Published September, 1982

Generation Mean Analyses of Various Allelochemics in Cottons[1]

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ABSTRACT

Several secondary plant metabolites are alleged to confer resistance of cotton, *Gossypium hirsutum* L., to various insects and mites. At the present time, only the inheritance of the sesquiterpenoid gossypol has been elucidated. This research was conducted to determine inheritance of several inferred/hypothesised allelochemics from cotton, in particular those alleged to confer resistance to the tobacco budworm, *Heliothis virescens* (F.).

Generation mean analysis (GMA) was used as the genetic design. The following GMA's were evaluated: 'DES-24' × 'SATU-65', DES-24 × 'BJA-592', and DES-24 × MOHG. The six populations (P_1, P_2, F_1, F_2, BCP_1, and BCP_2) comprising a GMA were planted as a randomized complete block with four replications. Analyses of young cotton leaves for condensed tannins, catechin, total phenols, $E_{1,1}$, aniline reacting terpenes, and phloroglucinol reactive compounds were obtained at intervals during the growing season. Analysis of variance was conducted on seasonal means.

The inheritance of gossypol, as measured by the CHEA extract of the phloroglucinol test, was predominately additive. Gossypol, as detected by the aniline reacting terpene test, was shown to be inherited by predominately dominant gene action. The genetic analysis indicates that the CHEA and aniline reacting terpene tests are measuring gene products which are inherited in different manners. The four tests used for the determination of condensed tannins also appeared to be measuring somewhat diverse genetic products. This was apparent because $E_{1,1}$ and fresh disc assay tannins, but not tannin and catechin tannins, showed significant variation among populations in the DES-24 × SATU-65. $E_{1,1}$ and catechin tannins, but not the tannin and fresh disc assay tannins, showed significant variation among populations in the DES-24 × BJA-592 cross. When condensed tannins as measured by any of these tests were genetically analyzed in a cross, inheritance was highly additive.

The high degree of additive gene action for condensed tannins, a flavonoid-anthocyanin mixture, gossypol by the CHEA test, and total phenolics indicate that it should be possible to fix and select for increased levels of these compounds.

Additional index words: Host-plant resistance, Generation mean analysis, *Heliothis virescens* (F.), *Gossypium hirsutum* L., Allelochemistry.

DAMAGE by the *Heliothis* complex (cotton bollworm, *Heliothis zea* (Boddie) and tobacco budworm (TBW) [*H. virescens* (F.)], to cultivated upland cotton, *Gossypium hirsutum* L., is documented in the literature (11). Gossypol has been shown to confer some resistance to insects in cotton (3). The genetics of gossypol in cotton has been studied extensively with the action of both the primary gland alleles and the modifiers being elucidated. Both additive and dominance factors are involved (9, 16, 17).

Chan et al. (4) demonstrated that condensed tannins were toxic to the bollworm, tobacco budworm, and pink bollworm, *Pectinophora gossypiella* (Saunders), by laboratory feeding tests. However, the inheritance of condensed tannins in cotton is not documented.

Lambert (8) characterized 35 foreign cotton cultivars for resistance to several insect pests. Five were found to produce TBW larvae significantly smaller than a commercial check 'Deltapine 16' (DPL-16). Among these 5 were 'BJA-592' (PI. 362158, Chad) and 'SATU-65' (PI. 365541, Uganda), and a Missouri breeding line MOHG. No explanation for their apparent resistance was given.

This study was designed to determine the inheritance of the condensed tannins, total phenolics, gossypol and its analogs and a flavonoid-anthocyanin mixture. These are all thought to condition resistance to *Heliothis* spp. and various other insect pests of cotton. We included gossypol, because its inheritance is well known, and thus could serve as a control in the experiment.

MATERIALS AND METHODS

As a result of Lambert's (8) findings the strains BJA-592, SATU-65, and MOHG were chosen as the resistant parents for the genetic studies. The commercial cultivar 'DES-24' was chosen as the susceptible parent and crossed to each of the resistant lines as female.

SATU-65 and BJA-592 are foreign cultivars. MOHG is not homozygous for some morphological traits but is probably homozygous for allelochemics since it is resistant to the TBW.

The genetic design used was the generation mean analysis (GMA) (6). The six populations made for each GMA (P_1, P_2, F_1, F_2, BCP_1, and BCP_2) were planted 10 May 1979 at Mississippi State Univ. in a randomized complete block design with four replications and with four row plots, 8 m long. Plants were thinned to one per 33 cm. Genetic analysis was performed using a modification of the GMA as suggested by Meredith and Bridge (10).

