ISOLATION AND IDENTIFICATION OF VOLATILE KAIROMONE THAT AFFECTS ACARINE PREDATOR–PREY INTERACTIONS

Involvement of Host Plant in Its Production

M. DICKE,[1] T.A. VAN BEEK,[2] M.A. POSTHUMUS,[2]
N. BEN DOM,[2] H. VAN BOKHOVEN,[1,2] and AE. DE GROOT[2]

[1]*Department of Entomology*
Agricultural University
P.O. Box 8031
6700 EH Wageningen,
The Netherlands

[2]*Department of Organic Chemistry*
Agricultural University
Dreijenplein 8
6703 HB Wageningen,
The Netherlands

Abstract—A volatile kairomone emitted from lima bean plants (*Phaseolus lunatus*) infested with the spider mite *Tetranychus urticae*, was collected on Tenax-TA and analyzed with GC-MS. Two components were identified as the methylene monoterpene (3E)-4,8-dimethyl-1,3,7-nonatriene and the methylene sesquiterpene (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, respectively, after purification by preparative GC on a megabore column and recording of UV, IR, and [1H]NMR spectra. The response of two species of predatory mites towards the identified chemicals was tested in a Y-tube olfactometer. Four of the compounds tested, linalool (3,7-dimethyl-1,6-octadien-3-ol), (E)-β-ocimene [(3E)-3,7-dimethyl-1,3,6-octatriene], (3E)-4,8-dimethyl-1,3,7-nonatriene, and methyl salicylate attracted females of *Phytoseiulus persimilis*. Linalool and methyl salicylate attracted females of *Amblyseius potentillae*. The response of *A. potentillae* to these two kairomone components was affected by the rearing diet of the predators in the same way as was reported for the response to the natural kairomone blend: when reared on a carotenoid-deficient diet, the predators responded to the volatile kairomone of *T. urticae*, but when reared on a carotenoid-containing diet they did not. The identified kairomone components are all known from the plant kingdom. They are not known to be produced by animals de novo.

381

In addition to biological evidence, this chemical evidence suggests that the
plant is involved in production of the kairomone. Based on the present study
and literature data on the response of *T. urticae* to infochemicals, it is con-
cluded that the kairomone component linalool is also a component of a vol-
atile spider-mite dispersing pheromone.

Key Words—Kairomone, spider mites, predatory mites, lima bean leaves,
tritrophic system, methylene monoterpene, methylene sesquiterpene, rearing
diet, carotene, identification.

INTRODUCTION

Since the first report on volatile kairomones (sensu Dicke and Sabelis, 1988) in
interactions between phytophagous mites and predatory mites (Sabelis and Van
de Baan, 1983), a lot of biological information has been obtained on many
aspects of these acarine allelochemicals. Volatile kairomones have been reported
from 13 acarine prey–predator interactions (Sabelis and Van de Baan, 1983;
Sabelis and Dicke, 1985; Dicke and Groeneveld, 1986; Dicke et al., 1986,
1988; Dong and Chant, 1986; Dicke, 1988). Studies have concentrated on the
effect of predator condition on the response (Sabelis and Van de Baan, 1983;
Dicke and Groeneveld, 1986; Dicke et al., 1986; Dong and Chant, 1986; Dicke,
1988), the type of behavior elicited in the predator by the kairomone (Sabelis
et al., 1984a), kairomone preference (Dicke and Groeneveld, 1986; Dicke,
1988), and extraction of the compounds from leaves with methylene chloride
and study of their origin (Sabelis et al., 1984b).

As a result of the increased biological knowledge on volatile kairomones
in spider mite—predatory mite interactions, questions have arisen that can only
be answered by identification of the chemicals involved:

1. Do different predator species, which all respond to the same prey spe-
cies, use the same chemicals as a source of information? The phytoseiid species
Phytoseiulus persimilis Athias-Henriot, *Typhlodromus occidentalis* (Nesbitt),
Amblyseius potentillae (Garman), and *Typhlodromus pyri* Scheuten, all respond
to a volatile kairomone of the two-spotted spider mite, *Tetranychus urticae*
Koch (Sabelis and Van de Baan, 1983; Dicke et al., 1986; Dicke, 1988).
Tetranychus urticae is a valuable prey species to the first two predator species,
but the latter two are severely hindered by the dense web structure of this prey,
which reduces predator reproductive success (Sabelis, 1981; Overmeer, 1981;
McMurtry et al., 1970). This difference in profitability is reflected in the
response of the predators to a volatile kairomone of *T. urticae*. *Phytoseiulus
persimilis* and *T. occidentalis* respond to the kairomone when satiated or starved
for 24 hr, respectively (Sabelis and Van de Baan, 1983), but severe quantitative
or qualitative food deficiencies are needed to demonstrate the response of *A.*

potentillae and *T. pyri* to the volatile kairomone of the two-spotted spider mite (Dicke et al., 1986; Dicke, 1988; Dicke, unpublished data). It is interesting to know whether the different effects of *T. urticae* on reproductive success of these four phytoseiid species are also reflected in differences in the chemical information used by the predators.

