

HERBIVORE-INDUCED VOLATILE EMISSIONS FROM COTTON (*Gossypium hirsutum* L.) SEEDLINGS

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Abstract—The effect of herbivory on the composition of the volatile blends released by cotton seedlings was investigated by collecting volatiles from undamaged, freshly damaged (0–2 hr after initiation of feeding), and old damaged (16–19 hr after initiation of feeding) plants on which corn earworm caterpillars (*Helicoverpa zea* Boddie) were actively feeding. A blend of 22 compounds was consistently observed to be emitted by the old damaged plants with nine occurring either only in, or in significantly greater amounts in old damaged, as compared with freshly damaged plants. These were (Z)-3-hexenyl acetate, hexyl acetate, (E)- β -ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene, (Z)-3-hexenyl butyrate, (E)-2-hexenyl butyrate, (Z)-3-hexenyl 2-methylbutyrate, (E)-2-hexenyl 2-methylbutyrate, and indole. The nature of this response is compared with other studies where herbivore-induced volatile responses are also known. The presence of large amounts of terpenes and aldehydes seen at the onset of feeding and the appearance of other compounds hours later suggest that cotton defense mechanisms may consist of a constitutive repertoire that is augmented by an induced mechanism mobilized in response to attack. A number of the induced compounds are common to many plants where, in addition to an immediate defensive function, they are known to be involved in the attraction of natural enemies.

Key Words—Cotton, *H. zea*, feeding deterrence, phytoalexin, semiochemicals, terpenes, tritrophic interactions.

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INTRODUCTION

In the past, studies on plant volatile production have generally dealt with the relationship between the healthy plant and its attractancy or repellency to potential herbivores, or with the effect on herbivore feeding of plant-produced chemicals (Carroll and Hoffman, 1980; Dickens, 1984; Gunasena et al., 1988; Mitchell et al., 1991). Intact healthy plants often produce specific odors that are recognized by certain herbivores searching for potential oviposition or feeding sites, but damage to these plants may result in the release of volatile anti-feedants that serve to reduce the damage inflicted by the herbivore (Mihaliak et al., 1987). Plant odors can also be attractive to parasitoids or predators, which then attack the herbivores. Moreover, it has recently been shown that some plants produce a specific blend of volatile chemicals, only in response to herbivore-induced damage, that attract either predators (Dicke and Sabelis, 1988) or parasitoids (Turlings et al., 1990), which then attack the injurious herbivores and relieve the plant. The predators and parasitoids appear to orient to these chemicals, recognizing them as marker cues for their prey or hosts.

This plant response to herbivore damage is likely to be part of a general chemical defense against the herbivores, which is exploited secondarily by natural enemies of the herbivores, thereby adding to the benefits that the plant gains from the emission of volatiles (Turlings and Tumlinson, 1991). In those cases so far described, the volatile blends released by plants in response to herbivore damage are unique to the particular plant species described but contain a number of commonly encountered plant volatiles (Turlings et al., 1993a). Furthermore, prior results with corn seedlings (Turlings et al., 1993b) and preliminary results with cotton (Turlings and McCall, unpublished) indicate that the composition of the induced blend of volatiles does not vary significantly when the plants are attacked by different species of herbivorous insects. This suggests that the plant is responding nonspecifically to insect damage and that this response is characteristic for that particular plant species or variety. Therefore, as part of a series of studies on the interactions among plants, herbivores, and parasitic wasps, the nature of this induced response in cotton plants was investigated. In the present study, the effect of damage by one caterpillar species, corn earworm, *Helioverpa zea* Boddie (Lepidoptera: Noctuidae), was examined. This was chosen as a complement to studies on the Braconid wasp *Microplitis croceipes* Cresson (Hymenoptera: Braconidae), a parasitoid specialist on corn earworm and related species, and its host location behavior when foraging in cotton and other plant species (McCall et al., 1993). To date, studies on cotton volatiles have dealt with the odors of intact plants (Thompson et al., 1971; Hedin et al., 1975b; Hedin, 1976), or with specific biosynthetic classes of compounds (Minyard et al., 1965, 1966; Pomonis et al., 1980; Chang et al., 1988). Here the complete

odor blend emitted by cotton under attack by caterpillars is described, and the delayed nature of the release is shown.

