Table III. Carotenoid Analyses of Pyrethrum Extract and Pyrethrum Residue

Carotenoid	Pyrethrum extract		Pyrethrum residue	
	wt %	% of total	wt %	% of total
α- and β-carotene	0.01	1.2	0.03	1.9
Neurosporene-like	0.02	2.4	0.04	2.5
Lutein diesters[a]	0.57	69.5	1.18	72.8
Flavoxanthin-like	0.05	6.1	0.09	5.5
Lutein monoester[a]	0.14	17.1	0.27	16.7
Lutein	0.03	3.7	0.01	0.6
Total carotenoids	0.82		1.62	

[a] Expressed as the free xanthophyll.

pollen. At stage 3 pollen formation in all florets is complete and the first row of disk florets opens. The rapid rise in carotenoid content is therefore associated with formation of carotenoids in the corolla of the disk florets and development of the carotenoid-rich pollen. As each disk floret develops, the corolla opens and the pollen is released. Loss of the bulk of pollen is surprisingly rapid and occurs within a few hours. Further carotenoid loss occurs with the fading of the open disk floret corolla; thus, as each disk floret matures, carotenoids are reduced by both physical loss of pollen and photolysis–oxidation of the corolla.

By collecting and analyzing pollen collected from flower heads grown from buds in 2% sucrose solution, it was found that at least 38% of the carotenoids present in the flower head are associated with pollen. Further, the lutein diester fraction isolated from pollen dissected from immature flower heads was found to contain more than 80% of cis isomer. The relative stability of the *cis*- and *trans*-lutein diesters was compared by exposing the separate compounds, impregnated on filter papers (approximately 0.5 $\mu g/cm^2$), to sunlight. Overall rates of decomposition were similar, 85 to 90% being decomposed after 30 min exposure. However, examination of the degradation products by tlc showed that the pattern of degradation differed between the two isomers. The cis isomer undergoes a roughly 50% conversion to the trans form; interconversion of the trans to the cis form is not nearly so marked. The effect of this difference in degradation in a mixture of the two isomers would be a differential loss of the cis component over the trans, similar to that observed in the developing flower head.

As the flower head matures, its pyrethrins content increases and is at a maximum when approximately half the florets are open (stage 4, Table II). It is now clear that inclusion of immature flower heads (stages 2 and 3, Table II) not only reduces the yield of pyrethrins but increases the carotenoid content of the oleoresin.

The carotenoid content of pyrethrum oleoresin ranges between 1 and 2% w/w, typical analyses of these materials being recorded in Table III. Refined extract containing 50% pyrethrins contains approximately 0.04% carotenoids. Pyrethrum residue, which is the material remaining after methanol extraction of pyrethrum oleoresin, contains increased proportions of carotene and lutein diesters compared with oleoresin. The residue, which is readily available, represents a valuable source of lutein for carotenoid studies.

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An Investigation of the Essential Oil of *Hibiscus syriacus* L.

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The essential oil of *Hibiscus syriacus* L., an alternate host plant of the boll weevil (*Anthonomus grandis* Boheman), the major pest of cotton (*Gossypium hirsutum* L.) was obtained by steam distillation of buds and flowers and analyzed with an integrated gas chromatography–mass spectrometry system. Structures for 65 components were proposed which included 12 hydrocarbons, 6 esters, 24 carbonyls, 10 alcohols, and 13 miscellaneous compounds. Significant differences were apparent between cotton essential oil and the essential oil of *H. syriacus*.

Until the discovery by Coad (1914) of the malvaceous plant *Hibiscus syriacus* L. (rose-of-Sharon) on which the boll weevil (*Anthonomus grandis* Boheman) could feed, oviposit, and develop, this insect was considered monophagous on cotton (*Gossypium hirsutum* L.). Since that time, several natural infestations of the boll weevil on *H. syriacus* have been reported (Bondy and Rainwater, 1935; Gaines 1933, 1934). For example, rose-of-Sharon near cotton fields has been observed to be infested in times of stress when cotton has matured or when weevil population pressures are high and food is in short supply.

