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Variations in Gossypol Concentration of Flower Buds of Cotton[1]

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ABSTRACT

Gossypol concentration of flower buds (squares) from selected medium to high gossypol lines of upland cotton, *Gossypium hirsutum* L., was studied both in greenhouse and field conditions.

Gossypol concentration of 20-day-old squares generally rose and fell during the growing period. Two cycles of fluctuation occurred in a high gossypol line. The gossypol concentration was independent of square size in the high gossypol line, but was negatively correlated with square size in four medium gossypol lines. Gossypol concentration of squares from greenhouse-grown plants were strongly correlated with those of squares from plants of the same genotype grown in the field. Samples of 3 to 5 squares/plant, collected during the same 12-day interval, gave reliable comparisons of the relative gossypol concentration of all lines tested.

Additional index words: ***Gossypium hirsutum,*** **Polyphenolics, Terpenoids, Insect tolerance,** ***Lepidoptera.***

COTTON (*Gossypium hirsutum* L.) possesses subepidermal glands dispersed in the aboveground plant parts and in the cotyledons of seeds. These glands have been referred to as oily bolls, oil glands, black glands, gland-dots, resin glands, and gossypol glands (11); recently workers have commonly called them pigment glands (1, 13, 15). The glands contain several pigments among which gossypol, a polyphenolic yellow pigment, is most important (8).

Gossypol has been recognized as a toxic substance since 1915 (16). It combines with some lysine during processing and thus affects the nutritive value of cottonseed meal. Extra effort for extraction or detoxification of gossypol is usually required in the processing of cottonseed oil and meal (16).

Pons et al. (10) showed variations in gossypol concentration of cottonseed from different cultivars. Lee et al. (4) later showed genetic control of gossypol concentration in cottonseed. Glandless cotton has very low gossypol concentration in the seeds (9, 12). However, glandless strains are preferred for feeding by certain insects (2, 3). Cottons with high gossypol concentration on the other hand, have increased resistance against certain insects (2, 6, 8, 11).

The growing demand for insect-resistant cotton has resulted in programs for developing varieties with high gossypol concentrations in plant tissues, a glabrous leaf surface, and nectariless leaves and flowers. Each of these characters increased resistance to certain harm-

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ful Lepidoptera species (2, 5, 7). Of the three characters, gossypol is the only antibiotic factor.

Total gossypol in the flower buds (squares) should exceed 1.2% on a dry weight basis to protect them from insect attack (6). No cultivated upland cotton contains gossypol at such a level; thus, the development of high gossypol upland cultivars is highly desirable. This objective, however, has been difficult to achieve. Accurate determination of gossypol concentration requires time-consuming chemical analysis. Also gossypol concentration in the square changes with environments and during the development of the cotton plant. Thus multiple determinations are usually required for single plants (10).

Lukefahr et al. (5) studied gossypol concentration in certain aerial parts in relation to the environment, growth stage, and variety. Gossypol concentration in the cotton squares dropped significantly at the peak fruiting stage (5). The gossypol concentration in cottonseed decreased at higher temperatures and increased with higher rainfall (10).

The present studies were undertaken to further explore changes in gossypol concentration of squares during plant development. The objective was to establish procedures for reliably distinguishing significant differences between cotton biotypes while minimizing the labor and expense of sampling and analysis.

MATERIALS AND METHODS

The present experiment was conducted during 1972. Selected strains were grown first in the greenhouse and then were transplanted to the field at the Plant Science Farm, New Mexico State University.

In the first experiment, five strains were selected:

69-102, a line developed from a single plant selection from 'Del Cerro 153' in 1968. During 1969 to 1971 this line was grown in the greenhouse and selected for type.

169-1, a line selected from segregating generations of 'XG-15' × 'Acala' and selfed for at least six generations. Selection 'XG-15' (*G. hirsutum* var. *punctatum* 'Socorro Island' × 'DPL-14') was obtained from M. J. Lukefahr, Brownsville, Tex.

489-10, a selection from 'Coker 100A' selfed for at least five generations, was obtained from J. A. Lee, North Carolina State Univ., Raleigh, N. C.

70-217-1, a selfed line selected from Lee's densely glanded 'TH-149,' which was derived from a triple-hybrid of *G. hirsutum, G. arboreum,* and *G. thurberi.*

1230-15-3, a line from Acala × 'XIII-F,' a selection from Lukefahr's (*G. hirsutum* var. *punctatum* Socorro Island × DPL-14). The segregating generations were placed under heavy selection pressure for high gossypol and large bolls; selected plants were selfed for at least four generations.

