Responses of Russian Wheat Aphid (Homoptera: Aphididae) to Aphid Alarm Pheromone

P. A. SHAH,[1] J. A. PICKETT,[2] AND J. D. VANDENBERG[3]

Environ. Entomol. 28(6): 983–985 (1999)

ABSTRACT In a series of laboratory tests, Russian wheat aphids, *Diuraphis noxia* (Mordvilko), responded to synthetic aphid alarm pheromone, (*E*)-β-farnesene, by removing stylets and leaving feeding sites or by crawling out of test arenas. Late instars and adults were more responsive than early instars. In dose–response assays, EC_{50} estimates ranged from 0.94 to 8.95 mg/ml among 3 experiments. In arenas, *D. noxia* also responded to the proximity of cornicle-damaged nymphs of either the green peach aphid, *Myzus persicae* (Sulzer), or of *D. noxia*, which suggests endogenous production of alarm pheromone by *D. noxia*. Combinations of (*E*)-β-farnesene and the aphid-pathogenic fungus *Paecilomyces fumosoroseus* (Wize) Brown & Smith did not enhance aphid mortality relative to controls treated with fungus only. Further studies involving appropriate formulations of (*E*)-β-farnesene are necessary before practical biorational strategies can be devised combining this semiochemical and biological control agents.

KEY WORDS *Diuraphis noxia*, *Myzus persicae*, *Paecilomyces fumosoroseus*, aphid, semiochemicals, alarm pheromone

(*E*)-β-FARNESENE IS A common alarm pheromone among aphids (Pickett et al. 1992) and is released from cornicle secretions when aphids are attacked by natural enemies of.arthropods. An elicited behavior, such as dropping off the host plant, may help aphids avoid natural enemies. Control measures using synthetic alarm pheromone have had some success in combination with fungal entomopathogens or contact insecticides (Griffiths and Pickett 1980, Pickett et al. 1992). Increased activity in response to alarm pheromone may increase the potential for contact between the aphid and fungal spores.

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), was identified in the United States in 1986 (Stoetzel 1987). It has since spread throughout western North America, infesting various small grains and grasses. Cumulative damage from aphid feeding exceeds $1 billion (Anonymous 1994). Control measures include cultural, biological and chemical methods (Halbert et al. 1992).

The objectives of the current work were to determine if *D. noxia* can respond to endogenous or synthetic alarm pheromone, and to evaluate the effectiveness of combinations of alarm pheromone and an aphid-pathogenic isolate of the fungus *Paecilomyces fumosoroseus* (Wize) Brown & Smith.

[1] Institute of Microbiology, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland.
[2] IACR Rothamsted, Department of Biological and Ecological Chemistry, Harpenden, Herts, AL5 2JQ, UK.
[3] U.S. Plant, Soil & Nutrition Laboratory, USDA–ARS, Ithaca, NY 14853.

Materials and Methods

Aphid and Fungus Cultures. *Diuraphis noxia* was maintained on barley (wintermalt or variety 8–12, U. S. National Small Grains Collection, Aberdeen, ID), *Hordeum vulgare* L., at 25°C and a photoperiod of 15:9 (L:D) h (Vandenberg 1996). *Myzus persicae* (Sulzer) was reared on turnip, *Brassica campestris* variety *rapifera* Metz, at 20–21°C and a photoperiod of 16:8 (L:D) h by M. Tauber, Cornell University (Ithaca, NY). *Paecilomyces fumosoroseus* strain M612 was obtained from Mycotech Corporation (Butte, MT). Cultures were maintained on Sabouraud's dextrose agar plus 2% yeast extract at 25°C and a photoperiod of 15:9 (L:D) h.

Response of *D. noxia* to Aphid Injury. Individual late-instar *D. noxia* were placed in the center of pieces of filter paper (25 mm diameter). Each aphid was observed after the introduction of one of the following 4 aphids: (1) a healthy *D. noxia* nymph, (2) a healthy *M. persicae* nymph, (3) an injured *D. noxia* nymph, (4) an injured *M. persicae* nymph. Injury to aphids used in treatments 3 and 4 was caused by gently pressing on the abdomen with a steel needle to stimulate release of potential endogenous pheromone from aphid cornicles. We recorded, to the nearest second, the time for each test aphid to respond to the presence of the other aphid by exiting the arena. Observations were made for a maximum of 3 min, after which the aphids were discarded. Each aphid was used only once. Five aphids were used for each treatment and each experiment was replicated 3 times. Analysis of variance (ANOVA) was done on $\log_{10}$-transformed exit times (SAS Institute 1990).

