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OLFACTORY RESPONSES TO APHID AND HOST PLANT VOLATILE RELEASES: (*E*)-β-FARNESENE AN EFFECTIVE KAIROMONE FOR THE PREDATOR *Adalia bipunctata*

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Abstract—The volatiles released from several aphid and host plant species, alone or associated, were studied for their infochemical role in prey location. Using a four-arm olfactometer, the attraction of several combinations of three aphid (*Myzus persicae*, *Acyrthosiphon pisum*, and *Brevicoryne brassicae*) and three plant (*Vicia faba*, *Brassica napus*, and *Sinapis alba*) species toward *Adalia bipunctata* larvae and adults was observed. Both predatory larvae and adults were attracted only by *A. pisum* and *M. persicae* when they were crushed, whatever the host plant. (*E*)-β-Farnesene, the aphid alarm pheromone, was the effective kairomone for the ladybird. Plant leaves alone (*V. faba*, *B. napus*, and *S. alba*) or in association with nonstressed whole aphids (the three species) did not have any attraction for the predator. The *B. brassicae* specialist aphid is the only prey that was not attracted to *A. bipunctata* larvae and adults, even if they were crushed. Release of *B. brassicae* molecules similar to the host plant allelochemicals was demonstrated by GC–MS analysis. The lack of behavioral response of the ladybird at short distance toward the cruciferous specialist aphid was related only to the absence of (*E*)-β-farnesene in the aphid prey volatile pattern.

Key Words—Infochemical, predator, prey localization, olfactometer, β-farnesene, kairomone.

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INTRODUCTION

Predators and parasitoids, with their complex biology, elaborate interactions with other organisms, and importance in pest control, are fascinating subjects for ecological studies. Beneficial insects are sensitive to chemical aspects of the multitrophic environment, particularly with regard to host location (Poppy, 1997). To localize prey in natural habitats, entomophagous insects use numerous chemical cues emitted by prey and host plants, alone or in association (Vet and Dicke, 1992). Many different chemical cues correspond to a diversity of associations between potential prey and host plant species. However, little is known about chemical communication among predatory insects. Recently, reports of electroantennogram (EAG) recordings from three predatory insect species, namely *Coleomegilla maculata* (Coleoptera, Coccinellidae), *Chrysoperla carnea* (Nevroptera, Chrysopidae; Zhu et al., 1999), and *Coccinella septempunctata* (Coleoptera, Coccinellidae; Al Abassi et al., 2000) showed significant EAG responses to semiochemicals released from potential prey and host plants. These predators possibly use such chemicals to locate their prey. Ninkovic et al. (2001) also demonstrated that the seven-spotted ladybird, *C. septempunctata*, responded positively to volatiles from the aphid, *Rhopalosiphum padi*, and to infested plants of *Hordeum vulgare*. Two molecules, namely (*E*)-β-farnesene and β-caryophyllene, were found to be a kairomone and an informative inhibitor, respectively, for the seven-spot ladybird by electroantennography and olfactometry methods (Al Abassi et al., 2000).

To study the relation between volatile emissions from aphids and host plant complexes, the foraging behavior of the predator and localization of the aphid prey by the predatory ladybird was studied using a four-way olfactometer. This work was designed to observe the predator response toward chemical cues emitted by the host plant, the aphid (nonstressed whole insect or crushed), alone or in combination. Different odor sources, corresponding to potential situations met by the ladybird in its natural habitat were tested. In parallel, GC–MS analyses of the tested odor source samples were performed to identify the volatile compounds affecting the predator behavior. The relationship between prey suitability, volatile release, and behavioral response of the predator are discussed in relation to the potential use of infochemicals in the biological control of aphids.

METHODS AND MATERIALS

Plant and Insect Rearing. Broad beans (*Vicia faba* L.), white mustard (*Sinapis alba* L.), and oilseed rape (*Brassica napus* L.) were grown in 10 cm diam. plastic pots in three separate controlled environmental rooms at 20 $\pm$ 2°C and under a 16/8 hr L/D photoperiod. While beans were cultivated in pots containing a 1:1 mixture of perlite:vermiculite, Brassicaceae species were sown in 20 $\times$ 30 cm plastic trays containing ordinary compost, and were transplanted into plastic pots with the same compost when the plants had two true leaves.

Acyrthosiphon pisum. (Harris) and *Myzus persicae* Sultzer were reared on the three host plant species, while *Brevicoryne brassicae* L. was reared only on crucifer species. Plants were inoculated at the 5–6 true leaf stage with one of the aphid species. Each combination of aphid and plant was isolated in separated conditioned rooms at 20 ± 2°C and under a 16/8 hr photoperiod. Mass rearing of *Adalia bipunctata* L. was maintained for many years in the laboratory. Both control adults and larvae were reared in aerated plastic boxes and fed with *A. pisum* on *V. faba*. From hatching, larvae that were used in olfactometry assays were individually reared in 5 cm diam. Petri dishes. Ladybirds (72-hr-old) were used to test the chemical cues at the larval stage. Other beetle larvae achieved the adult stage in Petri dishes. One-week-old adults were used to observe the infochemical role of the various tested odor sources. Ladybirds were fed with an excess of *A. pisum* reared on *V. faba* in individual Petri dishes.