METHODS AND MATERIALS

Insects. All of the insects used (*Helicoverpa zea* Boddie, CEW) were reared on a pinto bean-based artificial diet using the procedure described by King and Leppla (1984). In all studies, late-second to third-instar larvae were used.

Plant Treatments. Cotton (*Gossypium hirsutum* L., var. McNair 235) was grown in a greenhouse, in styrofoam cups (500 ml), with one seedling per cup. The seedlings used were approximately 20 cm tall at 2–3 weeks old (4 to 6-leaf stage). The plants were brought indoors between 1700 and 1900 hr. In cases where the plant treatment was classed as being old damage, five caterpillars were placed on each plant, and the whole plant and pot were covered with an inverted styrofoam cup in which the base was replaced by fine nylon mesh, and then fitted tightly to the plant pot to retain the caterpillars. On the following morning, the plant was cut close to the base of its stem with a razor blade, the cut end wrapped in wet cotton to prevent dehydration, and immediately placed with the caterpillars in a volatile collection chamber. All volatile collections were made between 0900 and 1200 hr, at 16–19 hr after feeding began. In cases classed as fresh damage, plants were stored overnight as described, but no larvae were introduced until the cut plants were placed in the volatile collection chamber. In both old and fresh damage, extra caterpillars that had been starved overnight were placed with the plants, so that active feeding was occurring during the volatile collections from both treatments.

Collection of Plant Volatiles. The volatile collection system has been described in detail by Turlings et al. (1991a). Briefly, humidified air, purified by an in-line activated charcoal filter, entered four parallel chambers consisting of Pyrex glass tubes (approx. 50 cm long and 115 mm in diameter), each with a glass frit to ensure laminar airflow through the tube. The tubes were assembled from three sections to facilitate cleaning and introduction of the seedlings. Air-flow at 300 ml/min was balanced with house air and vacuum, and maintained at slightly higher than atmospheric pressure inside each chamber. Air exited each chamber through a reusable 3.7-mm-ID \times 4-cm-long glass collection trap packed with 25 mg Super Q adsorbent (80–100 mesh) (Alltech, Deerfield, Illinois), which was prerinse prior to each volatile collection with 5–10 ml dichloromethane to remove impurities. Volatiles were collected for 2 hr, after which the collection traps were extracted immediately with 150 μ l dichloromethane, and internal standards added (1 μ g each of octane and nonyl acetate in 30 μ l dichloromethane). A total of four collections comprising a system blank (containing only wet cotton wool), undamaged seedlings (seedlings simply cut

at the base and wrapped in wet cotton wool), fresh damaged seedlings, and old damaged seedlings were run in parallel on each occasion and the airflows through each were equalized with Aalborg flowmeters at the downwind end of the collection traps. Each treatment involving plant damage consisted of three seedlings under attack by a total of 20–25 caterpillars. The experiment was replicated five times.

Chemical Analyses. Collected volatiles were analyzed with a Hewlett-Packard model 5890 GC, or a Varian model 3700 GC, equipped with split-splitless capillary injector systems and flame ionization detectors. All analyses were performed on two fused silica capillary columns, with helium as carrier gas (19 cm/sec). The columns were 50 m × 0.25 mm ID with a 0.25- μ m film of bonded methyl silicone (007) and 50 m × 0.25 mm ID with a 0.25- μ m film of bonded cyanopropyl methyl silicone CPS-1 (Quadrex Corporation, New Haven, Connecticut) and were operated at an initial temperature of 40°C for 3 min, then programmed at 5°C/min to 180°C. All injections of 2 μ l were made in the splitless mode, and split after 30 sec. Data were collected, analyzed, and stored with a Perkin-Elmer chromatographic data system.

