

NOTE

Subversion of alarm communication: Do plants habituate aphids to their own alarm signals?

Anca S. Petrescu, Edward B. Mondor, and Bernard D. Roitberg

Abstract: When attacked by a predator, pea aphids, *Acyrtosiphon pisum*, emit an alarm pheromone, (*E*)- β -farnesene, which causes nearby conspecifics to disperse from the area. However, herbivore-damaged plants also emit (*E*)- β -farnesene. We hypothesized that plants release farnesene to habituate aphids, i.e., to disrupt their alarm-pheromone responses, perhaps to reduce herbivory by increasing parasitoid or predator efficacy. Thus, we addressed two questions: (1) Do aphids habituate to (*E*)- β -farnesene, and (2) Are they habituated at levels produced by aphid-infested plants? On an artificial diet devoid of farnesene, aphids were exposed to 10 ng/cm³ of (*E*)- β -farnesene or a hexane control over 24 h. Habituation was achieved, as dropping responses to 50 ng/cm³ of (*E*)- β -farnesene decreased after exposure. We then exposed aphids to 0.8 ng/cm³ of (*E*)- β -farnesene, a pheromone concentration emitted by plants, or a hexane control for 24 h. Their reaction to 38 ng/cm³ of (*E*)- β -farnesene, the maximum pheromone concentration found in aphid-cornicle droplets, was not significantly different before and after exposure. Thus, our hypothesis that plants emit farnesene to disrupt aphid alarm communication remains unsupported.

Résumé : À l'attaque d'un prédateur, le Puceron du pois, *Acyrtosiphon pisum*, émet une phéromone d'alerte, le (*E*)- β -farnésène, qui déclenche la dispersion des autres pucerons de la même espèce qui sont dans le voisinage. Cependant, les plantes endommagées par les herbivores émettent aussi du (*E*)- β -farnésène. Nous avons posé en hypothèse que les plantes libèrent le farnésène pour conditionner les pucerons, i.e., pour perturber les réactions des pucerons aux phéromones d'alerte, peut-être pour diminuer l'herbivorie, en augmentant l'efficacité des parasitoïdes ou des prédateurs. Nous avons donc examiné deux questions : (1) les pucerons s'habituent-ils à la présence du (*E*)- β -farnésène? et (2) s'adaptent-ils aux concentrations que libèrent les plantes infestées de pucerons? Des pucerons soumis à un régime alimentaire artificiel sans farnésène ont été exposés soit à 10 ng/cm³ de (*E*)- β -farnésène, soit à un hexane témoin, pendant 24 h. Il y avait effectivement habituation puisque les réponses à une concentration de 50 ng/cm³ ont diminué après l'exposition. Nous avons par la suite exposé des pucerons à 0.8 ng/cm³ de (*E*)- β -farnésène, la concentration de phéromone émise par les plantes, ou à un hexane témoin pendant 24 h. Leur réaction à 38 ng/cm³, la concentration maximale de phéromone trouvée dans les gouttelettes émises par les cornicules des pucerons, n'était pas significativement différente avant ou après l'exposition. Notre hypothèse selon laquelle les plantes émettent du farnésène pour perturber la communication de la phéromone d'alerte chez les pucerons n'est donc pas corroborée.

[Traduit par la Rédaction]

Introduction

Habituation is decreased responsiveness to a signal after repeated presentation, as a result of neurophysiological changes (Domjan and Burkhard 1986). Habituation may help an organism to focus on salient stimuli by ignoring redundant information. While habituation may be adaptive for insects under natural conditions (e.g., to conserve energy upon not finding a mate quickly) (Mankin et al. 1999), it may allow

biocontrol practitioners to exploit these responses (e.g., mating disruption) (Carde and Minks 1995; Rumbo and Vickers 1997). One area in which an understanding of habituation may advance biocontrol strategies is the disruption of alarm communication between conspecifics of a pest species.