Olfactometer. The four-way olfactometer that was used to test the behavioral responses of second instars and adult ladybirds toward several stimuli was similar to the one described by Vet et al. (1983). Compressed air was circulated through active charcoal and a water bottle before entering the exposure chamber. Air left the latter through a hole in the chamber roof. Airflow in each of the four arms was adjusted with a flow meter to 60 ml min^{-1}, thereby creating four equal distinct fields in the chamber. Odor emitting samples were placed into a 25-ml airtight glass flask linked by plastic tube to one of the four olfactomer arms. The olfactometer system was placed into a controlled temperature room at 20 ± 2°C. Before the beginning of the assays, the system was cleaned with pure ethanol and rinsed with distilled water.

Odor Sources as Chemical Cues. Several stimuli were tested as odor sources:

1. Undamaged, nonstressed whole aphids (*A. pisum*, *M. persicae*, or *B. brassicae*): Aphid samples (250 mg) previously collected from one of the host plants were carefully placed into 25-ml glass flasks.
2. Crushed aphids: one of the three species (250 mg) having an odor source similar to a stressed aphid colony. Aphid samples were rapidly crushed with glass rods in 25-ml glass flasks and immediately covered with glass tops to be airtight.
3. Host plant sections (*V. faba*, *B. napus*, or *S. alba*): Stems (5 cm) with three leaves were cut from healthy uninfested plants and placed into glass flasks.
4. Aphid and host plant sections: combinations of the three plant and three aphid species having volatile production that resulted in aphid feeding on plants. Five-centimeter stems infested with aphids (250 mg) were placed into glass flasks.

Aphid and/or host plant samples in glass flasks were placed into the air stream of one of the four arms of the olfactometer. Complementary assays using pure (*E*)-β-farnesene, a well-known aphid alarm pheromone as odor source, were performed. The latter molecule was purified from *A. pisum* lipids using microcolumn

chromatography (40 × 5 mm, 70–230 mesh silica gel E60 column) with 2-hexane as eluent. The observation method for the use of (*E*)-β-farnesene was similar to the one described to test aphid or plant samples as chemical cues. (*E*)-β-Farnesene was injected into the glass flask using a Hamilton syringe. We waited 3 min before joining the odor source to the exposure chamber of the olfactometer to allow for evaporation of hexane prior to starting observations.

Behavioral Observations. Second instars (72-hr-old) and adults ($N = 20$ per stimulus) were individually observed for 20 min in the olfactometer. The olfactometer was divided into one central area and four others related to the four odor sources. Ladybird durations in each area were determined. Insect localization in one of the four areas at the end of the observation was considered the final behavioral choice of the predatory ladybird. After every five observations, the position of the odor fields was changed. The exposure chamber was cleaned with pure ethanol and rinsed with distilled water after each assay.

Analysis of Volatile Releases from Aphids and Plants. Aphid and plant samples (250 mg) were crushed with a glass pestle in a glass tube adapted to the SPME method. Each aphid species was tested at least in duplicate. Crushed samples were first maintained for 30 min at 30.0 ± 0.2°C in glass tubes adapted to SPME. The volatile metabolites were sampled for 30 min with 100-μm PDMS (polydimethylsiloxane) SPME fibers from Supelco® and immediately analyzed by GC–MS on an Hewlett-Packard HP5972 mass spectrometer coupled with an HP5890 series II gas chromatograph. The following analytical conditions were used: split–splitless injection at 250°C, HP5-MS (5% phenyl-dimethylpolysiloxane) column (30 m × 0.25 mm, df = 1 μm). Samples were purged with He at 4 ml min^{-1} for 11 min, and the temperature program was from 40°C (1 min hold) to 180°C at 6°C min^{-1} then to 280°C at 15°C min^{-1}. MS spectra were obtained in the EI mode at 70 eV (scanned mass range from 30 to 300 amu). The analytes were identified on the basis of their retention times and by interpretation of MS fragmentation patterns. Spectra were compared to those of the Wiley238.L spectral library. (E)-β-Farnesene in hexane was analyzed by the same procedure.

Statistical Analysis. Observed frequencies related to the final choice of *A. bipunctata* in localizing prey were compared to corresponding theorical frequencies (one odor source and three controls) using a χ^2 test. Relative stay durations of ladybirds were compared by the contrast method using the residual mean square from ANOVA after arcsin$\sqrt{x}$ transformation (Dagnelie, 1973).