2. What is the function of the volatile kairomone to the prey mite itself? The predators may cue in on any spider mite-related chemical, but as a result of natural selection, only a response to a chemical that is indispensable or inevitable to the prey mite will last long on an evolutionary time scale. From leaves infested with *T. urticae*, a volatile spider-mite dispersing pheromone is emitted, the biological characteristics of which suggest that it may be (partly) identical to the kairomone (Dicke, 1986). However, only identification of the chemicals involved in the intraspecific and interspecific interactions may demonstrate this.

3. What is the origin of the kairomone? An interaction of the host plant and the spider mite is a prerequisite for demonstration of a response by *P. persimilis* (Sabelis et al., 1984a; Dicke, unpublished data). The kairomone in the current tritrophic system is present on previously infested leaves from which all spider mites and visible spider-mite products have been removed (Sabelis et al., 1984a). This indicates that the kairomone may have been produced by the plant and/or by the spider mites that deposited it on the plant. Chemical identification of the kairomone may assist in revealing the origin of the kairomone and the role of the plant and the spider mite in its production.

Here we report on the isolation, by collection of airborne chemicals, and identification of components of the volatile *T. urticae* kairomone.

METHODS AND MATERIALS

Mites. The two-spotted spider mite (*T. urticae*) was reared on lima bean plants (*Phaseolus lunatus* L.) at 20–30°C, 50–70% relative humidity under continuous fluorescent light that was added to the natural daylight regime.

The predatory mite *P. persimilis* was obtained from Koppert BV (Berkel en Rodenrijs, The Netherlands) six years ago. It was reared at ca. 20°C on lima bean leaves infested with *T. urticae*, either on wet cotton wool or on clay flower pots that were put upside down in a water basin.

The predator *A. potentillae* was reared on either of two diets: *Vicia faba* (broad bean) pollen or *T. urticae*. In both cases, the food was provided on plastic arenas (McMurtry and Scriven, 1965; Overmeer, 1985). The predators of these two cultures will be indicated by the suffixes (Vf) and (Tu) respectively. For additional information on rearing history, see Dicke et al. (1989).

Isolation of Kairomone for Analytical Purposes. Twenty lima bean plants, with two to three leaves per plant, were cut and immediately placed in a 5-liter

glass jar with 1 cm of freshly distilled water at the bottom. Care was taken not to damage the plants except for cutting the stem. Three plant treatments were used: clean, infested with spider mites, or damaged mechanically by rubbing the leaves with carborundum on a wet cotton wool pad. A Pyrex glass tube (161 × 6.4 mm OD; ID 3 mm) with 90 mg of Tenax-TA in the center, held in place by two plugs of silanized glass wool, was connected to the outlet of the jar, and a charcoal filter to the inlet. Air was sucked through the system with a membrane pump at ca. 15 ml/min, for 1, 3, 5, 15, or 60 min.

The collected volatiles were released from the adsorbent by heating in a Thermodesorption Cold Trap Unit (Chrompack, Middelburg, The Netherlands) at 250°C for 3 min, with an N_2 flow of 20 ml/min. The desorbed compounds were collected in a cold trap (SIL5CB-coated, fused silica capillary) at -100°C. Flash heating of the cold trap provides sharp injection of the compounds into the capillary column of the gas chromatograph to which the cold trap is connected.

This desorption procedure is inapplicable when thermolabile compounds are present, but that was not the case here: no qualitative differences were observed between the chemicals identified with thermodesorption and those identified after solvent extraction of Tenax on which volatiles were collected from plants infested with *T. urticae*. Thermodesorption was used here because it provides a more sensitive analysis method: all desorbed volatiles are injected into the GC at the same time.

Isolation of Kairomone for Preparative Purposes. Two compounds that could not be identified by analytical GC and GC-MS were collected in larger amounts and purified by preparative GC for subsequent analysis by GC-IR and NMR.

Freshly picked infested lima bean leaves (500–600 g) were put in a stainless-steel cylinder (24.7 cm ID, 40.4 cm high) and flushed with 2.5 ml purified air per second overnight. The air was first pumped through glass tubes containing KOH pellets, mixed molecular sieves 5A and 13X, activated charcoal, and Tenax-TA before entering the cylinder at the center top. The kairomone-containing air left the cylinder at the bottom through a perforated stainless-steel ring near the wall of the cylinder. Via a Teflon tube, the air was led through a glass trap (4.8 mm ID) containing Tenax-TA (ca. 200 mg). The Tenax was cleaned before use by overnight extraction in a Soxhlet apparatus with MeOH, EtOAc, and hexane, respectively. The Tenax with the collected volatiles was extracted with 4 ml pentane–acetone (4 : 1) for 30 min in a micro-Soxhlet on a glass frit. The extract was poured in a conical 5-ml flask and an insulated 25-cm Vigreux column was put on top of the flask. The solution was concentrated to 0.3 ml in a water bath of ca. 42°C. The above procedure was repeated nine times. All the concentrated extracts were combined and concentrated to 0.6 ml. In ca. 20 injections of 25 μl each, the compounds were separated on a megabore

column. The two compounds of interest were trapped in two glass traps cooled to $-70°C$ (the FID was disconnected during trapping). When the traps were not connected to the GC, they were closed with teflon tubing.

