

Chapter 38

PHYSIOLOGY OF SECONDARY PRODUCTS

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INTRODUCTION

Cotton produces a large number of secondary products that often occur in specialized cells or tissues and serve diverse biological functions. The concentrations of these compounds may vary from a few ppb for some volatile terpenoids to more than 20 percent of the dry weight for condensed tannins and lignins. Some secondary products are undesirable because they present toxic hazards during fiber processing and seed utilization. Their synthesis also diverts photosynthate from the desired products. However, many secondary products have important desirable roles in resistance to pests and to environmental stress, and some also may be useful as pharmaceuticals. Thus, a thorough knowledge of secondary products chemistry and biology is needed to manipulate and use these compounds for optimal cotton production and utilization.

In this review the structure, biological activity, localization, genetic control, and interaction with environment will be discussed for different secondary products. The compounds and their derivatives are divided into the broad categories: phenolic acids, flavonoids, terpenoids and unique fatty acids.

PHENOLIC ACIDS

The cotton plant (*Gossypium* spp.), like most other plants, produces a number of derivatives of benzoic and cinnamic acids. Both groups of acids are probably derived from shikimate metabolism, although this has not been confirmed in cotton. Maga and Lorenz (1974) identified the most prominent phenolic acids in defatted cottonseed flour. The major benzoic acid derivatives, 3-methoxy-4-hydroxybenzoic (vanillic) and 3,5-dimethoxy-4-hydroxybenzoic (sinapic) acid, each occurred at 30 ppm. The major cinnamic acid derivatives, 4-hydroxycinnamic (*p*-coumaric), 3-methoxy-4-hydroxycinnamic (ferulic) and 3,5-dimethoxy-4-hydroxycinnamic (syringic) acid, occurred at 41, 45 and 21 ppm, respectively. Other phenolic acids, found at 4-11 ppm, included *p*-hydroxybenzoic, 2-hydroxybenzoic (salicylic), 2-hydroxycinnamic (*o*-coumaric), 2,5-dihydroxybenzoic (gentisic), 3,4-dihydroxycinnamic (caffeic), 3,4-dihydroxybenzoic (protocate-

chic) and chlorogenic acid. The concentrations of total phenolic acids and of vanillic acid alone were at or slightly above the taste threshold. Thus, they may contribute slight astringency or bitter taste to cottonseed products.

Benzoic acid has not been reported to occur in cotton. However, benzaldehyde and benzyl alcohol make up 0.2-1.0 percent of the volatile products collected by steam distillation of various cotton tissues, gin trash and mill dust (Hedin *et al.*, 1975a,b,c, 1976). These compounds occur in subfractions with potential byssinotic activity but have not been tested directly against animal cells.

The hydroxylated cinnamic acids appear to function largely as intermediates in the synthesis of coumarins, lignins and flavonoids. Scopoletin, a coumarin derivative of ferulic acid, occurs in trace amounts along with its glucoside scopolin in living tissues of the cotton plant (Hanny, 1980). Concentrations of scopoletin generally increase markedly during senescence or following infection and stress (Caldwell *et al.*, 1966; Wakelyn *et al.*, 1974). The compound persists in dead tissues and may be found in mill dust from bales stored for several years. Field dried bracts contained 5 ppm of scopoletin (Wakelyn *et al.*, 1974), and frost-killed bracts and leaves contained 22.6 and 21.6 ppm, respectively (Doolan *et al.*, 1982).

Wiese and DeVay (1970) found that caffeic acid, like scopoletin, increased in *Verticillium*-infected cotton. Caffeic acid at 10^{-4} M decreased IAA degradation by 30 percent in healthy cotton tissue, and scopoletin was mildly inhibitory. They concluded that these compounds might contribute to the increase of IAA and decrease of IAA decarboxylation that commonly occurs in diseased tissues. Terpenoid aldehydes and catechins, however, are probably more important because they accumulate to much greater concentrations than phenolic acids and coumarins (Bell and Stipanovic, 1978) and similarly affect IAA decarboxylation.

Lignin may constitute over 40 percent of the dry weight of mature cotton stems (Veksler *et al.*, 1978). *p*-Coumaryl aldehyde, coniferyl aldehyde (3-methoxy-4-hydroxycinnamic aldehyde) and syringyl aldehyde have been isolated from dried cotton stalks (Brauns and Brauns, 1960), and the corresponding *p*-coumaric, guaiacilic and syringic structural units have been shown in cotton dioxane lignins (Veksler *et al.*, 1977). Presumably most of the aldehydes are reduced to corresponding alcohols before incorporation into the lignin polymer. El-Hinnawy *et al.* (1980) concluded that cinnamyl units in lignin are linked mostly through *p*-aryl type linkages.

Lignin structure in cotton varies with plant age (El-Hinnawy *et al.*, 1980; Veksler *et al.*, 1977, 1978). In young plants lignin contains almost exclusively guaiacilic residues, indicating that it is synthesized from coniferyl alcohol. With age the degree of methylation of lignin first increases to a peak and then after several months may decrease slightly; corresponding changes occur in the percentage of syringyl units in the polymer. *p*-Coumaryl units never occur in more than minor amounts. C3 side chain substitution and condensation of aromatic nuclei in lignin are greatest in young vegetative plants and least in mature stems,

presumably due to the changes in methylation. Lignins extracted with dioxane contain some condensed proanthocyanidins (see section on flavanols) which decrease in percentage as the plant ages.

The percentage of syringyl units in cotton lignin varies considerably for different preparations, even from the same cultivar. This might be due to the influence of environment or pests on lignin composition. Recent studies have shown that rapid lignin synthesis in response to pests is an important mechanism of both cultivar and induced resistance to diseases and insects in various plants (Bell, 1981; Kuc, 1981). Infection-induced lignin, compared to that in healthy plants, often has more syringyl residues and little or no aldehyde content (indicated by negative reactions with acidic phloroglucinol). Numerous reports of increased yellow fluorescence in diseased tissues of cotton (Bell, 1973) may be due to induced lignin synthesis, because syringyl derivatives fluoresce yellow or yellow-green (Bell, 1981). Studies of the possible importance of lignins in the resistance of cotton to pests and stress are needed.

FLAVONOIDS

The flavonoids of cotton have the basic structure shown in Figure 1. Additional hydroxyl substitutions can occur at the 3, 8 or 3' carbons, and carbonyl oxygen can occur at the 4 carbon depending on the specific compound. Hydroxy groups can react further to form methyl ethers or glycosides at various positions. Variations also occur in the oxidative state of the heterocyclic ring, and these distinguish groups of compounds known as flavonols, flavones, anthocyanins and flavanols.

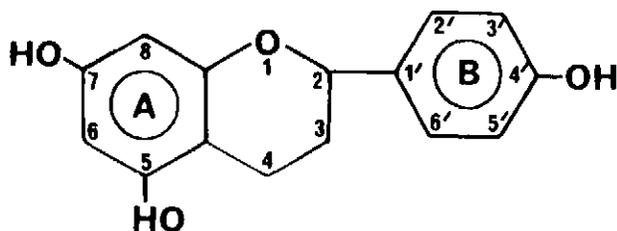


Figure 1. Ring structure of flavonoids, showing numbering of carbons and ring identification.

FLAVONOLS

Flavonols may make up 2-4 percent of the dry weight of flowers and 1 percent of leaves. The localization of these compounds among cells or tissues is uncertain, although it has been suggested that they occur in the epidermis of embryos in seed (see references in Pratt and Wender, 1959). I have also found the flavonol isoquercitrin in the capitate hairs of the epidermis of stems and leaves.

