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PLANT-DERIVED SYNERGISTS OF ALARM PHEROMONE FROM TURNIP APHID, *Lipaphis (Hyadaphis) erysimi* (HOMOPTERA, APHIDIDAE)

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Abstract—The turnip aphid, *Lipaphis (Hyadaphis) erysimi*, responds weakly to (*E*)-β-farnesene, the main component of the alarm pheromone, but the response is substantially increased by incorporating plant-derived isothiocyanates, identified in aphid volatiles by coupled gas chromatography–single-cell recording.

Key Words—Aphid, pheromone, alarm pheromone, plant components, electrophysiology, single-cell recording, isothiocyanate, *Lipaphis erysimi*, *Hyadaphis erysimi*, Homoptera, Aphididae, (*E*)-β-farnesene.

INTRODUCTION

The turnip aphid, *Lipaphis (Hyadaphis) erysimi*, has been reported to respond well to its own alarm pheromone but not to the pheromones from a series of other aphids, even though these responded well to pheromones from each other (Nault and Bowers, 1974). Pheromone from these aphids, including *L. erysimi*, contained the sesquiterpene (*E*)-β-farnesene (I, Figure 1). This compound elicited a high response for each aphid except *L. erysimi*. It was proposed that the alarm pheromone from *L. erysimi* contained other components, and the presence of another sesquiterpene was suggested (Nault and Montgomery, 1979; Nault and Phelan, 1984). The objective of the present study was to identify the compounds necessary for full alarm response by *L. erysimi*.

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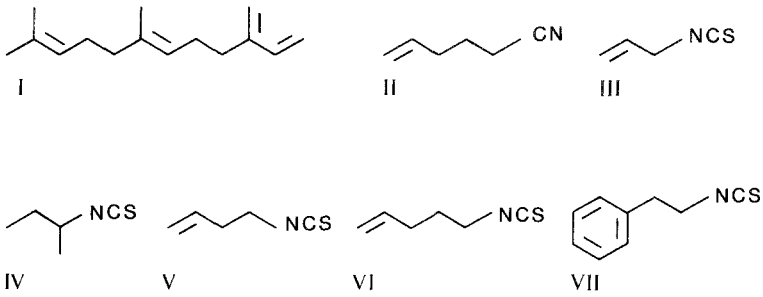

FIG. 1. Structures of (*E*)-β-farnesene (EBF) (I), 4-pentenyl cyanide (II), allyl isothiocyanate (III), 2-butyl isothiocyanate (IV), 3-butenyl isothiocyanate (V), 4-pentenyl isothiocyanate (VI), and 2-phenylethyl isothiocyanate (VII).

METHODS AND MATERIALS

Compounds. Farnesene, prepared by the method of Dawson et al. (1982) containing 40% of the active isomer, (*E*)-β-farnesene (EBF), was employed at a concentration of 4 μg/ml EBF in ether, or 1 μg/ml in water, and stored in glass ampoules under N_2. 4-Pentenyl cyanide (II), allyl isothiocyanate (III), 2-butyl isothiocyanate (IV), 3-butenyl isothiocyanate (V), 4-pentenyl isothiocyanate (VI), and 2-phenylethyl isothiocyanate (VII) were obtained commercially or synthesized by conventional methods. When tested in behavioral bioassays, the concentrations of these compounds were 0.2 μg/ml in ether or 1 mg/ml in water.

Insects. Lipaphis (Hyadaphis) erysimi (Kaltenbach), obtained on shepherd's purse [*Capsella bursa-pastoris* (L.) Medic.] in Hertfordshire in 1981, were maintained on turnip (*Brassica campestris* var. *rapifera* Metz.) unless otherwise stated, at 16 hr daylength and 20 ± 5°C.

Isolation of Volatiles. L. erysimi were extracted with ether and the extract dried and concentrated down from a fivefold dilution to give a solution equivalent to 1 g aphids/ml ethereal extract. The extract was then vacuum distilled as described previously (Pickett and Griffiths, 1980).

Aerial parts of turnip, Chinese cabbage [*Brassica campestris* var. *chinensis* (L.) Makino], and shepherd's purse, grown under glass, were cooled under N_2, broken up finely, covered, and allowed to stand for 85 min so that conversion of glucosinolates to volatile metabolites could take place. The volatiles were then isolated by condensation as described previously (Pickett and Stephenson, 1980) and the condensate extracted into ether to give a concentration equivalent to 20 g of plant material/ml ethereal extract. Solutions were sealed in glass ampoules under N_2 and stored at −20°C.

Bioassays. Colonies of third- to fourth-instar *L. erysimi* were established on leaves of Chinese cabbage by confining adults in clip cages on the plants for

three days. The adults were then removed and the young allowed to develop for a further two to three days before testing. Single aphids were reared in a similar manner, excess nymphs being removed at the same time as the adults. Numbers of aphids responding by moving from their feeding sites were recorded 60 sec after application of the test material (colonies of 50–60 insects, five replicates; single aphids, ten replicates).

