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Strain and Within-Season Variability of Various Allelochemics Within A Diverse Group of Cottons[1]

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ABSTRACT

Several secondary plant metabolites are alleged to confer resistance of cotton, *Gossypium hirsutum* L., to various insects. The effects of these compounds on the insects, variability among cotton strains, and inheritance have been studied by various workers. Research on seasonal variability and on geotype × environment interactions is sparse. These findings prompted our research to determine the effects of within-season environment and cotton strains on allelochemics alleged to be important in repelling the tobacco budworm, *Heliothis virescens* (F.).

Data were recorded on samples collected at 10 dates from 20 cotton strains in replicated field plots. The following allelochemics were quantified: total phenolics, gossypol and its analogs, a flavonoid-anthocyanin mixture, and three tests for condensed tannins (tannin, E1,1, and catechin). Significant differences among cotton strains, sample dates, and significant interaction between these main effects were detected. However, the interaction components were small relative to the main effects. These results suggest that differences in the levels of allelochemics are inherited and that genetic effects among cotton strains should be manageable in breeding programs.

Additional index words: Host plant resistance, Genotype × environment interaction, *Heliothis virescens* (F.), Allelochemistry.

THE development of cottons, *Gossypium hirsutum* L., with high levels of terpenoids and good agronomic characters has been a high priority of cotton breeders for many years (4, 5, 6). Recently another class of compounds, the condensed tannins, has attracted the attention of plant breeders (3). The apparent role of these compounds, terpenoid aldehydes and condensed tannins, in the protection of cotton from insects is documented in the literature (3, 5, 10).

Much of the early breeding work for these compounds was focused on identifying new sources of germplasm with high levels of terpenoid aldehydes (gossypol). Genetic research on the influence of condensed tannins on insects, however, is limited. Genotype × environment interaction research on the terpenoid aldehydes has recently been reported (4, 5, 6). Seasonal and genetic variability of condensed tannins is only beginning to be investigated (10, 13, 14). In 1980 we conducted research to determine the variation among a diverse group of cotton strains and the effects of within season environment (plant growth stage)

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on allelochemics alleged to be important in repelling the tobacco budworm, *Heliothis virescens* (F.). Broad sense heritability values were also calculated.

MATERIALS AND METHODS

Twenty cotton strains were used in this study (Table 1). These entries were planted 5 May 1980 in a randomized complete block with four replications on the Plant Science Farm at Mississippi State, Miss. Data were collected from 9 m, 2-row plots. The soil type was a Leeper silty clay loam (fine, montmorrillonitic, non-acid, thermic, vertic Haploquepts). Fifty terminal leaves (2.5 cm wide) were collected for chemical analyses weekly for 10 weeks from 13 June to 15 August. Collection of tissue began about 1 week prior to squaring. The tissue was freeze-dehydrated and ground in a Wiley Mill[4] for analytical purposes.

Six analytical tests were conducted on the tissue samples. The compound(s) quantified and references for analytical procedures are given in Table 2. Two of these tests measured condensed tannins, one test each measured total phenolics, gossypol and its analogs, condensed tannin and catechin, and a flavonoid-anthocyanin mixture.

The 20 cotton strains were grouped as follows: 1.) MOHG and five doubled haploids derived from MOHG; 2.) TX-LY-18-72 glandless, three doubled haploids derived from TX-LY-18-72 glandless, and Stoneville 7A glandless, nectarless, (ST-7AGN); 3.) four glanded commercial cultivars; and 4.) five upland cottons which have been shown to reduce tobacco budworm larval growth rates. The data were analyzed as a randomized complete block, split-plot in time design. The 19 degress of freedom for cotton strains were partitioned into five orthogonal comparisons as follows: 1.) groups; 2.) strains within group 1; 3.) strains within group 2; 4.) strains within group 3; and 5.) strains within group 4. The 171 degrees of freedom for strains × within season environments (week) were partitioned into five orthogonal comparisons as follows: 1.) weeks × groups; 2.) weeks × strains within group 1; 3.) weeks × strains within group 2; 4.) weeks × strains within group 3; and 5.) weeks × strains within group 4.

The genotypic variance among populations and the various interaction components of variation were obtained by algebraic manipulations of mean square expectations. The genetic model assumed that both weeks and strains were random effects. These data were used to calculate broad sense heritabilities, Hanson et al. (8), using the restricted phenotypic variance definition of Gordon et al. (7).

