Gossypol, Flavonoid, and Condensed Tannin Content of Cream and Yellow Anthers of Five Cotton (*Gossypium hirsutum* L.) Cultivars

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Cream and yellow anthers of five cotton (*Gossypium hirsutum* L.) cultivars were analyzed for gossypol and related terpenoid aldehydes, condensed tannin, and flavonoid content. Interest in the constituents of cream and yellow anthers stems from studies that indicate yellow anthers suppress *Heliothis virescens* larval growth. Yellow anthers of four glanded cultivars averaged 1.09% dry weight gossypol; cream anthers averaged 0.87%. Gossypol for a glandless cultivar, NM868, was barely detectable, averaging 0.02 and 0.03% for cream and yellow anthers, respectively. Gossypol was identified as the most prevalent terpenoid aldehyde. Condensed tannins account for 4.79 and 5.34% dry weight of yellow and cream anthers, respectively. Twenty flavonoids were isolated and 13 identified. Gossypetin-3',7-glucoside (25.7%) and quercetin-3-glucoside (25.2%) were the major flavonoids found in anthers regardless of anther color or cotton cultivar. Minor quantitative and no qualitative differences were found in flavonoid constituents between cream and yellow anthers of the five cotton cultivars. The higher gossypol percent in yellow vs. cream anthers is apparently responsible for the previously observed growth suppression of *Heliothis virescens* larvae.

Developing cotton, *Gossypium hirsutum* L., anthers are a primary food source of tobacco budworm larvae, *Heliothis virescens* F., a major insect pest of cotton (Burks and Earle, 1965; Shaver et al., 1977). In *Gossypium* spp. germ plasm, geneticists have identified at least two anther colors, pale cream and yellow (Stephens, 1954). Certain cotton cultivars have 7–15% incidence of yellow anthers occurring in their natural populations (Meredith, 1979).

Hanny et al. (1979) fed developing cream and yellow anthers of five cotton cultivars to larvae of the tobacco budworm to compare the effects of these two anther colors on larval growth. Growth was suppressed significantly (15%) when larvae were fed developing yellow anthers compared with larvae fed cream anthers.

Because anthers of the cotton bud are a preferred feeding site for tobacco budworm larvae, a comparative chemical analysis of cream and yellow anthers was undertaken to develop an understanding of the larval growth suppression associated with yellow anthers. Gossypol and related terpenoid aldehydes (Lukefahr et al., 1966; Bell and Stipanovic, 1977), condensed tannins (Chan and Waiss, 1978), and flavonoids (Shaver and Lukefahr, 1969) isolated from whole flowerbuds of cotton have been reported to inhibit the growth of tobacco budworm larvae in laboratory bioassays. This paper reports the results of a comparative analysis of these three chemical classes in cream and yellow anthers of five cotton cultivars; their relationship to tobacco budworm larval growth is discussed.

MATERIALS AND METHODS

Plant Material. Field plantings at Stoneville, MS, of cotton cultivars DES-24, CAMD-SM, TM-1, Tamcot-37, and NM 868 with genetic counterparts of cream and yellow anthers were used as the source of anthers. One–three days before anthesis, flower buds of each cultivar were harvested, brought to the laboratory, and immediately dissected. The anthers were frozen, lyophilized, weighed, and ground in a Wiley mill to pass a 40-mesh screen. Anthers of each color of each cultivar were analyzed separately. Subsamples of the anther powder were prepared for separate triplicate analysis of gossypol and related terpenoid aldehydes and condensed tannins. Fresh flower buds were collected, and anthers were dissected as above for separate triplicate analysis of flavonoids.

The anthers used for chemical analysis were collected from the same field plots at the same time as anthers used in the *Heliothis* larval growth study (Hanny et al., 1979).

Flavonoid Analysis. Ten grams each of fresh excised anthers were immersed in 50 mL of 0.01 N HCl in absolute ethanol and held at 0 °C for 2 days (Parks, 1965). The extracts were brought to room temperature and filtered, and gross flavonoid content of each sample was determined using the butanol–HCl test (Bell and Stipanovic, 1972).