Instruments Used. For analytical GC a Varian 3500 gas chromatograph equipped with a 30-m DB-17 (J&W) column was used; split ratio 1 : 100, carrier gas nitrogen; temperature program 60°C (0 min hold) to 210°C (0 min hold) with 2.5°C/min; FID. For preparative purposes a Carlo Erba gas chromatograph equipped with a 30-m DB-1 (J&W) column was used; 0.53 mm ID; 3.0 μm film thickness; no split; carrier gas nitrogen; temperature program 60°C (0 min hold) to 150°C at 5°C/min, then to 210°C at 10°C/min, 10 min hold.

All volatiles were analyzed by GC-MS using a Chrompack SIL19CB column (25 m × 0.25 mm ID). The column temperature was held at ambient for 2 min, then at 40°C for 4 min, and then programmed to 280°C at a rate of 4°C/min. Electron impact ionization was carried out at 70 eV on a VG MM7070F mass spectrometer (resolving power 1000).

The 300-MHz [^{1}H]NMR spectra were recorded in $CDCl_3$ on a Bruker CXP300. The C$\underline{H}Cl_3$ signal was used as an internal reference (7.26 ppm). Assignments were confirmed by homo-nuclear decoupling experiments.

For GC-IR, a Carlo-Erba gas chromatograph equipped with a 30-m DB-1 (J&W) column (film thickness 1 μm, split 1 : 5) connected with a Bruker IFS-85 FT-IR detector was used.

For HPLC, a Gilson 303 pump and a Gilson 116 UV detector were used in combination with a Rainin C18 column, 10 cm × 4.6 mm, 3 μm particle size. Solvent: MeOH–H_2O (9 : 1); flow rate 1 ml/min; detection UV 220 nm.

Synthesis. To synthesize (3E)-4,8-dimethyl-1,3,7-nonatriene, geraniol was oxidized with CrO_3 pyridine to geranial, which was converted to the desired product with the Wittig reagent methyltriphenylphosphonium iodide.

To synthesize (3E, 7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, (2E, 7E)-3,7,11-trimethyl-2,7,10-dodecatriene-1-ol (Aldrich) was oxidized with CrO_3 in pyridine to the corresponding aldehyde. This aldehyde was submitted to a Wittig reaction using CH_2I_2, Zn, and $TiCl_4$, yielding the desired product.

Solvents. All solvents used were of analytical or HPLC quality.

Separation of (Z)- and (E)-β-Ocimene. β-Ocimene (3,7-dimethyl-1,3,6-octatriene) could only be obtained as a mixture of the Z and E isomers. This mixture was separated by preparative GC on a 9.8% Carbowax on Chromosorb W-HP 100–120 column (200 × 0.2 cm), with a TCD detector and a column temperature of 80°C. The desired isomer was collected in a capillary tube which was cooled in Dry Ice. The ratio of the two isomers in the collected fractions was determined on the same column.

Bioassay. To determine whether extracts or synthetic chemicals had kairomonal activity, a Y-tube olfactometer was used. A glass Y-shaped tube held a Y-shaped iron wire in the center. At the end of both arms of the olfactometer,

filter paper (15 cm^2) with test or control solutions was placed in PVC cages. Air was sucked out at the base of the tube and led to the outside of the climate room in which the olfactometer was positioned. The air speed in both arms of the olfactometer was measured with a hot wire anemometer and standardized at 0.7 ± 0.1 m/sec by adding dry cotton wool in the PVC cages and/or changing the total airflow speed with a valve. Female predators were placed on the wire in the basal tube and observed individually. When the predator walked upwind and reached the far end of one of the arms, the experiment was terminated. A sign test was used to analyze the results ($\alpha = 0.05$). The experiments were performed at $25 \pm 1\,°C$ and $60 \pm 10\%$ relative humidity.

Only female predators were tested in the olfactometer. In the case of *P. persimilis*, they were well-fed, but unless stated otherwise, *A. potentillae* females were tested after a 20-hr period of starvation in a plastic tube at 25°C.

