EMISSION OF ALARM PHEROMONE IN APHIDS: A NON-CONTAGIOUS PHENOMENON

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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 studied aphid species is (*E*)-ß-farnesene. We recently demonstrated that the production and accumulation of (*E*)-ß-farnesene during development by juvenile aphids is stimulated by exposure to odor cues, most likely (*E*)-ß-farnesene itself, emitted by other colony members. Here we examined whether the release of (*E*)-ß-farnesene can be triggered by exposure to the alarm pheromone of other individuals and thereby amplify the signal. Such contagious emission might be adaptive under some conditions because the amount of (*E*)-ß-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. Using a push-pull headspace collection system, we quantified the (*E*)-ß-farnesene released from aphids exposed to conspecific alarm signals. Typical avoidance behavior was observed with exposure to (*E*)-ß-farnesene (i.e., they ceased feeding and dropped from host-plant); however, no additional alarm pheromone was detected, suggesting that contagious release of (*E*)-ß-farnesene does not occur.

Key Words – Aphid alarm pheromone production, *Acyrthosiphon pisum*, (*E*)-ß-farnesene, headspace collection system.

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, and in most aphid species, individuals respond to attack by natural enemies by releasing an alarm pheromone (Bowers et al., 1972) which induces perceiving individuals to stop feeding, disperse locally, and often drop from the host plant (Braendle and Weisser, 2001).

Like most insect species, aphids are highly dependent upon chemical signals (Pickett and Glinwood, 2007). Whereas alarm pheromones in other insects and mites usually consist of a mixture of chemicals (e.g. Verheggen et al., 2007a), the aphid alarm pheromone appears to contain a single chemical in most Aphidinae species (Bowers et al., 1972 ; Francis et al., 2005): the sesquiterpene (*E*)-ß-farnesene (EßF). EßF has been identified as a unique volatile compound in 13 aphid species, including the pea aphid, *Acyrthosiphon pisum* Harris (Francis et al., 2005). EßF also acts as a kairomone used by predators and parasitoids to locate their aphid prey (Pickett and Glinwood, 2007; Verheggen et al., 2007b; Verheggen et al., 2008). These recent findings highlight the possibility of direct negative effects of alarm pheromone production in the form of increased apparency to natural enemies. Beale et al. (2006) effectively exploited these properties by adding an EßF synthase gene to *Arabidopsis thaliana* plants, increasing their attraction of aphid parasitoids.

In a recent study, we found that juvenile aphids reared in social isolation on artificial diet release less EßF than those reared in colony or those reared in isolation but exposed to colony odors (Verheggen et al., submitted). We suggested that aphid, plant or aphid-induced plant volatiles may stimulate the production of additional EßF in downstream aphid signal recipients.

In this study we examined whether exposure to EßF stimulates the release of EßF by receiving individuals by measuring the pheromonal response of individuals exposed to EßF from conspecifics. Such a contagious phenomenon could be adaptive if there are benefits to disseminating the alarm farther than would be achieved by the release of EßF by a single individual.

MATERIALS AND METHODS

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 experiment. Plants were grown in square 9 x 9cm 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 diameter x 30 cm) made of glass and Teflon® (Figure 1). Chambers were sealed on both ends and 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 EßF emissions, we crushed 50 3rd instar aphids inside our volatile collection chambers using a glass pestle left inside the chamber after use. To quantify EßF 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 and sampled air was pulled through the filters from both the lead and downstream chambers at a rate of 0.75 L/min per chamber. Five experiments were conducted for 1 hr each with 9 replicates (Table 1). The first experiment (crushed – empty) was a positive control designed to document the EßF distribution in our system. The second experiment (empty – infested) measured the amount of EßF released by a colony of 50 *A. pisum* under our laboratory conditions. The third (empty – non infested) and fifth (crushed – non infested) experiments are controls, respectively devoted to the evaluation of the potential amount of EßF that could be released from an uninfested broad bean unexposed or exposed to EßF. The fourth experiment (crushed – infested) was conducted to show whether "Downstream" aphids emit additional alarm signal at the time they are exposed to an alarm signal from conspecifics.

