VISUAL FIELD SCREENING STRATEGY FOR PURITY AND SEED QUALITY IN CONVENTIONAL BREEDING NURSERIES

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Abstract

Commercial recombinant DNA technology (GE traits) within cotton (*Gossypium hirsutum*) cultivars has been rapidly and widely adopted across multiple cotton producing countries. Glyphosate resistance (GR) is the prominent technology in the U.S. and is often stacked with other GE traits. Genetically Engineered traits carry patents and have changed the legal structure of all major cotton breeding programs around the world. Cross pollination can readily transfer GE traits to unlicensed germplasm once a trait is commercialized and no longer under regulated status. Cotton is a self-pollinated crop, in which cross pollination can easily occur, and flowers over a long period due to its perennial growth habit. Volunteer plants and inadvertent mechanical mixture during seed processing are other potential sources of contamination. Currently GE traits do not alter the natural appearance of the plant; thus, adventitious presence (AP) can only be confirmed with expensive molecular analyses. A field method to visually identify GE trait carrying plants would be a beneficial tool to help manage AP in early generation breeding nurseries. Trials were conducted at Lubbock, TX in 2013 and 2014 to evaluate the potential use of low-rate broadcast glyphosate treatments to induce identifying visual differences between cotton plants "GR +" and "GR –" within a breeding nursery. Three synthetic mixtures were planted: 100% conventional cultivar, 50/50% mixed conventional and Genuity® Roundup Ready® Flex cultivars, and 100% Genuity® Roundup Ready® Flex cultivar. Multiple low rates of glyphosate were applied at multiple timings (Table 1). Four rows by 25 foot plots were arranged in a randomized complete block design, and treatments were structured as a 3^2X5 factorial. Plants were evaluated after application for incidence of herbicide response. Damaged and non-damaged plants were tested for GR trait presence. Incidence ratings were compared to actual trait presence numbers. Boll counts, yield, and multiple seed quality parameters were evaluated.

Introduction

Commercial recombinant DNA technology (GE traits) within cotton (*Gossypium hirsutum*) cultivars has been rapidly and widely adopted across multiple cotton producing countries. Glyphosate resistance (GR) is the prominent technology in the U.S. and is often stacked with other GE traits. Genetically Engineered traits carry patents and have changed the legal structure of all major cotton breeding programs around the world. Cross pollination can readily transfer GE traits to unlicensed germplasm once a trait is commercialized and no longer under regulated status. Cotton is a self-pollinated crop, in which cross pollination can easily occur, and flowers over a long period due to its perennial growth habit. Volunteer plants and inadvertent mechanical mixture during seed processing are other potential sources of contamination. Currently GE traits do not alter the natural appearance of the plant; thus, adventitious presence (AP) can only be confirmed with expensive molecular analyses. A field method to visually identify GE trait carrying plants would be a beneficial tool to help manage AP in early generation breeding nurseries.

Methods

Trials were conducted at Lubbock, TX in 2013 and 2014 to evaluate the potential use of low-rate broadcast glyphosate treatments to induce identifying visual differences between cotton plants "GR +" and "GR –" within a breeding nursery. Three synthetic mixtures were planted: 100% conventional cultivar, 50/50% mixed conventional and Genuity® Roundup Ready® Flex cultivars, and 100% Genuity® Roundup Ready® Flex cultivar. Multiple low rates of glyphosate were applied at multiple timings (Table 1). Four rows by 25 foot plots were arranged in a

randomized complete block design, and treatments were structured as a 32X5 factorial. Plants were evaluated after application for incidence of herbicide response. Damaged and non-damaged plants were tested for GR trait presence. Incidence ratings were compared to actual trait presence numbers. Boll counts, yield, and multiple seed quality parameters were evaluated.