Anther extracts were then examined by two-dimensional paper chromatography (PC) in butanol–acetic acid–water (4:1:5), followed by chloroform–butanol–water (2:4:4). Twenty district spots were visualized under UV, one of which was an anthocyanin (Parks, 1965). Six additional spots were visualized when the chromatograms were sprayed with $AlCl_3$ and Neu's reagent (Harbourne, 1973). These additional spots were present in only trace amounts in all chromatograms and could not be characterized.

The 20 major spots visualized under UV were excised from the chromatograms and eluted with 80% ethanol. The eluants were filtered, concentrated under vacuum to an aqueous solution, and lyophilized. Aliquots of the lyophilized materials were then hydrolyzed 30 min in 2 N HCl in a boiling water bath and subsequently extracted twice with ethyl acetate. The aqueous phase was frozen and lyophilized for subsequent sugar analysis. The ethyl acetate phases of the 20 hydrolysates were dried under vacuum and dissolved in methanol. A high-pressure liquid chromatography (LC) method was developed for analysis. A Waters 202 liquid chromatograph equipped with two pumps, a gradient solvent programmer, and dual-channel UV detector coupled to a Spectra-Physics 4000 integrator was used. The stationary phase was a 3.9 mm × 30 cm reverse-phase μ-Bondapak C_{18} column. The mobile phase consisted of 2% aqueous acetic acid and acetonitrile (70:30, v/v), with a flow rate of 1.5 mL/min at 105.46 kg/cm^2 at room temperature. Eluants were detected at 254 and 436 nm. Of the 20 hydrolysates, 13 were present in sufficient quantity for characterization. These 13 hydrolysates were identified by cochromatography with standards on LC. Identity was confirmed by comparing retention times of

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Table I. Gossypol, Condensed Tannin, and Flavonoid Content[a] of Cream and Yellow Anther Counterparts of Five Cotton Cultivars

cultivar	pollen color	gossypol, %	tannin, %	flavonoid, %
DES 24	cream	1.08	5.68**	0.58
DES 24	yellow	1.19**	5.08	0.59
CAMD-SM	cream	0.75	5.94**	0.57
CAMD-SM	yellow	1.00**	4.20	0.58
TM-1	cream	0.91	5.53*	0.52
TM-1	yellow	1.36**	5.13	0.59**
TAMCOT 37	cream	0.75	5.55	0.52
TAMCOT 37	yellow	0.83**	5.36	0.53
NM 868	cream	0.02	4.02	0.51
NM 868	yellow	0.03	4.16	0.52
LSD 0.01		0.08	0.49	0.03
glanded mean	cream	0.07	5.67**	0.55
	yellow	1.09**	4.94	0.57
glandless mean	cream	0.02	4.02	0.51
	yellow	0.03	4.16	0.52

[a] Content expressed as percent dry weight. [b] Significantly higher than the compared anther color counterpart at the 0.05 (*) and 0.01 (**) levels of probability, respectively.

samples with standards on two-dimensional PC in butanol–acetic acid–water (4:1:5), followed by chloroform–butanol–water (2:4:4), and spectrophotometric scans at 240 to 500 nm, followed by the spectral shifts obtained using 2 M NaOH, $AlCl_3$, powdered NaOAc, and powdered NaOAc and H_3BO_3 (Harbourne, 1967, 1973).

Sugars were identified by gas–liquid chromatography of their trimethylsilyl ethers (Kagan and Mabry, 1965).

The nature of glycosidation (i.e., monosides, biosides, diglycosides) was determined by methods of Randerath (1968). The position of glycosylation was determined by methods of Seikel (1962), Venkatamaran (1962), Jurd (1962), and Harbourne (1967, 1973).

Gossypol and Related Terpenoid Aldehyde Analysis. Quantitative analysis of gossypol and related terpenoid aldehydes was determined as previously described (Hanny et al., 1978). Qualitative analysis was obtained using thin-layer chromatography, nuclear magnetic resonance (NMR), and mass spectrometry (MS) (Stipanovic et al., 1974).