The most abundant flavonols occur as glycosides of one of the four aglycones shown in Figure 2. The 4'-methyl ethers of quercetin and kaempferol are the only other known aglycones (Struck and Kirk, 1970). The sugar composition and

FLAVONOLS

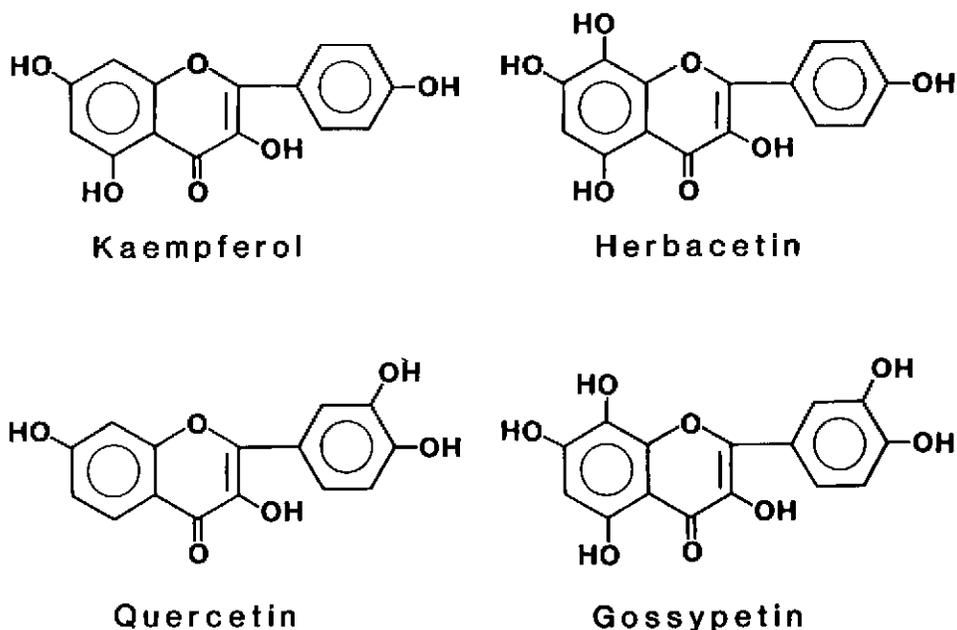


Figure 2. Structures of major flavonol aglycones from *Gossypium*.

occurrence of cotton flavonols in different tissues is given in Table 1. The absolute structures of the sugars is only partially known. Two different rhamnoglucosides of quercetin have been identified from seed. In the first, rutin, the sugar moiety is rutinose (6-O- α -L-rhamnosyl-D-glucose), whereas in the second the sugar is neohesperidose (2-O- α -L-rhamnosyl-D-glucose). Blouin *et al.* (1981a,b) concluded that the main rhamnoglucoside of kaempferol in seed was a neohesperidoside. The linkage between rhamnose and galactose in the rhamnogalactosides is apparently the same as in rutin. The 3-glucoside of quercetin usually has been identified as isoquercitrin, which contains glucose in the β -D-pyranose form. However, Sadykov (1972) reported that the 3-monoglucosides of kaempferol and quercetin from the cultivar '108 F' (*G. hirsutum*) had properties different than expected for the β -D-glucopyranosides and suggested that kaempferol-3- α -D-glucofuranoside was the correct structure for the kaempferol glucoside. The

Table 1. Flavonols reported to occur in various tissues of *Gossypium*.

Flavonol	Tissue			
	Petals	Anthers	Leaves	Seed
Kaempferol glycosides				
3-glucoside (astragalin)	+(O-H) ²		+(H)	
3-glucoglucoside				+(H)
3-rhamnoglucoside	+(O-H)		+(H)	+(H)
Quercetin glycosides:				
3-glucoside (isoquercitrin)	+(H)	+(H)	+(H)	+(H)
3-glucoglucoside		+(L)		+(H)
3-rhamnoglucoside	+(O-II)	+(L)	+(H)	+(H)
3-rhamnogalactoside				+(H)
3-xyloglucogalactoside			+(O-H)	
3'-glucoside	+(O-H)	+(L)		
4'-glucoside	+(O-H)			
7-glucoside (quercimetrin)	+(O-H)	+(L)		
7-glucoglucoside		+(L)		
7-rhamnoglucoside		+(L)		
3,7-diglucoside		+(L)		
4'-methyl-7-glucoside	+(O-H)			
Herbacetin glycoside:				
7-glucoside (herbacitrin)	+(O-H)			
Gossypetin glycosides:				
3-glucoside (gossytrin)	+(O-H)	+(H)		
7-glucoside (gossypitrin)	+(O-H)	+(L)		
8-glucoside (gossypin)	+(O-H)	+(VL)		
3 (or 7)-glucoglucoside		+(H)		

¹Data are adapted from published reports: petals (Hanny *et al.*, 1978; Hedin *et al.*, 1968; Parks *et al.*, 1972; Parks *et al.*, 1975; Sadykov, 1972); anthers (Hanny, 1980); leaves (Hanny *et al.*, 1978; Howell *et al.*, 1976; Parks *et al.*, 1972; Sadykov, 1972); seeds (Blouin *et al.*, 1981a,b; Pratt and Wender, 1959, 1961).

²Relative concentrations in parentheses: H=high, M=medium, L=low, VL=very low, O=none detected.

flavonol identified as trifolin by Parks *et al.* (1965a,b, 1972, 1975) most likely is the kaempferol-3-glucoside, australin. Trifolin is kaempferol-3-galactoside, whereas the glycoside from cotton contains glucose (Parks *et al.*, 1965a,b; Blouin, 1981a,b). Different conclusions have been reached about the location of glucose substitutions on the β ring of *Gossypium* flavonols. Hanny (1980), Hedin *et al.* (1968) and Sadykov (1972) reported only the 3'-glucoside of quercetin, whereas Park *et al.* (1975) reported only the 4'-glucoside in various *Gossypium* species.

Sadykov (1972) concluded that the sugar in quercetin-3-glucogluco-side from *G. barbadense* flower petals apparently was sophorose (2- β -D-glucosido-D-glucose); this sugar has not been identified in other studies. More detailed studies are needed to ascertain the structures of the sugar moieties in the *Gossypium* flavonols.

Flavonols appear to be important because they influence insect behavior. Low concentrations may act as feeding stimulants, whereas higher concentrations often inhibit larval growth and especially pupation. Guerra and Shaver (1969) found that ethanolic solutions (5 mg/ml) of isoquercitrin and rutin, when applied to surfaces of leaf disks, stimulated feeding by larvae of tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Heliothis zea*). The pupal weight and percentage of larvae pupating in these insects also increased slightly when concentrations of .05 to .10 percent of these compounds were added to artificial diets; 0.025 percent of these flavonols caused similar stimulation of the pink bollworm, *Pectinophora gossypiella* (Lukefahr and Martin, 1966; Shaver and Lukefahr, 1969). Aqueous solutions of 0.1 percent quercetin, quercetin-7-glucoside and quercetin-3'-glucoside applied to filter paper wrapped over water-agar plugs moderately stimulated feeding attempts by boll weevil (Hedin *et al.*, 1968). In contrast, kaempferol, quercetin-3-glucoside and cyanidin-3-glucoside had no effect or slightly inhibited boll weevil feeding. Thus, some specificity in stimulation may reside with different flavonol structures.

Rutin and isoquercitrin at concentrations above 0.2 percent inhibit larval growth and especially pupation in cotton bollworm, tobacco budworm, and pink bollworm (Chan *et al.*, 1978; Elliger *et al.*, 1980; Guerro and Shaver, 1969; Lukefahr and Martin, 1966; Shaver and Lukefahr, 1969). Concentrations of 0.05 to 0.1 percent of rutin added to 0.1 percent gossypol greatly increased toxicity to bollworms, indicating a synergistic interaction between flavonoids and terpenoids in natural resistance to insects (Lukefahr and Martin, 1966).

The toxicity of flavonols depends both on their specific structure and the insect species. Flavonols containing the 3',4'-*ortho*-dihydroxy structure are more toxic than those having only a 4'-hydroxyl group (Chan *et al.*, 1978; Elliger *et al.*, 1980). Addition of sugar moieties generally decreases toxicity; quercetin is more toxic than isoquercitrin, which is more toxic than rutin (Lukefahr and Martin, 1966; Shaver and Lukefahr, 1969). With most flavonols, the pink bollworm is more sensitive and the cotton bollworm less sensitive than the tobacco budworm. Of the numerous flavonols known in cotton only kaempferol, quercetin, isoquercitrin and rutin have been included in comparative tests. Appreciable concentrations of free kaempferol and quercetin have not been found in cotton tissue; instead these occur mostly as glucosides. There is a critical need for more toxicity data on most of the major glycosides that occur in cotton before a strategy concerning their genetic or cultural manipulation for insect control can be developed.

The flavonols also may be important for their adverse effects. Blouin *et al.*

(1981a,b) conclusively showed that the undesirable yellow color of baked products prepared with cottonseed flour is due to flavonols and can be duplicated by adding rutin to baked products. Several studies have implicated flavonols and their oxidation products (polyphenols) in byssinosis. Kilburn *et al.* (1973, 1974) showed that quercetin, its oxidation products and polyphenols extracted from cotton trash recruited polymorphonuclear leucocytes on airways when inhaled by hamsters. Polyphenols also cause aggregation of human red blood cells, and rutin at 0.018 to 0.100 percent stimulates 12 to 46 percent release of histamine from platelets of pig blood (Ainsworth *et al.*, 1979b). The composition of the polyphenol preparation in these studies is unclear, but most likely included oxidized derivatives of flavanols and lignins, as well as flavonols. Because of the potent activity of rutin at concentrations found in the cotton plant, other major flavonol glucosides known in cotton should be investigated for possible involvement in byssinosis.

