Published July, 1988

Volatile Monoterpenes Collected from the Air Surrounding Flower Buds of Seven Cotton Genotypes

J. F. Chang,* J. H. Benedict, T. L. Payne, and B. J. Camp

ABSTRACT

Some volatile monoterpenes released by cotton (*Gossypium hirsutum* L.) are olfactory cues to boll weevils (*Anthonomous grandis* Boheman) and some parasites. However, literature examined showed no studies that described or compared the monoterpene odor of different cotton genotypes at different stages of growth or in different environments. The objective of our study was to determine the monoterpene composition of the air surrounding flower buds of five cotton genotypes ('CAMD-E', 'SP-37', 'STV-213', STV-213 glandless, RDC-102 glandless, HG-1, and LEBO) grown under normal agronomic practices at College Station and Corpus Christi, TX. The monoterpenes were collected from the air surrounding excised buds of each genotype with a Porapak Q effluvial collection system. The quantity of each monoterpene was determined with capillary column gas chromatography utilizing purified standards. The most abundant monoterpenes collected were α-pinene, β-pinene, β-myrcene, d-limonene, and β-ocimene. The total quantity of these five monoterpenes was greater for buds of glanded genotypes than for buds of glandless genotypes. Ratios of the five monoterpenes provided a relatively characteristic chemical profile for each genotype. Quantities and ratios of the five monoterpenes collected from buds were dynamic in that they fluctuated with the age of the cotton plant and the environment in which the plants were grown. These results indicate that the five monoterpenes composing, in part, the odor of commercial upland cotton, may differ spatially, temporally, and genetically. This suggests that researchers attempting to identify attractive plant odors should simultaneously compare insect responses and plant odor composition.

Additional Index Words: **Allelochemicals, Effluvial collection method, *Gossypium hirsutum* L., Host plant resistance, Kairomones, Plant attractants, Semiochemicals.**

CULTIVATED COTTON can be chemically identified by the quality and quantity of isoprenoid compounds present in various plant organs compared to other species of the genus *Gossypium* (Bell, 1986). The isoprenoid compounds in upland cotton range from 10-carbon monoterpenes (Kumamoto et al., 1979; Minyard et al., 1965), through sesquiterpenes (Minyard et al., 1966), to the 30-carbon triterpene gossypol (Berardi and Goldblatt, 1980).

Phytochemical investigations by members of the USDA Boll Weevil Laboratory at Mississippi State University have identified a total of 252 secondary plant compounds in cotton (Hedin et al., 1976). Hedin et al. (1975) reported that 70 volatiles were emitted by 'Delta Pine Smoothleaf'. Fifteen of the 70 compounds were also found in cotton flower bud essential oil (Hedin, 1976). Terpenoid compounds are the main constituents of the essential oil in cotton that are responsible, in part, for the peculiar odor of upland cotton (Harborne, 1973). Glandless cotton lines are reported to be devoid of terpenoid compounds based on chemical analyses of plant tissue (Bell, 1986; Elzen et al., 1985) and thus would not be expected to emit volatile terpenes into the air.

Volatile terpenes are known to be olfactory attractants to certain insects (Hedin et al., 1977). Dickens (1984) and Hedin et al. (1976) found that boll weevils are responsive to terpenoid volatiles in cotton. Benedict et al. (1979) and Pieters and Bird (1977) found that boll weevils were more attracted to feed and oviposit on some cotton genotypes than others in field free-choice tests. Elzen et al. (1983) determined that volatile terpenoids from cotton attracted the insect parasite *Campoletis sonorensis* (Cameron), that attacks tobacco budworm larvae [*Heliothis virescens* (F).] on cotton. Further, the odors of different cotton genotypes and species are thought to influence the host-plant preference of the boll weevil and tobacco budworm (Benedict et al., 1987; Chang et al., 1986). In our review of the literature, however, we could not find studies characterizing the chemical composition of the air surrounding different cotton genotypes. We feel that identifying the composition of odors from cotton genotypes that are attractive to boll weevils is useful in understanding host-plant insect interactions and developing insect resistant genotypes.