The chemicals were dissolved in dichloromethane [(*Z/E*)-β-ocimene, linalool (3,7-dimethyl-1,6-octadien-3-ol) and methyl salicylate], paraffin oil [1-octen-3-ol, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate, 4,8-dimethyl 1,3,7-nonatriene] or hexane [(*Z/E*)-β-ocimene]. Aliquots of 0.1 ml of diluted chemicals were poured onto a piece of filter paper outside the climate room and transferred to the olfactometer immediately. A filter paper with 0.1 ml solvent served as control. In these tests, new filter papers were prepared for each predator tested. All chemicals were obtained from Aldrich except for (*Z*)-3-hexen-1-yl acetate, which was a Roth product; β-ocimene, which was obtained as a mixture of isomers from International Flavor and Fragrances Industries or from Dr. J. H. Tumlinson (USDA, Gainesville, Florida) and (3*E*)-4,8-dimethyl-1,3,7-nonatriene isomers and (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, which were synthesized (see above). All chemicals, except (*Z/E*)-β-ocimene (see Results) and the methylene terpenes, were $\geq 98\%$ pure. The methylene terpenes were ca. 90% pure, as a result of rapidly occurring racemization.

RESULTS

Collection of Kairomone and Identification. The suitability of Tenax-TA to collect the volatile kairomone of *T. urticae* was demonstrated in the olfactometer with satiated females of *P. persimilis*: 31 of 49 predators chose the arm with solvent extract of Tenax on which volatiles of spider mite-infested plants had been collected ($P = 0.04$). By GC-MS analysis of the mixture of volatiles collected from spider mite-infested plants on Tenax-TA, many components could be distinguished (Table 1, Figure 1). Only three of these (2-butanone, 2-hexanal 1-octen-3-ol) have been found in samples collected from clean intact bean plants (Table 1). Some others, such as the general green leaf volatiles (*Z*)-3-hexen-1-

TABLE 1. VOLATILES COLLECTED FROM INTACT, ARTIFICIALLY DAMAGED, AND T. urticae-INFESTED LIMA BEAN PLANTS AND THEIR FUNCTION AS KAIROMONES FOR PHYTOSEIID MITES P. persimilis OR Amblyseius potentillae REARED ON V. faba POLLEN (Vf) OR T. urticae (Tu) OR AS DISPERSING PHEROMONE FOR SPIDER MITE T. urticae[a]

		Identified in			Component of			
					Kairomone of			Dispersing pheromone of T. urticae
						A. potentillae		
Peak number	Tentative identification	T. urticae infested plants	Artificially damaged plants	Undamaged plants	P. persimilis	(Vf)	(Tu)	
1	2-Butanone	+	+	+				
2	2-Methyl-propan-1-ol	+	−	−				
3	1-Butanol	+	−	−				
4	3-Pentanone	+	−	−				
5	1-Penten-3-ol	+	−	−				
6	Hexanal	−	+	+				
7	Unidentified (MW = 98)	+	+	+				
8	2-Hexenal	+	+	+				
9	(Z)-3-Hexen-1-ol	+	+	−	−	−	−	
10	(Z)-3-Hexen-1-yl acetate	+	+	−	−	−	−	
11	1-Octen-3-ol	+	+	+	−	−	−	
12	(E)-β-Ocimene	+	−	−	+	?[b]	?[b]	
13	(3E)-4,8-Dimethyl-1,3,7-nonatriene	+	−	−	+			
14	Linalool	+	−	−	+	+	−	
15	(Z)-3-Hexen-1-yl butyrate	+	+	−	+	+	−	+
16	Methyl salicylate	+	−	−	+	+	−	
17	(3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	+	−	−				−

[a] P. persimilis were tested as satiated females. A. potentillae as females that were starved for 24 hr at 25°C. Data on T. urticae are based on the data of Dabrowski and Rodriguez (1971), Rodriguez et al. (1976) and Dicke (1986).
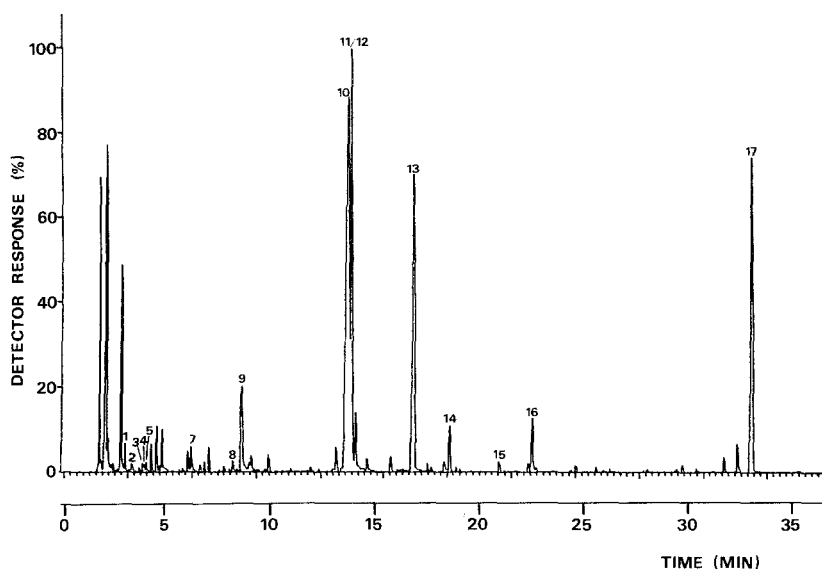[b] Uncertain because pure isomer was not tested.