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Table I. Hydrocarbons

I_k	M^+	Compd	Reference or fragmentation	%
930	120	Trimethylbenzene	Stenhagen *et al.* (1969)	1.1
1040	128	Naphthalene	Stenhagen *et al.* (1969)	0.4
980	140	1-Methyl-*cis*-4-isopropyl-cyclohexane	Stenhagen *et al.* (1969)	0.2
1150	142	( )-Methylnaphthalene	Stenhagen *et al.* (1969)	0.3
1100	156	Undecane	Stenhagen *et al.* (1969)	0.2
1200	170	Dodecane	Stenhagen *et al.* (1969)	0.2
1330	180	( )-Trideca-( )-diene	43, 57, 41, 71, 55	0.3
1560	204	δ-Cadinene	Stenhagen *et al.* (1969)	2.1
1700	240	*n*-Heptadecane	Stenhagen *et al.* (1969)	1.0
1880	248	( )-Octadecatriene	41, 55, 43, 67, 81	1.5
1850	250	( )-Octadecadiene	41, 55, 43, 69, 83	0.2
1990	260	( )-Nonadecatetraene	41, 55, 43, 67, 79	0.6

Table II. Carbonyls

I_k	M^+	Compd	Reference or fragmentation	%
1010	120	Phenylacetaldehyde	Stenhagen *et al.* (1969)	1.3
1055	124	Cyclohex-( )-enyl acetaldehyde	57, 44, 82, 81, 68	0.2
1060	138	2,4-Nonadienal	41, 81, 55, 43, 29	0.5
1110	138	1-(2-Furyl)-3-butanone	Stenhagen *et al.* (1969)	1.1
1030	140	*trans*-2-Nonenal	Stenhagen *et al.* (1969)	1.2
1105	150	3-(Cyclohexadienyl)-butyraldehyde	29, 55, 91, 107, 121	1.3
1245	150	Safranal	Stenhagen *et al.* (1969)	0.4
1070	152	4-(cyclohexen-1-yl)-butyraldehyde	68, 39, 42, 91, 96	1.2
1310	152	Pulegone	Stenhagen *et al.* (1969)	0.8
1315	152	Isopulegone	Stenhagen *et al.* (1969)	0.2
1165	156	2-Decanone	Stenhagen *et al.* (1969)	1.2
1185	156	*n*-Decanal	Stenhagen *et al.* (1969)	1.3
1285	170	2-Undecanone	Stenhagen *et al.* (1969)	0.2
1330	184	4-Dodecanone	Stenhagen *et al.* (1969)	0.3
1380	184	2-Methyl-5-undecanone	Stenhagen *et al.* (1969)	0.5
1375	190	7-Phenyl-2-heptanone	69, 81, 95, 67, 83	0.4
1440	190	2-Pentenyl-hydroxy-phenylketone	69, 121, 105, 91	3.2
1435	192	α-Ionone	Stenhagen *et al.* (1969)	1.3
1480	192	β-Ionone	Stenhagen *et al.* (1969)	0.9
1475	194	5-[( )-ethyl-(,) cyclohexadienyl]-2-pentanone	69, 53, 107, 67, 93	2.5
1480	198	2-Tridecanone	43, 58, 41, 59, 29	0.3
1605	210	8-(Cyclohexenyl) octanal	41, 54, 67, 81, 95	0.2
1580	218	2,4-Dimethyl-4[p-cuminyl]-valeraldehyde	145, 105, 91, 160, 117	2.7
1535	220	6-p-Tolyl-3-heptanone	41, 43, 55, 82, 72	1.6