J.F. Chang and J.H. Benedict, Texas A&M Univ. Agric. Res. and Ext. Ctr., Rt. 2, Box 589, Corpus Christi, TX 78410; T.L. Payne, Dep. of Entomology, B.J. Camp, Dep. of Veterinary Physiology and Pharmacology, Texas A&M Univ., College Station, TX 77843. Contribution TA-22704 from the Texas Agric. Exp. Stn., College Station, TX 77843. Received 15 May 1987. *Corresponding author.

Published in Crop Sci. 28:685–688 (1988).

The present work is the first step in establishing the chemical composition of the plant odor that is involved in host-plant selection by the insect fauna of cotton. The objective of this study was to determine the monoterpene composition of the flower bud odor from seven cotton genotypes planted in two different geographic environments. We do not propose in this study to show relationships between composition of flower bud odor and insect olfactory responses.

MATERIALS AND METHODS

Monoterpene volatiles were collected with a Porapak Q (Waters Associates, Inc., Farmingham, MA) effluvial collection system (Chang et al., 1986) from the air surrounding flower buds of the following cotton genotypes: (1) CAMD-E, a short-season cotton cultivar; (2) SP-37, a short-season cultivar; (3) STV-213, a full-season cultivar; (4) STV-213 gl, a glandless isoline of the cultivar STV-213 devoid of epidermal terpenoid glands; (5) RDC-102, a glandless short-season cotton breeder's strain; (6) HG-1, a full-season high gossypol breeder's strain; and (7) LEBO, a short-season breeder's strain known to be highly attractive to the boll weevil [unpublished data (Benedict, 1985)]. These cotton genotypes were chosen because they represented a range in quantity of terpenoid aldehydes (Zummo et al., 1984). Volatile chemicals were quantified by capillary column gas chromatography using purified commercial monoterpene standards (Aldrich, Milwaukee, WI) as previously described by us (Chang et al., 1986).

Cotton genotypes were planted on the Texas A&M University Research and Extension Center, Corpus Christi, and on the Texas A&M University farm, College Station, TX. The soil types at Corpus Christi were Victoria (fine, montmorillonitic, hyperthermic Udic Pellusterts) and Orelia (fine-loamy, mixed, hyperthermic Typic Ochraqualfs); and at College Station they were Burleson (fine, montmorrillonitic, thermic Udic Pellusterts), Heiden (fine, montmorillonitic, thermic Udic Chromusterts), and Crockett (fine, montmorillonitic, thermic Udertic Paleustalfs). At Corpus Christi, each genotype was grown in adjacent plots, four rows by 10 m per plot, replicated four times. At College Station, the genotypes were grown in adjacent plots, one row by 100 m per plot, replicated two times. Flower buds were systematically collected from all plots. The two locations differed in rainfall, soil type, soil and air temperatures, and date of planting. Flower bud volatiles were collected in Corpus Christi from plants in the one-third-grown flower bud stage through the second week of bloom (26 d); and in College Station for 4 d during the one-third-grown flower bud stage of growth.

Flower buds, whose calyx diameter was ca. 7 mm, were randomly harvested in the morning from each cultivar and directly placed into glass jars. A replicate was a jar with 300 flower buds. Each flower bud weighed approximately 1 g. Teflon tape was used to seal the jars, allowing entry of ambient air into the jar through an air-cleaning column. The air cleaning column was packed with the adsorbant Porapak Q. The volatiles released into the air within the system were trapped and concentrated on Porapak Q packed in a collection column. Air flow through the collection column was ca. 0.5 L/min. After 8 h of collection, the volatiles were eluted from the columns with 2 mL of n-pentane into 5-mL vials and stored at −45 °C until quantification was performed.

In order to evaluate the collection efficiency of the Porapak Q effluvial collection system (hereafter referred to as the system), a known quantity of each monoterpene was placed in the system collection jar and trapped. This was replicated eight times. A mean recovery efficiency of 98.6% for α-pinene, 87.9% for β-pinene, 84.5% for β-myrcene, 86.5% for d-limonene, and 73.8% for β-ocimene was obtained with the system utilizing an 8-h collection period and 2 mL of eluant. The coefficient of variability was 2.5% for these data.