Table 1. Glyphosate Application Treatment Factors

Seed Mixtures	
Mixture 1	100% Conventional Cultivar
Mixture 2	50% Conventional / 50% Flex Mixture (by weight)
Mixture 3	100% Roundup Ready Flex Cultivar
Glyphosate Application Timings	
Timing 1	5 Vegetative Nodes
Timing 2	8 Vegetative Nodes
Timing 3	11 Vegetative Nodes
Glyphosate Application Rate (g a.i./ha)	
Rate 1	280
Rate 2	560
Rate 3	840
Rate 4	1120
Rate 5	Unsprayed Control

Results

Chi-square Analyses

Chi-square goodness of fit analyses (Tables 2, 3, and 4) were conducted to see if herbicide injury incidence counts fit the expected distribution of glyphosate non-resistant and glyphosate resistant phenotypes in each plot. Analyses for 50/50 mixture plots were conducted for incidence counts determined at 7 days after application (DAA) and 16 DAA. Expected phenotypic values were derived by multiplying the plot stand count by 0.5 since it is expected that 50 percent of the plants were resistant and 50 percent were susceptible. All treatments that fit the expected phenotypic model at 7 DAA also fit the model at 16 DAA, and three additional treatments fit the model at 16 DAA than 7 DAA. These results suggest that 16 DAA may be a more conducive time to conduct accurate phenotyping than 7 DAA, which may be explained by the systemic action of glyphosate herbicides which results in long periods of visual injury development. Timing 1 Rate 2 (T1R2) and Timing 2 Rate 2 (T2R2) may be more advantageous than other treatments that fit the phenotypic model since these treatments have the lowest Rate, so they may be less harmful to susceptible plant yield and seed quality. Furthermore, T1R2 may be the most advantageous of all treatments since it fit the model at both 7 DAA and 16 DAA ratings, and the screening can be conducted at 5 nodes, well before bloom development occurs.

Table 2. Seed 50/50 mixture Chi-square analyses of plant glyphosate resistance phenotype calculated from 7 DAA injury incidence counts against expected ratios of planted phenotypes. Lubbock, TX - 2014

	Observed		Expected			
Treatment	**Susceptible**	**Resistant**	**Susceptible**	**Resistant**	**X^2**	**P value**
T1R1	70	154	108	116	26.12	< 0.05
T1R2	94	91	89	96	0.46	0.9 - 0.1
T1R3	98	52	72	78	17.40	< 0.05
T1R4	131	80	102	109	16.02	< 0.05
T2R1	37	176	103	110	81.68	<0 .05
T2R2	117	76	93	100	11.70	< 0.05
T2R3	117	87	99	105	6.67	< 0.05
T2R4	124	79	98	105	13.25	< 0.05
T3R1	16	116	64	68	69.25	< 0.05
T3R2	75	148	108	115	19.25	< 0.05
T3R3	76	89	80	85	0.34	0.9 -0.1
T3R4	79	79	76	82	0.18	0.9 -0.1

Table 3. Seed 50/50 mixture Chi-square analyses of plant glyphosate resistance phenotype calculated from 16 DAA injury incidence counts against expected ratios of planted phenotypes. Lubbock, TX - 2014

	Observed		Expected			
Treatment	**Susceptible**	**Resistant**	**Susceptible**	**Resistant**	**X^2**	**P value**
T1R1	63	161	108	116	36.57	< 0.05
T1R2	93	92	89	96	0.28	0.9 - 0.1
T1R3	93	57	72	78	11.25	< 0.05
T1R4	130	81	102	109	14.94	< 0.05
T2R1	19	194	103	110	132.38	< 0.05
T2R2	105	88	93	100	2.87	0.1 - 0.05
T2R3	110	94	99	105	2.57	0.9 - 0.1
T2R4	108	95	98	105	1.94	0.9 - 0.1
T3R1	26	106	64	68	43.29	< 0.05
T3R2	65	158	108	115	32.81	< 0.05
T3R3	75	90	80	85	0.54	0.9 - 0.1
T3R4	75	83	76	82	0.05	0.9 - 0.1

Table 4. Chi-square decision for null hypothesis (H_0)

Treatment	**7 DAA**	**16 DAA**
T1R1	Reject	Reject
T1R2	Don't Reject	Don't Reject
T1R3	Reject	Reject
T1R4	Reject	Reject
T2R1	Reject	Reject
T2R2	Reject	Don't Reject
T2R3	Reject	Don't Reject
T2R4	Reject	Don't Reject
T3R1	Reject	Reject
T3R2	Reject	Reject
T3R3	Don't Reject	Don't Reject
T3R4	Don't Reject	Don't Reject

Plant Height Measurements and Node Counts

Plant height (cm) was measured and total vegetative nodes were counted for five confirmed Resistant and five confirmed Susceptible plants in each 50/50 mixture plot. The plant height and node count data for each treatment as well as untreated controls are presented in Figures 1 and 2. All Timing 3 treatments had significantly greater height measurements and node counts for susceptible plants than the untreated control; possibly due to glyphosate application increasing fruit shed and resulting in increased vegetative growth of susceptible plants.

