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Identification and Quantitative Analysis of the Volatile Substances Emitted by Maturing Cotton in the Field

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ABSTRACT

When atmosphere from cotton plants (*Gossypium hirsutum* L., var. Deltapine Smoothleaf) was condensed by passing it over the expansion coil of an air conditioner and three 1-hour collections per day (early morning, noon, and late afternoon) were made, the total essential oils were found to consist of 50 to 60% β-bisabolol (I_k 1660) and γ-bisabolene (I_k 1550) and 30 to 40% geraniol (I_k 1250), myrtenal (I_k 1328), nerolidol (I_k 1520), and β-caryophyllene oxide (I_k 1590). As the plant matured, *trans*-2-hexanol was produced in concentrations of 7 to 27%. Before fruiting, β-bisabolol made up as much as 60% of the total essential oil transpired by the plants, and as the concentration of β-bisabolol increased, that of γ-bisabolene decreased.

Ample laboratory (3, 6) and field (12) evidence exists that a volatile substance(s) in the cotton plant, *Gossypium* sp., attracts the boll weevil, *Anthonomus grandis* Boheman. The Boll Weevil Research Laboratory at State College, Mississippi, is therefore investigating the constituents of cotton plants in an effort to identify those which attract or initiate other specific host plant-insect behavior. This continuing study of the aroma profile of the cotton plant (4, 9–11) has resulted in the isolation and identification of a number of carbonyls, terpenes, sesquiterpene hydrocarbons, oxides, and alcohols obtained from the essential oils by steam distillation. This report describes a qualitative and quantitative survey of the chemical components transpired by the growing plant.

MATERIALS AND METHODS

Equipment. An apparatus designed by Baker (1) to study apparent photosynthesis was used in the study. This system consisted of a plastic housing, 203.2 × 203.2 × 182.9 cm, with a 61-cm diameter flexible hose leading out of one end into a 42,000 BTU thermostated air conditioner. The air was returned to the opposite end of the system by a flexible hose of the same diameter. The chamber was covered and sealed at the ground with clear plastic. Leakage was found to be negligible when the chamber air contained a concentration of CO_2 of 0.03% at 35 to 40 C. The capacity of the system was 16,750 liters, and the wind velocity was spatially uniform, an average 0.8 mph around the leaves on the upper half of the plant.

For analysis of the rate of transpiration and the composition of the transpired components, the water condensed by the air conditioner was collected every 15 min from the expansion coils, and the volume was measured. Samples of water collected over a period of 1 hr three times a day, from 0800 to 0900, from 1300 to 1400, and from 1700 to 1800 hr CST were pooled for analysis. Measurements of humidity made at the end of each period permitted adjustment of the changes in transpiration by changes in the water content of the air.

The water from each hourly collection was extracted three times with 300-ml portions of dichloromethane. Then the combined extracts were dried over anhydrous sodium sulfate and filtered, and the solvent was removed at 35 C. The resulting oil was diluted to a constant volume for gas-liquid chromatography.

Gas Chromatographic Analysis. The oils were subjected to GLC[3] at the following conditions: 3.2 mm × 3.047 m stainless steel column packed with 10% SE-30 on 60/80 mesh Chromosorb-P treated with hexamethyl disilazane. Carrier gas flow (N_2) was at a rate of 50 ml/min; column temperature was 175 C; injector and detector temperatures were 180 and 195 C, respectively. Kováts' indices (7) were obtained by GLC of a series of normal hydrocarbons at the same conditions. These values, *e.g.*, decane = 1000, undecane = 1100, reflect GLC effective molecular weights (5) which aid in the evaluation of the GLC profile. The GLC peak assignments were made with reference to our previous structural work (8, 10, 11).

Quantitation. Standard samples of decane and β-bisabolol were diluted with heptane, and a known quantity was gas chromatographed using the same conditions as the essential oils. Peak area was measured by triangulation, and the total area for both standards was normalized to 100% so the value for each component was a percentage. Therefore, the nonlinear relationship of component concentration to peak area was adjusted by using two standards. Decane and heptadecane were added to the samples as internal standards.

RESULTS AND DISCUSSION

Table I gives the concentration of oil present in the water condensate from three daily collections from 23 plants in the closed system on 10 days within a period of 24 days from September 9 to October 2. At the time of initial samplings, the plant was beginning to set buds; the final collections were made when bolls had been set.