Statistical analyses of all data collected in the studies were conducted with transformed data ($\sqrt{x+1}$). Data were transformed to meet the assumptions of the analysis of variance. Means were separated with Duncan's multiple range test ($P < 0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

The most abundant volatiles collected from the air surrounding cotton flower buds at Corpus Christi and College Station were the monoterpenes: α-pinene, β-pinene, β-myrcene, d-limonene, and β-ocimene (hereafter referred to as total monoterpenes). At Corpus Christi, the largest quantity of total monoterpenes was captured from LEBO flower buds (Table 1). The total monoterpenes collected from the air surrounding flower buds of HG-1 and CAMD-E were significantly greater than those collected from SP-37, STV-213, STV-213 gl, or RDC-102. In contrast to the work of Bell (1986) and Elzen et al. (1985), we found that the glandless genotypes produced monoterpenes; however, they produced significantly less total monoterpenes than any of the glanded genotypes. Hedin et al. (1975) collected volatile compounds from whole cotton plants with Chromosorb 102 (Manville Products Corp., Lompoc, CA), but reported finding only the monoterpenes α-pinene, d-limonene, and β-myrcene. This suggests that Porapak Q is more efficient than Chromosorb 102 for trapping β-pinene and β-ocimene. Reineccius and Anandaraman (1984) reported Porapak Q to be more effective than a number of other adsorbants at trapping terpene volatiles from the air.

Differences in the quantity of individual monoterpenes were found among the genotypes at Corpus Christi (Table 1). On the average, α-pinene was the most abundant of the five monoterpenes collected from the glanded genotypes. The quantity of α-pinene from HG-1 was significantly greater than that from the other genotypes. The quantity of β-pinene from the air surrounding LEBO was significantly greater than that from the other genotypes. Possibly this monoterpene, in

Table 1. Quantity of volatile monoterpenes in the air surrounding flower buds from seven cotton genotypes grown at Corpus Christi, TX, 1985.

Genotype	Monoterpene					
	α-pinene	β-pinene	β-myrcene	d-limonene	β-ocimene	Total
	ng/bud					
LEBO glanded	88b*	69a	127a	13a	340a	638a
HG-1 glanded	145a	47b	74bc	12a	50bc	329b
CAMD-E glanded	101b	29c	99ab	9ab	88b	327b
SP-37 glanded	53c	46b	39cd	7abc	29c	173c
STV-213 glanded	80b	18c	29de	8ab	32c	166c
STV-213 gl glandless	2d	2d	14ef	5bc	32c	55d
RDC-102 glandless	1d	1d	5f	4c	24c	34d

* Means within columns followed by different letters are significantly different at the 0.05 probability level by Duncan's multiple range test. Each flower bud weighed ca. 1 g.

combination with the other monterpene volatiles, and the large quantity of total monoterpenes may be responsible for the greater attractiveness of LEBO to boll weevils. The quantities of β-myrcene were greatest from LEBO and CAMD-E. The largest quantity of β-ocimene was also collected from the air surrounding flower buds of LEBO. Of the five monoterpenes, d-limonene was collected in the lowest quantity from all genotypes. The most abundant monoterpene from the glandless genotypes was β-ocimene.

The quantities of the five monoterpenes collected at College Station (Table 2) showed similar relationships to one another as at Corpus Christi. However, there was a trend for reduced quantity of total monoterpenes from the air surrounding flower buds of all genotypes grown at College Station compared to that at Corpus Christi, except for SP-37.

The effect of plant age on individual and total monoterpenes collected from the air surrounding flower buds was determined by combining the data for 11 sample dates for the five glanded cotton genotypes grown at Corpus Christi (Table 3). The sampling dates covered the plant growth period from the one-third-grown flower bud stage through the second week of bloom (26 d). Monoterpene collection for each glanded genotype followed the same distribution pattern throughout the 11 sampling dates (4–30 June). These data demonstrated that the flower buds that were formed early (prebloom) or late in plant development produce lower amounts of monoterpenes than those flower buds formed during the early to mid-blooming period.

