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RAPID COMMUNICATION

Emission of Alarm Pheromone in Aphids: a Non-Contagious Phenomenon

F. J. Verheggen · M. C. Mescher · E. Haubruge · C. M. De Moraes · E. G. Schwartzberg

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Abstract In response to attack by natural enemies, most aphid species release an alarm pheromone that causes nearby conspecifics to cease feeding and disperse. The primary component of the alarm pheromone of most species studied is (*E*)-β-farnesene. We recently demonstrated that the production and accumulation of (*E*)-β-farnesene during development by juvenile aphids is stimulated by exposure to odor cues, most likely by (*E*)-β-farnesene emitted by other colony members. Here, we tested whether the release of (*E*)-β-farnesene can be triggered by exposure to the alarm pheromone of other individuals, thereby amplifying the signal. Such contagious emission might be adaptive under some conditions because the amount of (*E*)-β-farnesene released by a single aphid may not be sufficient to alert an appropriate number of individuals of the colony to the presence of a potential threat. By using a push–pull headspace collection system, we quantified (*E*)-β-farnesene released from *Acyrthosiphon pisum* aphids exposed to conspecific alarm signals. Typical avoidance behavior was observed following exposure to (*E*)-β-farnesene (i.e., aphids ceased feeding and dropped from host-plant); however, no increase in alarm pheromone amount was detected, suggesting that contagious release of (*E*)-β-farnesene does not occur.

Keywords Aphid alarm pheromone production · *Acyrthosiphon pisum* · (*E*)-β-farnesene · Headspace collection system

This paper and the following paper by Hatano et al. on the same topic were received and processed essentially simultaneously.

F. J. Verheggen · E. Haubruge
Department of Functional and Evolutionary Entomology,
Gembloux Agricultural University,
Passage des Déportés 2,
5030 Gembloux, Belgium

M. C. Mescher · C. M. De Moraes · E. G. Schwartzberg
Department of Entomology, The Pennsylvania State University,
University Park, PA 16801, USA

F. J. Verheggen (✉)
Unité d'Entomologie fonctionnelle et évolutive,
Faculté universitaire des Sciences agronomiques,
Passage des Déportés, 2,
5030 Gembloux, Belgium
e-mail: entomologie@fsagx.ac.be

Introduction

As a result of parthenogenetic reproduction, aphids typically have a clonal colony structure and are surrounded by other genetically identical individuals. This social environment favors communal defense mechanisms. In most species, individuals respond to attack by natural enemies by releasing an alarm pheromone that induces perceiving individuals to stop feeding, disperse locally, and often drop from the host plant (Braendle and Weisser 2001). Whereas alarm pheromones in other insects and mites usually consist of a mixture of chemicals (e.g., Verheggen et al. 2007a), the alarm pheromone of most Aphidinae appears to contain a single chemical, the sesquiterpene (*E*)-β-farnesene (EβF) (Francis et al. 2005). EβF has been identified as the only volatile compound in the alarm pheromone of 13 aphid species, including the pea aphid, *Acyrthosiphon pisum* Harris (Francis et al. 2005). EβF also acts as a kairomone, used by predators and parasitoids to locate their aphid prey (Pickett and Glinwood 2007, Verheggen et al. 2007b, 2008). These findings highlight the possibility of negative effects of alarm pheromone production in the form of increased appearance to natural enemies. Beale et al. (2006) exploited this by adding an EβF synthase gene to *Arabidopsis thaliana* plants, making them attract aphid parasitoids.

In a recent study, we found that juvenile aphids reared in social isolation on artificial diet release less EβF than those reared in a colony or in isolation but exposed to colony odors (Verheggen et al., unpublished). We suggested that aphid volatiles may stimulate other aphids to produce EβF. In this study, we tested this by stimulating release of EβF from aphids and measuring whether additional EβF is released by individuals that perceive these volatiles. Such a contagious phenomenon could be adaptive if there are benefits to disseminating the alarm farther than would be achieved by the release of EβF by an individual.

Methods and Materials

Insects and Plants Pea aphids were reared on broad beans, *Vicia faba*, in an environmentally controlled greenhouse (L16:D8, RH 35±5%, 25±2°C) for several months prior to the experiments. Plants were grown in 9×9 cm plastic pots, filled with a peat-based, general-purpose potting soil (Metro Mix 200 Series, SunGrow Agriculture Distribution Inc., Bellevue, WA, USA).

Push–pull Headspace Collection System The push–pull headspace collection system consisted of two cylindrical chambers (12 cm diam.×30 cm) made of glass and Teflon®. Chambers were connected to one another with Teflon® tubing. To maintain ambient humidity and normal atmospheric pressure within the chambers, activated-carbon-filtered air was pumped into the system at the same rate that air was removed via air-entrainment filters, in a manner consistent with push–pull headspace collection setups described elsewhere (e.g., Tholl et al. 2006).