Table III. Esters

I_k	M^+	Compd	Reference or fragmentation	%
1190	152	Methyl-p-hydroxy-benzoate	120, 91, 65, 63, 119	5.2
1170	172	Ethyl octanoate	Stenhagen *et al.* (1969)	1.1
1380	200	Ethyl decanoate	Stenhagen *et al.* (1969)	1.2
1575	228	Ethyl laurate	Stenhagen *et al.* (1969)	0.8
1675	228	Isopropyl undecanoate	60, 42, 61, 102, 228	3.1
1870	270	Methyl palmitate	Stenhagen *et al.* (1969)	6.5

However, rose-of-Sharon is normally dispersed among many plants that are unattractive to the boll weevil; nevertheless, the weevil is able to distinguish it as an alternate host. Then some mechanisms must direct the boll weevil to this host.

Various components of the essential oils of cotton, the boll weevil's primary host, have been isolated and identified (Hedin *et al.*, 1971a,b, 1972; Minyard *et al.*, 1965, 1966, 1967, 1968) and a combination of several were reported responsible for the attraction of the cotton plant to boll weevils (Minyard *et al.*, 1969). Hanny (1972) investigated the attractancy of the boll weevil to the essential oil of *H. syriacus* L. and found it to be 71% as potent as cotton at 10 ppm. An investigation of the constituents of the essential oil of rose-of-Sharon was therefore conducted to determine whether any correlation existed with the chemical components of cotton essential oil that might explain the status of rose-of-Sharon as an alternate host plant.

MATERIALS AND METHODS

Isolation and Fractionation. Buds and flowers of *H. syriacus* (6.28 kg) were steam distilled in an all-glass system, and the distillate was extracted with ethyl ether. Yields of oil was *ca.* 2.0 g (318 ppm). The oil was chromatographed on a 2 × 25-cm cold water jacketed Florisil column. The hydrocarbons were eluted with 100 ml of pentane, and the polar compounds were eluted successively with 100 ml each of 5, 10, 20, and 50% Et_2O in pentane and, finally, with 100% Et_2O. Progress of the elution and recombination of all fractions into five reconstructed fractions was monitored by silica gel tlc.

Analytical Glc–Ms. Fractions were introduced into a Hewlett-Packard 5930 quadrupole mass spectrometer from a 250 ft × 0.03 in. capillary column coated with OV-17. The glc unit was programmed from 120° to 160° at 1°/min; the final temperature was maintained for 60 min. Mass spectra were obtained at 70 eV. The gas chromatographic profile obtained with an FID was used to estimate the relative concentrations of the oil components. Material balance observations were made by peak triangulation and normalization to 100%. Comparisons were made with authentic samples where available.

RESULTS AND DISCUSSION

From the 96 maxima observed, structures for 65 components were proposed. In 41 instances, apparent matches with published spectra were obtained. Tentative identity for 24 of the components was made based on fragmentation patterns. Partial information was obtained for the other 31 components.

The compounds (grouped functionally) are listed in Tables I–V with their I_k (Kovats, 1961), mass, percentage content, and literature citation (Stenhagen *et al.*, 1969) or five most significant fragments. The mass spectral scans were obtained on chromatographic fractions of the oil. This approach was used to obtain cleaner spectra and to aid in interpretation, since the relative polarity could predict compound functionality. Relative retention indices (I_k) were obtained for most components as an aid in establishing proposed identities (Kovats, 1961).

Table I lists 12 hydrocarbons that comprise 8.1% of the oil. No terpene hydrocarbons are present although Minyard *et al.* (1965) reported that terpene hydrocarbons comprised 34.0% of the cotton square extract. Also, sesquiterpene hydrocarbons comprise 14.8% of the essential oil of the cotton bud (Minyard *et al.*, 1966) but only one sesquiterpene hydrocarbon, δ-cadinene (2.1%), was present in rose-of-Sharon oil. (This component is present in cotton but at a much lower percentage (0.24%).)