RESULTS

GC–MS chromatograms of volatiles from aphids and host plants are presented in Figure 1. Volatile compounds were not detected from nonstressed whole aphids of the three tested species. Only crushed *A. pisum* and *M. persicae* released (*E*)-β-farnesene whatever the host plant species. Molecules similar to host

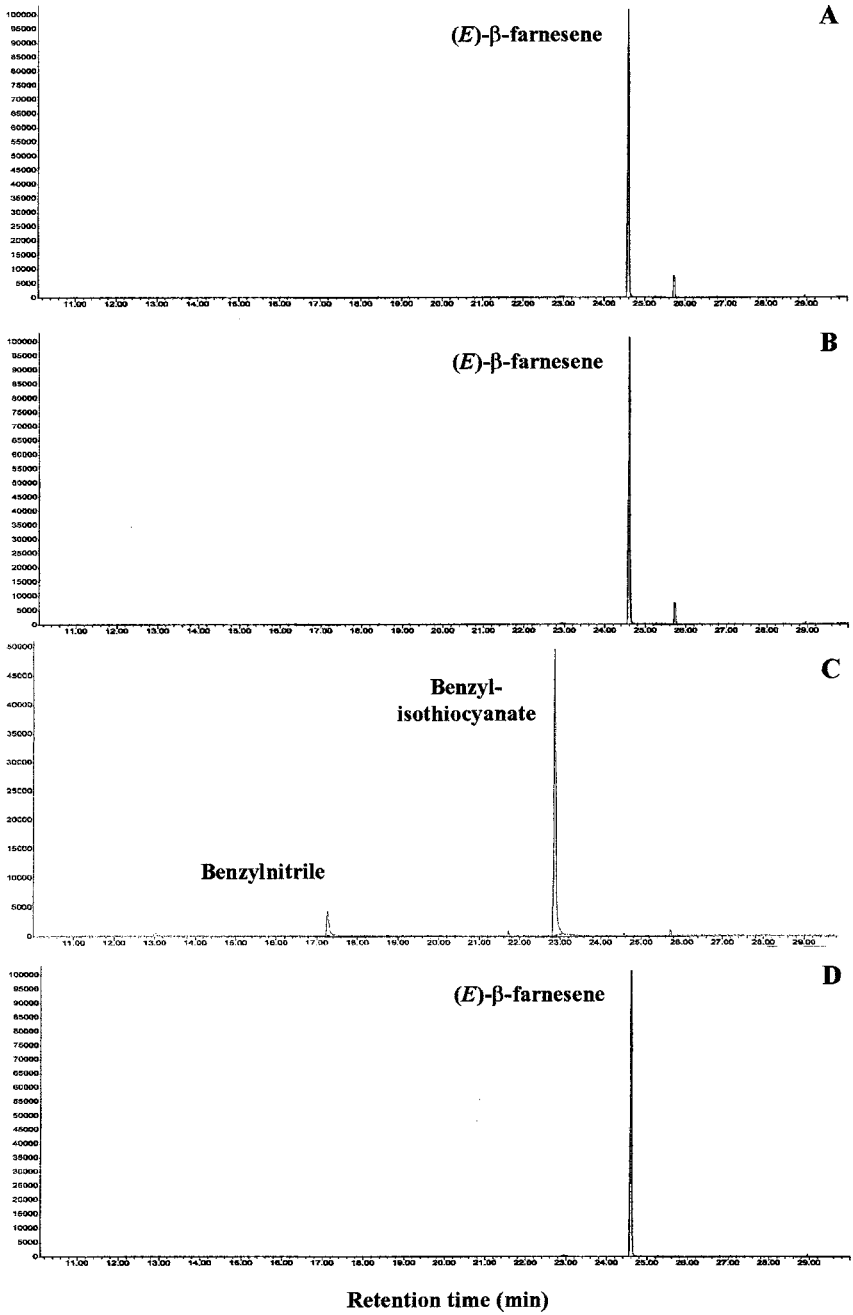

FIG. 1. GC–MS chromatograms of volatile releases from *Myzus persicae* (A) and *Acyrthosiphon pisum* (B) both reared on *Vicia faba*, *Brevicoryne brassicae* reared on *Sinapis alba* (C). The chromatogram of the (*E*)-β-farnesene purified solution in hexane is also presented (D).

plant allelochemicals, namely benzyl-isothiocyanate and the benzylnitrile, were found when crushed *B. brassicae* reared on *S. alba* was analyzed. Volatiles from *B. brassicae* reared on *B. napus* were not detected. This was linked to the low concentration of glucosinolate precursors in the aphid host plant. Both Brassicaceae plant species emitted degradation products of glucosinolates (nitriles and isothiocyanates); however, volatile were not detected from broad bean samples.