Flavonol composition may vary among tissues (Table 1), cultivars, species, and environments. Herbacetin and gossypetin glycosides and 3', 4'- and 7-glycosides of all aglucones have been reported only from anthers and petals of flowers; only 3-glycosides of quercetin and kaempferol are known in leaves and seed. High concentrations of herbacetin and gossypetin are closely associated with yellow flower color. These aglucones are abundant in yellow-flowered cultivars of *G. hirsutum*, but are missing from white-flowered cultivars. Likewise, they occur only among yellow-flowered species of the wild American diploid cottons. Distinct differences appear to occur in flavonols of *G. hirsutum* and *G. barbadense*. Quercetin-7-glycosides and kaempferol-3-glucoside are more abundant in *G. barbadense* than in *G. hirsutum*, whereas the rhamnoglucosides occur abundantly in *G. hirsutum* but only in trace quantities in *G. barbadense*. Other differences among species have been reviewed by Parks *et al.* (1975).

Parks *et al.* (1972) examined the effects of different growing environments across the U.S. Cotton Belt and of different nutritional levels, temperatures and photoperiods in controlled environments on flavonol contents of tissue. The flavonol content of petals was quite constant, regardless of environment. In contrast, flavonol composition of leaves varied markedly depending on age of tissue as well as environment. Howell *et al.* (1976) and Sadykov (1972) also observed that flavonol concentrations increase gradually in leaves with ageing, usually concurrently with decreases in flavanols (catechins and condensed tannins) that are synthesized earlier in leaf development. The greatest concentrations of flavanols occurred in the youngest unfolded leaves next to the terminal, whereas the greatest content of the flavonol, isoquercitrin, was in the third leaf back of the terminal.

Howell *et al.* (1976) also studied the relative ability of leaves of different ages to synthesize flavanols and flavonols in response to infection by *Verticillium* wilt. The ability of leaves to synthesize flavanols in response to infection quickly decreased with age, being negligible in leaves 5 and 6 back from the terminal. In contrast, induced flavonol synthesis was greatest in the 4th leaf, and 3- to 6-fold

increases in flavonol concentrations occurred in the 5th and 6th leaves, which were the oldest leaves studied. These studies indicate that the flow of flavonoid biosynthesis in leaves is largely to flavanols in young tissues but to flavonols in older tissue. It has not been determined whether the flavanols are directly converted to flavonols.

FLAVONES AND ANTHOCYANINS

Flavone and anthocyanidin aglucones normally occur bound in glycosides. The only clearly identified flavone and anthocyanidin aglucones in cotton are apigenin and cyanidin, respectively (Figure 3). Apigenin occurs in high concentrations as the 7-rutinoside in flowers of the wild Australian species, *G. australe*, *G. robin-*

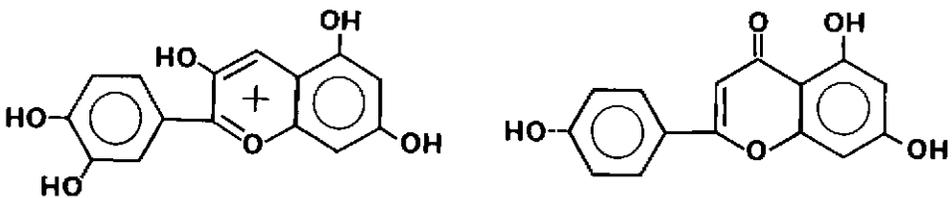


Figure 3. Structures of flavone and anthocyanidin aglucones from *Gossypium*.

sonii and *G. sturtianum*, but has not been found in other *Gossypium* species (Parks *et al.*, 1975). Eight other compounds with flavone characteristics have been found in the Australian species but have not been identified. Cyanidin-3-glucoside (chrysanthemine) is the major anthocyanin in flower buds, petals and leaves (Hedin *et al.*, 1967; Sadykov, 1972). Cyanidin-3-xyloglucoside (Sadykov, 1972) and an unidentified anthocyanin of *G. sturtianum* (Chan and Waiss, 1981) also have been reported to occur in flowers. Chrysanthemine is concentrated in the epithelial cells that make up the envelope of pigment glands in green tissues (Chan and Waiss, 1981). A second anthocyanidin associated with pigment glands in *G. hirsutum* may be pelargonidin (Hedin *et al.*, 1981). Delphinidin is obtained by acid hydrolysis of cotton condensed proanthocyanidins, but it has not been found as a glycoside.

Chan and Waiss (1981) obtained 4.5 mg of pure chrysanthemine from 135 mg of isolated pigment glands of 'Waukena White' (*G. barbadense*), and estimated that 10.4 percent and 9.4 percent of the dry weight of pigment glands from *G. arboreum* var. *sanguineum* and *G. barbadense*, respectively, were anthocyanin. However, some oil contents of the glands were lost during freeze drying, and further oil was undoubtedly lost during flotation of glands on methylene chloride. Thus, the estimates might be high for intact glands in the plant. Anthocyanins in glands apparently occur only in the epithelial cells surrounding the lysigenous cavity which contains terpenoids dissolved in oils. Sadykov (1972) reported anthocyanin contents of 2.1 and 5.5 percent in flower petals of two cultivars of *G.*

hirsutum. This anthocyanin apparently occurs mostly in vacuoles of parenchymous cells.

Hedin *et al.* (1982) recently reported that the growth of tobacco budworms on leaf terminals and flower petals of cotton was negatively correlated with chrysanthemins contents, as well as gossypol contents, indicating that this anthocyanin may have a role in resistance to insects. Anthocyanin both in pigment glands and in parenchyma cells of petals appeared to be effective. In feeding tests, cyanidin, delphinidin and chrysanthemins showed toxicity to budworms that was similar to that of flavonols, gossypol and tannin.

Various pesticides, temperature extremes and drought stress may cause reddening of cotton leaves and stems. Parrott and Lane (1980) showed that such reddening caused by the insecticide methomyl was correlated with increases in anthocyanin contents. The effects of other stresses on anthocyanins have not been determined.

FLAVANOLS

The flavanols of cotton have the structures shown in Figure 4. The monomeric flavanols are characterized by a reduced heterocyclic ring that gives them much greater water solubility than corresponding flavones or flavonols. They do not occur as glycosides but are polymerized via 3C-8C linkages to form flavanol polymers called condensed proanthocyanidins (or condensed tannins). The condensed tannins can be hydrolyzed by dilute HCl in butanol to yield cyanidin and delphinidin from catechin and gallicocatechin moieties, respectively, in the polymer. This reaction is frequently used for quantitative estimates of 'tannins' in cotton, but it only detects the flavanol polymer. Once oxidation of the heterocyclic ring and *ortho*-dihydroxy groups occurs, such as during seed ripening or death in diseased tissues, a nonhydrolyzable dark brown water-insoluble pigment (melanin) is formed.

Estimates of flavanol concentrations in tissues have varied considerably and have been presented in a variety of units. For purposes of uniformity, I have

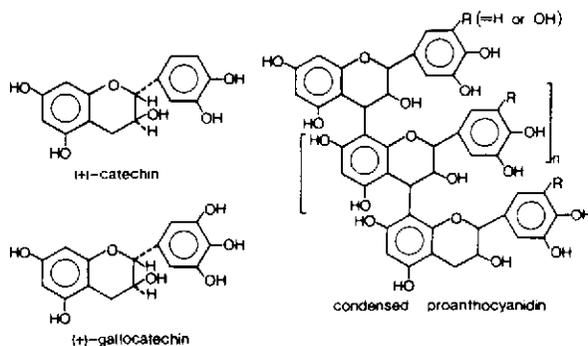


Figure 4. Structures of flavanols from *Gossypium*.

recalculated some data into percent dry weight, assuming that leaves and stems have dry weights of 10 and 20 percent, respectively, and that $E_{1\text{cm}}^{1\%} = 270$ for products of butanol-HCl hydrolysis of condensed tannin as suggested by Lane and Schuster (1981).

The variable results from different studies of flavanol concentrations probably are due largely to the different methods and different standards used. The best estimates of catechin and gallic catechin probably result from initial separation by thin-layer chromatography on silica gel followed by reaction with 2,4-dimethoxybenzaldehyde to estimate quantities (Howell *et al.*, 1976). Ethyl ether:formic acid (95:5) is an excellent developing solvent, and (+)-catechin should be used as a standard.

Condensed tannins are best extracted with 70 percent acetone: 30 percent water from finely ground (100-200 mesh) dry tissues (Lane and Schuster, 1981). They can best be estimated in extracts or fresh leaf disks by hydrolysis in butanol:conc. HCl (95:5) at 98C for 2 hours, followed by reading absorbance at 550 nm. Purified condensed tannin prepared by the methods of Chan *et al.* (1977) can be used as the standard. Different standards may be needed for different cultivars, because E for condensed gallic catechin is about twice as great as E for condensed catechin, and considerable variation in catechin:gallic catechin ratios may occur in tannins from different cottons. Ratios of 1:4, 1:1, 2:1 and 4:1 have been reported for condensed tannin of Texas 1055 and free catechins of Seabrook Sea Island 12B2, Acala 4-42 and Stardel, respectively (Bell and Stipanovic, 1978; Howell *et al.*, 1976; Lane and Schuster, 1981). Obviously, the same standard could not be used for accurate measurements of condensed tannins in both Texas 1055 and Stardel. Likewise, tannic acid should not be used as a standard because it has quite different chemical properties than the flavanol tannins of cotton.