Samples were also analyzed by GC–mass spectroscopy (GC–MS) with a Nermag model R1010 mass spectrometer in both electron impact and chemical ionization modes. Both of the columns and the conditions described above were used in GC–MS. Methane and isobutane were used as reagent gases for chemical ionization. The retention times on both columns and the spectra of the natural compounds were compared with those of candidate synthetic compounds. (3*E*)-4,8-Dimethyl-1,3,7-nonatriene and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatriene were synthesized by the Wittig reaction of geraniol and farnesal, respectively, with methylenetriphenyl phosphorane (analogous to Maurer et al., 1986). In the case of the (*Z*)-3-hexenyl and (*E*)-2-hexenyl esters of butanoic and 2-methyl butanoic acids, identifications were further confirmed by cochromatography of the natural compounds with authentic standards. All of the other synthetic standards were obtained from commercial sources.

RESULTS

Identity of Released Volatiles. In volatiles emitted by plants with old damage, 22 compounds were consistently present. Figure 1 shows typical chromatograms for volatile collections made from three seedlings over 2 hr, and Table 1 shows the calculated amounts released from one seedling per hour, based on an integration of the data from five replicates per treatment. None of these compounds were found in blank collections made from wetted cotton wool, and only minute amounts were found in collections from undamaged cotton, some of which may have arisen as a result of slight mechanical damage to the plants during handling.

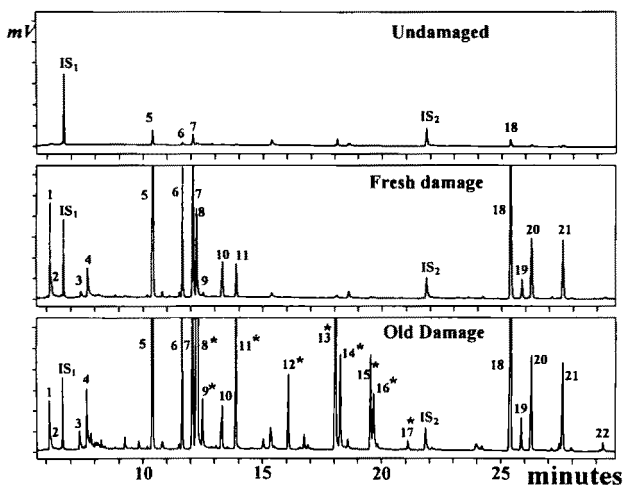


FIG. 1. Gas chromatographic analyses on a methyl silicone capillary column of volatiles collected from groups of three cotton seedlings for a period of 2 hr. Fresh damage: volatiles collected during the 2 hr immediately following initiation of feeding by 20–25 *H. zea* larvae. Old damage: volatile collection commenced at least 15 hr after initiation of feeding. Peak numbers correspond to the compounds named in Table 1 and those peaks marked with an asterisk are present in significantly greater amounts in old damage than in fresh damage. IS₁ and IS₂ represent *n*-octane and *n*-nonyl acetate, respectively.

It was possible to identify most of these compounds by comparison of the mass spectra and retention times on polar and nonpolar columns with the known synthetic compounds. In the case of α -guaiene and δ -guaiene however, authentic standards of sufficient purity to make positive identification were not available. The seven most intense ions in the electron impact mass spectra of these two tentatively identified compounds were 105 (100), 204 (M^+ , 85.4), 93 (84.4), 70 (83.3), 81 (78.1), 91 (77.1), and 107 (66.7) for α -guaiene; and 93 (100), 108 (81.6), 79 (75.5), 107 (68.4), 81 (63.3), 91 (57.1), and 95 (55.1) for δ -guaiene. Additionally, a number of other compounds were occasionally found in the volatile collections. Of these, certain compounds were matched with library mass spectra and provisionally identified as phellandrene, copaene, terpinene, bisabolol, benzaldehyde, and nonanol, but as they were not consistently found in most of the volatile collections, their identities were not confirmed.

Variations in Volatile Blends. Nine of the compounds were found to be released in significantly greater amounts from plants with old damage than from freshly damaged plants (Figure 1, Table 1). Of these, (*Z*)-3-hexenyl acetate, hexyl acetate, (*E*)- β -ocimene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and (*Z*)-3-hexenyl butyrate were also found in volatiles of freshly damaged plants (peaks

TABLE 1. AMOUNTS, STANDARD DEVIATIONS, AND RELATIVE PERCENTAGES OF COMPONENTS OF COMPLETE BLENDS FOR UNDATED PLANTS, FRESH AND OLD DAMAGED PLANTS RELEASED BY 1 COTTON SEEDLING PER HOUR, DETERMINED FROM DATA COLLECTED FROM 5 REPLICATES PER TREATMENT^a

