Published January, 1980

Variability in Flower-Bud Gossypol Content and Agronomic and Fiber Properties within the Primitive Race Collection of Cotton[1]

R. H. Dilday and T. N. Shaver[2]

ABSTRACT

Forty-one accessions from the "race" (Texas race stock) collection of cotton, *Gossypium hirsutum* L., were grown in Tuxpan, Veracruz, Mexico and analyzed for flower-bud gossypol content and agronomic and fiber properties. The selected accessions are from diverse points of origin and represented four distinct growth types ("races"). Flower buds were harvested seven times from November 1974 to March 1975 and seedcotton was harvested in April 1975. Terpenoid aldehyde percentages (including gossypol) from flower buds harvested on the fourth date were significantly higher than from those harvested at other dates. The highest mean terpenoid aldehyde percentages were shown by Texas 766 (T-766; 1.33%), T-197 (1.29%), T-663 (1.27%), T-805 (1.23%), T-1150 (1.20%), and T-216 (1.15%). Nineteen accessions produced significantly higher percentages ($\geq$ 0.88%) than the check, Stoneville 213 (0.74%), and 12 accessions showed levels above 1.0%. Seven accessions showed significantly lower levels ($\leq$ 0.60%) than the check. Correlation coefficients between terpenoid aldehyde percentages and both boll size and seed index were negative and significant, but correlations between terpenoid aldehyde percentages and seed/locule, lint index, lint percentage, and micronaire were not significant. However, two of the six accessions (T-197 and T-216) that produced the greatest terpenoid aldehyde contents from flower buds had significantly larger bolls, more seed/locule, and higher seed index than the others. These two accessions and T-805 had a higher lint index, and T-216 and T-805 had a significantly higher lint percentage than the other high terpenoid entries in the test. Thus, we selected good combinations of terpenoid aldehyde percentages and agronomic and fiber properties in spite of certain negative correlations.

Additional index words: Insect resistance, Breeding stocks, Terpenoids, Polyphenolics, *Gossypium hirsutum* L.

GOSSYPOL and related terpenoid aldehydes occur naturally in plants of the genus *Gossypium* and are usually enclosed in glands. It was suggested in the early 1900's that these glands were a source of natural resistance to insect pests (3, 9). Bottger et al. (2) showed a negative relationship between gossypol content in the plant and growth and development of larvae of the cotton bollworm, *Heliothis zea* (Boddie). Later tests showed that bollworm larval weights decreased and that developmental time and mortality percentages increased when the level of gossypol was increased in the bollworm diet (1, 8). Bottger and Patana (1) suggested that potential sources of high levels of gossypol were "Arizona Wild Cotton," *G. thurberi* Tod., and "Socorro Island Wild," a strain of *G. hirsutum* L. A high-gossypol strain from Socorro Island Wild (XG-15) was developed and tested in large screen cages for resistance to the tobacco budworm, *Heliothis virescens* (Fabricius). These tests showed that the high-gossypol material had fewer eggs, larvae, damaged flower buds (squares), and damaged bolls than the check material which had normal levels of flower-bud gossypol (7).

Since the relationship between gossypol content and resistance to the *Heliothis* complex has been established, extensive efforts have been made to develop agronomically competitive breeding stocks of cotton with flower buds containing at least 1.2% gossypol, about twice the amount found in current cultivars. Socorro Island Wild has been the single high gossypol or terpenoid aldehyde source used most often in *G. hirsutum* breeding programs. Although agronomic improvements have been achieved, the development of agronomically competitive breeding stocks has been slow (10), probably because of the limited genetic diversity of the high-gossypol material. Furthermore, developing resistance to one or two insects from a single germplasm source can lead to widespread susceptibility to other insects and diseases such as the corn leaf blight, *Helminthosporium maydis*, epidemic encountered in 1970. Therefore, Dilday and Shaver (4) surveyed the *G. hirsutum* "race" collection for flower-bud gossypol content and have identified several stocks that produce high levels.

The present study was designed to evaluate several *G. hirsutum* stocks grown under the same environment and to determine the following: 1) the variation in the flower-bud content of gossypol and related terpenoid aldehydes within a single season, and 2) the relationship of the flower-bud content of gossypol and related terpenoid aldehydes to agronomic and fiber properties of selected breeding stocks.