Flavanol concentrations vary considerably among various tissues and with age. The seed embryo contains only traces of flavanols in the palisade parenchyma, whereas high concentrations occur in the pigment layers of the seed coat (Holloin, 1982). Sadykov (1972) reported that seed hulls contained 12 percent catechin and related compounds just prior to ripening, but following ripening contained only 7 percent. Presumably the balance was oxidized into the brown pigments of the seed coat during ripening.

Following seed germination flavanol synthesis in the root begins within 1-2 days and appears first in the root cap and endodermis (Mace and Howell, 1974). Next, flavanols appear in the hypodermis and finally in parenchyma cells scattered throughout the root bark. Only a few scattered paravascular, pith and xylem ray cells form flavanols in healthy stele.

In young hypocotyls and stems, flavanols initially are synthesized in the epidermis and endodermis. Concentrations are low in new tissues but increase progressively over several weeks. Hunter (1974, 1978) found that flavanol concentrations increased linearly from 0.5-0.8 percent in 6-day-old hypocotyls to 1.1-2.3 percent in 12- to 14-day-old hypocotyls. These differences apparently account for the

marked increase of resistance to seedling diseases that occurs during this period of growth. Flavanol concentrations continue to increase with age in both root and stem bark (Bell and Stipanovic, 1978) until stationary levels of about 7-8 percent are reached (Sadykov, 1972).

In leaves, the lowest concentrations of flavanols occur in the cotyledonary and first true leaves. Concentrations increase progressively with each leaf formed until about the tenth leaf. Subsequent leaves have about the same amount as the tenth leaf, except considerable variation occurs among different leaves (Lane and Schuster, 1981). Finally, late in the growing season concentrations in leaves again decline (Sadykov, 1972).

Any given leaf has the highest flavanol concentration when first unfolded and only partially expanded. Concentrations in the leaf then decline progressively with age (Bell and Stipanovic, 1978; Lane and Schuster, 1981). Chan *et al.* (1978) and Hedin *et al.* (1981) reported flavanol levels of 22-50 percent in the shoot terminals (mostly leaf buds) of a few cultivars, but Hanny *et al.* (1978) found concentrations of only 7.5-24.3 percent in terminals in a survey of 39 cotton strains. Unfolding and young, expanding leaves are reported to contain 3-44 percent flavanol tannins with concentrations of 3-8 percent being most common (Chan *et al.*, 1978; Guinn, 1982; Hedin, 1981; Hanny *et al.*, 1978; Howell, 1976; Lane and Schuster, 1981; Schuster and Lane, 1980). The high concentrations usually have been found in exotic cotton strains selected for high levels of resistance to insects. Old leaves contain 1-11 percent flavanol tannin depending on cultivar and exact age. Free catechin and gallic acid concentrations ranged from 0.5 to 0.7 percent in young leaves to 0.3 to 0.4 percent in old leaves of Acala 4-42 (Howell *et al.*, 1976). Sadykov (1972) reported total flavanol concentrations of 7-11 percent in petioles of leaves, but Bell and Stipanovic (1978) found very low concentrations (<2%). Hedin *et al.* (1982) concluded that flavanol concentrations in leaf veins were higher than those in the leaf blade. Young capitate hairs on leaves initially contain flavanols but later contain flavanol glucosides.

Hanny *et al.* (1978) found condensed tannin concentrations of 6.0-11.7 percent in flower buds of 37 cotton strains. Parts of dissected mature flower buds from one cultivar contained the following percentages (%) of condensed tannins: receptacle, 11; calyx, 8; fused corolla tube, 18; corolla, 9; anthers, 3.4; carpels, 19; and pistil, 23 (Chan *et al.*, 1978). In more extensive studies, Hanny (1980) found condensed tannin concentrations of 4.8-5.3 percent in anthers of various glanded and glandless cultivars with yellow- or cream-colored pollen. Plants with cream colored pollen contained significantly more tannin in anthers than those with yellow pollen within each of several cultivars.

Guinn (1982) studied concentrations of flavanols in 4-day-old bolls and found concentrations of 2.0-4.6 percent of hot water-soluble and 3.7-8.1 percent of insoluble flavanols. Concentrations in the bolls and in middle-aged leaves did not vary significantly with moisture stress or irrigation regimes, but leaf concentrations were increased by artificial defruiting of plants.

Many pathogens and insects tend to attack tissues and organs of the plant that are low in flavanol content, i.e. root tips, stele, anthers and old leaves. Such attack on tissues may result in marked increases in flavanol content (Mace *et al.*, 1978). Hunter (1974) found that flavanol concentrations in hypocotyls doubled within 24 hours after being inoculated with *Rhizoctonia solani*. Likewise, Bell and Stipanovic (1978) found that flavanol concentrations in stele tissue increased from 0.2-0.8 to 4.2-8.0 percent at 10 days after inoculation with *Verticillium dahliae*. Benedict and Bird (1981) found that mixtures of nonpathogenic *Bacillus* species that increase resistance to boll weevil when sprayed on cotton plants stimulate increases of flavanol synthesis in cotton tissues.

Flavanols apparently have several important functions in cotton. Halloin (1982) found that flavanol polymers and especially their melanized products appear to be involved in the regulation of water uptake by seed. When seed were removed from bolls a few days prior to dehiscence and ripened under nitrogen, they were fully viable, but seed coats were white, fragile and imbibed water much more rapidly than normal seed. Such rapid imbibition would allow extensive deterioration of seed in the field during extended moist periods (see also Chapter 31). Thus, flavanol metabolites are important determinants of seed quality. Flavanols and melanins formed at wound sites and at diseased sites may also prevent dehydration or water congestion of tissues.

Another important role of flavanols is in disease resistance. Flavanols and their oxidation products formed with peroxidase act as bactericides (Vernere, 1980), fungicides (Howell *et al.*, 1976; Hunter, 1978), enzyme denaturants (Hunter, 1974, 1978) and antisporelants (Howell *et al.*, 1976). Hunter (1978) showed that low levels of catechin stimulated pectinase production by a strain of *Rhizoctonia solani* highly virulent to cotton, whereas only inhibitory effects occurred with moderately virulent strains. Thus, the response of pathogens to flavanols may also be important in determining their virulence. Most studies implicating flavanols as determinants of disease resistance in cotton have been reviewed by Bell and Stipanovic (1978). More recent studies indicate that flavanols contribute to increases of resistance with aging against seedling pathogens (Hunter, 1978) and to cultivar resistance against bacterial blight (Vernere, 1980).

Flavanols also contribute to insect resistance, but their precise role is still uncertain. Waiss *et al.* (1981), Hedin *et al.* (1981), Chan *et al.* (1977, 1978) and Elliger *et al.* (1980) showed that condensed tannin and catechin inhibit growth of young larvae of cotton bollworm, tobacco budworm and pink bollworm when incorporated into diets at concentrations greater than 0.1 percent wet weight. Larvae of the budworm quickly lose their sensitivity to condensed tannin with age and are not appreciably affected by even 0.4 percent after they are 7 days old (Waiss *et al.*, 1981). Klocke and Chan (1982) found that bollworms feeding on condensed tannin-treated diet exhibited decreased protease and invertase activities in the midgut caecal wall and lowered total protein and sugar levels in the hemolymph compared to controls. However, these differences apparently had no

effect on assimilation and efficiency of conversion of digested matter into animal biomass. They concluded that reduction in growth is due mostly to reduction in food consumption. Flavanols, therefore, might not be effective in decreasing feeding or growth on the intact plant, which also contains feeding stimulates, even though they inhibit feeding when added alone to synthetic diets.

Cotton stocks resistant to spider mites have consistently shown unusually high levels of condensed tannins in leaves. The isolated tannins from resistant cultivars also have shown greater levels of astringency per unit of tannin than those from susceptible lines, indicating qualitative differences of tannins and flavanols among cottons (Lane and Schuster, 1981). Schuster and Lane (1980) found that four high-tannin cotton lines also had fewer bollworms and less square damage than cotton lines with lower tannin levels. However, two other high tannin lines had no apparent resistance. Based on studies with five cultivars, Hedin *et al.* (1981) concluded that when concentrations of tannins and other phenols (terpenoid aldehydes) were high in cultivars, weights of bollworm and budworm feeding on terminals were low. After more extensive studies, however, Hedin *et al.* (1982) concluded that tobacco budworm larval growth in the field is slightly positively correlated with tannin concentrations. Likewise, Hanney (1980) found that cream-colored anthers in various cottons had higher condensed tannin concentrations than yellow-colored anthers but gave better growth of tobacco budworms. It is important that the interactions of flavanols with feeding stimulants, such as the flavonol glycosides, and with other toxicants, such as the terpenoid aldehydes, be determined. Then, their role in resistance to insects may be more clear.