Prey localization by the ladybird at both adult and larval stages using crushed or nonstressed whole aphids reared on three different host plants are presented in Table 1. Two aphids, *A. pisum* and *M. persicae*, attracted the predatory ladybirds at

TABLE 1. RESPONSES OF *Adalia bipunctata* ADULTS (A) AND SECOND INSTARS (B) TOWARD CRUSHED AND NONSTRESSED WHOLE APHIDS REARED ON DIFFERENT HOST PLANT SPECIES[a]

Host plant and aphid combinations			Observed frequencies	χ^2	P
(A)					
Vicia faba	*A. pisum*	Whole insects	0.4	2.40	0.121
		Crushed	0.45	4.27	0.039*
	M. persicae	Whole insects	0.35	1.07	0.301
		Crushed	0.50	6.67	0.010**
Brassica napus	*M. persicae*	Whole insects	0.40	2.40	0.121
		Crushed	0.45	4.27	0.039*
	B. brassicae	Whole insects	0.30	0.27	0.603
		Crushed	0.25	0.00	1.000
Sinapis alba	*M. persicae*	Whole insects	0.35	1.07	0.301
		Crushed	0.45	4.27	0.039*
	B. brassicae	Whole insects	0.20	0.27	0.603
		Crushed	0.30	0.27	0.603
(B)					
Vicia faba	*A. pisum*	Whole insects	0.40	2.40	0.121
		Crushed	0.50	6.67	0.010**
	M. persicae	Whole insects	0.30	0.27	0.603
		Crushed	0.45	4.27	0.039*
Brassica napus	*M. persicae*	Whole insects	0.30	0.27	0.603
		Crushed	0.50	6.67	0.010**
	B. brassicae	Whole insects	0.15	1.07	0.301
		Crushed	0.25	0.00	1.000
Sinapis alba	*M. persicae*	Whole insects	0.30	0.27	0.603
		Crushed	0.45	4.27	0.038*
	B. brassicae	Whole insects	0.25	0.00	1.000
		Crushed	0.20	0.27	0.603

[a] Observed frequencies related to the final choice of *A. bipunctata* ($N = 20$) to localize the odor sources were compared to the corresponding theorical frequency (one odor source and three controls) using a χ^2 test.

* and ** indicate significant differneces at $P < 0.05$ and $P < 0.01$, respectively.

larval and adult stages when the aphids were crushed regardless of their host plants. No significant effect was observed when the nonstressed whole aphids reared on one of the tested host plants was used as an odor source.

Plant species, alone or in association with aphids, were also tested as chemical cues. When leaves of *V. faba*, *B. napus*, or *S. alba* alone were used as odor sources, no significant attraction was observed on the final choice of the ladybird larvae ($\chi^2 = 0.27$ and $P = 0.603$, $\chi^2 = 0.27$ and $P = 0.603$, $\chi^2 = 1.07$ and $P = 0.301$) and adults ($\chi^2 = 0.27$ and $P = 0.603$, $\chi^2 = 1.07$ and $P = 0.301$, $\chi^2 = 2.40$ and $P = 0.121$).

Associations of plant leaves and nonstressed whole aphids were used without having any significant informative effect as semiochemicals on prey localization by the ladybird. *A. pisum* or *M. persicae* on *V. faba* ($\chi^2 = 0.27$ and $P = 0.603$, $\chi^2 = 0.00$ and $P = 1.000$), *M. persicae* or *B. brassicae* on *B. napus* ($\chi^2 = 1.07$ and $P = 0.301$ twice) and on *S. alba* ($\chi^2 = 1.07$ and $P = 0.301$, $\chi^2 = 0.27$ and $P = 0.603$) did not attract predatory beetle larvae. Adults of *A. bipunctata* did not respond to molecules released from nonstressed whole aphid and host plant combinations: *A. pisum* or *M. persicae* on *V. faba* ($\chi^2 = 1.07$ and $P = 0.301$, $\chi^2 = 0.27$ and $P = 0.603$), *M. persicae* or *B. brassicae* on *B. napus* ($\chi^2 = 1.07$ and $P = 0.301$, $\chi^2 = 0.27$ and $P = 0.603$) and on *S. alba* ($\chi^2 = 2.40$ and $P = 0.121$, $\chi^2 = 1.06$ and $P = 0.301$).

Observation of times spent by the ladybirds in each of the four olfactometer arms, corresponding to the four air arrivals including the tested odor source, confirmed the attractive effect of crushed *A. pisum* and *M. persicae* reared on *V. faba* (Figure 2), on *B. napus* (Figure 3), and on *S. alba* (Figure 4) for adults of *A. bipunctata*. Similar results were observed when testing the former aphid and host plant combinations on the two spot ladybird larvae (Table 2). Nonstressed whole aphids from *A. pisum* and *M. persicae* were not attractive for predator larvae or adults. The third prey species, *B. brassicae*, never attracted the predatory ladybirds even if the insect odor samples were crushed (Figure 4). Associations of nonstressed whole aphids and plant leaves, or the latter alone, were also used as chemical cues. When *V. faba*, *B. napus*, or *S. alba* leaves were tested as odor sources, attraction of ladybird larvae was not observed on the times spent in the olfactometer field related to the odor source samples ($F = 0.23$ and $P = 0.632$, $F = 0.26$ and $P = 0.578$, $F = 0.29$ and $P = 0.531$).