Synthetic Alarm Pheromone. (*E*)-β-Farnesene was synthesised by the method of Dawson et al. (1982),

packed under nitrogen in glass ampoules, and stored at 4°C until use. Ampoules were used within 7 d of opening and freshly prepared dilutions in hexane were used for each experiment. For experiments involving the use of synthetic (*E*)-β-farnesene (described below), a 1-μl drop was applied ≈2 mm away from each test colony. The numbers of aphids and the approximate area occupied by each colony were recorded just before application. Hexane-only treatments were performed as controls for each experiment.

Responses of Different Stages to Synthetic Alarm Pheromone. Age-group differences were tested in 2 ways. For the 1st experiment, pheromone applications were made near 5 groups of ≈25 *D. noxia* consisting of both early (1st and 2nd) and late (3rd and 4th) instars. This experiment was repeated 3 times. In the 2nd experiment, pheromone applications were made near 5 groups of ≈25 *D. noxia* of uniform age (late instars or adults). This experiment was done once. For all tests, the pheromone concentration was 1 mg ml^{-1}. The number of aphids withdrawing their stylets and moving away from their feeding sites were recorded at 30, 60, and 180 s after application. The effect of pheromone concentration on the proportion of aphids responding within each group was analyzed using logisitic regression (SAS Institute 1995).

Dose–Response to Synthetic Alarm Pheromone. Colonies of ≈6 late instars were treated with (*E*)-β-farnesene at concentrations of 1, 0.1, 0.01, 0.001, and 0.0001 mg ml^{-1}. Dosages were applied in a randomized order and 5 replicates were used for each dose. The experiment was performed 3 times. Logistic regression of the proportion of aphids responding in each group on the natural logarithm of dose was done and the concentration for 50% response (EC_{50}) was estimated (SAS Institute 1995).

Applications of Alarm Pheromone and *Paecilomyces fumosoroseus*. Suspensions of *P. fumosoroseus* conidia at a concentration of 4×10^7 conidia ml^{-1} were prepared as described by Vandenberg (1996). Five milliliters of the suspension were applied to barley (2-leaf stage) in an enclosed spray tower (Vandenberg 1996) either before or after either 1 μl of hexane or (*E*)-β-farnesene (1 mg ml^{-1}) had been applied near *D. noxia* colonies on the plants. Control plants were treated with 1 μl hexane or 1 mg ml^{-1} (*E*)-β-farnesene before being sprayed with 5 ml of 0.1% Tween 80. Conidial deposition on each replicate plant was estimated by sampling conidia deposited on plastic cover slips held in place near the plant canopy during the spray. The average dosage applied was 33 ± 5 (mean ± SD) conidia per square millimeter.

After spraying, barley plants were maintained at high humidity for 24 h and at ambient humidity (≈30–80%) thereafter. After 3 d, the number of living and infected late instars were counted on each plant. Living aphids were transferred to excised barley leaves embedded in 3% water agar and incubated 4 d before final assessment for mycosis (Vandenberg 1996). The experiment was done twice. ANOVA was used to determine the effect of treatment on arcsine-transformed percentage mycosis (SAS Institute 1990).

Table 1. Average (±SEM) response times for late instars of *D. noxia* exposed to one other aphid in an arena

D. noxia nymph exposed to	No. seconds to respond		
	Exp 1	Exp 2	Exp 3
Uninjured *D. noxia* nymph	180 ± 0a	180 ± 0a	130 ± 31a
Uninjured *D. persicae* nymph	180 ± 0a	180 ± 0a	180 ± 0a
Injured *D. noxia* nymph	13 ± 3b	85 ± 38b	151 ± 29a
Injured *M. persicae* nymph	21 ± 7b	116 ± 39b	31 ± 12b

Five aphids used for each treatment in each experiment. Untransformed means are shown; a $\log_{10}$ transformation was used for ANOVA. For Exp 1, $F = 61.0$; df = 3, 16; $P = 0.0001$; for exp 2, $F = 7.0$; df = 3, 16; $P = 0.003$; for exp 3, $F = 10.0$; df = 3, 16; $P = 0.001$. See text for combined ANOVA results. Means within a column followed by the same letter are not significantly different by the Tukey test ($P < 0.05$).