Collections on day 1 through day 4 were made on consecutive days (Table I). During the next week, a marked lowering in the plant volatile profile became apparent. On day 12 the concentration of oil was 255 times lower than that for the same time a week earlier (day 4). During this week, the plants had begun to produce large numbers of buds. During the

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[3] Abbreviations: GLC: gas-liquid chromatography; I_k: Kováts' indices.

week between day 12 and day 18, the plants began to set bolls. Collections on day 18 through day 21 were made on consecutive days, and during this period, the water released by the plants continued at the same general rate.

Table I shows that as the plants began to set buds (day 1–day 4), the greatest concentration of metabolic end products was produced in the afternoons, the time of day that follows a period of active photosynthesis. Baker (1) showed that photosynthetic activity is a maximum at 1000 to 1200 hr (CST), which corresponds to the maximal radiation load when the day is clear. Thereafter, the photosynthetic activity decreases, and it is after this maximal metabolic activity that the concentra-

Table I. *Concentration of Total Essential Oil in the Water Condensate from Mature Cotton Plants*

Time of Collection	Concn of Oil in Condensate on Day									
	1	2	3	4	12	18	19	20	21	24
hr	*micrograms*									
0800–0900	...[1]	0.9	2.7	6.2	0.8	...	0.4	...	1.1	1.2
1300–1400	10.8	11.0	12.1	12.3	0.6	0.4	0.8	1.0	1.1	0.2
1700–1800	18.2	14.4	...	16.6	...	1.3	...	0.3	1.4	1.1

[1] Samples not taken at these times.

Table II. *Identification and Percentage of Components Found in the Atmospheric Condensate from Cotton Plants Collected at 0800–0900 hr*

I_k	Identity	Percentage Composition on Day						
		2	3	4	12	19	21	24
1106	Nonanal	...[1]	7.1	6.8	...	...	...	2.7
1112	Unknown	...	9.8	...	...	7.5	7.8	8.6
1156	4-Terpinenol	3.9	3.6	3.1	6.3	...	2.1	2.6
1232	Neurol	1.9	1.0	...	3.0	...	2.7	...
1256	Geraniol	2.6	...	2.9	4.3	6.9	5.2	1.6
1328	Myrtenal	7.2	3.4	5.8	11.2	1.2	2.7	1.3
1400	Copaene	...	2.4	4.3	2.8	6.8	1.7	1.5
1430	*trans*-α-Bergamotene	...	1.3	3.8	5.2	5.0	2.0	3.1
1450	β-Caryophyllene	...	3.1	...	...	...	...	1.2
1520	Nerolidol	9.9	3.3	9.1	4.9	4.1	6.0	4.4
1550	γ-Bisabolene	24.7	7.6	17.9	34.7	...	6.2	4.5
1590	β-Caryophyllene oxide	...	2.7	18.8	5.5	4.4	2.5	3.7
1604	Unknown	...	3.8	13.8	12.1	6.7	7.6	...
1660	β-Bisabolol	35.2	48.0	...	9.5	55.5	30.4	45.5
1688	Unknown	11.2	1.7	...	4.2	...	7.6	4.0

[1] These components were absent on these days.

Table III. *Identification and Percentage Composition of Components Found in the Atmospheric Condensate from Cotton Plants Collected at 1300–1400 hr*

I_k	Identity	Percentage Composition on Day							
		1	2	3	4	12	19	20	21
824	*trans*-2-Hexanol	1.6	1.3	10.4	8.6	6.6	1.2	6.7	9.8
1106	Nonanal	...[1]	...	4.0	...	8.1	3.9	12.7	17.5
1156	4-Terpinenol	2.6	...	3.1	3.4	5.6	...	...	3.8
1208	Unknown	1.1	1.9	...	3.9	3.7	...	...	1.8
1256	Geraniol	4.0	2.5	3.0	5.1	2.4	...	3.2	10.7
1328	Myrtenal	3.9	7.9	9.2	8.9	9.5	7.2	3.0	24.6
1370	Unknown	...	7.8	3.7	2.9	8.4	8.4	...	...
1430	*trans*-α-Bergamotene	3.8	2.2	1.3	4.3	10.6	4.6	...	...
1520	Nerolidol	8.8	6.5	3.9	4.7	4.5	...	3.1	5.9
1550	γ-Bisabolene	16.3	19.8	13.8	22.5	28.0	10.4	4.3	...
1590	β-Caryophyllene oxide	6.6	9.4	5.1	5.4	...	29.2	1.8	...
1660	β-Bisabolol	31.4	37.4	22.8	17.2	6.0	28.5	48.5	10.9
1688	Unknown	11.2	...	4.0	9.0	6.0	...	2.7	5.9

[1] These components were absent on these days.