Peak	Compound	Undamaged		Fresh damage		Old damage	
		Amount, ng/hr (SD)	Relative %	Amount, ng/hr (SD)	Relative %	Amount, ng/hr (SD)	Relative %
1	(Z)-3-hexenal	2.2 (1.9)	1.2	109.7 (68.3)	2.8	122.5 (605.1)	1.6
2	hexanal	0.4 (0.9)	0.2	12.2 (8.6)	0.4	20.0 (13.0)	0.3
3	(E)-2-hexenal	0	0	11.2 (9.6)	0.3	41.6 (31.6)	0.5
4	(Z)-3-hexen-1-ol	0	0	63.5 (72.3)	1.6	117.2 (35.4)	1.5
5	α -pinene	65.6 (84.5)	35.9	1341.8 (1168)	34.4	1311.5 (371.3)	17.0
6	β -pinene	11.5 (15.1)	6.2	239.3 (203.7)	6.1	229.0 (66.0)	3.0
7	myrcene	27.9 (30.5)	15.3	522.5 (348)	13.4	673.9 (269.0)	8.7
8	(Z)-3-hexenyl acetate	11.5 (23.0)	6.3	104.5 (100.2)	2.7	1617.7 (707.3)	21.0 ^b
9	hexyl acetate	0	0	3.7 (4.7)	0.1	69.5 (56.4)	1.0 ^b
10	limonene	9.3 (10.5)	5.1	100.0 (80.5)	2.6	98.2 (52.0)	1.2
11	(E)- β -ocimene	8.2 (16.5)	4.5	46.6 (16.2)	1.2	394.4 (266.0)	5.1 ^b
12	(3E)-4,8-dimethyl-1,3,7-nonatriene	0	0	20.0 (37.6)	0.5	206.0 (92.2)	2.6 ^b
13	(Z)-3-hexenyl butyrate	0	0	1.7 (3.3)	0.1	494.6 (415.2)	6.4 ^b
14	(E)-2-hexenyl butyrate	0	0	0	0	130.9 (127.7)	1.6 ^b
15	(Z)-3-hexenyl 2-methylbutyrate	0	0	0	0	110.0 (131.2)	1.4 ^b
16	(E)-2-hexenyl 2-methylbutyrate	0	0	0	0	77.1 (98.5)	1.0 ^b
17	indole	0	0	0	0	26.8 (12.3)	0.4 ^b
18	(E)- β -caryophyllene	30.0 (35.6)	16.4	835.7 (420.6)	21.5	1231.5 (370.4)	16.0
19	α -guaiene	0.9 (1.8)	0.5	63.8 (51.6)	1.6	90.8 (34.2)	1.2
20	α -humulene	9.1 (11.3)	5.0	220.4 (102.9)	5.6	325.7 (98.5)	4.2
21	δ -guaiene	5.7 (5.6)	3.1	197.5 (180.3)	5.1	302.0 (101.7)	4.0
22	(3E, 7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene	0.6 (1.2)	0.3	6.7 (11.9)	0.2	19.5 (15.0)	0.3

^aDetails of each treatment are given in the text.

^bSignificantly different from fresh-damage values (Mann-Whitney test: $P < 0.05$).

8, 9, 11, 12, and 13), but in significantly lower quantities ($P < 0.05$, Mann-Whitney test). (*E*)-2-Hexenyl butyrate, (*Z*)-3-hexenyl 2-methylbutyrate, (*E*)-2-hexenyl 2-methylbutyrate, and indole (peaks 14, 15, 16, and 17) were never found in volatiles from fresh damage. Although peak 22, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, also occurred in increased amounts in the old damage, this difference was not significant ($P = 0.09$).