Test materials were either air (20 ml) from above *L. erysimi* crushed in a glass syringe (20 aphids containing a total amount of ca. 1 ng EBF by GC), blown slowly (ca. 10 sec) from the syringe, or ethereal or aqueous solutions (0.2 μl) applied as a single droplet to the leaf on which the aphids were settled. Thus, EBF was applied at 0.8 ng in ether or 0.2 ng in water and the isothiocyanates at 0.04 ng in ether or 0.2 μg in water. Ethereal *L. erysimi* extract and volatiles (1 μl) were also applied as single droplets to leaves at ca 0.3 ng of EBF.

Gas Chromatography (GC). GC employed fused SiO_2 columns, 24 m $\times$ 0.3 mm, with a bonded OV-101 stationary phase at 30°C (2 min), 20°/min to 100°C, 6°/min to 200°C. Confirmation of identity was by coinjection with authentic compounds.

Gas Chromatography–Mass Spectrometry (GC-MS). Ionization was by electron impact at 70 eV, 200°C, with the GC capillary column directly coupled to the source of an MM 70-70F mass spectrometer and the integrated data system 2025 (VG Analytical, Altringcham, U.K.). Spectral enhancement by means of the data system was used to produce mass spectra for regions of the chromatogram associated with electrophysiological activity but where peaks in the total ion current were not clearly discernible. Tentative identification was by comparison with published spectra (Kjaer et al., 1963).

Electrophysiology. Recordings from the cells associated with the olfactory receptors on the primary rhinaria of *L. erysimi* alates were made using tungsten microelectrodes (Boeckh, 1962). The indifferent electrode was placed in the first antennal segment, and the recording electrode was then brought into contact with the multiporous plate of the rhinaria until impulses were recorded. Permanent copies of the action potentials generated by the receptor cells were obtained by standard methods (Wadhams et al., 1982).

Most recordings showed the presence of a number of cells since there are up to 14 olfactory cells in the rhinarium (Bromley et al., 1979). Only those recordings in which the responding cells could be readily distinguished were used in these experiments.

Stimulation. The stimulus (2 sec duration) was delivered into a purified airstream (800 ml/min) which flowed continuously over the preparation. The delivery system, employing a filter paper strip in a disposable Pasteur pipet cartridge, has been described previously (Wadhams et al., 1982). Compounds III–VI in pentane (10 μl) were applied to the filter paper and, after evaporation of solvent, were presented twice to each preparation at intervals of 2–15 min;

the exact interval was dependent upon the concentration of the previous stimulus. Impulse frequency was determined as the number of impulses elicited during the first 1 sec after stimulus initiation.

Gas Chromatography–Single-Cell Recording (GC-SCR). The coupled GC-SCR system has been described previously (Wadhams, 1982). After each experiment, records of the flame ionization detector response and of the action potential frequency were obtained by detecting the impulses with a level discriminator and plotting them by means of a voltage/frequency converter.

RESULTS AND DISCUSSION

Alarm pheromone released by crushing turnip aphids, *L. erysimi*, caused most aphids to move away from feeding sites (Table 1). The presence of EBF was confirmed by GC and GC-MS studies, but no other major terpenoid component was detected. However, synthetic EBF elicited only a weak response at a level comparable to the amount produced by the aphid. Ethereal extract of *L. erysimi* gave rise to a good response, and the activity was found to be largely in the volatile fraction obtained by vacuum distillation.

The alarm pheromone is thought to be perceived principally by the rhinaria on the fifth (proximal) and sixth (distal) antennal segments (Nault et al., 1973; Wohlers and Tjallingii, 1983), and Bromley and Anderson (1982) implicated the former in host-plant volatile reception. Although olfactory cells which responded strongly to EBF were found on the distal rhinarium, it was not possible to elicit a similar response from the receptors associated with the proximal rhinarium.

Cells on the proximal rhinarium responded strongly to *L. erysimi* volatiles. Coupled GC-SCR of the *L. erysimi* distillate revealed the presence of a number of active components which, with the exception of VIII, were perceived by one cell type A in this rhinarium (Figure 2). These compounds were identified by

TABLE 1. ALARM BIOASSAY WITH COLONIES OF TURNIP APHIDS, *Lipaphis (Hyadaphis) erysimi*

Treatment[a]	Aphids moving (% ± SE)
1. Crushed *L. erysimi*	99 ± 0.9
2. *L. erysimi* extract	90 ± 3.2
3. *L. erysimi* volatiles	74 ± 5.5
4. *L. erysimi* residue	23 ± 10.5
5. EBF	20 ± 8.4

[a]Treatments 4 and 5 differ from 1–3 at $P < 0.01$, based on paired t tests.