[4]Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table 1. Strains of cotton evaluated for allelochemics.

Group	Strain	Source
I	S_1 (D.H. 118)	†
	S_1 (D.H. 121)	†
	S_3 (D.H. 126)	†
	S_2 (D.H. 128)	†
	S_2 (D.H. 128 Interspecific)	†
	MOHG	W. P. Sappenfield, Univ. of Mo.
II	S_1 (D.H. 66)	†
	S_2 (D.H. 36)	†
	S_2 (D.H. 40)	†
	TX-LY-18-72 glandless	Luther Bird, Texas A&M Univ.
	Stoneville, 7AGN	W. R. Meredith, USDA, Stoneville, Miss.
III	Stoneville 731N	Commercial
	Deltapine 61	Commercial
	Stoneville 213	Commercial
	DES-24	Commercial
IV	SATU-65	P.I. 365541
	BJA-592	P.I. 362158
	Laxmi	P.I. 367241
	T-1055	Regional Collection
	PD-8619	T. W. Culp, USDA, Florence, S.C.

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RESULTS AND DISCUSSION

Mean squares from the analysis of allelochemic data are presented in Table 3. This group of 20 cotton strains was very diverse and the effect for strains was highly signifi-

Table 2. Chemical analyses performed on cotton tissue.

Analysis	Compound(s) detected	Reference
Phenolic	All phenolic compounds	9
Phloroglucinol		11
Ethyl acetate cyclohexane (CHEA)	Gossypol and analogs	
70% acetone – 30% water (7A-3W)	Flavonoid-Anthocyanin	
$E_{1,1}$	Condensed tannin	1
Tannin	Condensed tannin	12
Catechin	Condensed tannin & free catechin	2

Table 3. Mean squares from analysis of variance of allelochemic data for a diverse group of cottons.

		Mean square					
		Condensed tannins					
Source of variation	df	Tannin	$E_{1,1}$	Catechin	Total phenolics	Gossypol	Flavonoid-Anthocyanin
Strains	19	40,084**	159.6**	9,889**	836	62.5**	313.0**
Groups	3	133,103**	542.2**	6,516**	1,342	297.5**	1,337.2**
Strains:G1	5	6,583	17.9*	723	886	11.2**	5.6
Strains:G2	4	7,167	14.7	617	411	0.3	1.6
Strains:G3	3	3,407	57.0**	4,722**	304	7.2*	26.7**
Strains:G4	4	72,622**	271.5**	37,024**	1,219	53.8**	455.4**
Replications	3	76,960**	45.6**	6,612**	17,102**	1.9*	18.3**
Error (a)	57	2,186	6.9	924	500	0.6	3.2
Weeks (W)	9	243,182**	830.4**	67,983**	243,004**	26.3**	68.1**
Strains × Weeks	171	2,787**	13.2**	1,259*	663	1.2**	6.3**
Weeks × Groups	27	6,163**	32.8**	3,627**	1,962**	3.8**	17.7**
W × Strains:G1	45	2,028	9.8	899	571	0.8	1.7
W × Strains:G2	36	2,132	7.4	511	396	0.2	0.4
W × Strains:G3	27	1,668	6.1	617	249	0.9	5.1**
W × Strains:G4	36	2,696	13.7*	1,163**	382	0.7	10.2**
Error (b)	540	2,103	9.9	1,158	1,043	0.7	3.1

*,** Significant at 0.05 and 0.01 levels of probability, respectively.

cant for all allelochemic tests except total phenolic content. Groups were also highly significant for all classes of allelochemics except total phenolics.

Differences among doubled haploids from MOHG (strains: G1) were found for tannin (E1,1) and gossypol. There were no differences among the glandless strains (strains; G2). In the commercial group (strains: G3) differences between strains in catechin, tannins by the E1,1 test, gossypol and flavonoid-anthocyanins were detected. Strains within group 4 showed significant differences for all chemical analyses except total phenolics. Strains in this group were unrelated and were of diverse growth habit and origin.

The week effect was highly significant for all chemical analyses (Table 3). These data suggest that the concentration of cotton allelochemics was influenced by factors associated with the age of the plants. The mean squares for the interaction of strains × weeks and groups × weeks were highly significant. This demonstrates that all strains do not behave alike with respect to allelochemic concentration as physiological stages of plant growth change during the season.