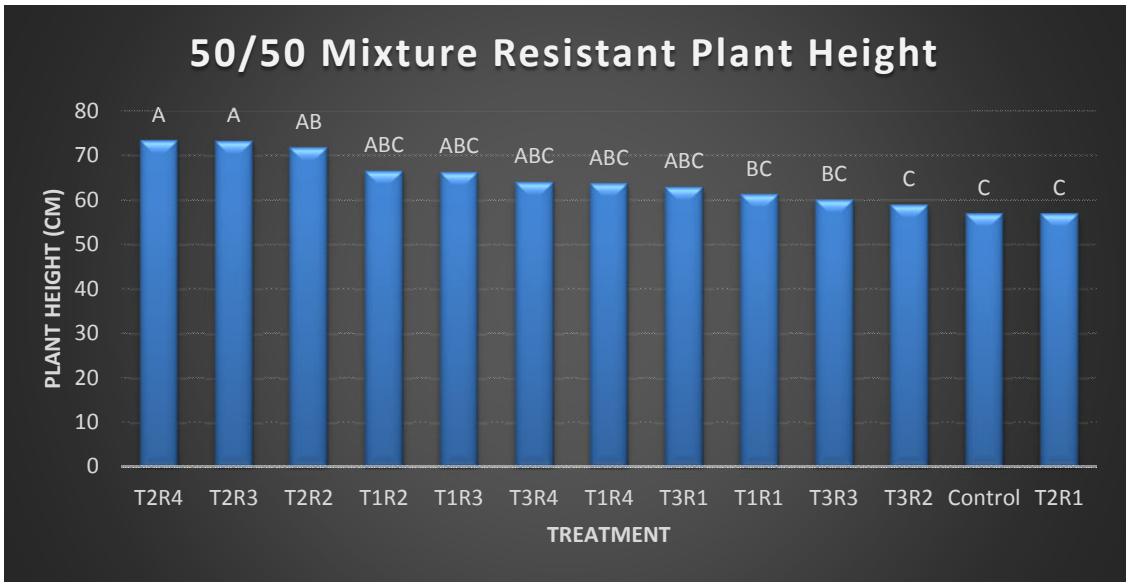

Figure 1. LS means separation of susceptible plant height among treatments.
*Bars associated with same letter are non-significant at P-value 0.05.