Adults of ladybirds were not attracted by the volatiles released from the three plant species when used alone ($F = 0.28$ and $P = 0.404$, $F = 1.87$ and $P = 0.825$, $F = 1.40$ and $P = 0.321$ for *V. faba*, *B. napus*, and *S. alba*, respectively). Association of leaves and nonstressed whole aphids were also tested, but did not present any significant effect on the duration ladybirds stayed in the olfactometer area corresponding to the odor source. *A. pisum* or *M. persicae* on *V. faba* ($F = 1.08$ and $P = 0.302$, $F = 1.43$ and $P = 0.289$), *M. persicae* or *B. brassicae* on *B. napus* ($F = 1.22$ and $P = 0.728$, $F = 1.45$ and $P = 0.768$), and on *S. alba*

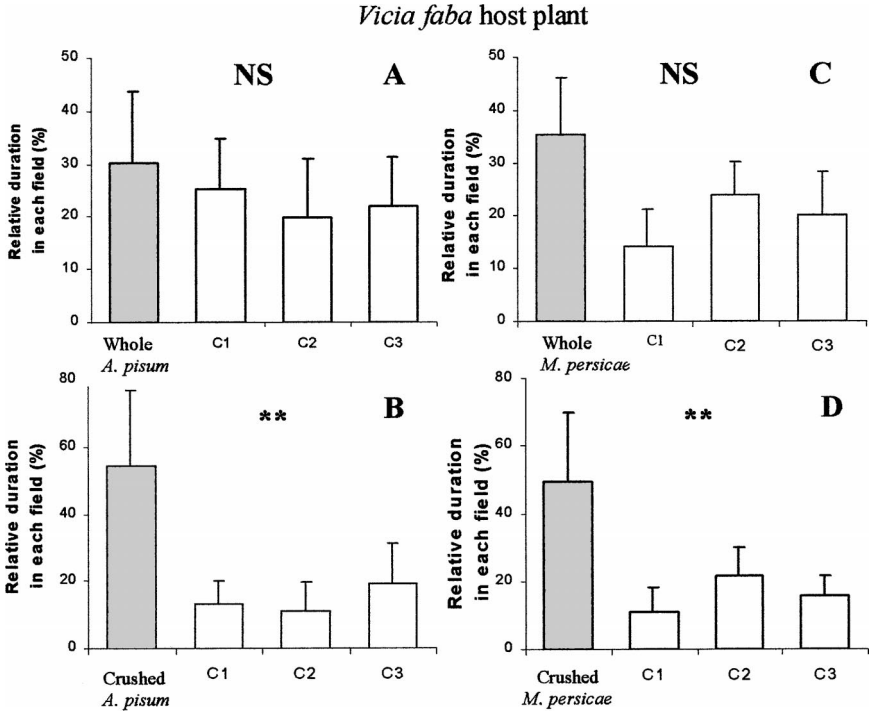

FIG. 2. Mean relative time (%) spent in each field of a four-arm olfactometer by *Adalia bipunctata* adults exposed to nonstressed whole or crushed *Acyrthosiphon pisum* (A and B, respectively) or *Myzus persicae* (C and D, respectively) both reared on *Vicia faba*. C1, C2, and C3 are the control air sources related to three of the four olfactometer arms. Error bars represent standard deviation of the mean. NS and ** indicate no significance and significant differences at $P < 0.01$, respectively.

($F = 0.25$ and $P = 0.382$, $F = 0.30$ and $P = 0.412$) did not attract predatory beetle larvae.

Molecules released by the plant and nonstressed whole aphid combinations did not have any significant effect on the time spent by *A. bipunctata* adults in the olfactometer exposure chamber. *A. pisum* or *M. persicae* on *V. faba* ($F = 0.97$ and $P = 0.408$, $F = 1.27$ and $P = 0.309$), *M. persicae* or *B. brassicae* on *B. napus* ($F = 1.53$ and $P = 0.215$, $F = 1.04$ and $P = 0.311$), and on *S. alba* ($F = 1.04$ and $P = 0.311$, $F = 0.21$ and $P = 0.644$) did not induce any significant difference of time spent by the predator adults in the field related to the tested odor source.