MATERIALS AND METHODS

The 56 accessions selected for this test came from the regional *G. hirsutum* primitive race collection, commonly called the Texas race collection. They represented: (a) diverse points of origin (Guatemala, El Salvador, Honduras, British Honduras, the Bahamas, USSR, and the Mexican States of Chiapas, Oaxaca, Guerrero, and Yucatan); (b) four distinct "races" (*punctatum, latifolium, morrilli*, and *richmondi*); and (c) various levels of flower-bud gossypol and related terpenoid aldehydes. The check materials selected for the test were two commercial cultivars ('Stoneville 213' and 'Deltapine 16'); a standard genetic marker (TM-1), and Socorro Island Wild, the original source of high flower-bud gossypol (*G. hirsutum*, "race" *punctatum*; Texas 934).

Seeds of the 56 accessions from the race collection and the four checks were planted at Brownsville, Texas, in expanded peat pellets in August 1974. Because of the photoperiodic growth habit of some of the accessions, the seedlings were transferred to Tuxpan, Veracruz, Mexico, when they were about 2 weeks old. Fifteen seedlings from each entry were transplanted to the field 57 cm apart in rows 1 m wide. Each entry was replicated four times (total of 60 seedlings/entry) in a randomized complete block design.

Flower buds were collected seven times: 22 Nov., 5 and 18 Dec. 1974; 8 and 18 Jan., 12 Feb., and 5 Mar. 1975. Approximate-

[1] Cooperative investigation of the SEA, USDA, and the Texas Agric. Exp. Stn., Texas A&M Univ., College Station, TX 77843. Received 8 June 1979.

[2] Research geneticist and research chemist, respectively, SEA, USDA, Brownsville, TX 78520. Present address of T. N. Shaver is Cotton Insects Research Laboratory, SEA, USDA, College Station, TX 77840.

ly 15 full-grown flower buds were harvested at random from each plant from each entry, quick-frozen on dry ice and transferred to Brownsville, Texas, where they were debracted, freeze-dried, and later analyzed for flower-bud gossypol and related terpenoid aldehyde percentage (dry weight basis). Total gossypol and related terpenoid compounds were determined by a colorimetric method involving reaction with aniline (12). The values obtained are referred to as gossypol percentages in this paper. Mean gossypol percentages in the entries from the race collection were compared statistically to percentages in the checks by Duncan's multiple range test. Seedcotton was harvested in April 1975. Boll size (g seedcotton/boll), number of seed/locule, seed index (g/100 seed), lint index (grams of lint from 100 seed), lint percentage and micronaire, were determined for each entry. Correlation coefficients for gossypol percentage and each of the agronomic and fiber properties were determined for the accessions. Coefficients fitting data points X and Y to $Y = AX^b$ based on the least-square-fit power curve for agronomic and fiber properties at varying levels of gossypol were calculated based on actual values of the accessions.

Because of the differences in maturity of the material, only 18 accessions and the 3 day-neutral checks were harvested on the first date (22 Nov. 1974). Fifteen additional accessions were harvested on the second date (5 Dec. 1974), and 41 accessions were harvested on the third date (18 Dec. 1974). Entries that did not produce flower buds from the third through the seventh harvest dates were not included in this test. Only 33 of the 41 accessions produced enough seedcotton by April 1975 for fiber analysis.

RESULTS AND DISCUSSION

Performance of Check Cultivars

The gossypol content of Stoneville 213 was significantly greater than that of Deltapine 16 or TM-1. The agronomic and fiber properties of the three checks were not significantly different from each other (Table 1). Data from Stoneville 213 were used as the standard for further comparisons with the accessions.

The check having high flower-bud gossypol, Socorro Island Wild, did not initiate flower buds during the growing season; therefore, direct comparisons between this entry and the remaining accessions were not possible. In another environment Fryxell and Moran's (6) description of Socorro Island Wild was: growth habit – prostrate; fruit – small (2.5 to 3.0 cm long, about 2 cm wide), three-loculed (4 to 5 seed/locule); lint – tan, short (3/4 in. staple), very fine and sparse; seed coat – impermeable. Flower-bud gossypol content (1.28%) and such additional characters as boll size (0.5 g/boll) and seed index (6.2), have been reported in different environments for Socorro Island Wild (7, 13).