When attacked by a predator, pea aphids, *Acyrtosiphon pisum*, emit an alarm pheromone, (*E*)- β -farnesene, from cornicles on the posterior abdomen (Dixon 1973). Neighbouring clones and conspecifics disperse from the source of the signal by walking or dropping from the plant (Nault and Phelan 1984). However, plants such as broad bean (Du et al. 1998), corn (Bernasconi and Turlings 1998), cotton (Rose et al. 1998), and potato (Gibson and Pickett 1983) also emit (*E*)- β -farnesene when insect-damaged. There are two possible interpretations of this phenomenon: (1) employment of like compounds across kingdoms is coincidental, or (2) plants emit (*E*)- β -farnesene for defensive purposes against insects. (*E*)- β -farnesene emitted from aphid-infested plants functions as a synomone for aphid parasitoids (Du et al. 1998). How-

Received August 4, 2000. Accepted January 15, 2001.
Published on the NRC Research Press Web site on April 11, 2001.

A.S. Petrescu, E.B. Mondor,¹ and B.D. Roitberg.
Behavioural Ecology Research Group, Department of
Biological Sciences, Simon Fraser University, Burnaby,
BC V5A 1S6, Canada.

¹Corresponding author (e-mail: emondor@sfu.ca).

ever, we hypothesize that plant-produced (*E*)- β -farnesene may also reduce insect feeding by habituating aphids to their own alarm pheromone, thus increasing aphid susceptibility to parasitoids and predators. A rigorous test of this hypothesis demands answers to two questions: (1) Do aphids habituate to (*E*)- β -farnesene, and (2) If they do, are they habituated at the levels produced by aphid-infested plants?

Methods and results

Pea aphids were collected from sweet pea, *Lathyrus odoratus* 'Cuthbertson,' in Burnaby, British Columbia. Aphids were reared in the laboratory on broad bean plants, *Vicia faba* 'Broad Windsor,' potted in garden soil. Two-week-old broad bean plants were infested with adult aphids and placed in a fume hood. The constant airflow over the leaf surfaces was intended to reduce the concentration of plant volatiles to which aphids were exposed prior to testing. Aphids born over a 24-h period were raised as a synchronous colony for 4 days until their third instar. An artificial diet (modified from Dill et al. 1990) was used as a growth medium for fourth-instar and adult aphids. The original diet was changed as follows: the pH was lowered to 7.6 from 8.3 with NaOH (aqueous), and yellow food colouring was used instead of green. Habituation and testing were carried out using the same artificial-diet system to eliminate plant-derived farnesene that may have unquantifiable effects on aphid responses.

Artificial leaves were made by sealing 2 circles of Parafilm™ 12 cm in diameter, which were injected with the artificial diet. Petri dishes 8.5 cm i.d. \times 3.5 cm high were covered with the artificial leaves. A 6.5-lb. clear glass plate was placed on top of 4 petri dishes to create pressure in the leaves, as aphids are passive feeders (Dixon 1998). Forty fourth-instar aphids were placed on each artificial leaf. The aphids were allowed to settle on the artificial leaves for 24 h. After this time, any aphids not feeding or in excess of 35 were taken out of the petri dish. A 1-cm² piece of Whatman™ filter paper was placed in the center of each dish to be subsequently used for (*E*)- β -farnesene application. Farnesene was diluted in hexane to the desired concentrations. In each experiment, 10 μ L of a farnesene solution or a hexane control was applied to the filter paper and allowed to diffuse throughout the 199-cm³ petri dish. The equilibrium concentration of (*E*)- β -farnesene per cubic centimetre,