Results and Discussion

Diuraphis noxia nymphs responded to the presence of injured nymphs of *D. noxia* or *M. persicae* by leaving the test arena (Table 1). The response of nymphs to the nearby presence of injured aphids is presumably caused by release of alarm pheromone by injured aphids of either species. Fastest average response times were observed when *D. noxia* nymphs were challenged with injured *M. persicae* nymphs. *Diuraphis noxia* nymphs did not respond when living, undamaged *D. noxia* or *M. persicae* nymphs were placed in the arena. Both treatment and experiment, and the interaction between them, significantly affected the exit times among aphids (for treatment: $F = 37.1$; df = 3, 48; $P < 0.001$; for experiment: $F = 7.3$; df = 2, 48; $P < 0.01$; for the interaction: $F = 6.5$; df = 6, 48; $P < 0.001$). The significant experiment and interaction effects were caused by the lack of response of some *D. noxia* individuals to the presence of injured *D. noxia* in the 3rd experiment (Table 1). This may have been because of the difficulty in standardizing the infliction of injury. Late instars of *D. noxia* exited from arena tests more quickly when confronted with injured *M. persicae* nymphs than when confronted with injured conspecific individuals. This may indicate greater or more rapid production of alarm pheromone by *M. persicae.*

Significantly more late than early instars responded to application of synthetic (*E*)-β-farnesene ($\chi^2 = 71.6$, df = 2, $P < 0.001$; $n = 255$). For all 3 experiments, 54% of late-instar *D. noxia* responded versus only 4% of early instars. Responses did not vary among the 3 response times (30, 60, and 180 s). Late instars and adults of *D. noxia* responded equally to pheromone treatments ($\chi^2 = 9.8$, df = 2, $P > 0.1$; $n = 79$). For this experiment, 25% of adults responded versus 14% of late instars.

Logistic regression revealed that aphid responsiveness depended on pheromone dose for all 3 experiments (Table 2). For the 1st 2 experiments, responses to pheromone application were in close agreement; however, a significant lack-of-fit to the logistic model for data from the 3rd experiment ($\chi^2 = 13.6$, df = 3, $P < 0.01$) made estimation of EC_{50} confidence limits impossible. The percentage responding at the highest dosage of 1 mg ml^{-1} was 39, 42, and 37% for the 3 experiments.

This is the first report to demonstrate the activity of synthetic (*E*)-β-farnesene in eliciting behavioral re-

Table 2. Regression parameters for the repsonse of late instars of *D. noxia* to varying concentrations of synthetic (*E*)-β-farnesene

Exp.	No. aphids	Slope ± SE	EC_{50} (95% CL)	EC_{50} (mg ml^{-1})[a]	χ^2
1	155	−0.39 ± 0.09	0.39 (−0.88–2.92)	1.48	27.0
2	170	−0.23 ± 0.06	−0.06 (−1.77–3.76)	0.94	18.1
3	142	−0.36 ± 0.11	2.19 (–)[b]	8.95	15.4

[a] Back-transformed after analysis.
[b] Confidence limits not estimable because of lack-of-fit of data to the model.

ponse by *D. noxia.* Response to (*E*)-β-farnesene has been shown to vary according to aphid species. In this study, late instars of *D. noxia* challenged with 1 mg ml^{-1} (*E*)-β-farnesene gave responses of 25–55% depending on the test run. Dawson et al. (1982) discharged air saturated with (*E*)-β-farnesene over groups of aphids and observed >70% responses in individuals of *M. persicae; Nasonovia ribis-nigri* (Mosley), and *Aphis fabae* Scopoli. No individuals of *Brevicoryne brassicae* (L.) responded, whereas average responses for the cereal aphids *Sitobion avenae* (F.), *Rhopalosiphum padi* (L.), and *Metopolophium dirhodum* (Walker) were 31, 47, and 61%, respectively.