Seasonal Variation in Gossypol Content

The 41 accessions harvested prior to the fourth harvest were analyzed as three distinct groups based on their consecutive harvest dates. Eighteen accessions (group 1) ranging from 0.54 to 1.30% ($\overline{X}$ = 0.86%) in gossypol were harvested on all seven dates. Fifteen accessions (group 2) ranging from 0.54 to 1.31% ($\overline{X}$ = 0.85%) in gossypol were harvested from the second through the seventh date, and eight accessions (group 3) ranging from 0.47 to 1.11% ($\overline{X}$ = 0.78%) were harvested from the third through the seventh harvest dates. The three checks were harvested on all seven dates and had a mean gossypol content of 0.66% (Fig. 1). Significant differences occurred among entries and harvest dates within each group and the check materials. Gossypol percentages of flower buds harvested on the fourth date were significantly greater than those harvested on all other dates in groups 1, 2, and the checks. In group 3, however, gossypol percentages in flower buds were not significantly different at the third and fourth dates. Gossypol percentages produced on these two dates were significantly greater than those produced on the other three dates. Furthermore, the gossypol percentages produced on the sixth harvest date were significantly lower than those produced on any other date in groups 1 and 2.

Because group 1 represented all seven harvest dates, it was separated further into three distinct groups, as follows: group 1-A (> 1.00% flower-bud gossypol, $\overline{X}$ = 1.11%); group 1-B (0.70-0.99%; $\overline{X}$ = 0.82%); and group 1-C (0.50-0.69%; $\overline{X}$ = 0.59%). Figure 2 shows that gossypol percentages differed from date to date. Maximum gossypol percentages were produced by flower buds harvested on the fourth date, and minimum percentages were produced by those harvested on the sixth date. Most differences between dates were significant. Because the three groups each initiated flower buds at different dates, these results suggested that the flower-bud gossypol content was not controlled by time of bud initiation but was influenced by environmental factors or plant age.

Table 1. Comparisons of flower-bud gossypol percentages and agronomic and fiber properties of three day-neutral cotton checks.

Designation	Gossypol	Boll size	Seed/locule	Seed index	Lint index	Lint	Micronaire
	%	g	no.	g	g	%	units
Stoneville 213	0.74 a*	5.00 a	6.89 a	11.03 a	8.10 a	42.3 a	4.85 a
Deltapine 16	0.62 b	4.51 a	5.51 a	10.80 a	7.45 a	40.5 a	4.75 a
TM-1	0.63 b	4.26 a	6.25 a	10.04 a	6.57 a	40.2 a	5.60 a

* Means in a column followed by the same letter are not significantly different as determined by Duncan's multiple range test at the 0.05 level of probability.

Table 2. Mean gossypol percentages from the third to seventh harvests, for 41 cotton accessions that produced flower buds and the check, Stoneville 213.

Texas race stock no.	Gossypol	Texas race stock no.	Gossypol
	%		%
766	1.33	490	0.83
197	1.29	606	0.83
663	1.27	481	0.75
805	1.23	489	0.75
1150	1.20	119	0.74
216	1.15	1134	0.73
1036	1.11	664	0.70
102	1.08	461	0.68
495	1.07	1161	0.66
187	1.04	605	0.65
665	1.04	194	0.65
487	1.02	932	0.63
114	0.94	163	0.61
115	0.94	171	0.59
165	0.93	27	0.58
497	0.93	674	0.56
144	0.92	707	0.55
258	0.92	301	0.55
152	0.88	146	0.54
231	0.86	306	0.47
642	0.86	Stoneville 213	0.74