DISCUSSION

This study represents the first attempt to describe the total blend of volatiles released by cotton plants following herbivore damage. Earlier studies described either the constituents of cotton essential oil (Minyard et al., 1965, 1966; Hedin et al., 1975a; Kumamoto et al., 1979), or volatile compounds from intact cotton plants (Thompson et al., 1971; Hedin et al., 1975b; Hedin, 1976; Pomonis et al., 1980; Chang et al., 1986, 1988). What is immediately apparent from this study is the enormous increase in volatile release following caterpillar feeding and the subsequent change in relative proportions of the compounds within the blend as feeding continues. The increase in the particular compounds in old damaged seedlings is very striking in some cases, with (*E*)-2-hexenyl butyrate, (*Z*)-3-hexenyl 2-methylbutyrate, (*E*)-2-hexenyl 2-methylbutyrate, and indole appearing only in older damaged plants, and others occurring in significantly greater amounts. The latter is best illustrated by the dramatic increase in (*Z*)-3-hexenyl acetate from 2.1% of the total blend in fresh damage to 21% in old damage (Table 1). Increases of this nature following herbivore damage have previously been recorded from corn seedlings following caterpillar damage (Turlings et al., 1990) and in lima beans following mite damage (Dicke et al., 1990a) (see below).

A number of the compounds recorded here are not mentioned in the previous literature on cotton volatiles (compounds 1, 8, 9, 12, 13, 14, 15, 16, 17, and 22 in Figure 1). The green leaf compounds hexyl acetate and (*Z*)-3-hexenyl acetate have been found in volatiles of cowpea (Lwande *et al.*, 1989) and corn (Buttery and Ling, 1984; Turlings *et al.* 1991a), respectively, and both are found in strawberry (Hamilton-Kemp et al., 1989). Peak 15, (*Z*)-3-hexenyl 2-methylbutyrate, is also known from strawberry leaves (Hamilton-Kemp et al., 1989). The related methylene monoterpene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene and methylene sesquiterpene, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene have been reported from cardamom oil (*Elettaria cardamomum*) (Maurer et al., 1986), from corn seedlings (Turlings et al., 1991a) under attack by caterpillars, and from lima beans leaves (Dicke et al., 1990a) and cucumber leaves (Dicke et al., 1990b) under attack by acarine herbivores. Many of the compounds identified in volatiles from cotton leaves in the present study are

also known to occur in the volatiles from flowers and bolls, although in different relative proportions (Turlings et al., 1993a), and the differences between fresh and old damage shown here are also seen in those plant parts (McCall and Turlings, unpublished).

(*Z*)-3-Hexenal is highly volatile and is seldom reported as a green leaf volatile elsewhere, although Turlings et al. (1991a) found high proportions in corn volatiles, probably as a result of using this volatile collection technique. This relatively simple, nondestructive, and highly efficient [as evidenced by the retrieval of (*Z*)-3-hexenal] procedure allows a more accurate determination of the identity and proportions of compounds emitted by the plant. This is preferable to identification of compounds obtained by extraction or more destructive methods, which lead to the identification of compounds present in the plant in various forms, or in the worst case, artifacts produced by degradation of plant-contained compounds. The composition of such extracts is unlikely to be relevant to the blend actually emitted by the plant and ultimately detected by insects.

Many of the compounds detected in cotton at the onset of, or induced by, herbivore infestation are known feeding deterrents (Zummo et al., 1984; Mihaliak et al., 1987; Gunasena et al., 1988; Hedin et al., 1991), or are known to have a defensive function against other unrelated pathogens (Zeringue and McCormick, 1989, 1990). The subsequent appearance of, or increase in, specific compounds only after 16 hr, suggests a more specific role for these induced compounds. Corn seedlings under attack by caterpillars show a dramatic increase in certain terpenoids 16 hr after the onset of damage caused by herbivory (Turlings et al., 1990), in response to the oral secretions of the herbivore (Turlings et al., 1993b). A similar response was also shown to occur in lima bean (Dicke and Sakelis, 1988). However, the presence within the cotton plant of high quantities of terpenoids at the initiation of herbivore feeding suggests that, unlike the faster growing annuals corn and lima bean, which may rely primarily on an induced response that is activated when attacked and becomes effective only after a number of hours (Turlings and Tumlinson, 1991), the relatively slow growing perennial cotton already has effective constitutive defense resources in place. Coley et al. (1985) suggested that slower growth rates favor larger investments in antiherbivore defenses, whereas plants with faster growth rates may utilize more mobile or flexible defenses. Results from the present study suggest cotton may utilize both means of defense. In fact, certain of the compounds induced in cotton, (*Z*)-3-hexenyl acetate and (3*E*)-4,8-dimethyl-1,3,7-nonatriene, are also found following herbivore damage in lima bean (Dicke and Sabelis, 1988), in corn (Turlings et al., 1991a), and in cowpea (Turlings et al., 1993a), and (*E*)- β -ocimene and indole are induced in lima bean and corn, respectively, suggesting a common role for these compounds. Moreover, there is evidence that production of terpenes and other antifeedants varies seasonally,