Table II lists 24 carbonyl compounds that comprise 24.8% of the total oil of rose-of-Sharon. Minyard *et al.* (1967) identified the 14 major carbonyl components in cotton buds, but they account for only 1.4% of the oil. Moreover, except for 2-nonenal (Minyard *et al.*, 1967) and β-ionone (Hedin *et al.*, 1971b), the components of the car-

Table IV. Alcohols

I_k	M^+	Compd	Reference or fragmentation	%
850	114	( )-Hepten-1-ol	Stenhagen *et al.* (1969)	0.2
1045	122	2-Phenylethanol	Stenhagen *et al.* (1969)	2.5
1005	124	2,4,7-Octatrien-1-ol	41, 55, 43, 56, 69	0.4
920	128	( )-Octen-1-ol	Stenhagen *et al.* (1969)	0.4
985	128	2-Ethyl-2-hexen-1-ol	Stenhagen *et al.* (1969)	0.8
870	130	1-Octanol	Stenhagen *et al.* (1969)	0.9
1330	150	Cuminyl alcohol	Stenhagen *et al.* (1969)	1.2
1355	150	Thymol	Stenhagen *et al.* (1969)	0.4
1365	164	Isoeugenol	Stenhagen *et al.* (1969)	2.1
1470	236	( )-Hexadecatrien-1-ol	43, 41, 57, 39, 69	0.3

bonyl fraction of rose-of-Sharon are different from those found in cotton. The presence of α- and β-ionone, safranal, and pulegone was interesting because they have been reported in the essential oil of unrelated genera (Robinson, 1969). Carbonyl compounds are important contributors to the flavor and aroma of food and plants. However, it is difficult to postulate their role, if any, as plant attractants for insects because these compounds are known to occur widely and are not characteristic of a particular plant or of plant groups.

Table III lists six esters present in rose-of-Sharon oil. No corresponding esters have been reported in cotton bud essential oil. However, methyl palmitate has been found (Hanny *et al.*, 1973) in the apical tip of cotton seedlings.

Table IV lists ten alcohols, only one of which (2-phenylethanol) has been reported as present in cotton bud essential oil (Hedin *et al.*, 1971b). The alcohols comprise 9.2% of rose-of-Sharon essential oil compared with 16.3% of the cotton bud essential oil (Hedin *et al.*, 1971b). β-Bisabolol, the major oxygenated component in cotton (Minyard *et al.*, 1968), has been reported as a major component of the boll weevil–cotton plant attractant complex (Minyard *et al.*, 1969). The absence of this sesquiterpene alcohol from rose-of-Sharon was somewhat unexpected because this plant is closely related. The aromatic alcohols, 2-phenylethanol, cuminyl alcohol, thymol, and isoeugenol, all of which are present in rose-of-Sharon, have been commonly reported in the essential oils of unrelated genera (Robinson, 1969) and the spectra have been amply reported.

Table V lists miscellaneous compounds including lactones, ethers, indole, phenylacetonitrile, di-2-furylmethane, quinoline, and several oxygenated compounds for which no identity is proposed. Indole is presumably a degradation product from the plant growth hormone. γ-Caprolactone was reported by Bondarovich *et al.* (1967) in black tea volatiles. Phenylacetonitrile is a stable volatile compound that may be an artifact formed during steam distillation; however, in some cases, it is a true natural product (Robinson *et al.*, 1967). Quinoline is reported as the parent compound for a group of quinoline alkaloids found in several plant families, an example of which is *Cinchona* spp. (Robinson, 1969). Its occurrence in rose-of-Sharon is of interest, but no comparable compound has been reported in cotton. However, the formation of volatile terpenes may somehow compete with the formation of alkaloids so plants having one lack the other (Treibs, 1953). For example, Tallent and Horning (1956) found that species of pine that contain alkaloids also have straight-chain aliphatic hydrocarbons rather than terpenes in their turpentines. The presence of quinoline, an alkaloid-type compound, in rose-of-Sharon may perhaps explain in part the presence of aliphatic hydrocarbons and the absence of terpene hydrocarbons in the essential oil of rose-of-Sharon. Apparently, none of the miscellaneous compounds found in rose-of-Sharon essential oil have been reported in cotton. Nevertheless, a more intense study is warranted because this group of compounds comprises 39.3% of the essential oil of rose-of-Sharon.