Condensed Tannin Analysis. Quantitative analysis of condensed tannins was performed as previously described (Hanny et al., 1978).

RESULTS AND DISCUSSION

Table I lists the gossypol, condensed tannin, and flavonoid content of cream and yellow anther counterparts of five cotton cultivars as percent dry weight. Yellow anthers averaged more gossypol (0.88 vs. 0.70%), less condensed tannin (4.79 vs. 5.34%), and essentially equal flavonoid (0.56 vs. 0.54%) content relative to cream anther counterparts of the five cotton cultivars. Qualitative analysis revealed gossypol as the primary constituent of the terpenoid aldehyde fraction. None of the related gossypol derivatives [i.e., hemigossypolone, methoxygossypol, and heliocides; Bell and Stipanovic (1977)] were found.

Table II lists the identities and amounts (as percent total flavonoids) of flavonoids identified in the two anther color counterparts of the five cultivars. Slight quantitative differences were found among individual components, but relative amounts were the same for both anther colors. No qualitative differences among the flavonoid constituents were found. On the basis of MS, PC retention times, and color reactions with spray reagents, compounds **15–20** (Table II) are believed to be scopoletin and a glycoside of scopoletin. However, because of the unstable nature of coumarins following acid hydrolysis, a more precise analysis will be required for positive identification.

In a previous 2-year study (Hanny et al., (1979), *Heliothis virescens* larvae fed on yellow anthers averaged about 15% lower weights than those fed on cream anthers. However, the analysis of variance for the 1978 study sug-

Table II. Flavonoid Content[a] of Cream and Yellow Anther Counterparts of Five Cotton (*Gossypium hirsutum*) Cultivars

	cultivar										
	Tamcot 37		TM-1		DES 24		CAMD-SM		NM 868		
no.	cream, %	yellow, %	cream, %	yellow, %	cream, %	yellow, %	cream, %	yellow, %	cream, %	yellow, %	LSD 0.05
01	0.3	0.4	0.2	0.1	0.4	0.2	0.3	0.6*	0.1	0.3	0.27
02	0.6	0.4	0.2	0.3	0.5	0.7	0.8	0.7	0.5	0.3	0.46
03	1.5	1.4	2.5	2.4	1.9	2.1	2.3	2.3	1.7	1.4	1.10
04	0.9	0.9	1.4	1.6	1.4	1.5	1.4	1.4	1.0	1.0	0.86
05	0.01	0.03	0.02	0.01	0.05	0.08*	0.01	0.03	0.04	0.06	0.03
06	24.3	22.7	25.2	24.4	28.6	25.5	27.9	27.8	24.8	25.5	9.61
07	0.1	0.2	0.05	0.2	0.1	0.5	0.2	0.4	0.3	0.5	0.40
08	1.2	1.4	1.1	1.3	1.9	2.5	2.2*	1.3	0.9	0.8	0.82
09	0.5	0.2	0.1	0.1	1.1	0.8	0.6	0.3	0.4	0.7	0.62
10	28.0	27.4	24.3	25.0	23.5	24.5	23.0	24.8	24.3	26.9	10.25
11	0.05	0.02	0.1	0.08	0.06*	0.01	0.07	0.09	0.05	0.03	0.05
12	0.5	0.7	0.1	0.6	0.2	0.2	0.1	0.5	0.8	0.6	0.63
13	2.2	1.8	1.6	3.5*	1.1	2.0	3.1	3.1	1.9	2.6	1.74
14	0.2	0.4	0.1	0.6	0.8	0.6	1.4	0.9	0.6	0.8	1.09
15	0.6	0.9	0.4	0.2	1.1	0.8	0.6	0.6	1.1	1.3	1.10
16	1.0	0.9	0.6	0.5	0.9	0.9	0.4	0.3	0.8	0.8	1.11
17	0.01	0.05	0.02	0.04	0.06	0.09	1.1**	0.5	0.3	0.2	0.35
18	2.3	2.4	3.5	3.8	2.5	2.7	1.8	3.2	1.4	1.1	1.61
19	33.7	35.4	37.0	34.0	33.5	33.3	35.0	33.6	37.6*	34.0	3.39
20	1.1	2.3*[e]	1.6	1.3	1.4	1.2	1.8	1.8	1.3	1.2	0.89