To generate natural EβF emissions, we crushed 50 third instar aphids inside the collection chambers with a glass pestle, which along with the aphids was left inside the chamber after crushing. To quantify EβF produced by the crushed (lead) and undisturbed (downstream) aphids, an adsorbent filter, containing 40 mg of SuperQ® (Alltech, Deerfield, IL, USA), was connected to each chamber. Clean air was pushed into the system at a rate of 1.5 L min^{-1} and sampled air was pulled through the filters from both the lead and downstream chambers at a rate of 0.75 L min^{-1} per chamber. Five experiments were conducted; each of 1 h with nine replicates (Table 1). The first experiment (crushed–empty) was a positive control designed to document the distribution of EβF, released upwind, in the two chambers. The second experiment (empty–infested) measured the amount of EβF released by a colony of 50 *A. pisum* under the laboratory conditions. The third (empty–non-infested) and fifth (crushed–non-infested) experiments were, respectively, controls devoted to the evaluation of the potential amount of EβF that could be released from an uninfested broad bean plant unexposed or exposed to EβF. The fourth experiment (crushed–infested) was conducted to show whether downstream aphids emit additional alarm signal when exposed to an alarm signal from upstream conspecifics.

Volatile Analysis Filters were eluted with 150 μl of dichloromethane and nonyl acetate (320 ng) was added to each sample as an internal standard. Extracts were analyzed by gas chromatography with flame ionization detection with a Hewlett–Packard 6890 series gas chromatograph equipped with a splitless injector (260°C). The column (Equity-1, Supelco, Bellefonte, PA, USA, 30 m×0.25 mm i.d.) was held at 40°C for 1 min before being heated to 260°C at 15°C min^{-1}. Quantification of compounds was carried out by comparing individual peak areas to that of the internal standard. Identification of EβF was made by comparison of its retention time with that of synthetic EβF (Bedoukian Research, Inc., Danbury, CT, USA) and confirmed by gas chromatography-mass spectrometry.

Table 1 Mean (*E*)-β-farnesene (EβF) emission amounts released by *Acyrthosiphon pisum* and detected in lead and downstream chambers, as well as average lead/downstream EβF ratios (±SE), in different experiments

Number	Lead chamber	Downstream chamber	Average EβF amounts (ng±SE)[d]		Average downstream/lead EβF ratios (±SE)[d]
			Lead chamber	Downstream chamber	
1	Crushed aphids[a]	Empty	1,295.74±261.43	1,130.25±148.87	1.056±0.190
2	Empty	Infested plant[b]	–	–	–
3	Empty	Non-infested plant[c]	–	–	–
4	Crushed aphids	Infested plant	1,585.06±288.37	957.69±153.83	0.769±0.094
5	Crushed aphids	Non-infested plant	1,384.22±275.00	1,048.26±133.65	0.859±0.113

[a] 50 crushed third Instar larvae *A. pisum*
[b] Single 20-cm-high *Vicia faba* infested with 50 third Instar *A. pisum*
[c] Single 20-cm-high non-infested *V. faba*
[d] Nine replicates were performed for each experiment

Results and Discussion

EβF was the only volatile released by *A. pisum* detected in our experiments, which is consistent with previous findings (Francis et al. 2005). In experiment one (crushed–empty), third instar *A. pisum* released an average (sum of upstream and downstream collections) of 48.5 ng of EβF per individual. The higher EβF levels observed in our study, compared to those found by Mondor et al. (2000) and Schwartzberg et al. (2008), may be explained by differences in eliciting EβF release (crushed vs. probed or natural attack). These EβF amounts are larger than what we might expect to see in a natural condition. However, within a colony, signaling and receiving aphids are much closer to each other than in our experiment and, therefore, the higher release rate in our experiment may compensate for this. Moreover, a higher release rate should be advantageous for testing our hypothesis.

The ratio of downstream aphid to lead aphid emission should be 1.0 if no additional EβF were produced from aphids in the downstream chamber. A ratio greater than 1.0 should, therefore, reflect emission of EβF from aphid/host plant complexes subjected to the alarm signal. No EβF was emitted from downstream plant and plant/aphid complexes in experiments with empty lead chambers (Table 1, experiment 2 [empty–infested] and 3 [empty–non-infested]). These data confirm that undisturbed aphids under the conditions of this experiment do not produce a detectable alarm signal. EβF was detected in experiments 1 (crushed–empty), 4 (crushed–infested), and 5 (crushed–non-infested). However, there was no difference in EβF ratios among these experiments (ANOVA, $F_{2,24}$=1.12, P=0.342). Although not significant, the small reduction in ratio in experiment 1 compared to experiments 4 and 5, may have been due to absorption of some EβF by the downstream plants used in the latter experiments. Interestingly, in experiment 4, the downstream aphids appeared to perceive EβF as 14% of these aphids dropped from their host plant.

These results indicate that the EβF alarm signal is not contagious. This conclusion is consistent with further observations that the amount of EβF released by a single aphid under attack is similar to the average amount of alarm pheromone released per consumed aphid in a colony (Schwartzberg et al., unpublished).

An understanding of how alarm pheromone is emitted in a natural setting may be important when studying the effects of alarm signaling among aphids, since a single alarm signal can influence aphid ecology through both inter- and intra-specific signaling. The way that such signals convey information in an aphid colony may be important for the effectiveness of a signaling alarm, as well as in reducing the costs of signal production in an environment in which signal eavesdropping by predators can add a fitness cost to signal production.

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