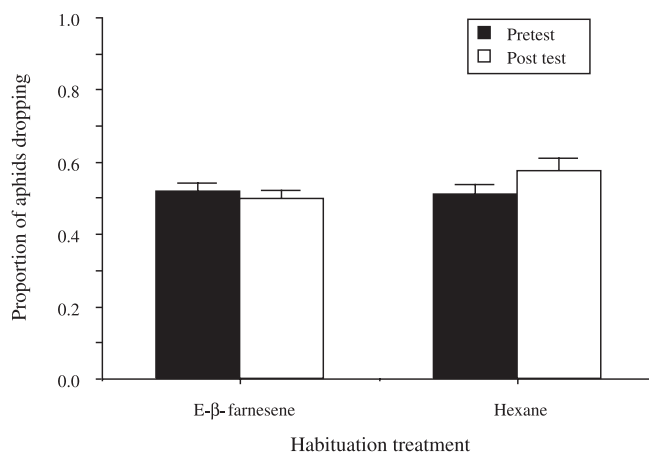
$$\begin{aligned} \text{equilibrium concentration (ng/cm}^3\text{)} \\ = \{[\text{solution concentration } (\mu\text{g}/\mu\text{L})] \\ \times (10 \mu\text{L}) \times (1000 \text{ ng}/\mu\text{g})\} / (199 \text{ cm}^3) \end{aligned}$$

was considered to be the exposure dose for the aphids.

Do aphids habituate to (*E*)- β -farnesene?

In the first experiment we determined whether aphids habituate to (*E*)- β -farnesene. In the pretest, dropping responses of aphids to 50 ng/cm³ (1.0 μ g/ μ L) of (*E*)- β -farnesene was recorded over 90 s, after which the artificial leaf was raised to let the farnesene escape and a new piece of filter paper was placed in the center of the dish. Aphids in 21 randomly chosen petri dishes were then exposed to 10 ng/cm³ (0.2 μ g/ μ L) of (*E*)- β -farnesene and aphids in 21 control dishes were exposed to hexane for 24 h. After this time, a 90-s post test of aphid dropping responses was conducted with 50 ng/cm³

Fig. 1. Proportions (mean \pm 1 SE) of pea aphids, *Acyrtosiphon pisum*, dropping from their feeding site during pretest and post-test exposure to 50 ng/cm³ (1.0 μ g/ μ L) of (*E*)- β -farnesene. The habituation stimulus was 10 ng/cm³ (0.2 μ g/ μ L) (*E*)- β -farnesene, or hexane.



(1.0 μ g/ μ L) of (*E*)- β -farnesene. Insects' reactions were recorded on videotape.

Habituation was evident, as aphids exposed to farnesene and hexane had significantly different post-test dropping responses ($F_{[1,42]} = 5.1325$, $P = 0.0287$) (Fig. 1). In this experiment the combined pretest means were not significantly different from the combined post-test means ($F_{[1,42]} = 1.4296$, $P = 0.2385$).

Do aphids habituate to (*E*)- β -farnesene levels produced by herbivore-infested plants?

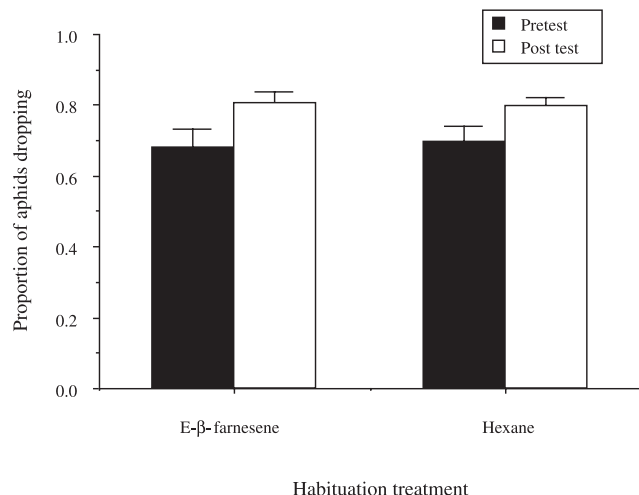
In the second experiment we determined whether aphids habituate to levels of (*E*)- β -farnesene emitted by aphid-infested plants; 35 aphids were used per artificial leaf ($n = 29$; $n = 15$ for (*E*)- β -farnesene, $n = 14$ for the hexane control). A pretest and post-test concentration of 38 ng/cm³ (0.76 μ g/ μ L) (*E*)- β -farnesene corresponds to the maximum pheromone level found in aphid-cornicle droplets (Mondor et al. 2000). Using Du et al. (1998), the (*E*)- β -farnesene concentration given off by aphid-infested bean plants over the initial 24 h of feeding was calculated to be 0.8 ng/cm³ (0.016 μ g/ μ L). This concentration was used to habituate aphids. The protocol was otherwise identical with that for experiment 1.