The lines BJA-592, SATU-65, and MOHG were originally selected for study because TBW larvae were reduced in weight at 5 days of age when grown on them (8). Larval growth studies are very time consuming and require large numbers of replications. Since larval weights were different it was reasoned that allelochemics may also differ between these lines. Terminal leaves were analyzed for putative allelochemics using the methods cited in Table 1. Eight analyses were performed on each sample. Four of these tests measured condensed tannins. One test each measured total phenolics, gossypol and its analogs, aromatic aldehydes (including gossypol), and flavonoid-anthocyanins.

For chemical analysis, 50 terminal leaves (2.5 cm) were collected from each plot. Nine samples were collected between 6 July and 27 September. Leaves were freeze dehydrated and ground in a Wiley®[4] mill to pass a 40-mesh sieve. Chemical analysis was then conducted on freeze dried leaf powder. Additional studies by White et al. (15) with a group of 20 diverse cotton lines showed that mean squares for the strain effects and week effects were 10 to 30 times greater than the strain by week

[1]Cooperative investigations of USDA-ARS, and Mississippi Agric. & For. Exp. Stn., Journal Article No. 5079 of the Mississippi Agric. & For. Exp. Sta. Part of a dissertation submitted by the Senior author. Received 5 Jan. 1982.

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[4]Mention of a trademark or proprietary product 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 that may also be suitable.

interactions. Thus, we decided to use the analysis of variance and generation mean analysis on the seasonal means averaged over the nine collection dates.

RESULTS AND DISCUSSION

Concentrations of allelochemics and mean squares from the GMA for the DES-24 × SATU-65 cross are presented in Table 2. Significant differences were found between parental lines for gossypol and flavonoid-anthocyanins (as determined from phloroglucinol reaction on the CHEA and 7A-3W extracts) and condensed tannins by the $E_{1,1}$ and fresh disc tests. Genetic analysis showed gossypol, flavonoid-anthocyanins, and condensed tannins to be inherited in an additive manner. The high degree of additivity and the difference between parents suggests that effective selection for gossypol, flavonoid-anthocyanins and condensed tannins should be possible in this cross.

No significant differences were found between parents for aromatic aldehydes (gossypols) as determined by the aniline reacting terpene test; however, there were significant differences among the means of the six populations. GMA showed that dominant gene action and residual epistasis made significant contributions to the inheritance of aromatic aldehydes.

Analysis of allelochemic data for the DES-24 × BJA-592 cross are presented in Table 3. Flavonoid-anthocyanins were found to differ among populations in this cross. As in the previous cross the inheritance of flavonoid-anthocyanins was found to be highly additive. There was variation in aniline reacting terpene concentration among populations. The inheritance was due to dominance gene action with epistatic gene action also making a significant contribution. The $E_{1,1}$ and catechin tests both produced significant differences for condensed tannins among the six populations. Additive gene action contributed more to the inheritance of condensed tannins than any of the other genetic components measured.

Results of the inheritance studies for the DES-24 × MOHG cross are presented in Table 4. In this cross significant differences were found among the six populations for total phenolics. Both additive and dominance gene action were found to make significant contributions to inheritance.

The only other class of allelochemics showing significant variation in this cross was gossypol as determined from the reaction of phloroglucinol on the CHEA extract. The inheritance was found to be additive with a significant contribution of residual epistasis.

Several conclusions can be made about the inheritance of the cotton allelochemics which were studied. First, the inheritance of gossypol, as measured by two different methods, gave different results. The CHEA extract of the phloroglucinol test was predominately additive with significant residual epistasis in the cross with MOHG. Gossypol as detected by the aniline reacting terpene test was shown to be inherited in a nonadditive manner. The results of these genetic analyses suggest that the CHEA and aniline reacting terpene chemical tests are measuring different biochemical gene products. If we consider that the aniline reacting terpenes also include gossypol, then the results from the two gossypol tests are comparable to those of other researchers in that both additive and dominance gene action are involved (9, 16, 17). Second, the four tests for the determination of condensed tannins also appeared to be measuring somewhat diverse genetic products. This was apparent because $E_{1,1}$ and fresh disc assay tannins, but not tannin and catechin tannins,

Table 1. Chemical analyses performed on cotton tissue.

Analysis	Compound(s) detected	Reference
Phenolic	All phenolic compounds	7
Phloroglucinol		13
Ethyl acetate cyclohexane (CHEA)	Gossypol and analogs	
70% acetone – 30% water (7A-3W)	Flavonoids-Anthocyanins	
Aniline reacting terpene	Aromatic aldehydes including the gossypols	12
$E_{1,1}$	Condensed Tannin	1
Tannin	Condensed Tannin	14
Catechin	Condensed Tannin & Free Catechin	2
Fresh disc assay	Condensed Tannin	Lane, H. C. (pers. comm.)†

† Lane, H. C., plant physiologist, USDA-ARS, Boll Weevil Res. Lab., Mississippi State, MS 39762.