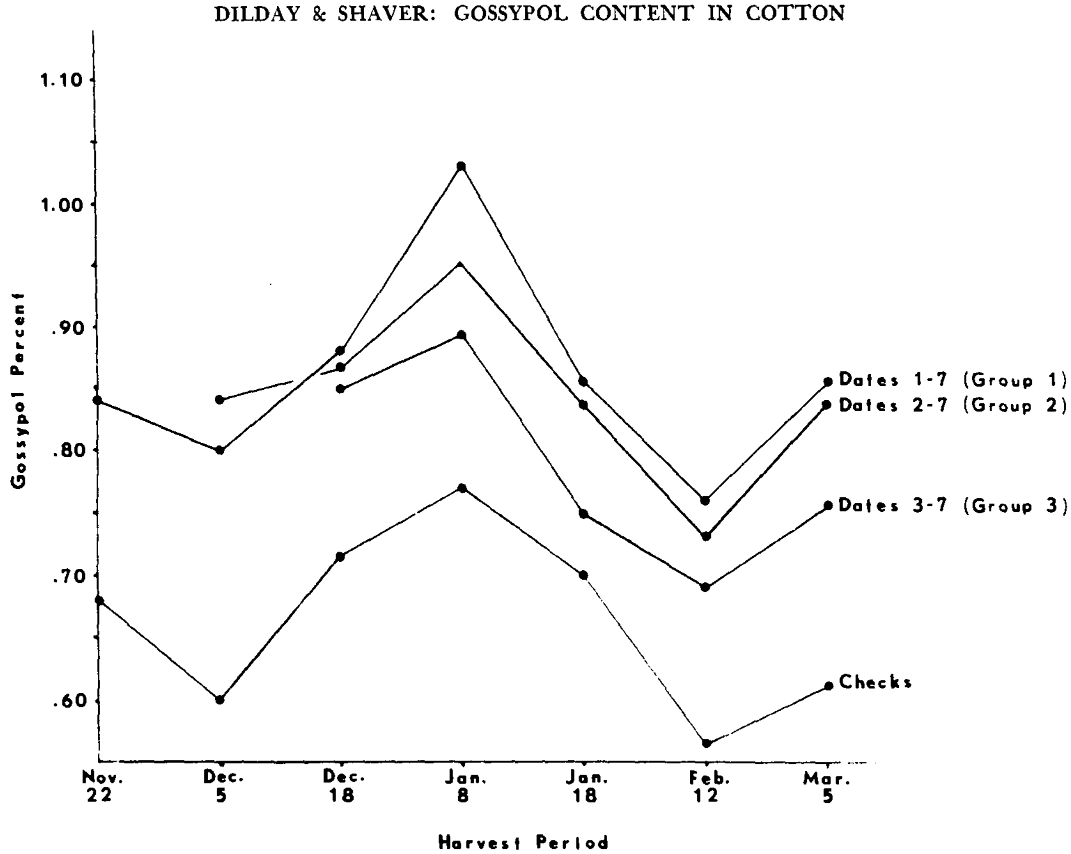

Fig. 1. Variation in flower-bud gossypol content in three groups of cotton accessions that initiated flowerbuds during three separate periods and in three checks, harvested at different dates during the growing season.

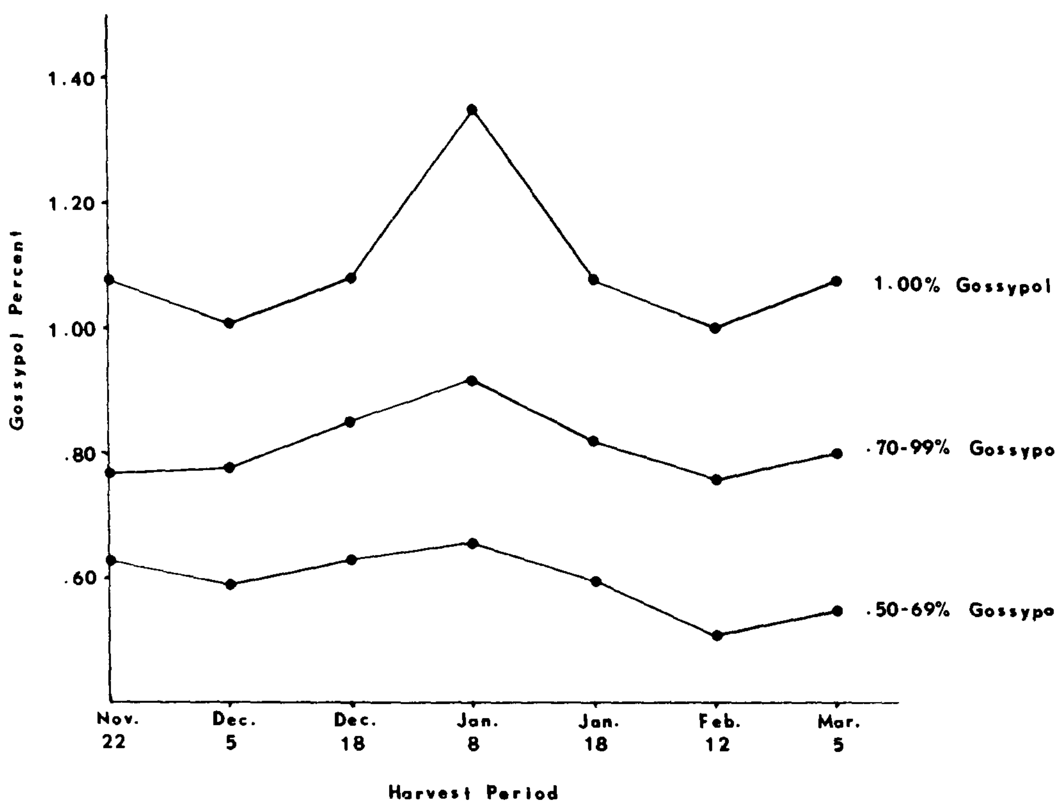

Fig. 2. Within-season variation in flower-bud gossypol percentages of 18 cotton accessions harvested on different dates and divided into three groups based upon their seasonal gossypol average.