A hexane solution of natural (E)-β-farnesene purified from *A. pisum* only includes this molecule (Figure 1). Before beginning the olfactometry assays with the aphid alarm pheromone, a hexane control was used. The foraging behavior of the ladybird was not affected by hexane as a chemical cue ($0.27 < \chi^2 < 1.07$ and

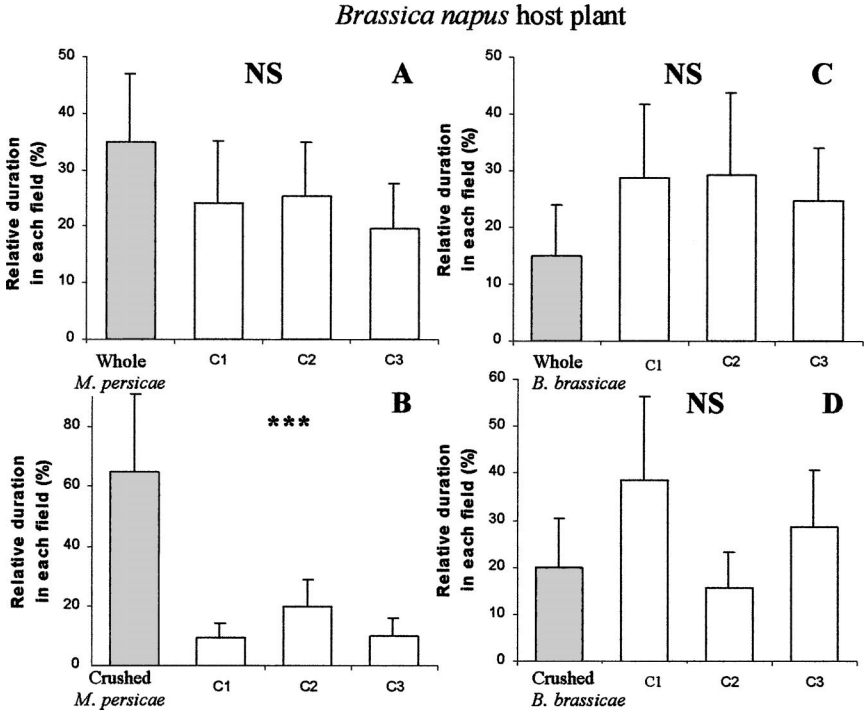

FIG. 3. Mean relative time (%) spent in each field of a four-arm olfactometer by *Adalia bipunctata* adults exposed to nonstressed whole or crushed *Myzus persicae* (A and B, respectively) or *Brevicoryne brassicae* (C and D, respectively) both reared on *Brassica napus*. C1, C2, and C3 are the control air sources related to three of the four olfactometer arms. Error bars represent standard deviation of the mean. NS and *** indicate no significance and significant differences at $P < 0.001$, respectively.

$0.603 < P < 0.301$). In contrast, both predatory larvae and adults were attracted by (*E*)-β-farnesene if the solution included more than 2 μg of the informative molecule ($\chi^2 = 6.67$ and $P = 0.010$, $\chi^2 = 8.47$ and $P < 0.006$, respectively). The stay durations of the ladybirds in the olfactometer area related to the aphid alarm pheromone solution was significantly higher than the durations corresponding to the other areas ($F = 11.23$ and $P < 0.001$, $F = 9.89$ and $P = 0.002$ for the larvae and adults, respectively).

DISCUSSION

Comprehension of the chemical ecology of plant–insect relations is a key factor in determining the way entomophagous beneficial insects localize host plants

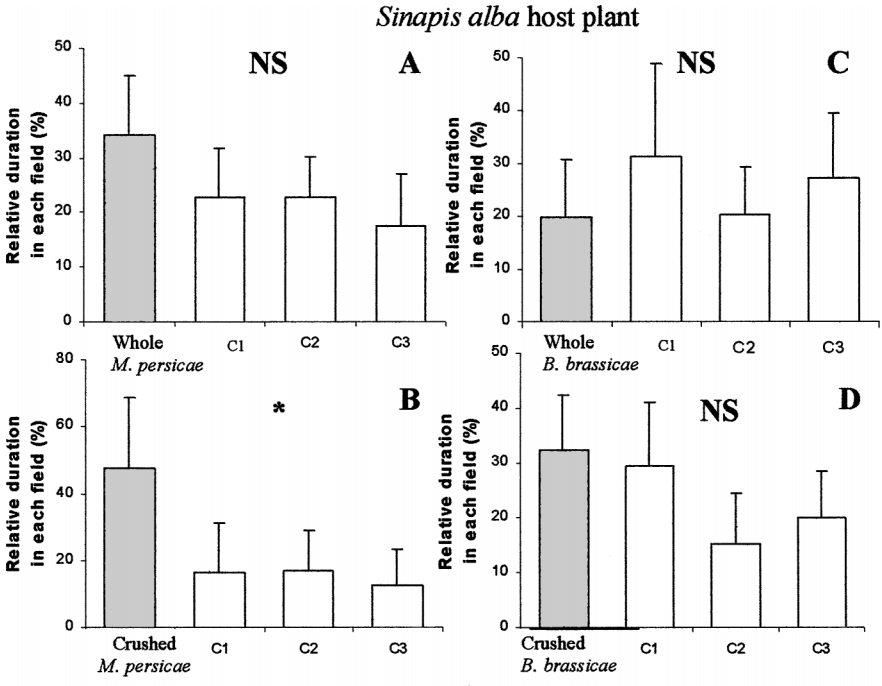

FIG. 4. Mean relative time (%) spent in each field of a four-arm olfactometer by *Adalia bipunctata* adults exposed to nonstressed whole or crushed *Myzus persicae* (A and B, respectively) or *Brevicoryne brassicae* (C and D, respectively) both reared on *Sinapis alba*. C1, C2, and C3 are the control air sources related to three of the four olfactometer arms. Error bars represent standard deviation of the mean. NS and * indicate no significance and significant differences at $P < 0.05$, respectively.