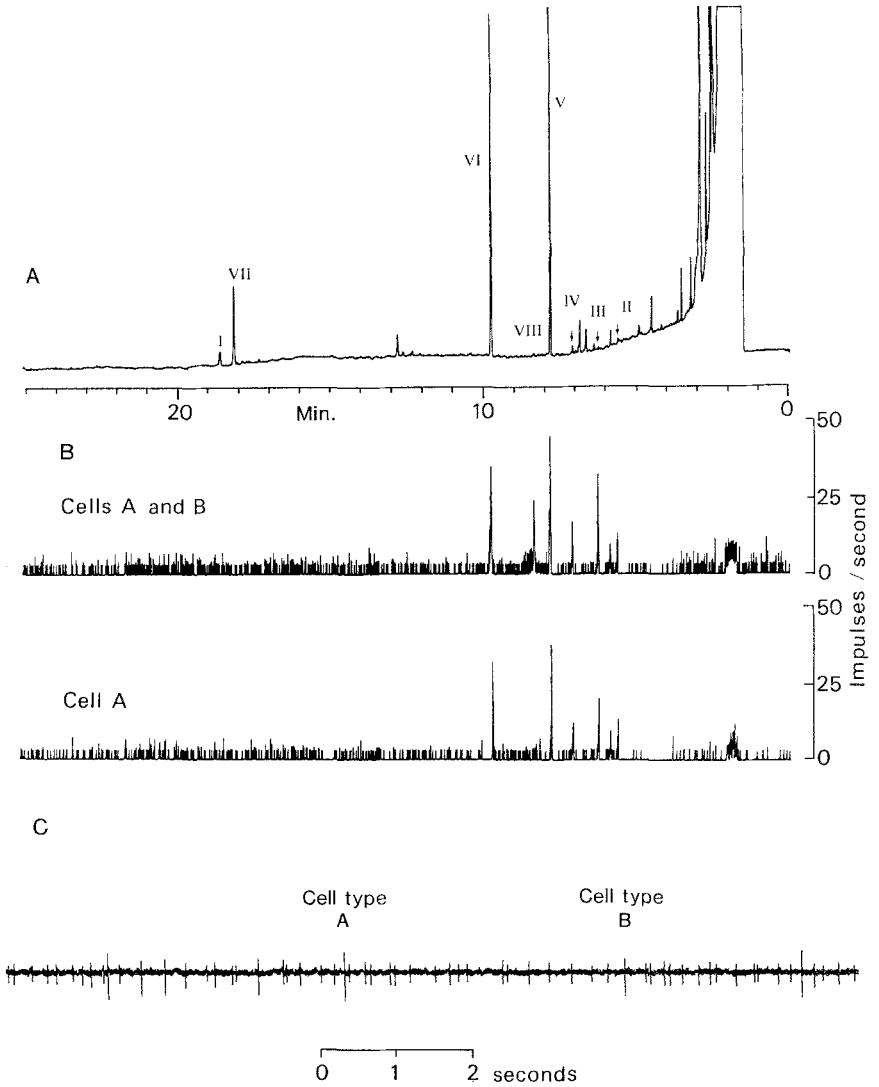

FIG. 2. GC-SCR: (A) Gas chromatogram of the vacuum distillate of *L. erysimi* extract. (B) Corresponding impulse frequency responses of two olfactory cells to stimulation with the *L. erysimi* extract. (C) Typical spontaneous activity of the cells recorded from the primary rhinarium on the fifth antennal segment.

GC and GC-MS as 4-pentenyl cyanide (II), allyl isothiocyanate (III), 2-butyl isothiocyanate (IV), 3-butenyl isothiocyanate (V), and 4-pentenyl isothiocyanate (VI) (Figure 1). 2-Phenylethyl isothiocyanate (VII), a major component of the extract, was inactive, and peak VIII, which was detected by cell type B, was not identified. Isothiocyanates are well-known components of cruciferous plants, including the turnip; they arise from enzymic decomposition of glucosinolate precursors (Ju et al., 1982) and were therefore considered likely to have arisen from the host plant.