Flavanols also have several adverse effects. The brown discoloration of baked products prepared from glandless cottonseed flour probably is due to flavanols (Blouin, 1981a,b; Halloin, 1982). Interspecific hybrid plants undergoing genetic lethal reactions show massive spontaneous flavanol synthesis in stele tissues, cambium and phloem where little or no synthesis normally occurs. This response closely resembles the normal induction of flavanols in response to pathogens. Thus, Mace and Bell (1981) concluded that this genetic lethality may be analogous to autoimmune death in animals. The relationships between yield and flavanol content have not been determined. However, it is apparent that increasing flavanol concentrations in leaves from 3-5 to 20-25 percent for insect resistance would cause a considerable drain of photosynthate away from desired products.

TERPENES

VOLATILE TERPENES

The cotton plant produces a variety of monoterpenes and sesquiterpenes that are found in essential oils collected by steam distillation of plant parts comminuted in water. Extensive surveys are available of the volatile constituents in flower buds (Hedin *et al.*, 1975a,b; Minyard *et al.*, 1966, 1968, 1969), leaves (Hedin *et*

al., 1972; McKibben *et al.*, 1977), green hulls and bracts (Hedin *et al.*, 1975a), whole plants (Thompson *et al.*, 1971), air space above plants (Hedin, 1976; Hedin *et al.*, 1975c) and cotton lint and waste (Hedin *et al.*, 1975a). The concentrations of essential oils obtained from buds, leaves, whole flowering plants and whole mature plants have usually been 100-150 ppm (fresh weight). Concentrations obtained from young seedlings were 20-30 ppm.

Hedin (1976) studied seasonal variations in the emission of volatiles by cotton plants in the field in Mississippi. Peak emissions (10-26 $\mu\text{g}/4000$ liters of air/8 hr) were produced between July 17 and August 11 when plants were squaring and flowering. Emissions by very young or old plants were less than 5 μg .

Both similarities and distinct differences occur in the volatile terpenes (Figure 5) of *G. hirsutum* and *G. barbadense*. α -Pinene and *trans*- β -ocimene are major monoterpene hydrocarbons and (-)- β -carophyllene and α -humulene are major sesquiterpene hydrocarbons in both species. Terpenoid alcohols found in

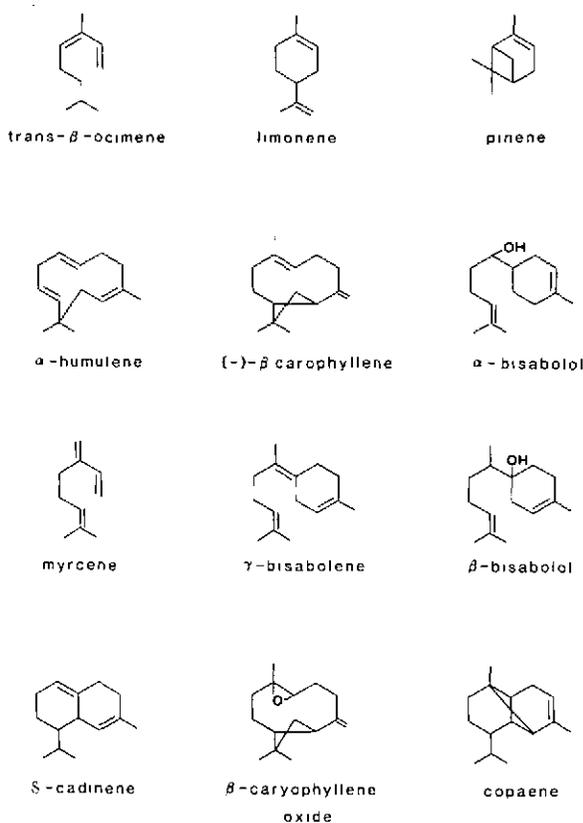


Figure 5. Structures of volatile terpenes from *Gossypium*.

minor concentrations (0.2-2.0 percent of the essential oil) in both species include linalool, α -terpineol, isoborneol, α -bisabolol and geraniol. Myrcene is a major monoterpene in *G. hirsutum* but has not been found in *G. barbadense*. Accordingly, terpenoid aldehyde derivatives of ocimene occur in both species, whereas derivatives of myrcene have been found only in *G. hirsutum* (Bell *et al.*, 1978). *G. barbadense* apparently lacks the ability to synthesize myrcene. The sesquiterpenes, *cis*- γ -bisabolene and β -bisabolol, occur in concentrations of 12.41 and 13.71% in oils from whole plants of *G. hirsutum* (Thompson *et al.*, 1971) compared to only 0.7 and 0.0 percent in those from leaves of *G. barbadense*. This is another major difference between the volatile terpenoids from the two species. Further differences occur in copacene, β -caryophyllene oxide and (–)- δ -cadinene, which occur at 14.3, 8.0 and 7.8 percent in *G. barbadense* volatile leaf oil compared to only 0.7, 0.4 and 0.3 percent, respectively, in oils from *G. hirsutum*. These differences might contribute to differences in insect-host relationships between the two species.

Volatile oils have been shown to attract both the boll weevil, *Anthonomus grandis* Boheman (McKibben *et al.*, 1977) and the Egyptian cotton leaf worm, *Spodoptera littoralis* Boisduval (Hedin *et al.*, 1972). The cotton constituents, (+)-limonene, (+)- α -pinene, β -caryophyllene oxide, (–)- α -caryophyllene and (+)- β -bisabolol, were effective attractants of boll weevils when used as single compounds (Minyard *et al.*, 1969). Maximal activity of the first three occurred at 0.3-3.0 ppm, while that of the last two occurred at 6-10 ppm in water. A mixture containing 10, 3, 100, 100, and 130 ppb of (+)- α -pinene, (+)-limonene, (–)- β -caryophyllene, (+)- β -bisabolol and (–)- β -caryophyllene oxide, respectively, was 124 percent as attractive as the most attractive volatile oil from cotton buds. McKibben *et al.* (1977) concluded that the volatile oils are sufficiently attractive to guide overwintering boll weevils to fields of seedling cotton. Pheromones from the male weevil are more important in influencing migration later in the season.

Recently, S.B. Vinson and H.J. Williams (personal communication) at Texas A&M University have found that volatile sesquiterpenoids may also attract wasp parasitoids of tobacco budworms. Wasps were attracted especially by β -bisabolol and to a lesser extent by γ -bisabolene, (–)- β -caryophyllene oxide and α -humulene. Thus, certain terpenoids may be useful to facilitate biological control.

No attempt has been made to manipulate the volatile terpenes with cultural or genetic techniques. The distinct differences in the volatile terpenoids of *G. hirsutum* and *G. barbadense* indicate that at least breeding approaches may be practical.

SESQUITERPENOID NAPHTHOLS AND KETONES

The compounds 2,7-dihydroxycadalene, lacinilene C and their respective 7-methyl ethers were first isolated from cotton bracts (Lynn and Jeffs, 1975; Stipanovic *et al.*, 1975, 1981). The probable biosynthetic relationships among

these compounds are shown in Figure 6. Lacinilene C-7-methyl ether causes a number of responses in animal cells that suggest it as a causative of byssinosis, a respiratory disease of cotton mill workers (Ainsworth *et al.*, 1979b; Kilburn *et al.*, 1979). This compound also is toxic to tobacco budworm (Stipanovic and H. Williams, personal communication). Lacinilene C and its cadalene precursor are bactericides and have been implicated in the resistance of cotton plants to infection by the bacterium, *Xanthomonas campestris* pv. *malvacearum* (Essenberg *et al.*, 1982). Thus, compounds in this group probably serve a normal function of protection of the plant against pests.