Strains within group 4 showed a significant strains × weeks interaction for catechin, tannins by the E1,1, and flavonoid-anthocyanins. The major differences among strains within groups 1, 2, and 3 were due to strain and week effects with the interaction significant only for flavonoid-anthocyanins in group 3.

Estimates of components of variance for allelochemic concentration are presented in Table 4. The most conspicuous features of these data are the small sizes of the genetic (strain) variances compared with the week and error variances for condensed tannins and phenolics. Genetic variances for gossypol and the flavonoid-anthocyanins mixture were the largest. It is also important to note the small size of the interaction components in relation to the main effects of strain and week. These data, in addition to the nonsignificance of the week × strain within

Table 4. Estimates of components of variance for cotton allelochemics.

Character	Variance components				
	Strains	Week	Strains × week	Strains × Rep	H_B‡
Condensed tannins					%
Tannin	930.3**	3,004.9**	170.9**	8.2	97
$E_{1,1}$	3.7**	10.2**	0.8**	0.0†	97
Catechin	221.5**	834.0**	25.3**	0.0†	98
Phenolics	17.8	3,029.2**	0.0†	0.0†	93
Gossypol	1.5**	0.3**	0.1**	0.0†	99
Flavonoid-anthocyanin	7.6**	0.7**	0.8**	0.0	98

*,** Corresponding mean square significant at 0.05 and 0.01 levels of probability, respectively.
† Negative estimates for which the most reasonable value is zero.
‡ Broad-sense heritability.

Table 5. Allelochemic concentration (dry wt. basis) by weeks for four groups of cottons.

Chemical or test	Cotton strain group†	Sampling date (weeks post-emergence)										Seasonal $\bar{X}$‡
		5	6	7	8	9	10	11	12	13	14	
							%					
Tannin	I	5.1	7.5	13.3	20.7	17.2	15.9	16.2	17.5	17.2	15.1	14.6 c
	II	6.6	9.2	16.3	24.3	21.5	19.4	21.1	26.2	26.7	22.2	19.3 a
	III	6.4	6.5	12.6	20.9	19.5	17.6	18.1	22.1	24.3	19.4	16.7 b
	IV	5.1	6.0	9.8	14.9	14.8	13.4	15.1	18.8	18.2	19.7	13.6 d
	$\bar{X}$	5.8	7.4	13.0	20.2	18.1	16.5	17.5	20.9	21.3	18.9	16.1
$E_{1,1}$	I	6.1	8.7	10.6	13.7	13.7	14.4	13.0	11.0	13.2	12.3	11.7 c
	II	6.8	9.8	11.9	15.0	15.8	16.7	15.5	15.5	20.0	18.4	14.5 a
	III	6.5	8.3	9.8	14.3	14.8	14.9	15.6	14.2	18.9	14.4	13.2 b
	IV	4.4	7.3	8.2	10.6	11.2	12.1	12.2	13.5	13.7	15.3	10.8 d
	$\bar{X}$	5.9	8.5	10.2	13.4	13.8	14.5	13.9	13.4	16.2	15.0	12.6
Catechin	I	7.4	9.7	13.0	14.3	14.9	12.2	12.6	14.1	12.0	12.9	12.3 b
	II	5.5	6.6	10.6	14.1	13.8	11.7	11.6	14.8	14.6	17.5	12.1 b
	III	7.7	9.2	12.9	15.5	16.0	12.8	13.6	17.2	15.0	14.7	13.5 a
	IV	6.4	9.5	11.2	12.6	13.5	10.8	12.1	17.2	14.4	18.2	12.6 b
	$\bar{X}$	6.7	8.8	11.9	14.1	14.5	11.8	12.4	15.7	13.9	15.7	12.6
Phenolics	I	5.0	5.0	5.9	8.2	8.3	8.6	11.7	14.3	17.6	16.0	_0.0 a
	II	4.4	4.2	5.1	7.6	7.2	7.2	12.5	16.3	22.4	19.3	10.6 a
	III	5.0	4.8	5.4	8.0	7.6	7.6	11.6	14.8	19.2	17.1	10.1 a
	IV	4.5	4.8	5.6	7.7	7.2	6.9	10.4	15.1	20.0	18.6	10.1 a
	$\bar{X}$	4.7	4.7	5.5	7.9	7.6	7.6	11.6	15.1	19.7	17.7	10.2
Gossypol	I	0.40	0.43	0.51	0.43	0.42	0.35	0.32	0.36	0.22	0.21	0.36 a
	II	0.07	0.09	0.13	0.08	0.09	0.10	0.12	0.08	0.07	0.09	0.09 c
	III	0.33	0.34	0.42	0.32	0.28	0.27	0.24	0.26	0.21	0.19	0.29 b
	IV	0.30	0.38	0.45	0.35	0.30	0.30	0.25	0.33	0.30	0.24	0.32 ab
	$\bar{X}$	0.28	0.31	0.38	0.30	0.28	0.26	0.24	0.26	0.20	0.18	0.27
Flavonoid-anthocyanin	I	0.55	0.72	0.75	0.46	0.66	0.46	0.59	0.61	0.40	0.39	0.56 b
	II	0.05	0.06	0.06	0.04	0.09	0.09	0.05	0.05	0.04	0.16	0.07 c
	III	0.44	0.63	0.84	0.46	0.59	0.42	0.57	0.57	0.41	0.33	0.53 b
	IV	0.45	0.73	0.87	0.55	0.58	0.58	0.60	0.91	0.61	0.55	0.64 a
	$\bar{X}$	0.38	0.54	0.63	0.38	0.48	0.39	0.45	0.54	0.36	0.36	0.45