Volatile Analysis. Filters were eluted using 150 µl of dichloromethane. Nonyl acetate (320 ng) was added to each sample as an internal standard. Extracts were analyzed by GC-FID using a Hewlett-Packard 6890 series gas chromatograph. Aliquots of 1 µL were injected with a splitless injector held at 260°C. The column (Equity-1, Supelco, Bellefonte, PA, USA, 30 m x 0.25 mm i.d.) was maintained at 40°C for 1 min before being heated to 260°C at a constant rate of 15°C/min. This final temperature was maintained for 10 min. Quantifications of compounds were obtained by comparing individual peak areas to the internal standard. Identification of EßF was made by comparison of its retention time with that of synthetic EßF (Bedoukian Research, Inc., Danbury, CT, USA) and confirmed by GC-MS.

RESULTS AND DISCUSSION

EßF was the only detectable volatile released by *A. pisum* in our experiments, which is consistent with previous findings (Francis et al., 2005). In experiment one (crushed – empty), an average of 48.52 ng of EßF per 3rd instar *A. pisum* larva was found. The higher EßF 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 EßF elicitation techniques (crushing versus probing or natural attack). These EßF doses are larger than what we would expect to see in a natural condition; however we feel that these doses would be better to show the effects of a response by receiving aphids. Within a colony, signaling and receiving aphids are much closer to each other and if we had lower emission from signaling aphids in our experiments we may have underexposed aphids as compared to a natural setting.

The ratio of downstream aphid to lead aphid emission would be equal to 1.0 if no additional EßF was produced from the downstream chamber. Any increases in the amount of EßF collected from the downstream chamber therefore reflect emission of EßF from aphid/host plant complexes subjected to the alarm signal. Amounts are listed in Table 1 as downstream and lead aphid emissions and downstream/lead aphid emission ratios.

No EßF 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 observations confirm that *V. faba* do not emit EßF and demonstrate that undisturbed aphids under the conditions of this experiment do not produce a detectable alarm signal.

EßF was detected in experiments 1 (crushed – empty), 4 (crushed – infested) and 5 (crushed – non infested). Analysis of variance demonstrated the equivalence of the EßF ratios

obtained in these three experiments (ANOVA, $F_{2,24}$=1.12, P=0.342). The downstream/lead ratio found in experiment 1 was close to 1.0 as predicted. This ratio was not significantly different from the ratio obtained with a non-infested *V. faba* plant in the downstream chamber (*Tukey*, α=0.05). The very small reduction in the EßF ratio is likely due to the presence of the plant, which may act as an absorbent surface for airborne compounds to adhere to. In the fourth experiment (crushed – infested) aphids were present in the downstream chamber, yet there was no significant difference in the EßF ratio compared to that observed in experiment 5 (crushed – non infested) (*Tukey*, α=0.05). The downstream aphids did appear to perceive the EßF coming from the lead chamber, as the number of aphids in the downstream chamber that dropped from their host plant increased from 0 to 14%. These results indicate that amplification of the EßF alarm signal does not occur. This result is consistent with further observations that the amount of EßF 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., In press).

An understanding of how alarm pheromone is emitted in a natural setting, or at least an intact aphid colony subject to environmental cues, may be important when studying the effects of alarm signaling among aphids and their predators. We have seen that a single, environmentally ubiquitous alarm signal can influence aphid ecology in the form of both inter- and intra-specific signaling. The way that such signals convey information in an aphid colony may be important in both the effectiveness of alarm signals within a colony as well as in reducing the costs of signal production in an environment where signal eavesdropping by prey can add a fitness cost to signal production.

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Table 1. Five experiments were conducted to demonstrate whether unstressed aphids respond to the alarm pheromone of conspecifics by emitting additional alarm pheromone. Volatiles were collected in both chambers for 1 hr. (E)-ß-farnesene emission by unstressed aphids exposed to EßF from crushed conspecifics are presented as well as average Lead/Downstream EßF ratios (+/- SE). These average ratios were calculated as the mean the amount of EßF collected in the second chamber divided by the amount collected in the first chamber

n°	Lead chamber	Downstream chamber	Average EßF amounts (± SE) [d]		Average Downstream/Lead EßF ratios (± SE) [d]
			Lead chamber	Downstream chamber	
1	Crushed aphids [a]	Empty	1295.74 ± 261.43	1130.25 ± 148.87	1.056 ± 0.190
2	Empty	Infested plant [b]	/	/	/
3	Empty	Non infested plant [c]	/	/	/
4	Crushed aphids	Infested plant	1585.06 ± 288.37	957.69 ± 153.83	0.769 ± 0.094
5	Crushed aphids	Non infested plant	1384.22 ± 275.00	1048.26 ± 133.65	0.859 ± 0.113

[a] 50 crushed 3rd Instar larvae *A. pisum*
[b] Single 20 cm high *V. faba* infested with 50 3rd Instar larvae *A. pisum*
[c] Single 20 cm high non infested *V. faba*
[d] Nine replicates were performed for each experimentation

Figure legend

Figure 1. Push-pulled headspace collection set-up. Pumps are used to push and pull air through this system, maintaining normal atmospheric pressure in both chambers while allowing air to pass from the lead chamber (A) to the downstream chamber (B).