with peak production coinciding with fruiting and consequential *Heliothis zea* attack (Hedin, 1976; Zummo et al., 1984).

Turlings and Tumlinson (1992) showed that the induced response to herbivores in corn was not limited to the sites of damage but occurred throughout the plant, with undamaged leaves also releasing induced compounds. In this study a systemic response by cotton was not investigated. However, Karban and Carey (1984) showed that new growth on cotton seedlings that had been infested previously with mites was more resistant to subsequent infestations by the same and novel mite species than were seedlings that had never been infested. Thus, cotton may be capable of a systemic chemical response to herbivore damage. Further research will be required to determine whether release of volatiles by herbivore damaged cotton is systemic.

We also have shown that damaged cotton plants were the most important source of volatile cues that foraging parasitic wasps, *M. croceipes*, used to locate potential host sites (McCall et al., 1993). Other studies on lima bean (Dicke and Sabelis, 1988) and corn (Turlings et al., 1990, 1991b) found that it is the herbivore-induced volatiles in particular that are exploited by natural enemies to locate their hosts or prey. Although other workers have shown that parasitic wasps respond to volatiles of cotton (Elzen et al., 1984, 1986; Baehrecke et al., 1989; Li et al., 1992), they did not take into account the delay in the appearance of the induced response in the plant. Thus, whereas *M. croceipes* will respond in simple flight tests to individual green leaf odors (Whitman and Eller, 1990), characteristic of fresh herbivore or simple mechanical damage, they prefer old damage to fresh damage in choice tests, regardless of previous experience (McCall et al., 1993). The results of both that and the present studies suggest that this parasitic wasp has an innate predisposition to orient to herbivore-induced volatiles, which overrides positive learning experiences. Considering, then, the role of delayed herbivore-induced volatile responses in host-location by natural enemies on cotton and other plants, precise timing of plant damage must be considered crucial in such behavioral studies. The compounds making up the green leafy odor are common to many different plant species (Visser et al., 1979). Although they may be of secondary importance in the close range location of the freshest damage, where the hosts are currently likely to be, it is the induced compounds that are the more reliable and consistent indicators of the presence of herbivores. Natural enemies are faced with a problem in obtaining cues for hosts or prey, which need to be both reliable and detectable for efficient foraging (Vet and Dicke, 1992). Within the complex of odors originating from the plant, the herbivore, and the herbivore's waste products, the plant-derived odors are by far the most abundant and are the most attractive to foraging parasitic wasps (Turlings et al., 1991a). In nature the herbivore-specific volatiles within the plant odor blend would allow the endan-

gered plant to advertise itself more accurately and prominently to searching beneficial insects.