or prey. While the semiochemicals emitted by plants can explain the orientation and distribution of aphids, these substances also play an infochemical role for the aphidophagous natural enemies. In the first steps of prey searching, predators localize prey habitat by using chemical cues emitted by plants (Tumlinson et al., 1992). In our experiments, plant volatiles did not attract either adults or larvae of *A. bipunctata* at short distances. Other examples also illustrate that isolated plants are not universal sources of infochemicals for entomophagous beneficials. Indeed, *Diaeretella rapae*, an aphid parasitoid is not attracted by noninfested Brassicaceae leaves (Reed et al., 1995). Whether *C. septempunctata* responds positively to volatiles from aphids, *R. padi*, from *H. vulgare* infested plants, ladybirds do not react to volatiles from uninfested plants in olfactometer bioassays (Ninkovic et al., 2001). Neveu et al. (2002) also demonstrated that the parasitoid, *Trybliographa rapae* (Hymenoptera: Figitidae) is not attracted to volatiles emanating

TABLE 2. RELATIVE DURATION (%) SPENT BY *Adalia bipunctata* SECOND INSTARS IN THE OLFACTOMETER ARM CORRESPONDING TO THE TESTED ODOR SOURCE (CRUSHED OR NONSTRESSED) WHOLE APHIDS REARED ON DIFFERENT HOST PLANT SPEICES[a]

Host plant and aphid combinations			Relative duration in odor field (%)	*F*	*P*
Vicia faba	*A. pisum*	Whole insects	30.4 ± 21.5	0.37	0.544
		Crushed	45.4 ± 17.3	4.9	0.030*
	M. persicae	Whole insects	27.9 ± 20.8	0.15	0.704
		Crushed	43.2 ± 16.1	4.75	0.032*
Brassica napus	*M. persicae*	Whole insects	14.1 ± 6.3	1.22	0.272
		Crushed	45.8 ± 20.7	4.15	0.045*
	B. brassicae	Whole insects	13.2 ± 7.7	1.45	0.232
		Crushed	17.9 ± 9.2	0.37	0.544
Sinapis alba	*M. persicae*	Whole insects	29.7 ± 10.6	0.25	0.617
		Crushed	46.1 ± 19.1	4.27	0.039*
	B. brassicae	Whole insects	19.1 ± 7.9	0.30	0.588
		Crushed	28.6 ± 12.2	0.38	0.538

[a] Relative stay durations of ladybrids ($N = 20$) were compared by the contrast method using the residual mean square from ANOVA after $\arcsin\sqrt{x}$ transformation.
* indicates significants differences at $P < 0.05$.

from uninfested turnips either as whole plants, roots, or leaves. The lack of response of both entomophagous predators and parasitoids was attributed to the low reliability of this signal to inform the beneficial species of host preference (Vet and Dicke, 1992).

None of the plant–aphid complexes that were tested was attractive to *A. bipunctata*. These results are in accordance with previous work on beneficials when a too low concentration of volatile liberation by the aphid and host plant samples was used. The foraging behavior of the aphid parasitoid *Aphidius ervi* was influenced by semiochemicals emitted by aphid infested plants when a certain threshold of infestation, in terms of number of aphids and hours of feeding activity, was reached (Guerrieri et al., 1999). A volatile dose-dependent response of another predatory beetle, *C. septempunctata*, toward prey–host plant complex was also observed. Significant differences toward odor source and control in a Y olfactometer were only observed when the seven spot ladybird was exposed to at least 30 aphid damaged shoots with 1200 tea aphids. Below this amount of volatile emitting biological sample, no significant attraction of the predator was observed (Han and Chen, 2002).

Aphid samples alone were tested as potential kairomone cues for *A. bipunctata*. Our results allowed us to demonstrate that there was no systematic response of a polyphagous aphid predator toward volatiles released from several potential

prey. When crushed *M. persicae* and *A. pisum* reared on several host plant species were used, predatory ladybird responded positively to the emitted chemical cues. When whole aphids were used alone, no informative effect was observed. Similar results have been obtained by Du et al. (1996). The parasitoid *A. ervi* was not attracted by whole *A. pisum* aphids. An hypothesis was proposed to explain these observations: crushed aphids release higher levels of volatile substances. The amount of emitted molecules is then sufficient to be perceived by the predators. The chemical cues released by whole aphids were not sufficient to allow prey localization by ladybirds. Volatile molecules were not detected by GC–MS analysis of whole aphid samples in our experiments. Larger amounts of whole aphids had to be used as odor sources to be localized by predators in our olfactometry assays. Using another prey species, Han and Chen (2002) showed that at least 2000 tea aphids were needed to emit enough odor to attract *C. septempunctata*. Moreover, these authors found that *C. septempunctata* was the most sensitive species of three tested natural enemies.