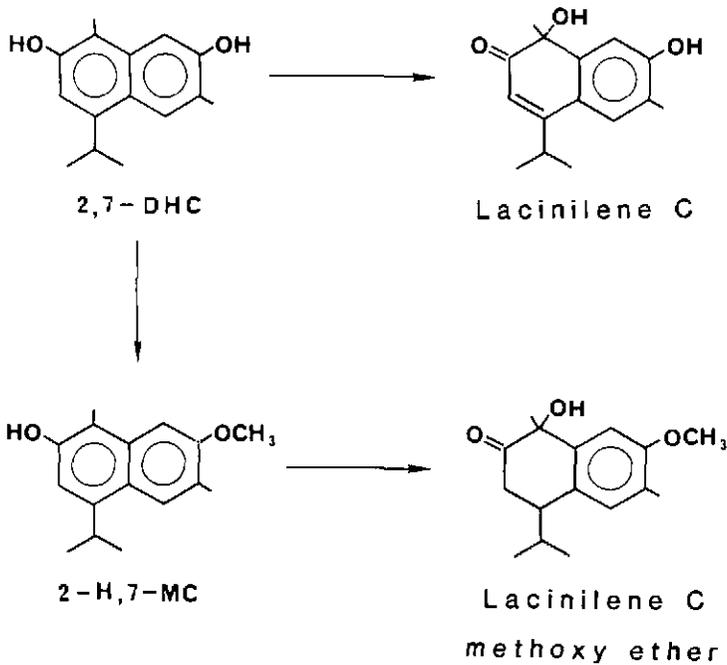


Figure 6. Structures and biosynthetic relationships of sesquiterpenoid naphthols and ketones from *Gossypium*.

The hydroxycadalenes and lacinilenes appear to be formed largely in response to stress in leaves, bracts and surface tissues of stems and bolls. Enhanced synthesis of these compounds has been elicited by inoculations with incompatible (avirulent) bacteria (Essenberg *et al.*, 1982), the boll rot fungus, *Diplodia gossypina*, and the defoliant, DROPP (Holloin and Greenblatt, 1982). "Field-dried" and "frost-killed" bracts and leaves often contain higher levels of lacinilenes than comparable "green-dried" tissues. Thus, synthesis might also be activated by normal senescence or chilling injury. Old green bract tissue, however, is frequent-

ly invaded by weak fungal pathogens, so that lacinilenes in "field-dried" tissues still may be elicited by infection.

Essenberg *et al.* (1982, personal communication) found intense yellow fluorescence, characteristic of lacinilenes, in both palisade and spongy mesophyll cells of bacterial-inoculated leaves, indicating that these are possible sites of lacinilene synthesis. Halloin and Greenblatt (1982) subdivided fungal-inoculated and DROPP-treated bolls into various tissue layers and found hydroxycadalenes and lacinilenes mostly in the outer epicarp tissue. It has not been clearly determined whether synthesis occurs in the epidermis as well as the underlying parenchyma cell layers.

Quantification of lacinilenes is complicated by difficulties in extraction and purification. Several days of extraction apparently are required for complete removal of lacinilenes from tissues with water, ether or ethanol. Beier and Greenblatt (1981, personal communication), Doolan *et al.* (1982), Gilbert *et al.* (1980) and Wall *et al.* (1980a,b) have developed various techniques to clean up cadalenes and lacinilenes and to separate and quantitate them by HPLC. Most of these investigators have reported concentrations of 5-50 ppm (dry weight) of lacinilene C methyl ether in bracts and leaves, and Beier and Greenblatt (1981) found similar concentrations of the other individual cadalenes and lacinilenes. Wall *et al.* (1980a), however, reported concentrations of lacinilene C methyl ether over 200 ppm in gin trash, over 500 ppm in bract, and 36-54 ppm in dust. The reasons for these higher values are not obvious. However, it should be noted that Wall *et al.* (1980a) used natural lacinilene C-methyl ether isolated from gin trash as a standard, whereas other studies have used a chemically-synthesized standard (McCormick *et al.*, 1978) or have calculated concentrations based on the extinction coefficients of the pure crystalline compound (Stipanovic *et al.*, 1975). Lacinilene C-methyl ether supplied by Wall had only about one-third of the biological activity as that of 85 percent pure chemically-synthesized material in tests by Ainsworth *et al.* (1979b). However, it is not known whether this natural lacinilene C-methyl ether was prepared in the same way as the standard used for quantification by Wall *et al.* (1980a). Complete purity of standards is essential, if erroneously high values are to be avoided.

Different cotton cultivars apparently make different amounts of the lacinilenes. G. Greenblatt and I found that the Asiatic cottons, *G. arboreum* and *G. herbaceum*, lack the ability to methylate either 2,7-dihydroxycadalene or lacinilene C. Consequently they make only lacinilene C and its precursor. Stipanovic *et al.* (1981) found that 2 cultivars of *G. hirsutum* had distinctly lower levels of hydroxycadalenes and lacinilenes than three other cultivars. G. Greenblatt and R. Beier (personal communication) further found that Rodgers GL-6 contained only 6 ppm lacinilene C methyl ether in bracts compared to 20-50 ppm in several other cultivars. Results from the latter two studies, however, might reflect different degrees of pest attack, rather than different genetic potential, if the lacinilenes are synthesized primarily in response to pests.

Essenberg *et al.* (1982) found that lacinilene C preparations from the cultivars WbM (0.0) and Im 216 had ellipticities of opposite signs at 331 nm. (+)-Lacinilene C was about 3X more toxic to bacteria than the (-)-lacinilene C. Thus, the toxicity of lacinilenes in tissues apparently can be altered by changing both isomerism and concentration. Detailed studies of environmental and genetic control of hydroxycadalenes and lacinilenes are needed to ascertain their importance and potential usefulness in pest resistance, as well as their role in byssinosis.

TERPENOID ALDEHYDES

Genera in the plant tribe Gossypae characteristically produce lysigenous glands located below the palisade cells of leaves and the hypodermal cells of stems and capsules (bolls). In older plants, glands are also found in the phloem rays of the bark. Lysigenous glands are composed of a large central cavity containing yellow to orange oily substances surrounded by a single layer of flattened epithelial cells. In green tissues of most *Gossypium* species the epithelial cells are red or purple because they contain high levels of anthocyanins (Chan and Waiss, 1981). In glands of seed, internal bark, staminal tissue and petals the epithelial cells generally do not contain anthocyanins but might contain other flavonoids. The oily substance within the gland cavity contains high concentrations of the terpenoid aldehydes shown in Figures 7 and 8. The occurrence and distribution of these terpenoids among glands of different *Gossypium* species and tissues has been reported by Bell *et al.* (1975, 1978) and Stipanovic *et al.* (1980).

The terpenoid aldehydes, gossypol and its methyl ethers, also accumulate in epidermal cells and a few scattered cortical cells of young roots after they are a few days old (Mace *et al.*, 1974) and later may be exuded from root surfaces (Hunter *et al.*, 1978a). In older roots, these terpenoid aldehydes occur throughout the phelloderm of the root bark. When plants are several months old, the same aldehydes may accumulate in xylem ray cells in the wood. Cells other than those mentioned remain free of terpenoid aldehydes in healthy plants.

Cotton tissues stressed by microbial infections, toxic chemicals or adverse environment synthesize terpenoid aldehydes apparently as a defense response. Examples include the induction of terpenoid aldehyde synthesis in cortical parenchyma of young roots by chilling (Bell and Christensen, 1968), in pericycle of roots by nematodes (Veech, 1978, 1979), in stem cortical tissue by *Rhizoctonia* (Hunter *et al.*, 1978b), in paravascular parenchyma by vascular fungal pathogens and bacteria (Bell and Stipanovic, 1978; Mace *et al.*, 1976), in boll endocarp tissue by boll rotting fungi (Bell, 1967), in germinating seed by fungi (Halloin and Bell, 1979) and in cambial tissue by various pathogenic organisms (Bell and Stipanovic, 1978). Cupric ions and other toxic chemicals also may stimulate terpenoid aldehyde synthesis in these tissues (Bell, 1967; Bell and Stipanovic, 1978). The major terpenoids that accumulate in stressed tissues are hemigossypol, hemigossypol methyl ether and their desoxy precursors. The mechanism of induced terpenoid aldehyde synthesis is not known. However, it has been shown