Table IV. *Identification and Percentage Composition of Components Found in the Atmospheric Condensate from Cotton Plants Collected at 1700–1800 hr*

I_k	Identity	Percentage Composition on Day						
		1	2	4	18	20	21	24
824	*trans*-2-Hexanol	...[1]	10.1	5.2	24.6	21.0	7.0	11.4
1112	Unknown	...	...	...	9.5	13.9	4.3	22.6
1130	Unknown	9.3	4.3	...	3.2	9.1	2.0	13.0
1208	Unknown	10.0	...	2.0	3.2	4.6	4.6	6.8
1256	Geraniol	0.7	...	2.0	13.9	20.8	4.4	6.8
1328	Myrtenal	1.5	2.6	4.1	7.1	10.4	1.9	7.3
1430	*trans*-α-Bergamotene	2.1	2.1	1.0	1.1	...	7.2	1.0
1420	Nerolidol	2.3	4.2	3.6	...	10.1	...	5.9
1550	γ-Bisabolene	3.9	4.7	32.9	13.1	...	20.2	2.2
1590	β-Caryophyllene oxide	3.8	3.8	2.7	...	...	4.5	2.6
1606	Unknown	1.8	3.3	3.6	11.2	...	10.3	4.2
1660	β-Bisabolol	60.3	53.0	30.3	11.3	9.8	30.4	4.2
1688	Unknown	3.4	5.2	7.7	...	...	...	5.1

[1] These components were absent on these days.

tion of end products increases and is subsequently dissipated by the plant. It does not appear that the release of essential oil is related to stomatal aperture, since the plants show the greatest transpiration rate in the 0800 to 0900 hr unit, and the smallest amount of essential oil produced. As the plant begins to set bolls, the energy requirements change so the amount of end products decreases.

An alternate explanation for the decrease in concentration of end products by the plant as it matures is that the photosynthetic activity generally begins to decrease in the leaves after 10 days (D. N. Baker, unpublished data).

Tables II, III, and IV give the I_k values, component identification, and percentage composition of organic volatile components of the water condensate obtained from plant transpiration. In the early morning collections (Table II), β-bisabolol (I_k 1660), the sesquiterpene alcohol which was active in the laboratory biosassay as an attractant for the boll weevil (9), and γ-bisabolene (I_k 1550) comprised 50 to 60% of the total volatile substances; and geraniol (I_k 1256), myrtenal (I_k 1328), nerolidol (I_k 1520), and β-caryophyllene oxide (I_k 1590), comprised an additional 30 to 40%. However, in most instances, β-caryophyllene was absent, which indicated that it was probably converted to its oxide as an end product. β-Caryophyllene oxide (I_k 1590) was as attractive to the boll weevil as β-bisabolol (9). γ-Bisabolene (I_k 1550) is one of the products of β-bisabolol when it is chemically dehydrated (4); however, its presence was probably not an artifact since 7,8-dihydrocurcumene and 8,11-dihydrocurcumene, the other two chemical dehydration products of β-bisabolol (5, 8), are absent.

Table III shows that the quantitative and qualitative composition of components at midday was generally similar to that of the early morning collections. However, when the plant matured to the boll setting stage (day 4), *trans*-2-hexanol (I_k 824) was emitted at midday in concentrations of 7 to 27%, though it was absent from the early morning collections.

Table IV gives the component composition for the late afternoon collections. In the maturing plant before the setting of bolls, the concentration of β-bisabolol reached 60% of the total oil. Moreover, there appears to be some relationship between β-bisabolol and γ-bisabolene because as the concentration of β-bisabolol decreased, that of γ-bisabolene increased. The over-all tendency for both constituents was to decrease in concentration as the plant matured through the fruiting stage.

Hedin *et al.* (4) and Gueldner *et al.* (2) showed in laboratory bioassays of cotton constituents that attractancy to the weevil is a "profile" effect. The host plant-insect specificity of natural stands of cotton for the boll weevil should therefore be attributed largely to its volatile profile.

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