[a] Expressed as percent of total flavonoid. [b] 01, unknown; 02, quercetin-3-diglucoside; 03, quercetin-7-rhamnoglucoside; 04, quercetin-3′-glucoside; 05, unknown;[c] 06, gossypetin-3-glucoside; 07, cyanidin-3-β-glucoside; 08, quercetin 7-rhamnoglucoside; 09, unknown; 10, quercetin-3-glucoside; 11, gossypetin-8-glucoside; 12, quercetin-3,7-diglucosidoglucoside;[d] 13, quercetin-3-rhamnoglucoside; 14, gossypetin-7-glucoside; 15, unknown;[c] 16, quercetin-3,7-diglucosidoglucoside;[d] 17, unknown; 18, unknown; 19, gossypetin-3′,7-diglucosidoglucoside;[d] 20, unknown.[c] [c] Blue fluorescence and R_f values suggest coumarin derivatives. [d] Position of diglucoside undetermined. [e] Significantly higher than the compared anther color counterpart at the 0.05 (*) and 0.01 (**) level of probability, respectively.

gested a cultivar × anther color interaction. One genetic comparison of interest was to consider the performance of the four glanded cultivars, DES 24, CAMD-SM, TM-1, and Tamcot 37, with the glandless (no gossypol glands) cultivar, NM 868. For the four glanded cultivars used in 1978, larvae fed on cream and yellow anthers averaged 204 and 167 mg, respectively, or a reduction of 19% due to feeding yellow anthers. The average larval weights for cream and yellow glandless NM 868 was 238 and 227 mg, respectively, or a 5% reduction due to feeding yellow anthers. To relate this differential response of glanded and glandless cultivars, the mean performance of the four glanded cultivars are also given in Table I.

The average gossypol content of the glanded cultivars was 1.09 and 0.87% for cream and yellow anthers, respectively. The glandless cultivar was almost devoid of detectable gossypol, averaging only 0.02 and 0.03%, respectively, for cream and yellow pollen. Shaver et al. (1978) reported a 50% reduction in tobacco budworm larval weight was obtained by increasing gossypol content of cotton from 0.6 to 0.8% (a 33% increase). In this study for the four glanded cultivars, the 25% increase in gossypol of yellow over cream anthers could explain the 19% larval growth suppression of yellow anthers. The small differences in larval weights and gossypol content of the yellow and cream anthers reinforces the hypothesis that higher gossypol in yellow vs. cream anthers is an important contributor to reducing larval weights.

Condensed tannins averaged 4.79 and 5.34% for the yellow and cream anther counterparts, respectively (Table I). The lower condensed tannin content of yellow anthers suggest that these constituents are not involved in growth suppression of tobacco budworm larvae by yellow cotton anthers. This is based on the premise that the mode of action of tannins is to reduce the availability of food protein by forming relative indigestible complexes with the protein. Therefore, their inhibitory effect on larval growth would be "dosage dependent": the greater the concentration of tannins, the greater the inhibition of larval growth (Feeny, 1968).

There were only slight quantitative differences in flavonoid content in either glanded or glandless types. Yellow and cream anthers averaged 0.56 and 0.54, respectively (Table I). TM-1 yellow anthers averaged 0.59%, significantly greater than TM-1 cream, which averaged 0.52%. The slight quantitative differences and no qualitative differences (Table II) found in flavonoid contents of cream and yellow anther counterparts of the five cultivars suggest that flavonoids are probably not the primary chemical constituents responsible for the suppression of tobacco budworm larval growth.

Results of this study indicate that condensed tannins and flavonoids are probably not involved in growth suppression of tobacco budworm larvae fed yellow anthers. The results suggest that the higher gossypol content of yellow pollen caused the previously observed reduction in larval weights.

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