Table 3. Correlation coefficients between flower-bud gossypol percentages and six agronomic and fiber properties for 33 Texas race stocks.

Character correlated with gossypol percentage	Correlation coefficient
Micronaire	0.330
Lint percentage	−0.037
Seed per locule	−0.187
Lint index	−0.248
Boll size	−0.387*
Seed index	−0.506*

* Significant at the 0.05 level of probability.

Table 4. Agronomic and fiber properties of the six cotton accessions having the highest flower-bud gossypol percentages and the check, Stoneville 213.

Texas race stock no.	Gossy-pol	Boll size	Seed/ locule	Seed index	Lint index	Lint	Micro-naire
	%	g	no	g	g	%	units
197	1.29 a*	3.70 b	7.12 a	9.62 b	4.20 bc	30.32 c	5.65 a
216	1.15 a	3.38 b	7.32 a	9.31 b	4.68 b	33.60 b	5.25 a
663	1.27 a	1.45 d	3.80 b	6.74 d	2.44 d	26.62 d	--
766	1.33 a	1.51 cd	4.15 b	6.60 d	2.56 d	28.40 cd	5.65 a
805	1.23 a	1.94 c	3.92 b	8.26 c	4.07 c	33.10 b	4.40 a
1150	1.20 a	1.33 d	4.75 b	6.05 d	2.29 d	27.90 cd	4.75 a
Check:							
Stoneville 213	0.74 b	5.00 a	6.82 a	11.03 a	8.10 a	42.30 a	4.85 a

* Means in a column followed by the same letter are not significantly different as determined by Duncan's multiple range test at the 0.05 level of probability.

Gossypol Content

The mean gossypol percentages of all the entries were calculated from harvest dates three through seven because of seasonal variation. Nineteen accessions had significantly higher gossypol percentages (≥ 0.88%) than Stoneville 213 (0.74% gossypol with 95% confidence limits of ± 0.14%) and 12 accessions produced over 1.0% gossypol (Table 2). Seven accessions had significantly lower gossypol percentages (<0.60%) than Stoneville 213.

The accessions which produced the highest gossypol percentages also had the greatest seasonal range. For example, the average seasonal range of gossypol was 0.44% for the six accessions that produced the highest percentages, and it was 0.20% for the six accessions that produced the lowest percentages. Furthermore, gossypol percentages of the six highest accessions ranged from 1.33 to 1.72% at the fourth harvest, but from 0.96 to 1.11% at the sixth harvest. These results emphasize the desirability of calculating gossypol percentages as means of several harvests rather than of a single harvest, especially for high-gossypol breeding stocks. These results also emphasize the importance of not comparing gossypol percentages from material harvested at different times during the growing season.

Previous screening trials at Veracruz, Veracruz, Mexico, in 1971-72 and 1972-73 revealed a significant difference between seasons in gossypol percentages (5). Included in the present test were 24 accessions screened at Veracruz in 1971-72 and eight accessions screened in 1972-73. Their seasonal gossypol percentages were compared to those from the same accessions grown at Tuxpan in 1974-75 for these tests. The 24 accessions averaged 1.35% gossypol at Veracruz, whereas they averaged 0.83% at Tuxpan. The difference between seasons was significant, but gossypol percentages of the 24 accessions from 1971-72 were significantly correlated ($r = 0.69$) with the same entries from 1974-75. The eight accessions at Veracruz averaged 0.94% gossypol and the same accessions grown at Tuxpan averaged 0.97% gossypol. The difference between seasons was not significant and the gossypol levels were significantly correlated ($r = 0.92$) for the two seasons.

The reported minimum level of gossypol needed to suppress *Heliothis* populations is 1.2% (7, 8). However, lower concentrations of gossypol have affected *Heliothis* larval growth adversely (11). Our results, which add another dimension, show that the range in flower-bud gossypol percentages during the growing season, as well as the variation between seasons also could be important factors in plant-insect interrelationships.