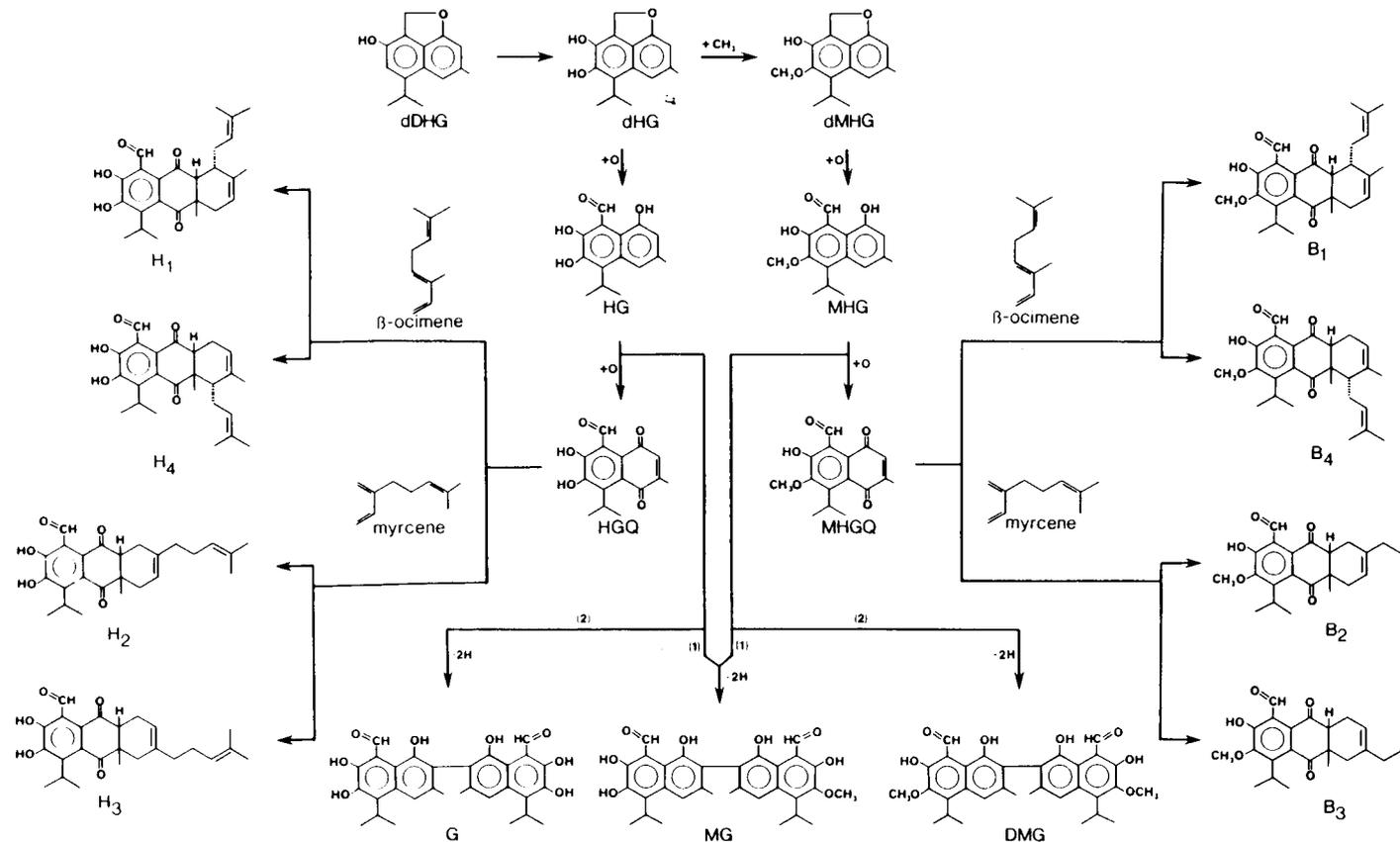
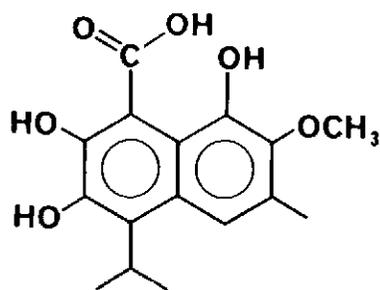
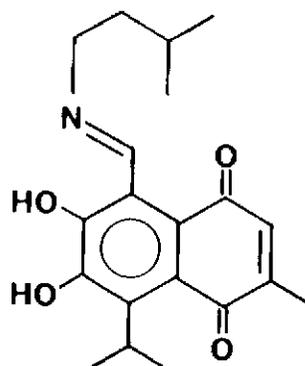


Figure 7. Structures and biosynthetic relationships among terpenoid aldehydes from *Gossypium*.



Raimondal



Gossyrubilon

Figure 8. Structures of raimondal and gossyrubilon.

that dead microbial cells and heteropolymers from cell walls also elicit synthesis, especially in resistant cultivars (Bell and Stipanovic, 1978; Heinstejn, 1980; Stepanichenko *et al.*, 1980)

Heinstejn *et al.* (1979) have reviewed studies of the biosynthesis of gossypol. They concluded that *cis-cis* farnesyl pyrophosphate (FPP) is a precursor of gossypol, and that the prenyltransferase enzyme complex (Widmaier *et al.*, 1980) is probably a key regulator of terpenoid aldehyde synthesis. The first products of cyclization from FPP have not been identified, but these probably are converted to desoxyhemigossypol and then to hemigossypol. Veech *et al.* (1976) have shown that peroxidase converts hemigossypol to gossypol. The heliocides apparently are formed by spontaneous Diels-Alder reactions between terpenoid aldehyde quinones and the monoterpenes ocimene or myrcene (Figure 7; Stipanovic *et al.*, 1977). Other enzymes involved in terpenoid aldehyde synthesis have not been isolated and characterized. However, the recent development of cell suspension cultures of cotton that synthesize gossypol (Heinstejn and El-Shagi, 1981) should facilitate biosynthetic studies.

The terpenoid aldehydes show a wide range of pesticidal activities and are poisonous to most monogastric animals. Many of the studies on biological activity have been reviewed by Bell and Stipanovic (1977, 1978). Recently demonstrated biological activities of terpenoid aldehydes include:

- 1) Toxicity to tobacco budworm, cotton bollworm and pink bollworm, (Chan *et al.*, 1978; Elliger *et al.*, 1978; Hedin *et al.*, 1981; Stipanovic *et al.*, 1977);
- 2) Toxicity to the spiny bollworm, *Earias insulana*, and the cotton leafworm, *Spodoptera littoralis* (Meisner *et al.*, 1977a,b,c,d);
- 3) Toxicity to the root-knot nematode, *Meloidogyne incognita* (Veech, 1979);
- 4) Toxicity to fungi: *Verticillium dahliae* (Paizieva *et al.*, 1977) and *Fusarium*

- oxysporum* (Kaufman *et al.*, 1981; Kumar and Subramanian, 1980);
- 5) Spermaticidal activity (Waller *et al.*, 1980) and menostasis and atrophy of the uterus (Kuo-Fen, 1980) in humans;
 - 6) Toxicity to detrimental gut bacteria in the boll weevil, *Anthonomus grandis* Boheman (Hedin *et al.*, 1978);
 - 7) Histamine release and, at higher concentrations, lysis of blood platelet cells from pig (Ainsworth *et al.*, 1979a) and of mast cells from rat (Elissalde *et al.*, 1983);
 - 8) Uncoupling of oxidative phosphorylation and inhibition of oxygen uptake by rat mitochondria (Paizieva *et al.*, 1977); and,
 - 9) Inhibition of production of pectic enzymes by *F. oxysporum* (Kumar and Subramanian, 1980).

The relative activity of different terpenoid aldehydes has not been studied extensively. Stipanovic *et al.* (1977) found that heliocides formed from ocimene (heliocides H₁ and B₁) were more toxic to *Heliothis* spp. than those formed from myrcene (heliocide H₂ and B₂). Others, however, have failed to confirm this observation (Hedin *et al.*, 1981; Elliger *et al.*, 1978). H.H.S. Fong (personal communication) found that the (+)-enantiomer of gossypol from *Thespesia* had no spermaticidal activity even though racemic gossypol from cotton was highly active. The active agent apparently is (-)-gossypol. This observation could be extremely important because most studies of biological activity have involved racemic gossypol acetate prepared by precipitation of gossypol with acetic acid from crude solutions in ethyl ether. Cotton cultivars apparently contain mostly (+)-gossypol plus variable proportions of (-)-gossypol (Dechary and Pradel, 1971). More attention needs to be given to the enantiomer composition of natural mixtures of gossypol and the activity of these natural mixtures.

Terpenoid aldehydes in pigment glands are an important source of resistance to herbivores and insects (Bell and Stipanovic, 1977). Glandless mutants of cotton are attacked by several insect species that normally do not feed on glanded cotton. Likewise, damage by many normal insect pests, rodents and birds is more extensive on glandless cottons. Terpenoid aldehyde contents of tissues generally correlate negatively with insect damage. For example, Hanny (1980) showed that damage by tobacco budworm (*Heliothis virescens*) in cotton cultivars was negatively correlated with the gossypol content of anthers. Seaman *et al.* (1977) and Shaver *et al.* (1980) showed that gossypol, heliocide H₁, heliocide H₂ and total terpenoid aldehyde concentrations in flower buds were negatively correlated with larval growth of *Heliothis* spp. fed on artificial diets containing extracts of buds. Similar relationships between terpenoid aldehyde contents in plant terminals and leaves of cotton cultivars and inhibition of larval growth has been shown for tobacco budworm, (Hedin *et al.*, 1981, 1982), the leafworm *S. littoralis* (Meisner *et al.*, 1977a,b,d) and cabbage looper (Hanny *et al.*, 1978). The importance of terpenoid aldehydes is further demonstrated by elevated insect resistance in 'high terpenoid' breeding stocks of cotton (Bell and Stipanovic, 1977; Sappenfield and Dilday, 1980).