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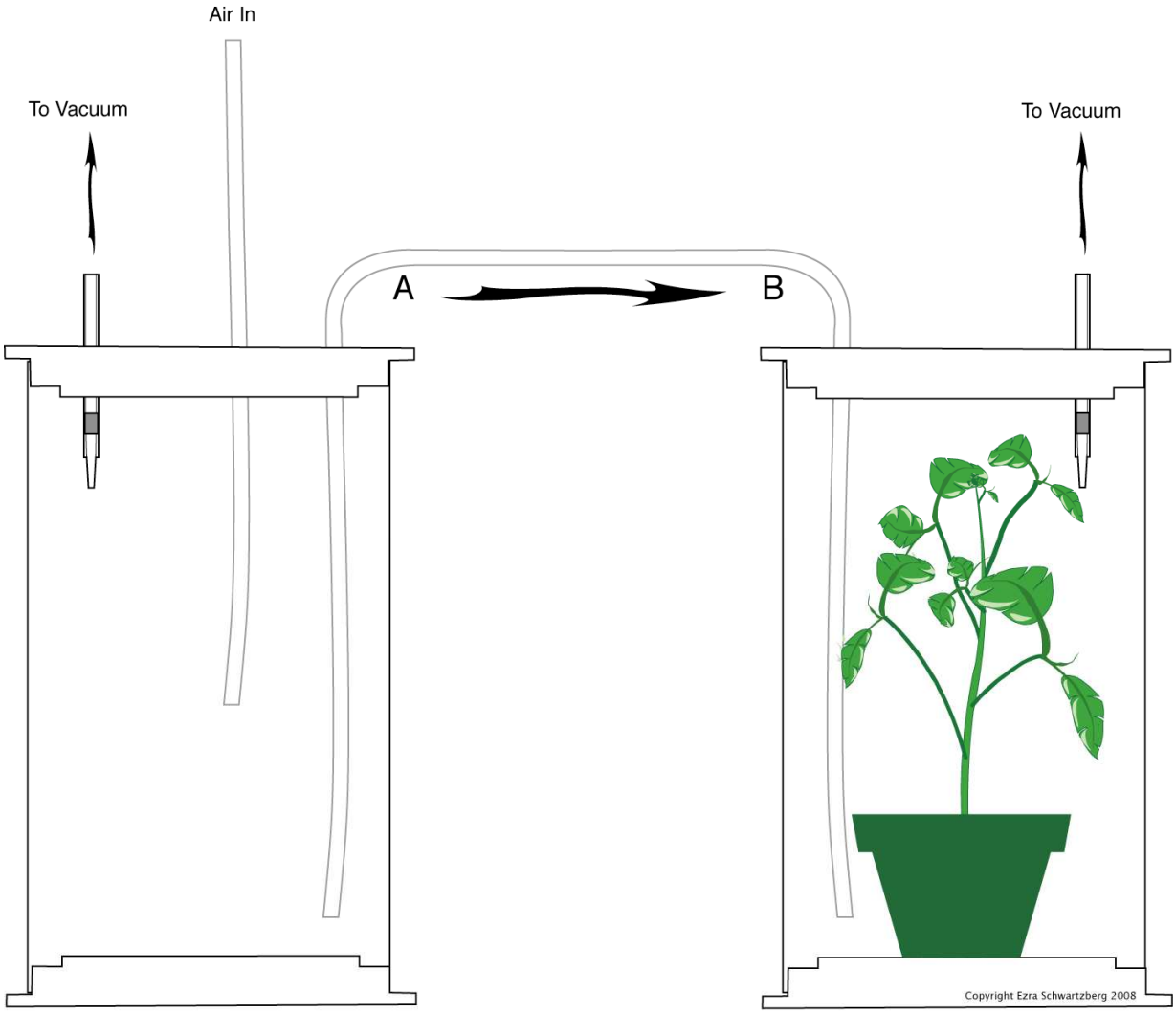
200 **Figure 1.**



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