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REFERENCES

- BAEHRECKE, E.H., WILLIAMS, H.J., and VINSON, S.B. 1989. Electroantennogram responses of *Campoletis sonorensis* to chemicals in cotton. *J. Chem. Ecol.* 15:37–45.
- BUTTERY, R.G., and LING, L.C. 1984. Corn leaf volatiles: Identification using tenax trapping for possible insect attractants. *J. Agric. Food Chem.* 32:1104–1106.
- CARROLL, C.R., and HOFFMAN, C.A. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilichna tredecimnotata*. *Science* 209:414–416.
- CHANG, J.F., BENEDICT, T.L., PAYNE, T.L., and CAMP, B.J. 1986. Methods for the collection and identification of volatile terpenes from cotton and evaluation of their attractiveness to boll weevils. *Southwest Entomol.* 11:233–241.
- CHANG, J.F., BENEDICT, T.L., PAYNE, T.L., and CAMP, B.J. 1988. Volatile monoterpenes collected from the air surrounding flower buds of seven cotton genotypes. *Crop Sci.* 28:685–688.
- COLEY, P.D., BRYANT, J.P., and CHAPIN, F.S., III. 1985. Resource availability and plant anti-herbivore defense. *Science* 230:895–899.
- DICKE, M., and SABELIS, M. 1988. How plants obtain predatory mites as bodyguards. *Neth. J. Zool.* 38:148–165.
- DICKE, M., VAN BEEK, T.A., POSTHUMUS, M.A., BEN DOM, N., VAN BOKHOVEN, H., and DE GROOT, A.E. 1990a. Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. Involvement of host plant in its production. *J. Chem. Ecol.* 16:381–396.
- DICKE, M., SABELIS, M.W., TAKABAYASHI, J., BRUIN, J., and POSTHUMUS, M.A. 1990b. Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. *J. Chem. Ecol.* 16:3091–3118.
- DICKENS, J.C. 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): electroantennogram studies. *J. Chem. Ecol.* 10:1759–1785.
- ELZEN, G.W., WILLIAMS, H.J., and VINSON, S.B. 1984. Isolation and identification of cotton synomones mediating searching behaviour by parasitoid *Campoletis sonorensis*. *J. Chem. Ecol.* 10:1251–1264.
- ELZEN, G.W., WILLIAMS, H.J., and VINSON, S.B. 1986. Wind tunnel flight response by hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. *Entomol. Exp. Appl.* 42:285–289.
- GUNASENA, G.H., VINSON, S.B., WILLIAMS, H.J., and STIPANOVIC, R.D. 1988. Effects of caryophyllene, caryophyllene oxide, and their interaction with gossypol on the growth and development of *Heliothis virescens*. *J. Econ. Entomol.* 81:93–97.
- HAMILTON-KEMP, T.R., RODRIGUEZ, J.G., ARCHBOLD, D.D., ANDERSEN, R.A., LOUGHRIN, J.H., PATTERSON, C.G., and LOWRY, S.R. 1989. Strawberry resistance to *Tetranychus urticae* Koch: Effects of flower, fruit and foliage removal-comparisons of air- vs. nitrogen-entrained volatile compounds. *J. Chem. Ecol.* 15:1465–1473.