FIG. 1. Gas chromatogram of volatiles from spider mite-infested bean plants collected on Tenax-TA. Peak numbers correspond to numbers given in Table 1. Most unlabeled peaks are artifactual peaks.

ol and (Z)-3-hexen-1-yl acetate are expected to be emitted by lima bean plants, but if so, the quantities were very small compared to the amounts of these compounds that were obtained from spider mite-infested bean plants. An analysis of the volatiles released by artificially damaged plants revealed several compounds that are also emitted by spider mite-infested plants (Table 1). Thus, emission of these compounds from spider mite-infested plants presumably is the result of mechanical damage of the plant. The other volatiles emitted from *T. urticae*-infested bean plants are caused by other spider-mite activities or are emitted from the spider mites themselves.

Two major components of the volatile kairomone could not be readily identified. Their mass spectra showed the molecular ion at m/z 150 and m/z 218, respectively, with similar fragmentation patterns (base peak for both at m/z 69). The difference between the two compounds was most likely one isoprene unit. Initially they were thought to be the furanoterpenes perillene and dendrolasin because of the almost identical mass spectra. However, a sample of a ginger oil, which was investigated at the same time, contained both perillene and the unknown compound. As it was not possible to deduce the correct structures from MS alone, we tried to isolate the two compounds in larger quantities and to record [^{1}H]NMR and IR spectra.

Initial attempts to separate and purify these two compounds by means of GC on a packed column failed. However, no separation could be obtained. The reason for this could be the low sensitivity of the TCD or the low inertness (leading to excessive tailing or decomposition of the compounds involved) of the column used. A separation by reverse-phase-HPLC was successful, but during isolation of the very small quantities of apolar pure compounds from the aqueous solvents, too many impurities were introduced for recording of high-quality NMR spectra. Also, evaporation of the solvent inevitably resulted in loss of the volatile compounds. Separation and purification of the two compounds was successful by preparative capillary GC on a megabore column, a relatively new technique. This technique combines good inertness with high separation power and a relatively high loadability. [^{1}H]NMR spectra showed that the resulting samples consisted of pure compounds. Altogether an estimated 300 μg (mol wt = 150) and 200 μg (mol wt = 218) were collected. The IR spectra, recorded immediately before NMR analysis, most closely resembled the IR spectra of monoterpene hydrocarbons. No evidence for the presence of any oxygen-containing functional group could be found. The most likely molecular formulas were thus $C_{11}H_{18}$ and $C_{16}H_{26}$ instead of $C_{10}H_{14}O$ (perillene) and $C_{15}H_{22}O$ (dendrolasin). From the NMR data, structure 1 (Figure 2) or its Z isomer followed for the lower molecular weight compound. Especially the signal at 6.58 ppm with coupling constants of 17, 10.5, and 10.5 Hz (corresponding with H-2) was most helpful in elucidating the structure. As it was not possible to determine whether the compound possessed the E or Z configuration, both were synthesized. By comparison of the spectral data and retention times of the isolated compound and the synthesized isomers, it was clear that the isolated compound had the E configuration. The higher-molecular-
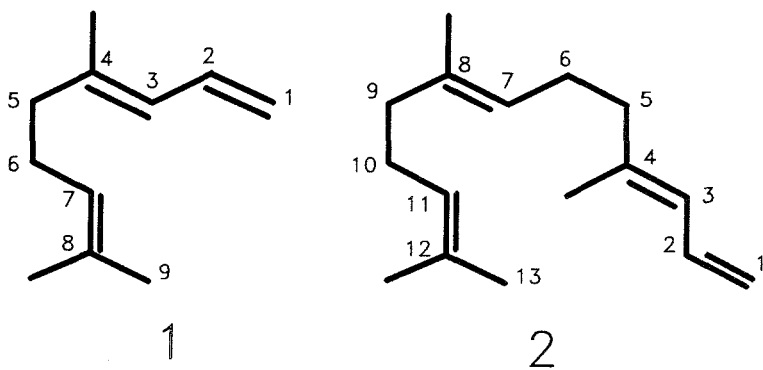

FIG. 2. Structures of the methylene terpenes (3E)-4,8-dimethyl-1,3,7-nonatriene (1) and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (2).

weight compound was now assigned structure **2** (Figure 2). The configuration around the 3,4 double bond is the same as in **1,** because of the identical chemical shifts and coupling constants. The configuration around the 7,8 double bond was deduced to be *E* from the chemical shift of the 7-methyl group at 1.61 ppm. In the case of the opposite configuration, the 7-methyl group would have appeared around 1.68 ppm. The structure of compound **2** was also confirmed by synthesis.