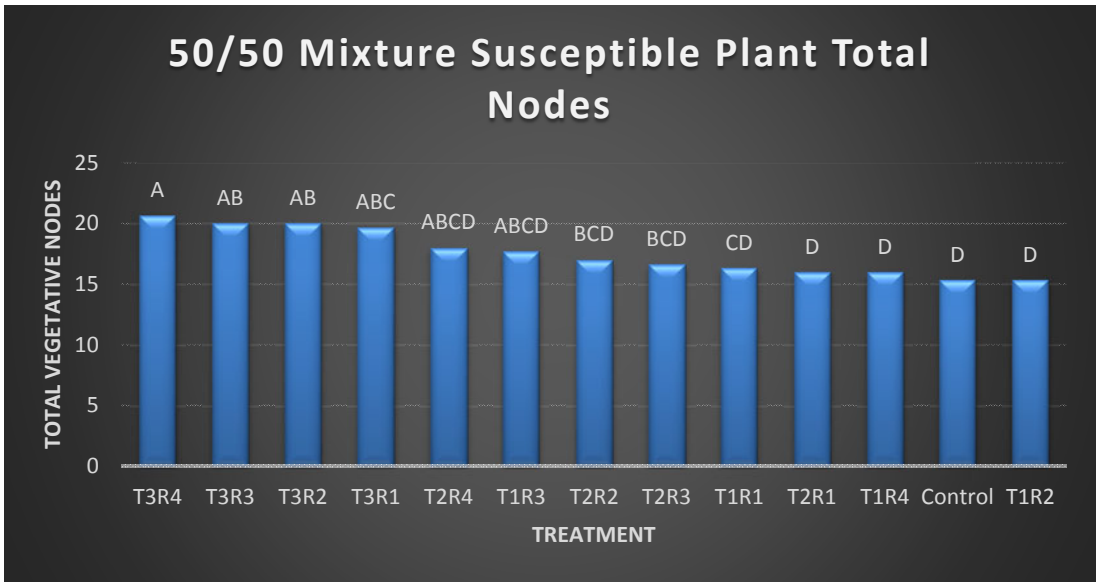

Figure 2. LS means separation of susceptible plants total vegetative node counts among treatments.
*Bars associated with same letter are non-significant at P-value 0.05.

Discussion

None of the Rate 1 treatments had phenotyping results that fit the Chi-Square model, suggesting that 280 g a.i./ha of glyphosate is not a concentrated enough rate to induce clearly visible herbicide injury symptoms that can be used for visually phenotyping resistance. Timing 1 Rate 2 and Timing 2 Rate 2 are clear candidate treatments for a screening method thus far in the study, since both treatments fit the chi-square model and Rate 2 has less potential to damage seed quality than Rates 3 and 4. Both of these candidate treatments have their advantages and disadvantages. Timing 1 Rate 2 is advantageous over Timing 2 Rate 2 since it fit the Chi-Square model at both 7 DAA and 16 DAA ratings while Timing 2 Rate 2 only fit the model at 16 DAA, and Timing 1 is earlier in the season which allows more time

to screen plots before flowering and gives damaged plants more time to recover from herbicide injury throughout the season. However, Timing 1 Rate 2 may be more damaging to susceptible plant growth than Timing 2 Rate 2 since Timing 1 susceptible plants were significantly shorter than resistant plants at the 0.05 level and Timing 2 plants were not (Table 5), suggesting that Timing 2 Rate 2 may be a more advantageous treatment in terms of less plant injury. Neither of the two treatments had significant effects on susceptible plant node counts compared to resistant plants (Table 6). Seedcotton yield and seed quality results will assist in further narrowing down a screening candidate treatment.

Table 5. Plant height (cm) comparisons between treatment phenotypes

Treatment	Resistant	Susceptible	P value
T1R1	61.33	56.80	> 0.1
T1R2	66.57	48.83	< 0.05
T1R3	66.37	56.07	> 0.1
T1R4	63.77	50.27	> 0.1
T2R1	56.97	44.80	0.1 - 0.05
T2R2	71.80	57.67	> 0.1
T2R3	73.33	52.60	0.1 - 0.05
T2R4	73.40	56.37	> 0.1
T3R1	70.10	62.93	> 0.1
T3R2	71.90	58.93	> 0.1
T3R3	72.73	60.10	0.1 - 0.05
T3R4	73.97	64.07	> 0.1
Control	56.97	52.67	< 0.05

Table 6. Vegetative nodes comparisons between treatment phenotypes

Treatment	Resistant	Susceptible	P value
T1R1	16.00	16.33	> 0.1
T1R2	17.33	15.33	> 0.1
T1R3	18.67	17.67	> 0.1
T1R4	18.33	16.00	> 0.1
T2R1	17.00	16.00	> 0.1
T2R2	18.33	17.00	> 0.1
T2R3	18.33	16.67	> 0.1
T2R4	19.00	18.00	> 0.1
T3R1	19.67	17.33	> 0.1
T3R2	20.00	16.00	0.1 - 0.05
T3R3	20.00	18.33	> 0.1
T3R4	20.67	17.33	> 0.1
Control	17.00	15.33	> 0.1

Summary

Overall these results suggest that non-lethal glyphosate applications to mixed plots of glyphosate non-resistant and glyphosate resistant cotton plants can result in altered morphological phenotypes of the different GR + and GR – genotypes that can be detected with the naked eye. Additional research is being conducted to assess glyphosate treatment effects on phenotyping accuracy, seed quality and seedcotton yield to assist in the selection of a screening strategy candidate treatment.