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CONDENSED TANNINS OF COTTON LEAVES

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Key Word Index—*Gossypium hirsutum*; cotton; Malvaceae; two-spotted spider mites; condensed tannins; resistance.

Abstract—Some primitive races of cotton (*Gossypium hirsutum*) are almost immune to spider mites. These strains contain condensed tannins of about 20% of dry wt. The tannins accumulate in leaves and their concentration increases in successive leaves until about the 10th true leaf. The upper leaves maintain this quantity until early fall. The condensed tannins give a moderate astringency to the leaf, and are mixed polymers hydrolysing in acidic digests to 1 part cyanidin and 4 parts delphinidin.

INTRODUCTION

The condensed tannins appear to be important agents in the protection of plants from insects, but conclusive evidence on this point is yet incomplete [1–3]. Feeny [4] reported that the severest attack by the winter moth (*Operophtheria brumata* L.) on oak leaves occurred when hydrolysable tannins were in high concentration, and condensed tannins low. Later in the season, the concentration of condensed tannin increased and feeding decreased. The locust (*Schistocera gregaria* Forskal) feeds much less on the bracken [*Pteridium aquilinum* (L.) Kuhn] during August and September presumably because of increased concentrations of condensed tannin [5]. A condensed tannin isolated from flower buds of cotton (*Gossypium hirsutum* L., Tx 254) retards larval growth of the tobacco budworm (*Heliothis virescens* F.) when added to artificial diets [6].

Several primitive strains of cotton are almost immune to two-spotted spider mites (*Tetranychus urticae* Koch). Spider mites placed on these plants do not increase in numbers sufficiently to harm the plants. The same mites placed on susceptible plants reproduce rapidly and soon kill the plants. We have reported previously that resistant plants contain a relatively high content of condensed tannins [7]. There was also a significant negative correlation between the procyanidin content expressed as $E^{1\%}_{1cm}$ values and the feeding of *Heliothis* spp. on breeder selections [8].

This study was undertaken to show the seasonal variation in tannin content of cotton, and to relate the chemical nature of the tannins to spider mite activity.

RESULTS AND DISCUSSION

The concentration of tannin is very low in the cotyledons and first true leaves of the primitive cottons. The concentration increases with successive leaves, and reaches a maximum about the 10th mainstem leaf. During mid- and late summer, the concentration in upper leaves averages that of the 10th leaf, but there is considerable leaf-to-leaf variation and variation among plants. Young expanding leaves contain more tannin on an area basis than expanded leaves, but expanded leaves continue synthesis for a period of at least 2 weeks after full expansion.

Condensed tannin extracts made from leaves gathered during summer (hot weather) months produce a light reddish extract that yields, after chromatography and drying, a light brown spongy material. Extracts made of leaves gathered during the winter and early spring are deep red, and separate on chromatography into a dark red material and the light brown tannin as above. These observations indicate that condensed tannin synthesis depends primarily on long photosynthetic periods and higher temperature.

Not all of the condensed tannin is extracted by acetone–water (7:3) or even butanol–HCl. The residues of the powdered leaves after extraction showed the presence of proanthocyanins by heating in butanol–HCl. Extractions of 100 mg samples of 16 strains gave a mean of 80% of the total in extract and residue with a standard deviation of 6%.

The λ_{max} of butanol–HCl digests of extracts and residues was 556 nm. Since cyanidin has a λ_{max} of 547 nm in butanol–HCl, delphinidin 558 nm [9], Bate-Smith [9] has shown that the λ_{max} can be used to calculate the cyanidin/deliphidin ratio. On this basis, the λ_{max} observed indicates that cotton leaf condensed tannin yields 20% cyanidin and 80% delphinidin. Bate-Smith [9] reports an average procyanidin has an $E^{1\%}_{1cm}$ of 150 and a prodelphinidin one of 300. These values can be used to calculate the percentage of condensed tannin. We used a value of 270 for cotton (30 from the 20% cyanidin, 240 from the 80% delphinidin). The range of concentrations shown in Table 1 (after addition of that in residue) is from *ca* 5% to *ca* 20% by dry wt.

The relationship between spider mite activity, $E^{1\%}_{1cm}$ and tannic acid equivalent (TAE) values is shown in Table 1. The high amount of prodelphinidin makes the tannin poorly soluble in water. The TAE values for the highest

Table 1. Cotton materials, tolerance and condensed tannin content

Materials			Condensed tannins	
Source*	Use	Tolerance to spider mite†	$E^{1\%}_{1cm}$‡	TAE
Tx 401	primitive stock	(very) S	6	4.7
Lankart 57	cv	S	11.2	5.3
Des 24	cv	S	22.5	7.8
St7A(GN)	glandless-nectariless deriv. of cv	S	32.8	10.2
Tx 254	primitive stock	R	34.6	10.0
Tx 1055	primitive stock	R	39.9	11.3
Tx 1123	primitive stock	(very) R	42	11.0
Tx 1124	primitive stock	(very) R	46	11.0

* Seed of the Tx material can be obtained from the Cotton Genetics Research Unit, SEA, AR, College Station, Texas. Seed of other material can be obtained from commercial seed producers.