Bioassay of Identified Components. The effect on the predatory mite *P. persimilis* of those identified chemicals that were available was tested in the Y-tube olfactometer. Figure 3 shows that linalool is attractive to *P. persimilis* females at a wide dose range, whereas methyl salicylate proved to be attractive only when offered at an amount of 20 µg. The dose range was based on the finding that the amounts of each compound recovered from 20 plants during 60 min of sampling were in the range of one to several tens of micrograms. (Z)-3-Hexen-1-ol, (Z)-3-hexen-1-yl acetate, and 1-octen-3-ol did not attract the predators. These latter compounds had been identified in samples obtained from both artificially damaged and spider mite-infested plants.

The data for ocimene are presented in Figure 4. β-Ocimene was obtained as mixtures of the *E* and *Z* isomers at ratios of 90:10 and 70:30. Gas chromatography on 3% OV-17 showed that some minor compounds were also present. When tested in the olfactometer, only the 90:10 (E, Z)-β-ocimene mixture proved attractive to *P. persimilis*, either when 20, 2, or 0.2 µg were offered. When the dose offered was only 0.02 µg, no response was observed. The difference in response of *P. persimilis* to the two β-ocimene samples could be
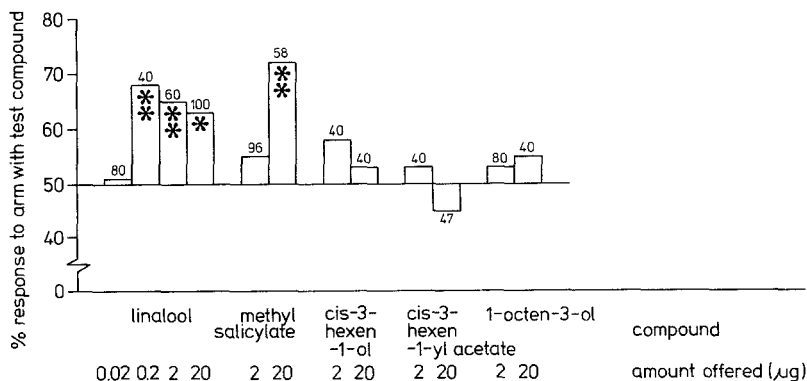

FIG. 3. Choice of *P. persimilis* females in Y-tube olfactometer to various doses of chemicals identified after collection of volatiles emitted from *T. urticae*-infested lima bean plants. Numbers under and above columns indicate the number of predators tested. **0.001 < P < 0.01; *0.01 < P < 0.05.
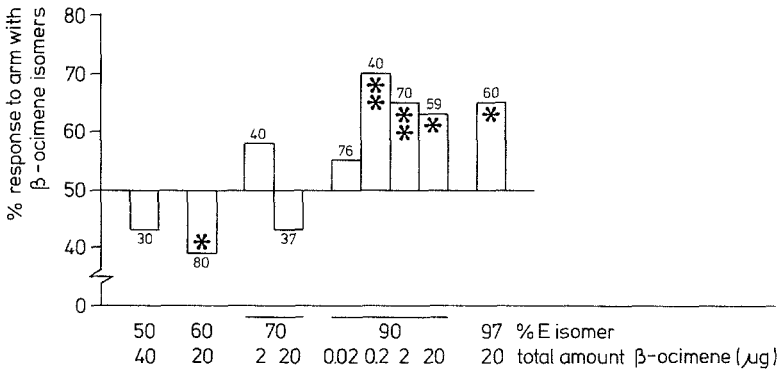
FIG. 4. Choice of *P. persimilis* females in Y-tube olfactometer to β-ocimene, offered at various *E/Z* ratios and various doses. Numbers under and above columns indicate the number of predators tested. For explanation of asterisks, see Figure 3.

caused by the impurities or by the difference in *E/Z* ratio. To investigate this, *E/Z* ratios of 50:50 and 60:40 were prepared from the 90:10 sample and a 97:3 *E/Z* ratio from the 70:30 sample. The 97:3 sample was attractive, but the 50:50 sample was not. The 60:40 *E/Z* ratio was even repellent. These data show that (*Z*)-β-ocimene reduced the attractivity of (*E*)-β-ocimene to *P. per-similis*.

Experiments with the methylene terpenes (*3E*)-4,8-dimethyl-1,3,7-nona-triene and (*3E*, *7E*)-1,4,8-trimethyl,1,3,7,11-tridecatetraene show that 20 μg of the former compound is also attractive to *P. persimilis*, whereas its *Z* isomer is not (Figure 5). No response of *P. persimilis* towards (*3E*, *7E*)-1,4,8-tri-methyl-1,3,7,11-tridecatetraene was recorded (Figure 5).

Amblyseius potentillae responded to the natural kairomone blend when reared on a carotenoid-deficient diet such as *V. faba* pollen, but not when reared on the carotenoid-containing spider mite *T. urticae* (Dicke et al., 1986). Pred-ators that differed in qualitative feeding history were offered the identified chemicals. The results (Figure 6) show that when amounts of 20 μg were offered, no chemical was attractive to *A. potentillae* (Tu). However, when the predator was reared on *V. faba* pollen, methyl salicylate and linalool were attractive, which resembles the data obtained for *P. persimilis*. However, in contrast to *P. persimilis*, *A. potentillae* (Vf) did not respond to 90:10 *E/Z* β-ocimene. Either (*E*)-β-ocimene is not attractive or the presence of 10% of the *Z* isomer or the other impurities disturbed the response of *A. potentillae* to the *E* isomer.