These five lines represent a broad genetical base containing a number of different gland alleles. Each has been selected for four to five generations and should be fairly homozygous for genes governing the percentage of gossypol. Among the five lines, 169-1. 70-217-1, and 1230-15-3 were originally considered high gossypol lines.

Seeds of each selected line were planted in four "Jiffy pots" (3.8 cm peat pots). When about 10 cm tall, these were transplanted to 16-cm clay pots which were randomly arranged on the greenhouse benches. The plants received both artificial light and sunlight for a total photoperiod of 14 hours.

At the beginning of the experiment, all squares were removed from the plants. Thereafter, new squares of pin-head size (1 to 1.5 mm in diameter) were tagged and dated on the day they emerged. The squares were picked 20 days after emergence. The bracts, calyx and base were removed, and the squares were placed separately in vials. The squares were then lyophilized for about 4 days. Biochemical degradation during the drying period was presumed to be minimal. Dried squares were ground to powder in a mortar and stored in a desiccator until analyzed. Each square was prepared and analyzed separately.

The procedure for gossypol analysis was suggested by the late Dr. Carl M. Lyman (personal communication).

The reagent contained (in order of addition): 200 ml isopropanol, 50 ml acetone, 600 mg p-anisidine, 150 ml isopropanol, 100 ml distilled water, and 1 ml glacial acetic acid.

The solvent contained: 350 ml isopropanol, 50 ml acetone, 100 ml distilled water, and 1 ml glacial acetic acid.

Fifty milligrams of ground square powder were placed in a test tube and 10 ml reagent added. The tube was capped and heated in a water bath for 1 hour at 65 C. The tube was cooled to room temperature and the solution filtered through Whatman #1 paper into a clean test tube. One ml of the filtrate was diluted with 9 ml of solvent, and the absorbance was read at 447 nm.

A standard curve was prepared using pure gossypol obtained from USDA Southern Regional Laboratory. New Orleans, La.

The p-anisidine method gives total gossypol readings slightly lower than Smith's aniline test (14). Readings obtained from the two methods are very highly correlated $r = 0.99^{**}$ (bud gossypol) and 0.93^{*} (seed gossypol). (*, ** means significant at the 0.05 and 0.01 levels, respectively.)

In the second experiment, all the plants except those of 489-10 were cut back to 10 to 15 cm and transplanted to a field of light sandy soil free of verticillium wilt.

Plants were arranged in the field in 102 cm rows with a 30.5 cm spacing between each plant in the row. Again, all the squares were removed at the beginning of the experiment. Tagging of squares and gossypol determination were the same as in the previous experiment. Samples of squares were kept on ice in a polystyrene chest, until they were carried to the laboratory for lyophilization. One or two squares per plant were tagged for each time period, but only one 20-day-old square was collected from each plant every 4 days.

RESULTS AND DISCUSSION

Experiment 1: Gossypol Content of Greenhouse-grown Cotton Squares

All cotton lines irregularly produced new squares. Throughout the growing season there were unproductive periods of 2 to 3 days followed by prolific periods of 5 to 7 days. After 3 weeks of fruiting, the size and number of squares produced were greatly reduced.

The gossypol concentration of squares from individual plants varied at different stages of development. A gradual increase of gossypol concentration was generally observed in squares initiated during the first 2 weeks of square differentiation. Thereafter the gossypol concentration of additional squares decreased.

Lines 169-1 and 1230-15-3, previously considered moderately high gossypol lines, had only a moderate concentration of gossypol in this experiment. Line 70-217-1 had the highest gossypol concentration. However, it also showed the most variation in gossypol concentration ranging from 0.93% to as high as 3.83% for individual squares with a mean value of 2.22%. By contrast, gossypol concentration in squares of the other four lines showed less variation throughout the growing period: 0.91% to 1.86% for 169-1, 0.98% to 1.81% for 69-102, 1.00% to 1.67% for 1230-15-3, and 0.88% to 1.57% for 489-10; means were 1.41%, 1.36%, 1.34%, and 1.22%, respectively. Gossypol concentration in squares of the high gossypol cotton plants might be more strongly affected by changes in metabolism as the plants develop.

Analysis of variance was performed using the means from each 12-day period of square development (Table

Table 1. Average gossypol concentration of 20-day-old flower buds from four individual plants of five lines of cotton initiated during intervals after initial square formation.