Table V. Miscellaneous Compounds

I_k	M^+	Compd	Reference or fragmentation	%
940	114	γ-Caprolactone	Stenhagen *et al.* (1969)	0.4
1455	117	Indole	Stenhagen *et al.* (1969)	1.7
	117	Phenylacetonitrile	Stenhagen *et al.* (1969)	1.2
	124	$C_8H_{12}O$	95, 53, 56, 81, 95	0.5
	126	$C_8H_{14}O$	42, 56, 31, 79, 67	0.8
1035	128	γ-Heptalactone	Stenhagen *et al.* (1969)	0.3
1115	129	Quinoline	Stenhagen *et al.* (1969)	0.2
1510	148	Di-2-furylmethane	Stenhagen *et al.* (1969)	0.4
	148	1-Methoxy-4-(1-propenyl)benzene	Stenhagen *et al.* (1969)	0.5
	152	$C_{10}H_{16}O$	81, 67, 91, 120, 152	0.3
	152	$C_{10}H_{14}O$	39, 91, 77, 121, 120	0.4
	152		81, 55, 91, 79, 77	0.3
1200	154	$C_{10}H_{18}O$	41, 43, 55, 56, 71	1.1
	162	$C_{11}H_{14}O$	120, 39, 91, 55, 65	0.2
1430	164	( )-Acetyl benzaldehyde	91, 69, 120, 121, 149	0.7
	166	$C_{11}H_{18}O$	81, 91, 53, 39, 120	0.1
1620	168	Isopropyl-methyl-cyclohexenyl-methyl ether	73, 70, 83, 97, 111	2.3
	168	( )-Methyl-cyclohexenylbutyl ether	97, 41, 69, 55, 81	0.4
1665	170		169, 168, 51, 59, 167	1.1
	180	$C_{12}H_{20}O$	81, 124, 55, 39, 137	0.3
	180		111, 109, 67, 69, 81	0.2
	182		83, 55, 111, 53, 112	1.1
1425	190	Phenethyl-3-methyl-2-butenyl ether	69, 91, 121, 120, 105	1.2
1470	190	Isopropyl-4-phenyl-2-butenyl ether	91, 105, 117, 77, 147	1.5
1580	192	$C_{13}H_{22}O$	69, 70, 71, 56, 85	0.5
	194		83, 107, 91, 93, 121	0.6
	202		69, 149, 81, 91, 105	0.6
1430	206	$C_{14}H_{22}O$	113, 43, 191, 149, 127	0.8
	206	$C_{14}H_{22}O$	41, 43, 55, 57, 69	0.8
1645	208		41, 43, 57, 55, 82	0.3
	208		69, 121, 175, 193, 208	0.6
1510	218	$C_{15}H_{22}O$	41, 69, 93, 82, 67	1.2
1490	220	$C_{15}H_{24}O$	43, 41, 57, 39, 58	5.3
1510	220	2,6-Di-*tert*-butyl-*p*-cresol	Stenhagen *et al.* (1969)	2.1
1525	220	$C_{15}H_{24}O$	57, 41, 205, 220, 82	1.2
1550	220	$C_{15}H_{24}O$	69, 41, 43, 55, 57	1.3
	220	$C_{15}H_{24}O$	43, 123, 39, 29, 159	0.5
	220	$C_{15}H_{24}O$	69, 81, 91, 109, 159	0.1
1520	222	$C_{15}H_{26}O$	41, 69, 93, 82, 67	1.6
1590	222	$C_{15}H_{26}O$	109, 81, 93, 67, 79	0.8
	222	$C_{15}H_{26}O$	91, 39, 148, 120, 105	0.1
1760	250		43, 58, 41, 55, 59	1.9
1660	264		43, 41, 57, 55, 69	1.5

The components of rose-of-Sharon essential oil therefore have little in common with the essential oil of the cotton bud although both are members of the Malvaceae family and attract the boll weevil. A similarity of these constituents would have lent support to Fraenkel's theory (1959) that essential oils are plant attractants for insects. The dissimilarity found suggests that other compounds in rose-of-Sharon may be responsible for the response of the boll weevil.