The combination of aphid inoculation with *P. fumosoroseus* and administration of (*E*)-β-farnesene did not result in increased fungal infection levels in the 2 experiments ($F = 1.3$; df = 3, 33; $P = 0.30$). The average percentage infection among the fungus treatments ranged from 21 to 62%. No infection was observed among hexane- or (*E*)-β-farnesene-treated controls. Hockland et al. (1986) showed increased fungal infection among *Aphis gossypii* (Glover) following sequential application of *Verticillium lecanii* spore suspensions and air saturated with (*E*)-β-farnesene. They concluded that aphid movement in response to the pheromone increased aphid exposure to fungus spores. We used 2 methods to combine pathogen and pheromone applications, varying the order of application of (*E*)-β-farnesene and *P. fumosoroseus,* but did not observe significant differences in infection compared with fungus-only controls. Perhaps the volatilized pheromone became dissipated too quickly to cause sufficient movement of aphids to ensure increased pick-up of fungal conidia.

Our study indicates *D. noxia* is responsive to synthetic or endogenous aphid alarm pheromone. Further investigations are necessary to determine whether *D. noxia* actively secretes alarm pheromones in response to disturbance or injury by natural enemies. The presence of injured aphids has been reported to affect feeding responses in *Lipaphis erysimi* (Kaltenbach) (Dawson et al. 1987). We did not test for altered feeding behaviors among apparently undisturbed aphids in the presence of injured aphids. Studies also are needed to explore the possible synergy between pheromone applications and other control agents (Smart et al. 1994, Furlong et al. 1995).

Acknowledgments

The authors thank J. McManus and L. Marriot for excellent technical assistance. Statistical advice was provided by L. Pritscher. J. Pell, B. Pye, and L. Smart and L. Wadhams contributed useful advice and discussions. J. Burd, C. Katsar, and J. Losey provided useful critical reviews of the manuscript.

References Cited

Anonymous. 1994. Economic impact of the Russian wheat aphid in the western United States: 1992–1993. Great Plains Agricultural Council Publication 152, Stillwater, OK.

Dawson, G. W., D. C. Griffiths, J. A. Pickett,. M. C. Smith, and C. M. Woodcock. 1982. Improved preparation of (*E*)-β-farnesene and its activity with economically important aphids. J. Chem. Ecol. 8: 1111–1117.

Dawson, G. W., D. C. Griffiths, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1987. Plant-derived synergists of alarm pheromone from turnip aphid, *Lipaphis (Hydaphis) erysimi* (Homoptera: Aphididae). J. Chem. Ecol. 13: 1663–1671.

Furlong, M. J., J. K. Pell, P. C. Ong, and A. R. Syed. 1995. Field and laboratory evaluation of a sex pheromone trap for the autodissemination of the fungal entomopathogen *Zoophthora radicans* (Entomophthorales) by the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Bull. Entomol. Res. 85: 331–337.

Griffiths, D. C., and J. A. Pickett. 1980. A potential application of aphid alarm pheromones. Entomol. Exp. Appl. 27: 199–201.

Halbert, S. E., L. E. Sandvol, R. L. Stoltz, and H. W. Homan. 1992. Russian wheat aphid. Univ. Idaho Curr. Inf. Ser. 817: 1–4.

Hockland, S. H., G. W. Dawson, D. C Griffiths, B. Marples, J. A. Pickett, and C. M. Woodcock. 1986. The use of aphid alarm pheromone (*E*)-β-farnesene to increase effectiveness of the entomophilic fungus *Verticillium lecanii* in controlling aphids on chrysanthemums under glass, p. 252. *In* R. A. Samson, J. M. Vlak, and R. Peters [eds.], Fundamental and applied aspects of invertebrate pathology. Society for Invertebrate Pathology, Wageningen, The Netherlands.

Pickett, J. A., L. J. Wadhams, and C. M. Woodcock. 1992. The chemical ecology of aphids. Annu. Rev. Entomol. 37: 67–90.

SAS Institute. 1990. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, NC.

SAS Institute. 1995. JMP statistics and graphics user's guide, version 3.1. SAS Institute, Cary, NC.

Smart, L. E., M. M. Blight, J. A. Pickett, and B. J. Pye. 1994. Development of field strategies incorporating semiochemicals for the control of the pea and bean weevil, *Sitona lineatus* L. Crop Prot. 13: 127–135.

Stoetzel, M. B. 1987. Information on and identification of *Diuraphis noxia* (Homoptera: Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. J. Econ. Entomol. 80: 696–704.

Vandenberg, J. D. 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against the Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 89: 1418–1423.

Received for publication 1 April 1999; accepted 15 June 1999.