The response of *A. potentillae* to linalool was studied in more detail (Fig-ure 7). Three different concentrations were tested: 20, 2, and 0.2 μg. When reared on *V. faba* pollen, all three concentrations attracted predators that were
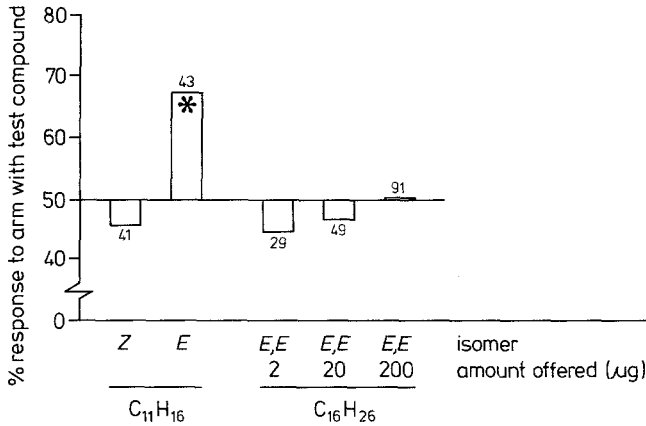
FIG. 5. Choice of *P. persimilis* females in Y-tube olfactometer to 4,8-dimethyl-1,3,7-nonatriene isomers and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Numbers under and above columns indicate the number of predators tested. For explanation of asterisks, see Figure 3.

starved for 20 hr. When satiated, these predators also responded to 2 μg linalool. A response to the volatile *T. urticae* kairomone in well-fed *A. potentillae* has only been observed when the predators were reared on *V. faba* pollen (Dicke, unpublished data). In contrast to the data for *A. potentillae* (Vf), amounts of
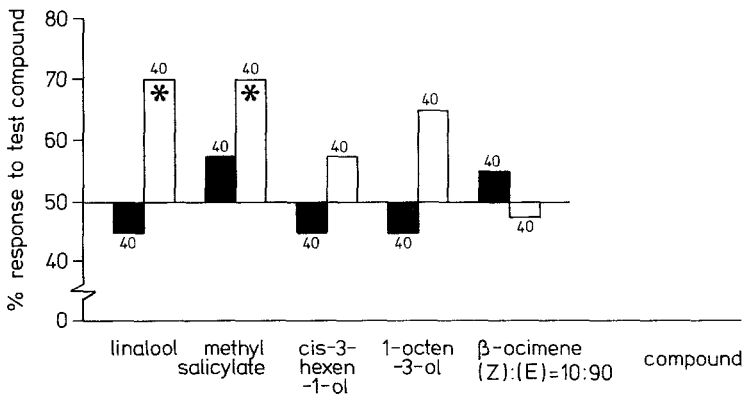

FIG. 6. Choice of starved female *A. potentillae* (Tu) (black bars) and *A. potentillae* (Vf) (white bars) in Y-tube olfactometer to synthetic chemicals (dose: 20 μg), identified after collection of volatiles from *T. urticae*-infested lima bean plants. For explanation of asterisks, see Figure 3. Numbers under and above columns indicate the number of predators tested.
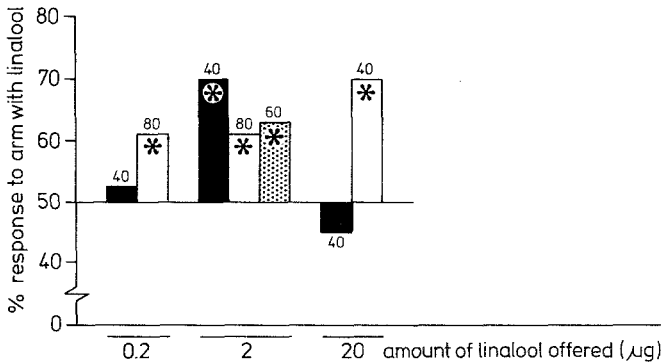
FIG. 7. Choice of starved female *A. potentillae* (Tu) (black bars) and starved and satiated *A. potentillae* (Vf) (white bars: starved; shaded bar: satiated) in Y-tube olfactometer to linalool at various doses. For explanation of asterisks, see Figure 3. Numbers under and above columns indicate the number of predators tested.

20 and 0.2 μg linalool were not attractive to *A. potentillae* (Tu) that were starved for 20 hr, but when 2 μg linalool was offered, a significant response was observed in these predators. The data for the responses of both predator species are summarized in Table 1.