We feel there were several limitations to the methods used in this study that can perhaps be overcome in future studies on air space volatiles of plants. The major limitation was that our studies did not measure the odor from whole, undamaged plants in the field. A second limitation is that the flower buds were excised from the plant and, thus, the odors we report here may be, in part, the response of a wounded, excised bud rather than that of an intact bud on field-grown plants. Therefore, we suggest that any attempts by readers to relate our findings with insect responses be made with these limitations in mind.

In summary, the characteristic odor produced by flower buds of each cotton genotype was attributed, in part, to the specific quantities of each of the five monoterpene volatiles discussed in this paper. Further, monoterpene quantity differed with the stage of growth and the specific environment in which the genotype was grown. Other volatile compounds not studied here may also contribute to the dynamic and characteristic composition of cotton air space volatiles. Understanding the quantities, ratios, and synchrony in release of volatile secondary compounds into the air surrounding cotton plants may help research scientists in their investigations of the plant attractants involved in the specific relationships between a cotton genotype and its insect fauna.

Table 2. Quantity of volatile monoterpenes in the air surrounding flower buds from seven cotton genotypes grown at College Station, TX, 1985.

Genotype	Monoterpene					
	α-pinene	β-pinene†	β-myrcene†	d-limonene†	β-ocimene	Total
	ng/bud					
LEBO glanded	73a*	50a	131a	6a	266a	527a
CAMD-E glanded	101a	21b	90a	7a	58b	277b
SP-37 glanded	78a	58a	46b	7a	22c	211bc
HG-1 glanded	106a	24b	42bd	7a	25c	204bc
STV-213 glanded	82a	15b	20c	6a	15c	138c
STV-213 gl glandless	1b	0c	0d	0b	10c	12d
RDC-102 glandless	1b	1c	0d	0b	10c	12d

* Means within columns followed by different letters are significantly different at the 0.05 probability level by Duncan's multiple range test. Each flower bud weighed ca. 1 g.

† The concentration of 0 indicates that the integrator detected < 0.5 ng.

Table 3. Quantity of monoterpene volatiles collected over 26 d of plant growth from the air surrounding flower buds of five glanded cotton genotypes. Collections were made from the one-third-grown flower bud stage through the second week of bloom at Corpus Christi, TX, 1985.

Day	Growth stage‡	Monoterpene					
		α-pinene	β-pinene	β-myrcene	d-limonene	β-ocimene	Total
		ng/bud					
1	Bud	82bc*	30cd	42c	11bc	71cd	236cd
3	Bud	75bc	31cd	46c	17ab	134abc	303bcd
4	Bud	55c	25cd	36c	4c	64cd	183d
5	Bud	47c	19d	25c	4c	92bcd	187d
6	Bud	59c	26cd	43cd	5c	99bcd	232cd
7	Bloom	87bc	55bc	106b	10bc	216a	474abc
10	Bloom	176a	94a	193a	24a	188a	675a
17	Bloom	155a	69ab	140ab	14ab	140abc	518ab
21	Bloom	130ab	49bcd	120ab	11bc	178ab	488ab
23	Boll	82bc	33cd	34c	5c	25d	179d
25	Boll	86bc	32cd	47c	6bc	57cd	228cd

* Means within columns followed by different letters differ significantly at the 0.05 level of probability based on Duncan's multiple range test.

† *Bud* is the growth stage from one-third-grown flower bud to first bloom. *Bloom* is the growth stage from first bloom to late bloom when flowers have been pollinated and are starting to mature. *Boll* is the growth stage from late bloom to early boll maturity when pollinated fruit are developing into mature capsules.

ACKNOWLEDGMENTS

We thank the following for their support: Cotton Inc., Grant 81-496; USEPA, Grant CR806277030; USDA, Grant 71-59-2481-1-2-030-1; Texas Agricultural Experiment Station, under Expanded Research; and Natural Fiber and Food Protein Commission. We also thank: L. Bird, for advice and assistance with field plots at College Station; E. Hejtmancik, for her guidance in the use of analytical instrumentation; K. Schmidt, J. Segers, M. Treacy, and M. Walmsley, for advice, assistance, and encouragement; and C. Villanueva, for word processing and editorial assistance.