At these pheromone levels there was no indication of habituation, as aphids exposed to farnesene and hexane had similar pretest and post-test dropping responses ($F_{[1,29]} = 0.0876$, $P = 0.7694$) (Fig. 2). An increase in post-habituation dropping responses for both treatments ($F_{[1,29]} = 6.4442$, $P = 0.0168$) may be attributed either to the age difference of 1 day or to a decrease in food quality (Dill et al. 1990).

Discussion

The concentration and presentation schedule, which was meant to mimic a biological scenario (i.e., the concentrations of (*E*)- β -farnesene emitted by aphid-infested plants), did not lead to habituation. Thus, our hypothesis that plants emit farnesene to interfere with aphid alarm communication remains unsupported. Habituation was obtained only at much

Fig. 2. Proportions (mean \pm 1 SE) of pea aphids dropping from their feeding site during pretest and post-test exposure to 38 ng/cm^3 ($0.76 \text{ }\mu\text{g}/\mu\text{L}$) of (*E*)- β -farnesene. The habituation stimulus was 0.8 ng/cm^3 ($0.016 \text{ }\mu\text{g}/\mu\text{L}$) (*E*)- β -farnesene, or hexane.



higher pheromone concentrations. However, a minimum habituation concentration remains to be determined.

The use of aphid alarm pheromone combined with pesticides and (or) natural enemies has been investigated as a pest-control tactic. El-Agamy and Haynes (1992) tested the effect of a strong single dose of (*E*)- β -farnesene (1 mg) on aphid survivorship in the presence of Malathion and (or) the predator *Nabis americanoferus* Caryon. Farnesene did not affect aphid survival, prompting those authors to suggest that either the insects were not habituated or the experimental design was not sensitive enough to detect such an effect. As habituation occurs at a concentration of 10 ng/cm^3 for 24 h, their results may indicate a sensitization effect rather than habituation. Sensitization, an increase in responsiveness, usually occurs in response to an intense stimulus (Domjan and Burkhard 1986). Thus, aphids may have been equally, if not more, likely to respond to the presence of a predator.

While habituation is a complex phenomenon, it generally involves exposure to a weak stimulus with a high frequency of repetition (Domjan and Burkhard 1986). In a closed system, concentrations of (*E*)- β -farnesene from aphid-infested broad bean plants increase over 4 successive days (Du et al. 1998). In an open system, such as under field conditions, emission of low levels of farnesene combined with air movement may result in a low-intensity but high-frequency scenario. Future research should explore the complex interaction between intensity and frequency of (*E*)- β -farnesene emission in plants and its role in the habituation of aphids.

To avoid predators, aphids also respond to vibration (Roitberg and Myers 1978; Clegg and Barlow 1982) and visual cues (Dixon 1958; Brown 1974; Dixon and Stewart 1975; Hajek and Dahlsten 1987; E.B. Mondor and B.D. Roitberg, unpublished data). Habituated or sensitized aphids may show increased responsiveness to these other cues. Additionally, novel stimuli (e.g., leaf vibration or visual cues) could restore the insects' level of response to the original stimulus (e.g., farnesene) (Domjan and Burkhard 1986). These considerations complicate pest-management strategies, which

are based on the idea of increasing parasitoid or predator efficacy.

Acknowledgments

We thank Scott Baird for preparing (*E*)- β -farnesene concentrations, Alex Fraser for assistance with the artificial-diet system, Graham Murrell for providing access to laboratory facilities, Linda Takahashi for technical assistance, and Jabus Tyerman for comments on previous versions of the manuscript. Funding for this project was supplied by a Natural Sciences and Engineering Research Council of Canada (NSERC) Undergraduate Student Research Award to A.S.P., an NSERC Post Graduate Scholarship B grant to E.B.M., and an NSERC operating grant to B.D.R.

