.5 a	1.7 a	1.4 a y
1445	1.1 b	4.6 a	4.6 a x	1.3 c	2.6 b	3.6 a x
9910	1.6 a	1.4 a	1.4 a y	1.4 a	1.7 a	1.2 a y
9920	1.3 a	1.2 a	1.1 a y	1.2 a	1.7 a	1.5 a y
10371	1.2 a	1.4 a	1.5 a y	1.4 a	1.6 a	1.9 a y

Table 2. Effect of four topical glyphosate applications on the yield of various genetic lines at the Maricopa Agricultural Center in the 2002 tolerance experiment. Values are means of four replications; means within a column (i.e., the same glyphosate rate) followed by the same letter are not significantly different at P=0.05 according to the Ryan-Einot-Gabriel-Welsch multiple range test.

	Glyphosate Rate		
Genetic line	**0**	**1.5**	**2.25**
	(lb ae/A)	*(lb ae/A)*	*(lb ae/A)*
161	1221 c	908 b	924 b
601	2024 ab	1675 a	1756 a
1445	2145 a	548 b	368 c
9910	1837 ab	1873 a	1858 a
9920	1550 bc	1498 a	1477 a
10371	1952 ab	1978 a	1894 a

Table 3. Application timing, cotton growth stage and the date glyphosate applications were made in the 2003 RR Flex tolerance trial at the Maricopa Agricultural Center. Cotton was planted on April 8, 2003. Following each application date, percent chlorosis and necrosis were visually assessed in all plots on the evaluation dates listed in the table. No chlorosis or necrosis symptoms were found at any time in any treatment. NAWF = nodes above white flower.

Applica-tion code	Growth stage	Cotton height (in)	Application Date	Evaluation Date
A	1 to 2 true leaves	2-3	7 May	16 May
B	4 to 5 true leaves	4-6	16 May	23 May
C	8 node	9-12	2 June	9 June
D	9 node	12-18	9 June	17 June
E	1st flower	18-24	13 June	20 June
F	17 days after 1st flower	24-36	30 June	7 July
G	31 days after 1st flower	42-48	16 July	23 July
H	5 NAWF	48-54	11 August	18 August
I	20% cracked bolls	54-60	2 September	9 September

Table 4. Yield of RR Flex cotton following multiple topical glyphosate applications at the Maricopa Agricultural. Center in 2003. All Roundup Weathermax (glyphosate) applications included ammonium sulfate (2% v/v). Values are means of four replications; means within a column followed by the same letter are not significantly different at P=0.05 according to the Ryan-Einot-Gabriel-Welsch multiple range test. NAWF = nodes above white flower.

Treatment Number	Treatment	Rate (lb ae/A)	Application Code	Growth Stage	Date
	glyphosate	1.5	A	1 to 2 true leaves	
	glyphosate	1.5	B	4 to 5 true leaves	
	glyphosate	1.5	C	8 node	
1	glyphosate	1.5	E	1st flower	915 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	1.5	C	8 node	
2	glyphosate	1.5	D	9 node	913 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	3.0	C	8 node	
3	glyphosate	3.0	D	9 node	915 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	3.0	C	8 node	
4	glyphosate	3.0	E	1st flower	895 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	3.0	C	8 node	
5	glyphosate	3.0	F	17 days after 1st flower	865 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	0.75	C	8 node	
	glyphosate	0.75	D	9 node	
6	glyphosate	3.0	G	31 days after 1st flower	902 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	0.75	C	8 node	
	glyphosate	0.75	E	1st flower	
7	glyphosate	3.0	H	5 NAWF	835 a
	glyphosate	0.75	A	1 to 2 true leaves	
	glyphosate	0.75	B	4 to 5 true leaves	
	glyphosate	0.75	C	8 node	
	glyphosate	0.75	E	1st flower	
8	glyphosate	3.0	I	20% cracked bolls	893 a
9	untreated check	0			895 a

Table 5. Cotton growth stage and height, Palmer Amaranth (AMAPA) height, and number of Ivyleaf morningglory (IPOHE) leaves at the time of the initial Roundup Weathermax topical applications along with the resulting weed control, early season cotton stunting, and mid-season cotton plant height (inches). The RR Flex cotton weed management study field at the Maricopa Agricultural Center in 2003 received a preplant-incorporated application of Prowl at 0.412 lb ai/A but was not cultivated during the season. Values are means of five replications; means within a column followed by the same letter are not significantly different at P=0.05 according to the Ryan-Einot-Gabriel-Welsch multiple range test. Means followed by (2) indicated the treatments received a second application of glyphosate by the time of the June 26 rating.