† Data for individual cotton strains are available from the authors upon written request.
‡ Means only comparable within a specific analytical test. Any two means not sharing the same letter are significantly different at the 0.05 probability level as determined by the Student-Newman-Kuels Range Test.

group interactions (Table 3) indicate the relative unimportance of the genotype × week interactions for most strains.

Therefore, interpretation of allelochemic data can generally be made for the main effects of strain and week. Because of the variation from week to week a population of cotton strains should be sampled for allelochemics on the same date and preferably across several sample dates. Our results for tannins, catechin, gossypol, and flavonoid-anthocyanins agree with the conclusions of Dilday and Shaver (5, 6) for gossypol. These authors emphasize the desirability of calculating gossypol percentages as means of several harvests rather than of a single harvest, especially for breeding stocks with high gossypol.

The analysis of the allelochemic data (Table 3) showed the effect of cotton strains to be highly significant indicating that considerable genetic variation existed within this group of strains. Broad sense heritability was high for all characters tested (Table 4). These two facts suggest that considerable variation exists within these groups of cottons and almost all of this variation is genotypic.

Weekly concentrations of allelochemics and seasonal means of allelochemics by groups are shown in Table 5.. In general, the concentrations of condensed tannins (tannin, catechin, E1,1) were low at the beginning of the season and increased rapidly until the 4th sampling data and then remained at that level for the remainder of the season.

Significant differences among groups were found for condensed tannins as measured by the three chemical procedures. Amounts in the tannin test ranged from 13.6 to 19.3%, for the E1,1 test 10.8 to 14.5%, and the catechin test from 12.1 to 13.5% on a dry weight basis. Total phenolics tended to increase linearly over the season but a decrease was noted at the last sample date, and no differences were found among groups. Gossypol and flavonoid-anthocyanins showed a slight trend to decrease across the season. Significant differences among the groups for gossypol were found (0.09 to 0.36%). Flavonoid-anthocyanin differences were significant and ranged from 0.07 to 0.64% among groups of lines.

The results of this study indicated significant genetic variation exists among cotton strains, and the genotype × week interaction within a season was small. Selection of germplasm high in these allelochemics would not be greatly complicated because of sampling dates or age of plants; however, all strains to be compared should be sampled at the same time. The amount of work required for a study of this type is immense. It was therefore impractical for us to repeat this test over locations and years. Dilday and Shaver (5, 6) quantified gossypol over years and found a significant difference between years for the levels of gossypol, but the year × entry interaction was not significant, suggesting that environmental factors uniformly influence the level of gossypol produced.

Success in selecting for increased levels of the allelochemics would therefore depend upon inheritance of the differences detected. Hopefully the differences are additive, or else generate additive × additive variance in hybrid populations.

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