References

- Bernasconi, M.L., and Turlings, C.J. 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomol. Exp. Appl.* **87**: 133–142.
- Brown, H.D. 1974. Defensive behaviour of the wheat aphid, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae), against Coccinellidae. *J. Entomol. Ser. A Gen. Entomol.* **48**: 157–165.
- Carde, R.T., and Minks, A.K. 1995. Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol.* **40**: 559–585.
- Clegg, J.M., and Barlow, C.A. 1982. Escape behaviour of the pea aphid *Acyrtosiphon pisum* (Harris) in response to alarm pheromone and vibration. *Can. J. Zool.* **60**: 2245–2252.
- Dill, L.M., Fraser, A.H.G., and Roitberg, B.D. 1990. The economics of escape behaviour in the pea aphid, *Acyrtosiphon pisum*. *Oecologia*, **83**: 473–478.
- Dixon, A.F.G. 1958. The escape responses shown by certain aphids to the presence of the coccinellid *Adalia decempunctata* (L.). *Trans. R. Entomol. Soc. Lond.* **110**: 319–334.
- Dixon, A.F.G. 1973. *Biology of aphids*. Edward Arnold, London.
- Dixon, A.F.G. 1998. *Aphid ecology: an optimization approach*. 2nd ed. Chapman and Hall, London.
- Dixon, A.F.G., and Stewart, W.A. 1975. Function of the siphunculi in aphids with particular reference to the sycamore aphid, *Drepanosiphum platanoideis*. *J. Zool.* (1965–1984), **175**: 279–289.
- Domjan, M., and Burkhard, B. 1986. *The principles of learning and behavior*. 2nd ed. Brooks/Cole Publishing Co., Belmont, Calif.
- Du, Y.J., Poppy, G.M., Powell, W., Pickett, J.A., Williams, L.J., and Woodcock, C.M. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *J. Chem. Ecol.* **24**: 1355–1367.
- El-Agamy, F.M., and Haynes, K.F. 1992. Susceptibility of the pea aphid (Homoptera: Aphididae) to an insecticide and a predator in the presence of synthetic aphid alarm pheromone. *Ann. Entomol. Soc. Am.* **85**: 794–798.
- Gibson, R.W., and Pickett, J.A. 1983. Wild potato repels aphids by release of aphid alarm pheromone. *Nature (Lond.)*, **302**: 608–609.
- Hajek, A.E., and Dahlsten, D.L. 1987. Behavioral interactions between three birch aphid species and *Adalia bipunctata* larvae. *Entomol. Exp. Appl.* **45**: 81–87.
- Mankin, R.W., Arbogast, R.T., Kendra, P.E., and Weaver, D.K. 1999. Active spaces of pheromone traps for *Plodia interpunctella* (Lepidoptera: Pyralidae) in enclosed environments. *Environ. Entomol.* **28**: 557–565.

- Mondor, E.B., Baird, D.S., Slessor, K.N., and Roitberg, B.D. 2000. Ontogeny of alarm pheromone secretion in the pea aphid, *Acyrtosiphon pisum*. *J. Chem. Ecol.* **26**: 2875–2881.
- Nault, L.R., and Phelan, P.L. 1984. Alarm pheromones and sociality in pre-social insects. In *Chemical ecology of insects. Edited by W.J. Bell and R.T. Carde*. Sinauer Associates, Inc., Sunderland, Mass. pp. 238–256.
- Roitberg, B.D., and Myers, J.H. 1978. Adaptation of alarm pheromone responses of the pea aphid, *Acyrtosiphon pisum* (Harris). *Can. J. Zool.* **56**: 103–108.
- Rose, U.S.R., Lewis, W.J., and Tumlinson, J.H. 1998. Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *J. Chem. Ecol.* **24**: 303–319.
- Rumbo, E.R., and Vickers, R.A. 1997. Prolonged adaptation as possible mating disruption mechanism in oriental fruit moth, *Cydia* (equals *Grapholita*) *molesta*. *J. Chem. Ecol.* **23**: 445–457.