Table 2. Concentration of allelochemics and mean squares from GMA for the DES-24 × SATU-65 cross.

Population	Total phenolics	Phloroglucinol: Gossypol	Phloroglucinol: Flavonoid-anthocyanins	Aniline reacting terpenes	Condensed tannins: $E_{1,1}$	Condensed tannins: Tannin	Condensed tannins: Catechin	Condensed tannins: Fresh disc
	mg/g dry wt.							
P_1 (DES-24)	53.78	1.79	3.67	5.29	66.40	164	174	39.73
P_2 (SATU-65)	54.14	2.41	4.27	5.31	81.20	157	174	34.84
F_1	53.84	2.23	3.78	4.37	74.90	160	177	38.84
F_2	53.29	2.28	3.81	4.52	71.60	157	178	38.80
BCP_1	52.28	1.96	3.39	4.02	68.30	159	170	43.33
BCP_2	54.01	2.38	3.87	4.97	74.50	153	177	39.38
L.S.D. (0.05)	NS	0.22	0.42	0.50	5.00	NS	NS	4.17
Source (df)		Mean squares						
Population (5)		2.23*	2.94*	9.85*	0.0010*			204.92*
Additive (A) (1)		9.99*	10.10*	3.50	0.0046*			527.15*
Dominance (1)		0.59	1.72	27.71*	0.0000			115.70
A × A (1)		0.31	0.73	0.02	0.0000			121.83
Residual epistasis (2)		0.13	1.09	9.01*	0.0002			129.97
Error (15)		0.19	0.71	1.01	0.0001			53.60

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Table 3. Concentration of allelochemics and mean squares from GMA for the DES-24 × BJA-592 cross.

Population	Total phenolics	Phloroglucinol		Aniline reacting terpenes	Condensed tannins			Fresh disc
		Gossypol	Flavonoid-anthocyanins		$E_{1,1}$	Tannin	Catechin	
	mg/g dry wt.							
P_1 (DES-24)	50.22	1.95	3.37	6.06	64.70	143	151	39.43
P_2 (BJA-592)	49.55	2.23	3.98	5.57	76.60	161	184	36.66
F_1	51.16	2.07	3.72	5.00	70.10	153	170	37.21
F_2	49.76	2.04	3.61	4.62	71.50	160	186	40.31
BCP_1	49.96	2.08	3.43	4.46	65.80	154	173	40.10
BCP_2	50.56	2.05	3.55	4.81	71.90	154	179	38.79
L.S.D. (0.05)	NS	NS	0.30	0.54	10.00	NS	10	NS

Source	df	Mean squares					
Population	5		1.76*	13.37*	0.0006*		5780.35*
Additive (A)	1		6.47*	1.43	0.0032*		18739.36*
Dominance	1		0.00	29.95*	0.0000		867.25
A × A	1		0.33	0.01	0.0002		2279.79
Residual epistasis	2		1.00	17.73*	0.0000		3507.88
Error	15		0.36	1.15	0.0002		1457.79

Table 4. Concentration of allelochemics and mean squares from GMA for the DES-24 × MOHG cross.

Population	Total phenolics	Phloroglucinol		Aniline reacting terpenes	Condensed tannins			Fresh disc
		Gossypol	Flavonoid-anthocyanins		$E_{1,1}$	Tannin	Catechin	
	mg/g dry wt.							
P_1 (DES-24)	51.16	1.75	2.95	5.28	66.50	141	153	36.73
P_2 (MOHG)	55.05	2.24	3.46	5.27	72.80	140	160	40.39
F_1	51.22	1.81	3.32	4.87	64.00	136	149	36.24
F_2	52.29	2.29	3.36	5.35	69.00	129	148	36.47
BCP_1	52.03	2.04	3.41	4.96	65.00	137	155	38.60
BCP_2	51.90	2.16	3.41	5.35	69.60	130	144	38.06
L.S.D. (0.05)	2.48	0.25	NS	NS	NS	NS	NS	NS

Source	df	Mean squares	
Population	5	73.75*	1.78*
Additive (A)	1	210.54*	4.22*
Dominance	1	93.39*	0.22
A × A	1	2.44	0.81
Residual epistasis	2	31.19	1.82*
Error	15	16.30	0.27

showed significant variation in the DES-24 × SATU-65 cross, and $E_{1,1}$ and catechin, but not the tannin and fresh disc assay tannins in the DES-24 × BJA-592 cross. However, when condensed tannins, as measured by any of these tests, were genetically analyzed, inheritance was highly additive.