The induced synthesis of terpenoid aldehydes is an important resistance mechanism against microbial pathogens (Bell and Stipanovic, 1978). However, efficacy is determined by how quickly the terpenoids are synthesized in response to the pathogen rather than by the concentration that eventually accumulates.

More rapid synthesis of terpenoid aldehydes in resistant than in susceptible cultivars has been demonstrated against the fungal pathogens *Fusarium oxysporum* (Kaufman *et al.*, 1981; Harrison and Beckman, 1982; Kumad and Subramanian, 1980) and *Verticillium dahliae* (Bell, 1969; Mace, 1978), the root knot nematode *Meloidogyne incognita* (Veech, 1978, 1979) and the bacterial pathogen *Xanthomonas malvacearum* (Bell and Stipanovic, 1978). Changes of resistance resulting from tissue aging also are associated with how quickly toxic levels of terpenoid aldehydes are accumulated (Bell, 1969; Bell and Stipanovic, 1978; Hunter *et al.*, 1978). The physiological bases for quick synthesis of terpenoids in response to pathogens have not been determined.

While terpenoid aldehydes are important for pest resistance, they also may have adverse effects. Gossypol, in lysigenous glands, makes up 0.5 to 1.0 percent of the dry weight of cottonseed from most cultivars. This concentration is highly toxic to most monogastric animals. Thus, cottonseed can not be used for food, and only small amounts can be used in poultry and swine feed. Appreciable concentrations of terpenoid aldehydes also occur in dried bract tissue and in mill dust which contains bract residues. Loewenschuss and Wakelyn (1972) found 440 to 650 ppm free gossypol in dry bracts of cotton. Stipanovic and Bell (unpublished) recently found that mill dust contained over 400 ppm free gossypol and 250-300 ppm of each heliocides H₁ and H₂. These concentrations are far above those required to cause lysis of pig blood platelets (Ainsworth *et al.*, 1979a) and release histamine from rat mast cells (Elissalde *et al.*, 1983). Thus, terpenoid aldehydes in dust need to be evaluated as possible contributing factors to byssinosis.

Variability of terpenoid aldehyde content in different tissues has been studied in considerable detail. Dilday and Shaver (1976a,b, 1980) surveyed terpenoid aldehyde concentrations in flower buds from more than 200 primitive stocks of *Gossypium hirsutum* during three different years. They found significant variations in terpenoid concentrations among stocks and between seasons. Subsequently, Dilday and Shaver (1981) also found significant variations between different sampling dates during a single season. They concluded that comparisons of genotypes at least should be based on samples taken on the same date, and for best results means should be obtained from several sampling dates. Flowerbuds from several race stocks had terpenoid concentrations about twice as high as those in prevailing commercial varieties. These stocks should be useful to increase resistance against insects that feed on flower buds.

Hanny *et al.* (1978) surveyed terpenoid aldehyde and tannin content in seed, flowerbuds, terminals and leaves of 39 cotton genotypes. Terpenoid aldehyde content ranged from 0.18 to 1.29 percent in seed, 0.11 to 0.83 percent in flowerbuds, 0.09 to 0.44 percent in terminals, and 0.11 to 0.24 percent in leaves.

Damage from cabbage loopers (*Trichoplusia ni* Hubner) was negatively correlated (-0.60) with terpenoid concentrations in terminals. Two genotypes HG-BR-BN and HG-6N-1 had desirable combinations of high flowerbud and terminal terpenoid content with moderate levels in seed.

The genetics of pigment gland formation and terpenoid aldehyde synthesis has been reviewed by Bell and Stipanovic (1977). Six different genes have been shown to control gland density, but the major controlling genes are $G1_2$ and $G1_3$. Lee (1977) has shown that the expressions of $G1_2$ and $G1_3$ depend on the overall genetic background of a given cotton stock. The monomers $G1_2G1_2g1_3g1_3$ and $g1_2g1_2G1_3G1_3$ produced 3.03 and 1.18 percent gossypol in seed in the '3-T' genetic background but only 0.69 and 0.24 percent in the Acala 4-42 background, respectively. Lee (1978) and Wilson and Smith (1977) have shown that $G1_3$ alleles from different cotton species or stocks may give different levels of terpenoid aldehydes and pigment gland density in bolls and flower buds. The allele designated as $G1'_3$ or $G1r_3$ was more potent than $G1^{rm}_3$, which in turn was more potent than $G1_3$. Monomeric $G1r_3$ gave 97.2 glands/cm² on bolls compared to only 19.6 for monomeric $G1_3$. $G1'_3$ and $G1r_3$ probably both designate the $G1_3$ allele obtained originally from the Socorro Island wild accession of *G. hirsutum*. Progress in using various genes to breed high terpenoid cottons for insect resistance has been reviewed by Sappenfield and Dilday (1980).

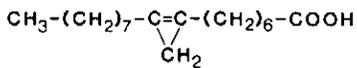
UNIQUE FATTY ACIDS AND LIPIDS

Cotton, like several other malvaceous plants, produces the cyclopropene fatty acids, malvalic and sterculic acid (Figure 9), and the cyclopropane fatty acids, dehydromalvalic and dehydrosterculic acid. These fatty acids make up 1-2 percent of the weight of oil from cottonseed (Bianchini *et al.*, 1981) and 0.02-0.10 percent of total dry weight (5-8 percent of the total fatty acids) of flower buds (Chan *et al.*, 1978). Similar amounts of each of the four acids, with a slight preponderance of malvalic, occur in cottonseed oils. The methyl esters of cyclopropene fatty acids are toxic to insects, but only at concentrations (0.3-0.6 percent) far greater than found in foliage and flowers. Cyclopropene fatty acids enhance the toxicity of terpenoid aldehydes to animals when the compounds are mixed (see references in Bell and Stipanovic, 1977). A possible similar role in pest resistance should be evaluated. Nothing is known about the effects of genotype or environment on the cyclopropenoid fatty acid content of cotton tissues.

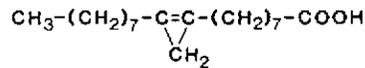
Vick and Zimmerman (1981) have shown that young cotton seedlings contain the enzymes lipoxygenase, hydroperoxide isomerase and hydroperoxide cyclase. These enzymes convert linolenic acid to the compounds shown in Figure 9. When linolenic acid was reacted with crude enzymes from 4-day-old etiolated cotton seedlings, 10 percent 9-hydroxy-12-oxo-*cis*-15, *trans*-10-octadecadienoic acid (γ -ketol; Figure 9), 60 percent 12-oxo-13-hydroxy-*cis*-9, *cis*-15-octadecadienoic

acid (α -ketol; Figure 9) and 25 percent 12-oxo-phytodienoic acid (12-oxo-PDA; Figure 9) were obtained. These compounds are formed by a number of plant species, but their physiological importance is unknown. Their resemblance to prostaglandins and leukotrienes formed from arachidonic acid by similar enzymes in animals is striking. The latter compounds are extremely potent regulators of immune reactions and other biological functions.

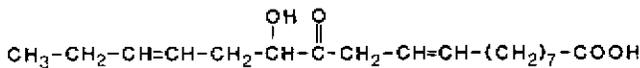
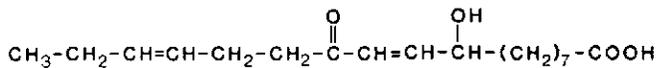
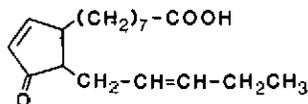
The surface wax of glabrous 'Bayou SM1' (*G. hirsutum*) was analyzed by Hanny and Gueldner (1976). They recovered 0.68 mg of wax/g fresh terminal shoot. The wax contained 49.9 percent n-alkanes, 5.5 percent n-primary alcohols and 44.6 percent sterols and related triterpenoids. The predominant alkane was n-



malvalic acid



sterculic acid

 α -ketol γ -ketol

12-oxo-PDA

Figure 9. Structures of cyclopropene fatty acids and fatty acid derivatives of linolenic acid.

nonacosane ($C_{29}H_{60}$) and the predominant alcohol was n-octacosanol ($C_{28}H_{58}O$). Various sterols each made up 0.4 to 6.5 percent of the wax. Nine of nineteen detected sterols and triterpenoids were identified. Cuticular extracts from cotton are toxic to various fungi (Wang and Pinckard 1973), but the specific toxic compounds have not been identified. Terpenoid aldehydes from pigment glands might also occur in such preparations, because these are readily eluted by solvents used to extract waxes.

SUMMARY

The secondary products of cotton make up a considerable percentage of the weight of the plant and apparently are essential for the plant to cope with pests and stress. Many of these compounds have been identified, but we know very little about their biochemistry or physiology. These compounds can be manipulated by genetic hybridization and selection and by environmental manipulation. Studies on the physiology of secondary products should be a fruitful area of future research and are essential before secondary products can be used judiciously for pest control.