REFERENCES

Bell, A.A. 1986. Physiology of secondary products in cotton. p. 597–762. *In* J.R. Mauney and J.M. Stewart (ed.) Cotton physiology. The Cotton Foundation, Memphis, TN.

Benedict, J.H., L.S. Bird, and C. Liverman. 1979. Bacterial flora of MAR cottons as a boll weevil resisting character. p. 228–230. *In* J.M. Brown (ed.) Proc. Beltwide Cotton Prod. Res. Conf. Phoenix, AZ. 7–11 Jan. Natl. Cotton Council of America, Memphis, TN.

Benedict, J.H., M.F. Treacy, D.W. Altman, and K.M. Schmidt. 1987. Preference of boll weevils and tobacco budworms for five species of *Gossypium*. p. 92–93. *In* T.C. Nelson (ed.) Proc. Beltwide Cotton Prod. Res. Conf. Dallas, TX. 4–8 Jan. Natl. Cotton Council of America, Memphis, TN.

Berardi, L.C., and L.A. Goldblatt. 1980. Gossypol. p. 183–237. *In* I.E. Liener (ed.) Toxic constituents of plant foodstuffs. Academic Press, New York.

Chang, J.F., J.H. Benedict, T.L. Payne, and B.J. Camp. 1986. Methods for collection and identification of volatile terpenes from cotton, and evaluation of their attractiveness to boll weevils. Southwest. Entomol. 11:233–241.

Dickens, J.C. 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boheman, (Coleoptera: Curculionidae): electroantennogram studies. J. Chem. Ecol. 10:1759–1785.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11:1–41.

Elzen, G.W., H.J. Williams, A.A. Bell, R.D. Stipanovic, and S.B. Vinson. 1985. Quantification of volatile terpenes of glanded and glandless *Gossypium hirsutum* L. cultivars and lines by gas chromatography. J. Agric. Food Chem. 33:1079–1082.

----, ----, and S.B. Vinson. 1983. Response by the parasitoid, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: Implications for host habitat location. Environ. Entomol. 12:1873–1877.

Harborne, J.B. 1973. Phytochemical methods. A guide to modern techniques of plant analysis. Chapman and Hall, London.

Hedin, P.A. 1976. Seasonal variations in the emissions of volatiles by cotton plants growing in the field. Environ. Entomol. 5:1234–1238.

----, J.N. Jenkins, and F.G. Maxwell. 1977. Behavioral and developmental factors affecting host plant resistance to insects. p. 231–275. *In* P.A. Hedin (ed.) Host plant resistance to pests. Symp. Series 62. American Chemical Society, Washington, DC.

----, A.C. Thompson, and R.C. Gueldner. 1975. Survey of the air space volatiles of the cotton plant. Phytochemistry 14:2088–2090.

----, ----, and ----. 1976. Cotton plant and insect constituents that control boll weevil behavior and development. Recent Adv. Phytochem. 10:271–350.

Kumamoto, J., J.G. Waines, J.L. Hollenberg, and R.W. Scora. 1979. Identification of the major monoterpenes in the leaf oil of *Gossypium sturnianum* var. *Nandewarense* (Der.) Fryx. J. Agric. Food Chem. 27:203–204.

Minyard, J.P., J.H. Tumlinson, P.A. Hedin, and A.C. Thompson. 1965. Constituents of the cotton bud. Terpene hydrocarbons. J. Agric. Food Chem. 13:599–602.

----, ----, A.C. Thompson, and P.A. Hedin. 1966. Constituents of the cotton bud. Sesquiterpene hydrocarbons. J. Agric. Food Chem. 14:332–335.

Pieters, E.P., and L.S. Bird. 1977. Field studies of boll weevil resistant cotton lines possessing the okra leaf-frego bract characters. Crop Sci. 17:431–433.

Reineccius, G.A., and S. Anandaraman. 1984. Analysis of volatile flavors. p. 195–293. *In* J.F. Lawrence (ed.) Food constituents and food residues: Their chromatographic determination. Marcel Dekker Inc., New York.

Zummo, G.R., J.C. Segers, and J.H. Benedict. 1984. Seasonal phenology of allelochemicals in cotton and resistance to bollworm. Environ. Entomol. 13:1287–1290.