Strain	Plant	Developmental period† 1-12 days	13-24 days	25-36 days	37-48 days	Strain means
		Gossypol (% dry wt)				
70-217-1	1	2.00	2.10	2.68	2.24	
	2	1.76	1.68	2.18	1.95	
	3	2.18	1.68	2.73	1.93	
	4	2.74	2.34	2.92	2.34	2.22
169-1	1	0.93	1.32	1.77	--	
	2	1.21	1.20	--	1.63	
	3	1.37	1.49	--	1.55	
	4	1.00	1.16	1.61	1.38	1.41
69-102	1	1.19	1.43	1.73	1.31	
	2	1.21	1.51	1.46	1.28	
	3	1.08	1.09	1.48	1.37	
	4	1.23	1.45	1.59	1.31	1.36
1230-15-3	1	1.16	1.34	1.56	1.27	
	2	1.17	1.36	1.70	--	
	3	1.15	1.37	1.58	--	
	4	1.09	1.37	1.22	1.44	1.34
489-10	1	0.99	1.04	1.37	1.15	
	2	1.07	1.23	1.58	1.23	
	3	1.10	1.14	--	1.26	
	4	0.99	1.08	1.46	1.37	1.22
Period means		1.33	1.42	1.77	1.51	

† Gossypol level shown in % based on 50 mg dry weight samples. All squares were stripped from the plant at the beginning of the experiment (0 days). Subsequent squares were tagged when initiated (1 to 1.5 mm diam.) and harvested when 20 days old to determine gossypol. The developmental period indicates when the square was tagged.

1). The specific interval of the periods was selected arbitrarily but was used uniformly for each period and line. The least squares method was used in the calculations because no reading could be obtained for some plants in some periods (Table 1).

The analysis of variance (Table 2) revealed highly significant differences in gossypol concentration among both lines and periods. The means of lines and periods were compared using FLSD (Fisher's least significant difference) procedures. Four lines were divided into three significantly different categories: 70-217-1 was alone as the high gossypol line; 169-1 and 69-102 formed an intermediate group; and 489-10 was the low gossypol line. Line 1230-15-3 contained gossypol amounts insignificantly different from either of the last two groups. The concentration of gossypol in the line 70-217-1 was 60 and 82% higher than the intermediate and the lowest groups, respectively, while the latter two groups differed by only 14%.

The gossypol concentration in the squares initiated at 0 to 12, 25 to 36 and 37 to 48 days after initial bud formation were significantly different and was highest in squares initiated at 25 to 36 days. Squares initiated at 13 to 24 days had concentration between those initiated at 0 to 12 and 36 to 48 days but were not significantly different.

The non-significant interaction between lines and periods suggests that sampling during the first 12 days after fully grown squares appear is a valid method to compare lines. All lines were affected by periods to an approximately equal degree. Analysis of the gossypol variations in squares suggests that several samples should be collected during the 12-day period. Other periods of development also may be used as long as lines are in the same stage of development.

Table 2. Analysis of variance of mean bud gossypol concentrations at four different 12-day periods in five cotton lines in the greenhouse.

Source of variation	df	Mean square	F
Total	73	0.2047	
Reduction	19	0.6865	
Lines	4	2.5238	71.90**
Periods	3	0.6390	18.21**
Lines X Periods	12	0.0464	1.32
Error	54	0.0351	

** Indicate statistical significance at the 0.01 level.

$$C.V. = \frac{\sqrt{0.0351}}{1.51} \times 100 = 12.38$$

Table 3. Correlation coefficients between gossypol concentrations and fresh weights of 20-day-old flower buds.

Line	r	n	Mean gossypol	Mean fresh sq. wt.
			%	g
169-1	-0.533**	30	1.315	0.173
489-10	-0.526**	39	1.148	0.229
69-102	-0.599**	59	1.322	0.297
70-217-1	-0.080	75	2.171	0.267
1230-15-3	-0.566**	54	1.291	0.247

** Indicates statistical significance at the 0.01 level.

Our results agree with those of Lukefahr et al. (5), if what they have called the peak fruiting stage is considered to be equivalent to the 37 to 48-day period of bud formation in the present study. In their study the squares from the peak fruiting stage were found to contain less gossypol than those collected earlier. "Earlier" in their study was not precisely defined, but would appear to correspond to the 25 to 36-day period in our study, because squares from the 0 to 12 and 13 to 25-day periods contained no more gossypol than those from the 37 to 48-day period.