To investigate further the activity of the newly identified compounds, the bioassay was modified to employ single aphids, thus avoiding release of isothiocyanates through leaf damage caused by large numbers of feeding aphids. Results (Table 2) again showed that the pheromone and the volatiles isolated from *L. erysimi* were more active than synthetic EBF alone. It was also demonstrated that the pheromone from *L. erysimi* bred on Chinese cabbage was equally active. 3-Butenyl isothiocyanate alone elicited only a weak response, but when it was applied together with EBF, all the test aphids responded. Allyl isothiocyanate and 2-butyl isothiocyanate gave a similar increase in the alarm response when applied in admixture with EBF. Dose–response data for these compounds were established using the electrophysiological bioassay and showed that, at the receptor level, allyl and 3-butenyl isothiocyanates were significantly more active than the 2-butyl and 4-pentenyl isothiocyanates (Figure 3). The

TABLE 2. ALARM BIOASSAY WITH SINGLE TURNIP APHIDS, *Lipaphis (Hyadaphis) erysimi*

Treatment	Aphids moving (%)[a]
Crushed *L. erysimi* from turnip	100a
Crushed *L. erysimi* from Chinese cabbage	100a
L. erysimi volatiles	100a
EBF	20b
3-Butenyl isothiocyanate	20b
EBF + 3-butenyl isothiocyanate	90a
Allyl isothiocyanate	0b
EBF + allyl isothiocyanate	100a
EBF + 2-butyl isothiocyanate	90a
Turnip volatiles	20b
EBF + turnip volatiles	100a
EBF + shepherd's purse volatiles	100a
EBF + Chinese cabbage volatiles	90a
EBF (aqueous)	0b
EBF + allyl isothiocyanate (aqueous)	70a

[a]Difference a from b, $P < 0.05$, based on chi-square test.

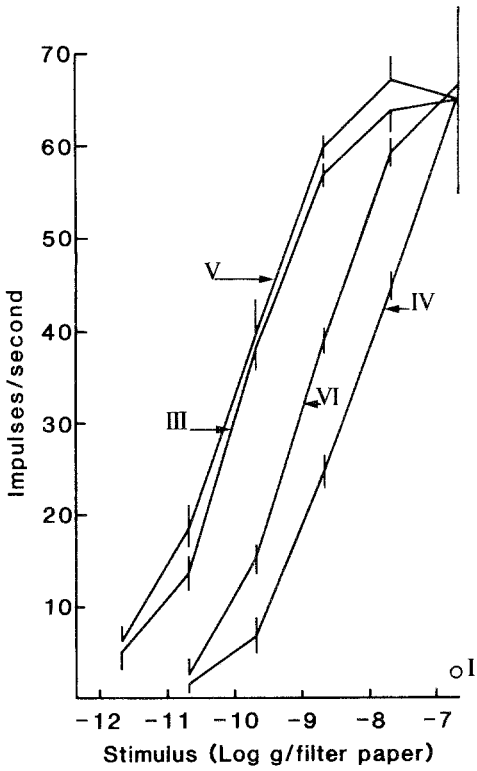

FIG. 3. Dose–response curves of *L. erysimi* olfactory cells to (*E*)-β-farnesene (I), allyl isothiocyanate (III), 2-butyl isothiocyanate (IV), 3-butenyl isothiocyanate (V), and 4-pentenyl isothiocyanate (VI). The cells were recorded from the primary rhinarium on the fifth antennal segment. Each point is the mean response (five preparations) ± SE. Where standard errors overlap only half the SE bar is shown.

olfactory receptor exhibits considerable selectivity towards these compounds: no response was observed to either EBF or the common leaf volatiles (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, and linalol when tested at high stimulus concentrations (2×10^{-7} g per filter paper).

Although the volatiles obtained from turnip, Chinese cabbage, and the weed host of *L. erysimi* in the U.K., shepherd's purse, increased activity of EBF (Table 2), only the turnip volatiles contained relatively large amounts of 3-butenyl and 4-pentenyl isothiocyanates. However, the relative proportions of these compounds obtained from *L. erysimi* bred on Chinese cabbage were similar to those from *L. erysimi* bred on turnip. This suggested that *L. erysimi* plays an active role in the production of these compounds. Indeed, when sinigrin, the glucosinolate precursor of allyl isothiocyanate, was added to an homogenate of

L. erysimi, allyl isothiocyanate was released at 37% of the theoretical amount after 5 hr. It is interesting to note that while the glucosinolate, sinigrin, serves as a feeding deterrent for non-Cruciferae feeding aphids, it serves as a powerful phagostimulant for *L. erysimi* (Nault and Styer, 1972). Thus, the glucosinolates or their isothiocyanate products play a dual key role in the biology of this species.

When sinigrin was added to an homogenate of *Myzus persicae*, a closely related aphid also in the Aphididae, release of allyl isothiocyanate was negligible. Also, the isothiocyanates III, IV, and V did not significantly increase the response of this aphid to the alarm pheromone.

EBF can be used to improve the efficiency of contact pesticides and biological control agents against aphids (Pickett et al., 1986), but the response of aphids to aqueous formulations of EBF is very weak. Indeed, only special formulations containing hydrocarbon propellents, applied using electrostatic spraying systems, have so far given good results (Pickett et al., 1984). However, the readily available allyl isothiocyanate, when applied together with EBF in water, causes a good response from *L. erysimi* (Table 2). Further studies on such alarm pheromone synergists may lead to the use of aqueous formulations of EBF against other aphids in the field.

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