The inconsistencies in the chemical analyses present special problems to breeders. Until the actual role of these allelochemics in the defense of cotton to the TBW is elucidated and definitive chemical analyses are developed, no real progress in developing TBW resistant cottons based on chemical analyses can be expected.

The high degree of additive gene action for condensed tannins, flavonoid-anthocyanins, gossypol by the CHEA test, and total phenolics indicate that it should be possible to fix and select for increased levels of these compounds. Although this study represents only a one-year field study, Dilday and Shaver (5) quantified gossypol over years and found a significant difference between years for the levels of gossypol, but year × entry interaction was not significant, suggesting that environmental factors uniformly influence the level of gossypol produced by different genotypes. White (15) collected allelochemic data from a diverse group of cottons and found very large broad sense heritabilities; again, suggesting that most variation in allelochemic concentration to be genetic rather than environmental. Research, therefore, indicates that a workable system should exist for increasing allelochemics in cotton once the detailed mechanism for the resistance of cotton to the TBW has been elucidated and decisions can be made relative to the value of the allelochemics to resistance.

REFERENCES

1. Bate-Smith, E. C. 1975. Phytochemistry of proanthocyanidins. Phytochemistry. 14:1107-1113.
2. Bell, A. A., and R. D. Stipanovic. 1972. Chemistry and nature of fungitoxic compounds in diseased cotton. p. 87-88. *In* Proceedings Beltwide Cotton Prod. Res. Conf.
3. Bottger, G. T., E. T. Sheehan, and M. J. Lukefahr. 1964. Relation of gossypol content of cotton plants to insect resistance. J. Econ. Entomol. 57:283-285.
4. Chan, B. C., A. C. Waiss, Jr., and M. J. Lukefahr. 1978. Condensed tannin, an antibiotic chemical from *Gossypium hirsutum*. J. Insect Physiol. 24:113-118.
5. Dilday, R. H., and T. N. Shaver. 1976. Survey of the regional *Gos-*

14350653, 1982, 5, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.2135/cropsci1982.0011183X002200050036x by North Carolina State Universit, Wiley Online Library on [21/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

sypium hirsutum L. primitive race collection for flowerbud gossypol and seasonal variation between years in gossypol percentage USDA-ARS Pub. ARS-S-146.
6. Hayman, B. I. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12:371-390.
7. Horwitz, W. 1975. Official Methods of Analysis of the Association of Official Analytical Chemists. p. 164-165. *In* W. Horwitz (ed.) Association of Official Analytical Chemists. Washington, D.C.
8. Lambert, L. 1977. Characterization of thirty-five foreign cultivars of cotton for resistance to boll weevil, tobacco budworm, tarnished plant bug and bandedwing whitefly. Ph.D. Dissertation, Mississippi State University. (Libr. Congr. Card No. Mic. 77-28, 555) Xerox Univ. Microfilms, Ann Arbor, Mich. (Diss. Abstr. 38B:3034).
9. Lee, J. A., C. C. Cockerham, and F. H. Smith. 1968. The inheritance of gossypol level in *Gossypium*. I. Additive, dominance, epistatic, and maternal effects associated with seed gossypol in two varieties of *Gossypium hirsutum* L. Genetics 59:285-298.
10. Meredith, W. R., Jr., and R. R. Bridge. 1972. Heterosis and gene action in cotton, *Gossypium hirsutum* L. Crop Sci. 12:304-310.
11. Parrott, W. L., J. N. Jenkins, J. C. McCarty, Jr., and L. Lambert. 1978. A procedure to evaluate for antibiosis in cotton to the tobacco budworm. J. Econ. Entomol. 71:310-312.
12. Smith, F. H. 1967. Determination of gossypol in the leaves and flowerbuds of *Gossypium*. J. Am. Oil Chem. Soc. 44:267-269.
13. Storherr, R. W., and K. T. Holley. 1954. Determination of free gossypol in mixed feeds. J. Agric. Food Chem. 2:745-47.
14. Swain, T., and W. E. Hillis. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10:63-68.
15. White, W. H., J. N. Jenkins, W. L. Parrott, J. C. McCarty, D. H. Collum, and P. A. Hedin. 1982. Genetics and within season variability of various allelochemics within a diverse group of cotton. Crop Sci. (In Press).
16. Wilson, F. D., and J. A. Lee. 1971. Genetic relationship between tobacco budworm feeding response and gland number in cotton seedlings. Crop Sci. 11:419-421.
17. Yang, H. C., and D. D. Davis. 1977. Heritability and combining ability for gossypol content in six lines of upland cotton. Crop Sci. 17:305-307.