Cotton growth stage	Cotton height	AMAPA height	IPOHE leaves	Rate (lb ae/A)	% IPOHE Control 22 May	% IPOHE Control 26 June	% Cotton stunting 26 June	Cotton height 16 July
1.5 lf	2.5 in	2.5 in	2-4	0.75	66 c	90 ab (2)	0 b	40.2 a
				1.125	79 b	96 a (2)	0 b	39.4 a
				1.5	93 a	96 a (2)	0 b	39.2 a
4 lf	4 in	6 in	6-8	0.75	–	82 b (2)	6 b	37.2 a
				1.125	–	89 ab (2)	4 b	36.2 a
				1.5	–	97 a (2)	0 b	38.2 a
10 node	10 in	10-18 in	10-20	1.125	–	69 c	28 a	35.7 a
				1.5	–	75 c	28 a	36.0 a

Table 6. Ivyleaf morningglory (IPOHE) control and cotton lint yield after all herbicide applications were completed. Large morningglory (>8 leaves) were plants that emerged with cotton or early in the season while small morningglory (<4 leaf) were those that emerged mid-season or later. The percent canopy cover measure was a visual estimate of the percent of the area of the cotton canopy that had morningglory tendrils and leaves at the top of the cotton canopy. The topical applications were broadcast treatments, the post-direct applications were applied in a 24 inch band along the crop row (i.e., 16 inches across the bottom of the furrow were not treated) and the layby applications were directed-broadcast treatments. The application dates and cotton growth stages and sizes were: 1 to 2 true leaf, 11 May 2003; 4 true leaf, 22 May 2003; 10 node and 10 inches tall, 4 June 2003; 12 node and 16 inches tall, 13 June 2003; 15 node and 18 inches tall, 24 June 2003; 17 node and 24 inches tall, 27 June 2003; and 20 node and 32 inches tall, 7 July 2003. Values are means of five replications; means within a column followed by the same letter are not significantly

Trt.	Herbicide	Rate lb ae/A	Growth stage (node/in)	Appl. method	% Control large IPOHE 30 July	% Control small IPOHE 30 July	% IPOHE Canopy Cover 13 Oct.	Lint Yield (lb/A) 15 Nov.
	glyphosate	1.125	1.5 leaf	Topical				
	glyphosate	1.125	10n/10"	Topical				
2	glyphosate	1.125	15n/18"	Topical	97 a	59 ab	0 b	623 ab
	glyphosate	1.125	4 leaf	Topical				
	glyphosate	1.125	12n/16"	Topical				
4	glyphosate	1.125	15n/18"	Topical	93 a	83 ab	0 b	527 ab
	glyphosate	1.5	4 leaf	Topical				
	glyphosate	1.5	12n/16"	Topical				
5	glyphosate	0.75	15n/18"	Topical	98 a	88 ab	0 b	514 ab
	glyphosate	1.125	10n/10"	Topical				
	glyphosate	1.125	15n/18"	Topical				
6	glyphosate	1.125	20n/32"	Topical	79 b	71 ab	0 b	494 b
	glyphosate	1.5	10n/10"	Topical				
	glyphosate	1.5	15n/18"	Topical				
7	glyphosate	0.75	20n/32"	Topical	80 b	88 ab	0 b	536 ab
	glyphosate	0.75	1.5 leaf	Topical				
	glyphosate	1.5	10n/10"	Post-Direct				
8	glyphosate	1.5	17n/24"	Layby	94 a	47 bc	10 a	595 ab
	glyphosate	1.125	1.5 leaf	Topical				
	glyphosate	1.125	10n/10"	Post-Direct				
10	glyphosate	1.5	17n/24"	Layby	93 a	24 cd	6 ab	602 ab
	glyphosate	0.75	1.5 leaf	Topical				
	glyphosate	1.5	10n/10"	Post-Direct				
	glyphosate	1.5	17n/24"	Layby				
11	prometyrn	1.5*	17n/24"	Layby	95 a	89 ab	0 b	645 a
	glyphosate	1.5	1.5 leaf	Topical				
	glyphosate	1.5	12n/16"	Post-Direct				
	glyphosate	0.75	17n/24"	Layby				
	Caparol	1.5*	17n/24"	Layby				
12	Aim	0.0161*	17n/24"	Layby	95 a	85 ab	0 b	603 ab

*Application rate in lb ai/A different at P=0.05 according to the Ryan-Einot-Gabriel-Welsch multiple range test.