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Constituents of Cotton Seedlings; an Investigation of the Preference of Male Boll Weevils for the Epicotyl Tips

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The essential oil obtained from the epicotyls (apical tips, 5–8-mm section from apex) of cotton seedlings (*Gossypium hirsutum* L. var. Deltapine Smoothleaf) was analyzed with an integrated gas chromatography–mass spectrometry system. Structures for 36 compounds are proposed: six sesquiterpene hydrocarbons, seven esters, four alcohols, 14 oxygen-containing compounds, and five miscellaneous. Four of the five miscellaneous compounds are believed to be metabolic products of the fungicide Terraclor with which the cotton seed had been treated. Partial structural data for some additional compounds are also given. A number of the compounds are present in cotton bud essential oil, but there are significant differences. The degree of preference of boll weevils, *Anthonomus grandis* Boheman, for cotton seedlings and their constituent parts was evaluated. Primarily males were attracted to the epicotyls and hypocotyls, but they fed exclusively on the epicotyls and were strongly deterred from feeding on the hypocotyls. Although the epicotyls appear to possess some unique quality that encourages feeding and pheromone production, none of the identified components seemed adequate to explain these properties.

Maxwell *et al.* (1963) reported that the flowers and buds were the most favored parts of the cotton plant for feeding by boll weevils (*Anthonomus grandis* Boheman), but they also fed well on whole seedlings. Later, a definite feeding preference was shown for the epicotyl of the seedling over that for the cotyledon, hypocotyl, and radical (Jenkins and Parrott, 1972). Then, since male boll weevils fed fresh cotton or cotton seedlings are more attractive than those fed artificial diets, this tissue appeared to be suitable for investigation because it might contain a specific precursor of the boll weevil sex attractant complex identified by Tumlinson *et al.* (1969); also, it might not contain as large a spectrum of compounds as the mature plant since it is a tissue which appears at an early stage in the development of the plant.

The present investigation was therefore made to identify as many of the volatile components in the epicotyl essential oil as possible so that they might be compared with the components of the sex attractant (Tumlinson *et al.*, 1969) and those of the essential oil of the cotton bud (Hedin *et al.*, 1971a,b, 1972; Minyard *et al.*, 1965, 1966, 1967, 1968). Feeding stimulant bioassays were performed to confirm the previous unreported data, this time with both sexes separately. Plant attractant bioassays were also performed with both sexes separately to further define the insect feeding preference.

MATERIALS AND METHODS

Plant Material. Treated delinted commercial cotton seeds were germinated in the greenhouse in flats of vermiculite and harvested 6 days after planting (total of 5 kg). When the cotyledons had been detached, the epicotyl sections of the seedlings were removed and immediately immersed in absolute methanol to prevent further enzymatic activity. They were then stored at 0° until an adequate quantity was obtained for steam distillation.

Feeding Stimulant Bioassays. Assays of the components as feeding stimulants were conducted as described by Hedin *et al.* (1968). Aqueous solutions (0.5 g/ml of concentration) of total seedlings, cotyledons, epicotyls, hypocotyls, and radicles were assayed. Ten 5-day-old laboratory-reared and sexed boll weevils (sexes were tested separately) were placed in a 100-mm petri dish with two agar plugs, one wrapped with paper impregnated with the test compound and the other with paper that was not impregnated. The dishes were placed in an incubator that was maintained at 28° and equipped with balanced lighting, and the weevils were allowed to feed for 4 hr. When the papers were unwrapped from the plugs, any punctures

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