† Key: S = susceptible; R = resistant (see ref. [12]).

‡ Tx 1055 produced the most variable $E^{1\%}_{1cm}$ values. The mean of 10 samples had a standard deviation of 3.6.

$E^{1\%}_{1cm}$ samples indicate only a moderate astrigency. Undoubtedly the values are low because of the poor extraction of tannin into cold blood. The properties of condensed tannins which could affect antibiotic activity include series (A or B), kind (procyanidins, prodelphinidins, or mixed polymers), MW, concentration and the resulting astringency [9]. The results in Table 1 indicate that quantity (concentration) and the resulting astringency are the key elements in providing resistance to cotton plants. However, the effectiveness of a given set of the above properties as spider mite (or insect) deterrents depends on other factors (concentration of other secondary compounds, growth conditions, etc.) such that the relationships are very complex. Some cultivars (such as St7A^{GN}) contain quantities of condensed tannins nearly equal to that of resistant plants but nevertheless are susceptible.

Some of the variables which could reduce the effectiveness of a condensed tannin are the following. Simple phenols are reported to affect the spider mite in several ways, although all ultimately are detrimental [10] The resistant plants (Table 1) are short-day plants. They are equally resistant from seedling stages through the fruiting period of the winter, but their fruitfulness is much lower than that of day-neutral cultivars. We found no changes in the λ_{max} of butanol–HCl digests of the plants examined. The changes in condensed tannins between summer and winter may mean that MWs are heterogeneous within cultivars.

Swain [3] concludes that feeding of insects (and spider mites?) is deterred by tannins as the concentration approaches 2% dry wt. Cotton in a 'high phenolic' plant. Protein extraction from the leaves is difficult because of phenolic compounds including the tannins [11]. The concentration necessary to adequately deter cotton pests is higher.

EXPERIMENTAL

Plant material. Leaves were harvested from cotton plants grown in greenhouses and fields. The samples were either freeze-dried or dried at 40° in a forced-draft oven, and ground to pass a 100 mesh sieve. Most of the chemical studies were done with Tx 1055 and Tx 401, accessions of primitive races one of which (Tx 1055) is very resistant to two-spotted spider mites, and the other a very susceptible strain [12]. Other measurements were made on cultivars and breeder selections.

Extraction and assay. Leaf powder (100 mg) was extracted with 4 × 5 ml Me_2CO–H_2O (7:3) containing 0.1% ascorbic acid. The vol. of the combined extracts was brought to 25 ml with H_2O. Aliquots (usually 0.2 ml) were heated in 10 ml *n*-BuOH–HCl (19:1) at 96° for 1 hr in sealed tubes. The anthocyanidins produced were measured at 550 nm [13]. The absorbencies were calculated to give an $E^{1\%}_{1cm}$ value. The extracted leaf powder residues were washed into a tube with 10 ml BuOH–HCl and heated 2 hr [14]. Qualitative assays for condensed tannin were made by heating fresh leaf discs (95 mm^2) in BuOH–HCl. The absorption at 550 nm was used to quantitatively estimate leaf-to-leaf variation on a plant. The λ_{max} of BuOH–HCl digests was determined for the extracts and residues of several resistant and susceptible strains.

Qualitative identification of anthocyanidins. The anthocyanidins, after removal of BuOH–HCl and eventual transfer to MeOH–1% HCl, were compared by PC to isolates of cyanidin from blackberries, and delphinidin from the rind of eggplant [15]. Spectra and HPLC measurements [16] were made.

Preparation and partial purification of condensed tannin. Dried leaf powder (50 g) was extracted twice with 500 ml Me_2CO–H_2O (7:3) containing 0.1% ascorbic acid. The Me_2CO of the combined extracts was salted out (upper layer) with NaCl. The Me_2CO was evapd under vacuum at 37°. The aq. soln remaining was washed twice with cyclohexane–EtOAc (1:1) and desalted by dialysis in 3500 MW cut-off tubing in several changes of 4 l. of

H_2O through which N_2 was bubbled [17]. The dialysed soln was freeze-dried. The cork-like red-tinted material obtained by freeze-drying was dissolved in a small vol. of Me_2CO–H_2O (7:3) and chromatographed on a Sephadex G-50-150 column (5 × 32 cm) that was swollen and developed with Me_2CO–H_2O (1:1) [17]. The brownish, condensed tannins eluted near the void vol.—long before any yellowish or red materials. Further purification was done on 3 × 15 cm columns of Sephadex LH-20 washed with 70% MeOH in H_2O and eluted with Me_2CO–MeOH–H_2O (5:2:3).

Astringency tests. The effective astringency of the leaf samples was measured by adding *ca* 15 mg of the powder to 5 ml or more of human blood diluted 1:100 with H_2O. After thorough mixing and chilling in ice-H_2O for 10 min, the samples were centrifuged 10 min at 3000 *g*. The absorption at 578 nm was measured for control and samples. The per cent precipitation of hemoglobin was used to calculate a tannic acid equivalent (TAE) using the formula 0.015 + 0.00024 (% ppt.) divided by the % powder in the blood sample [18].

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