Therefore, the correlation coefficients between gossypol concentration and the fresh square weight were calculated for each line. All the lines showed highly significant negative correlations (Table 3), except 70-217-1 which showed a negative, but non-significant, correlation. Thus, within lines small squares had higher gossypol concentration than larger squares of the same age produced at the same stage of plant development. To avoid any complications due to square size all squares collected for gossypol determination should be about the same size.

Experiment 2: Gossypol Content of Field-grown Cotton Squares

The samples produced in the field were collected every 4 days from each plant for 24 days. Results from samples collected on the first and last three dates were also grouped and analyzed as two periods. Table 4 shows the results of the gossypol determination. As most plants ceased abundant square production after six samples were collected, sampling was terminated 24 days after the date the first sample was collected.

The comparison of the results between those from the greenhouse and from the field for the five lines which were common in the two trials showed considerable similarity in their relative gossypol concentrations. The correlation coefficient between mean

Table 4. Gossypol concentrations of 20-day-old squares sampled in the field at 4-day intervals in two periods on seven breeding lines.†

Line	Plant	Date of sampling			Period mean	Date of sampling			Period mean	Mean % gossypol
		25 July	29 July	2 Aug.		6 Aug.	10 Aug.	14 Aug.		
		%				%				
70-217-1	1	2.36	2.62	2.40	2.46	1.59	1.56	2.11	1.75	
	2	2.52	--	1.85	2.19	2.07	1.33	1.42	1.61	
	3	2.88	3.40	2.47	3.92	1.36	1.97	2.40	1.91	2.14
169-1	1	1.64	1.58	1.44	1.55	1.59	1.23	1.32	1.38	
	2	1.64	1.90	1.76	1.77	1.03	1.19	1.10	1.11	
	3	2.14	2.30	1.51	1.98	1.99	1.41	1.46	1.62	1.57
1230-15-3	1	1.50	1.48	1.41	1.46	1.39	1.26	1.58	1.41	
	2	1.48	1.55	1.31	1.45	1.08	1.81	1.32	1.07	
	3	1.50	1.72	1.41	1.54	1.09	1.17	1.36	1.21	1.36
69-102	1	1.48	1.48	1.44	1.47	1.36	1.07	1.27	1.23	
	2	1.26	1.45	1.28	1.33	0.90	1.87	1.12	1.96	
	3	1.55	1.60	1.53	1.56	--	1.11	1.27	1.19	1.30
489-10	1	1.22	1.24	1.16	1.21	1.24	1.35	1.28	1.29	
	2	1.22	1.55	1.64	1.47	1.35	1.05	1.26	1.22	
	3	--	--	1.30	1.30	1.29	1.07	1.35	1.24	1.29

† % gossypol based on 50 mg dry weight samples.

gossypol concentration of squares from lines in the greenhouse and field ($r = 0.93$) was highly significant. Thus, the relative gossypol content of the cotton plant in the field can be estimated accurately from the gossypol levels determined in the greenhouse.

Analysis of variance was also performed using gossypol concentration for period means and lines corresponding to those tested in the greenhouse (Table 5). Again, differences between lines and periods were highly significant, and the interaction between the lines and periods was not significant. The mean gossypol concentration of lines 69-102 and 1230-15-3 were reversed in the field compared to the greenhouse, but these two means did not differ significantly in either test. This difference suggests that some change in the gossypol concentration of the squares occurs if the environment of the cotton plant is changed or if you grow it from a "stubbed-off" transplanted plant.

Based on our tests we would conclude that differences in square gossypol concentration between plants can best be determined by collecting and analyzing 3 to 5 squares produced on each plant during the same 12-day interval from the first initiation of flower buds. Plants may be artificially equalized in square development by debudding early lines until squares are initiated in late lines. Selection of significant small differences in gossypol concentration of less than 0.2% dry weight are not possible by this method.

Table 5. Analysis of variance of period means of gossypol level from the field test of five lines previously used in the greenhouse test.

Source of variation	df	Mean square	F
Total	29		
Lines	4	0.7747	22.85**
Periods	1	0.9953	29.36**
Lines X Periods	4	0.0961	2.83
Residual	20	0.0339	

** Indicate statistical significance at the 0.01 level.

$$C.V. = \frac{\sqrt{0.0339}}{1.53} \times 100 = 12.03$$

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