DISCUSSION

Damage inflicted on lima bean plants by rubbing the leaves with carborundum or by infestation with spider mites caused emission of many volatiles. However, a clear difference was observed between the volatile mixture emitted from plants damaged in either way. Most of the major volatiles emitted from clean lima bean plants, plants damaged mechanically, or plants infested with *T. urticae* were identified. The amounts of volatiles emitted from spider mite-infested plants were large enough to allow short sampling times in our investigation. However, minor compounds may have been missed.

Shortly after our identification of the two methylene terpenes (Figure 2), Maurer et al. (1986) reported the isolation of these compounds from *Elettaria cardamomum* (cardamom) oil, which, like ginger, is a species from the Zingiberaceae family. The spectral data reported by Maurer et al. (1986) are identical to the data recorded by us. In 1987, independent of our work, the same compounds were reported by Kaiser (1987) from flowers of many night-scented plant species of different families that are pollinated by moths.

The odor differences observed between plants that are in different condition, will allow many organisms to distinguish between them. This will be discussed according to the three questions posed in the Introduction.

1. Do different predator species use similar chemicals as volatile *T. urticae* kairomone? Four compounds elicited a significantly attractive response from at least one of the predator populations tested: these compounds are the terpenes linalool and (*E*)-β-ocimene, the methylene terpene (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and methyl salicylate.

All four compounds attracted *P. persimilis* females although methyl salicylate only elicited this response when offered at a dose of 20 μg. The effect of linalool and methyl salicylate on *A. potentillae* (Vf) is similar to that on *P. persimilis*. With respect to (*E*)-β-ocimene, no definitive conclusion can yet be made, because it is not known whether the presence of 10% *Z* isomer affected the behavior of *A. potentillae* (Vf) towards (*E*)-β-ocimene.

With regard to the kairomone components linalool and methyl salicylate, the data obtained for *A. potentillae* reared on either of the two diets are in agreement with those of Dicke et al. (1986) and Dicke (in preparation) on the response of *A. potentillae* to the volatiles emitted by *T. urticae*-infested plants. The only exception is the response of *A. potentillae* (Tu) to 2 μg linalool. This may indicate that *A. potentillae* (Tu) are sensitive to this component of the volatile *T. urticae* kairomone, although much less than *A. potentillae* (Vf), which respond much more consistently to linalool.

Our observations provide no reason to suppose that different predator species use different volatiles emitted from *T. urticae*-infested plants as kairomone. Investigations with other phytoseiid species, such as *T. occidentalis* or *T. pyri* are needed.

2. Do kairomone components function as dispersing pheromone for spider mites? The spider mite *T. urticae* also distinguishes between undamaged and *T. urticae*-damaged lima bean plants. Clean leaves emit a volatile kairomone that attracts spider-mite females. After infestation with *T. urticae*, the leaves emit a dispersing pheromone in addition. At a high ratio of dispersing pheromone to plant kairomone, the spider mites are repelled (Dicke, 1986).

It is interesting to investigate whether the chemicals that act as a kairomone to predatory mites act as a spider-mite dispersing pheromone. Some indications for this can be found in a study on host plant–spider mite interaction. Dabrowski and Rodriguez (1971) studied the response of *T. urticae* females to essential oil components of strawberry foliage. In a static air olfactometer, linalool repelled the mites at several concentrations. Methyl salicylate was indifferent, whereas (*E*)-β-ocimene or (3*E*)-4,8-dimethyl-1,3,7-nonatriene were not included in their study. In a later study by Rodriguez et al. (1976), mixtures of compounds were used. A mixture resembling strawberry essential oil attracted *T. urticae* females. An increase in the proportion of nonanal, one of the components, reduced the attractive effect. However, an increase in the proportion of methyl salicylate had no effect. Unfortunately, the effect of a change in the proportion of linalool was not studied.

In the context of the present study and the one by Dicke (1986), the data of Dabrowski and Rodriguez (1971) indicate that linalool is not only a component of the *T. urticae* kairomone that attracts predatory mites, but also of the *T. urticae* dispersing pheromone. This supports the hypothesis that the dispersing pheromone and the spider-mite kairomone have components in common (Dicke, 1986). There is no evidence that the kairomone component methyl salicylate is a component of the dispersing pheromone (Rodriguez et al., 1976). These data on the identity of components of the *T. urticae* dispersing pheromone are incorporated into Table 1. It will be interesting to investigate the effect of (E)-β-ocimene and $(3E)$-4,8-dimethyl-1,3,7-nonatriene on *T. urticae* females to know whether these kairomone components are also a component of the dispersing pheromone.

3. What is the origin of the volatile kairomone? The above considerations raise the question of the origin of the allelochemicals that function in plant–spider mite–predatory mite interactions. The chemicals presumably are of plant origin, as can be inferred from the classes of compounds identified (e.g., Karrer, 1976; Maurer et al., 1986). However, it cannot be concluded yet that the plant has active control over the production or emission of the volatiles or that the plant offers a substrate for spider-mite enzymes that subsequently produce the kairomone components. Investigation of, e.g., site and moment of production and possible storage of precursors are needed for that. Chemical identification of kairomone components was an essential first step for this.

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