The other aphid species, *B. brassicae*, was not attractive to ladybirds. *B. brassicae* (whole insects or crushed), alone or in association with host plant leaves, did not attract larvae or adults of *A. bipunctata*. In this case, two hypotheses might explain the lack of infochemical effect. Substances emitted by plants and/or the crucifer specialist aphids modified the kairomonal composition to the predatory ladybirds when compared to chemical cues from *M. persicae* and *A. pisum*. Al Abassi et al. (2000) demonstrated that the attractivity of (*E*)-β-farnesene for *C. septempunctata* decreased with increasing amounts of β-caryophyllene. The isothiocyanate emission that was detected by GC–MS from *B. brassicae* could act as a kairomone inhibitor such as the β-caryophyllene that informs the predator of prey unsuitability. For example, volatile isothiocyanates released from cruciferous plants stimulate the olfactory receptors of generalist herbivore insects such as *Aphis fabae*. The pentenyl- and butyl-ITC were repellent to this aphid species (Isaacs et al., 1993). These molecules could also repel generalist predators such as *A. bipunctata*. To confirm the presence of plant secondary substances in *B. brassicae*, analysis of molecules released by the tested aphid and host plant species, each alone or in association, were partially performed in previous work (Francis et al., 2001a) and completed here. The alternative hypothesis is that volatile molecules released by whole or crushed *B. brassicae* do not present any informative effect. As (*E*)-β-farnesene was not the major molecule emitted by the aphid (as from *A. pisum* and *M. persicae*), *B. brassicae* did not produce efficient kairomone for natural enemies, but developed a system of defense similar to its Brassicaceae host plants (Francis et al., 2000a,b, 2001b).

GC–MS analyses allowed us to show the attractive effect of (*E*)-β-farnesene over short distances when emitted alone by *A. pisum* and *M. persicae*, regardless of the host plant they fed upon, when in sufficient concentration. Other volatiles such as some isothiocyanates and nitriles from *B. brassicae* provide different chemical

cues to ladybirds. The beetles do not respond to a mixture of volatiles similar to the ones from Brassica host plants. Although the crucifer specialist *Lipaphis erysimi* responded to a mixture of (*E*)-β-farnesene and isothiocyanate, (*E*)-β-farnesene alone did not act as alarm pheromone (Dawson et al., 1987).

Ladybirds were attracted by (*E*)-β-farnesene as an olfactory cue with a dose-dependent factor. When the amount of (*E*)-β-farnesene was less than 2 μg, a significant attraction was not observed for the coccinellid. Absence of an informational role of whole *M. persicae* and *A. pisum* seemed to be due to the lower liberation of (*E*)-β-farnesene from undamaged aphids. Moreover, the kairomone role of (*E*)-β-farnesene on beneficial insects was not systematic. While the predatory species *A. bipunctata* was attracted by that latter terpene molecule, *Chrysopa cognata*, another aphid predator did not react to it as an olfactory cue (Boo et al., 1998). In contrast, flight assays in tunnels allowed observation of the (*E*)-β-farnesene attractive effect on the parasitoid *A. ervi* (Du et al., 1998). Moreover, prey searching activity of polyphagous predators from the Carabidae family was increased by the monoterpene release from *Sitobion avenae* aphid (Kirkland et al., 1998). Two carabid beetle species also are highly sensitive to (*E*)-β-farnesene: *Pterostichus melanarius* and *Harpalus rufipes* (Kielty et al., 1996).

In summary, this work shows that *A. bipunctata* only reacts to semiochemical cues from prey. Host plant and aphid–host plant complexes did not represent effective infochemical sources at short distances under our experimental conditions. Identification of potential (*E*)-β-farnesene synergists or inhibitors for the two spot ladybird is in progress by studying different ratios of plant volatiles (nitriles, isothiocyanates, and several terpenes) as odor sources. The results of complementary studies using plant–insect volatile releases will allow us to determine the effective infochemicals on predator flights. (*E*)-β-Farnesene, a well-known aphid alarm pheromone, alone was an effective kairomone for the two spot ladybird. The differential response of a polyphagous aphid predator to several potential prey demonstrates that biological control cannot be generalized. Each pest and cultivated plant species must be considered as a unique situation. Nevertheless, (*E*)-β-farnesene might be a promising molecule for use as a biopesticide attractant for several aphidophagous predators including *A. bipunctata*.

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