Table 5. Expected values of agronomic and fiber properties of the six cotton accessions with the highest gossypol percentages estimated with the "least-square-fit power curve."

Texas race stock no.	Boll size	Seed/ locule	Seed index	Lint index	Lint	Micro-naire
	g	no.	g	g	%	units
197	1.71	4.86	6.57	2.83	27.89	5.0
216	1.83	4.94	6.92	2.96	27.96	4.9
663	1.73	4.87	6.61	2.85	27.90	5.0
766	1.69	4.84	6.51	2.80	27.88	5.0
805	1.75	4.89	6.70	2.88	27.92	4.9
1150	1.78	4.91	6.77	2.90	27.93	4.9

Agronomic and Fiber Properties

Gossypol percentage was significantly negatively correlated with boll size and seed index (Table 3). Stoneville 213 had significantly larger bolls, higher lint index, higher seed index, and higher lint percentage than the six accessions that produced the highest gossypol percentages (Table 4). However, comparisons among the six accessions revealed that T-197 and T-216 differed significantly from each other in lint percentage only and that both of these accessions had significantly larger bolls, more seed/locule, and a higher seed index than the other four accessions. Lint index was significantly higher in T-197, T-216, and T-805 than in the other three accessions, and lint percentage was higher in these latter two than in the other accessions.

Because the six accessions that produced the highest level of flower-bud gossypol differed significantly in agronomic and fiber properties, estimated values based on the least-square-fit power curve for various levels of gossypol were calculated from the actual values of the accessions for the agronomic and fiber properties. Among the six accessions with the highest gossypol percentages, the actual values of T-197 and T-216 exceeded the calculated values for each of the agronomic and fiber traits (Table 5). Thus we selected new germplasm possessing high levels of terpenoid aldehydes combined with agronomic and fiber properties (larger bolls, seed index, and lint index, greater lint percentage, and more seed/locule) that were significantly greater than those expected in spite of certain negative correlations.

ACKNOWLEDGMENTS

The authors thank R. J. Kohel and P. A. Fryxell for furnishing the cottonseed; J. N. Brevard and T. J. Proske, for fiber analysis; and R. C. Toledo for assistance in maintaining the nursery.

REFERENCES

1. Bottger, G. T., and R. Patana. 1966. Growth, development, and survival of certain Lepidoptera fed gossypol in the diet. J. Econ. Entomol. 59:1166-1168.
2. ———, E. T. Sheehan, and M. J. Lukefahr. 1964. Relation of gossypol content of cotton plants to insect resistance. J. Econ. Entomol. 57:283-285.
3. Cook, O. F. 1906. Weevil-resisting adaptations of the cotton plant. USDA Bull. 88.
4. Dilday, R. H., and T. N. Shaver. 1976. Survey of the regional *Gossypium hirsutum* L. primitive race collection for flowerbud gossypol. USDA Rep ARS-S-80.
5. ———, and ———. 1976. Survey of the regional *Gossypium hirsutum* L. primitive race collection for flowerbud gossypol and seasonal variation between years in gossypol percentage. USDA Rep. ARS-S-146.
6. Fryxell, P. A., and R. Moran. 1963. Neglected form of *Gossypium hirsutum* on Socorro Island, Mexico. Emp. Cotton Grow. Rev. 49:289-291.
7. Lukefahr, M. J., and J. E. Houghtaling. 1969. Resistance of cotton strains with high gossypol content to *Heliothis* spp. J. Econ. Entomol. 62:588-591.
8. ———, and D. F. Martin. 1966. Cotton plant pigments as a source of resistance to the bollworm and tobacco budworm. J. Econ. Entomol. 59:176-179.
9. Quaintance, A. L., and C. T. Brues. 1905. The cotton bollworm. USDA Bur. Entomol. Bull. 50.
10. Sappenfield, W. P., L. G. Stokes, and K. Harrendorf. 1974. Selecting cotton plants with high square gossypol. Proc. Beltwide Cotton Prod. Res. Conf. p. 87-92.
11. Shaver, T. N., R. H. Dilday, and J. A. Garcia. 1978. Interference of gossypol in bioassay for resistance to tobacco budworm in cotton. Crop Sci. 18:55-57.
12. Smith, F. H. 1967. Determination of gossypol in leaves and flowerbuds of *Gossypium*. J. Am. Oil Chem. Soc. 44:267-269.
13. U.S. Agricultural Research Service. 1974. The regional collection of *Gossypium* germplasm. USDA Rep. ARS-H-2.