- HEDIN, P.A. 1976. Seasonal variations in the emissions of volatiles by cotton plants growing in the field. *Environ. Entomol.* 5:1234-1238.
- HEDIN, P.A., THOMPSON, A.C., and GUELDER, R.C. 1975a. Constituents of cotton bud essential oil. *Phytochemistry* 14:2087-2088.
- HEDIN, P.A., THOMPSON, A.C., and GUELDER, R.C. 1975b. Survey of air space volatiles of the cotton plant. *Phytochemistry* 14:2088-2090.
- HEDIN, P.A., PARROTT, W.L., and JENKINS, J.N. 1991. Effects of cotton plant allelochemicals and nutrients on behavior and development of tobacco budworm. *J. Chem. Ecol.* 17:1107-1122.
- KARBAN, R., and CAREY, J.R. 1984. Induced resistance of cotton seedlings to mites. *Science* 225:53-54.
- KING, E.G., and LEPPLA, N.C. 1984. Advances and Challenges in Insect Rearing. Agricultural Research Service, USDA, U.S. Government Printing Office, Washington, D.C.
- KUMAMOTO, J., WAINES, J.G., HOLLENBERG, J.L., and SCORA, R.W. 1979. Identification of the major monoterpenes in the leaf oil of *Gossypium sturtianum* var. *nandewarense*. *J. Agric. Food Chem.* 27:203-204.
- LI, Y., DICKENS, J.C., and STEINER, W.W.M. 1992. Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera: Braconidae) to cotton plant volatiles. *J. Chem. Ecol.* 18:1761-1773.
- LWNADA, W., McDOWELL, P.G., AMIANI, H., and AMOKE, P. 1989. Analysis of airborne volatiles of cowpea. *Phytochemistry* 28:421-423.
- MAURER, B., HAUSER, A., and FROIDEVAUX, J.C. 1986. (E)-4,8-Dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, two unusual hydrocarbons from cardamom oil. *Tetrahedron Lett.* 27:2111-2112.
- MCCALL, P.J., TURLINGS, T.C.J., LEWIS, W.J., and TURLINSON, J.H. 1993. Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae: Hymenoptera). *J. Insect Behav.* 6:625-639.
- MIHALIAK, C.A., COUVET, D., and LINCOLN, D.E. 1987. Inhibition of feeding by a generalist insect due to increase volatile leaf terpenes under nitrate-limiting conditions. *J. Chem. Ecol.* 13:2059-2067.
- MINYARD, J.P., TURLINSON, J.H., THOMPSON, A.C., and HEDIN, P.A. 1965. Constituents of the cotton bud. Terpene hydrocarbons. *Agric. Food Chem.* 13:599-602.
- MINYARD, J.P., TURLINSON, J.H., THOMPSON, A.C., and HEDIN, P.A. 1966. Constituents of the cotton bud. Sesquiterpene hydrocarbons. *Agric. Food Chem.* 14:332-336.
- MITCHELL, E.R., TINGLE, F.C., and HEATH, R.R. 1991. Flight activity of *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae) with reference to host plant volatiles. *J. Chem. Ecol.* 17:259-266.
- POMONIS, J.G., FLINT, H.M., and SMITH, R.L. 1980. Analysis of volatiles from host and nonhost plants of the pink bollworm. *J. Econ. Entomol.* 73:783-786.
- THOMPSON, A.C., BAKER, D.N., GUELDER, R.C., and HEDIN, P.A. 1971. Identification and quantitative analysis of the volatile substances emitted by maturing cotton in the field. *Plant Physiol.* 48:50-52.
- TURLINGS, T.C.J., and TURLINSON, J.H. 1991. Do parasitoids use herbivore-induced plant chemical defenses to locate hosts? *Fla. Entomol.* 74:42-50.
- TURLINGS, T.C.J., and TURLINSON, J.H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U.S.A.* 89:8399-8402.
- TURLINGS, T.C.J., TURLINSON, J.H., and LEWIS, W.J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251-1253.
- TURLINGS, T.C.J., TURLINSON, J.H., HEATH, R.R., PROVEAUX, A.T., and DOOLITTLE, R.E. 1991a. Isolation and identification of allelochemicals that attract the larval parasitoid *Cotesia marginiventris* (cresson) to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17:2235-2251.

- TURLINGS, T.C.J., TURLINSON, J.H., ELLER, F.J., and LEWIS, W.J. 1991b. Larval-damaged plants: Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomol. Exp. Appl.* 58:75-82.
- TURLINGS, T.C.J., WÄCKERS, F.L., VET, L.E.M., LEWIS, W.J., and TURLINSON, J.H. 1993a. Learning of host-finding cues by hymenopterous parasitoids, pp. 51-78, in D.R. Papaj and A.C. Lewis (eds.). *Insect learning: Ecological and evolutionary perspectives*. Chapman and Hall, New York.
- TURLINGS, T.C.J., MCCALL, P.J., ALBORN, H.A., and TURLINSON, J.H. 1993b. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *J. Chem. Ecol.* 19:411-425.
- VET, L.E.M., and DICKE, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37:141-172.
- VISSER, J.H., VAN STRATEN, S., and MAARSE, H. 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the colorado beetle, *Leptinotarsa decemlineata*. *J. Chem. Ecol.* 5:13-25.
- WHITMAN, D.W., and ELLER, F.J. 1990. Parasitic wasps orient to green leaf volatiles. *Chemoecology* 1:69-75.
- ZERINGUE, H.J., JR., and MCCORMICK, S.P. 1989. Relationships between cotton leaf-derived volatiles and growth of *Aspergillus flavus*. *J. Am. Oil Chem. Soc.* 66:581-585.
- ZERINGUE, H.J., JR., and MCCORMICK, S.P. 1990. Aflatoxin production in cultures of *Aspergillus flavus* incubated in atmospheres containing selected cotton leaf-derived volatiles. *Toxicon* 28:445-448.
- ZUMMO, G.R., SEGERS, J.C., and BENEDICT, J.H. 1984. Seasonal phenology of allelochemicals in cotton and resistance to bollworm. *Environ. Entomol.* 13:1287-1290.