Studies on the Scent of Stink Bugs (Hemiptera: Pentatomidae)
I. Alarm Pheromone Activity

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(Received February 25, 1974)

Existence of alarm pheromone in stink bugs was demonstrated. The scent discharged from the stink bugs for the purpose of defense against their enemies, made the other individuals of the same species alert and disperse. trans-2-Hexenal, one of the major components of the scent, had the same alarm effect. The activity was not species-specific among at least three species of Pentatomid bugs, Eurydema rugosa, E. pulchra and Nezara viridula.

INTRODUCTION

Existence of alarm pheromones in various hymenopterous and isopterous taxa is well known (Blum, 1968). There are, however, only a few reports on alarm pheromone in gregarious non-social arthropods. Calam and Youdeowei (1968) reported on larval defensive secretion of Pyrrohocorid, Dysdercus intermedius, which dispersed aggregated larvae. Apterous adults of the green peach aphid, Myzus persicae, were dispersed by scent of other individuals of the same species (Kislow and Edward, 1972).

Young instar larvae of many kinds of stink bugs show a strong tendency to aggregate and discharge a disagreeable scent composed of unsaturated aldehydes as the characteristic components when they are disturbed. This scent has a defensive effect against ants, and also disperse the aggregated larvae of the same species both in the field and in the laboratory.

Present report deals with the function of the scent as an alarm pheromone in three species of Pentatomid bugs, Eurydema rugosa, E. pulchra and Nezara viridula.

MATERIALS AND METHODS

Insects. First to third instar larvae of the cabbage bug, Eurydema rugosa were mostly used. They were reared on wild cruciferous plants such as Rorippa indica, R. pulsatia and Thlaspi arvense, at 23°C under 14 hr photoperiod. Other two species, E. pulchra and Nezara viridula were reared on cruciferous plants and sun-flower seeds, respectively, at 23°C under 15 hr photoperiod.

Extraction of scent. The scent of stink bugs was extracted with dichloromethane. The whole bodies of 200 larvae (2nd and 3rd instar) of each species were immersed in about 40 ml dichloromethane for two days, filtered, and washed with the same solvent. trans-2-Hexenal (Tokyo Kasei Co. Ltd., reagent grade), one of the major components of the stink bug scent was detected by gas chromatography.

1 Present address: Otsuka Pharmaceutical Co., Ltd., 463–10 Kagasuno, Kawauchi-cho, Tokushima, Japan
components of the scent discharged from the larvae of *Eurydema rugosa*, was also used as dichloromethane solution.

**Bioassay.** A sheet of filter paper (7 cm in diameter) was treated with 0.5 ml of dichloromethane solution of the body extract or commercial trans-2-hexenal, and the solvent was evaporated. The filter paper was put into a small glass vial (3 cm in diameter, 5 cm in depth). The air in the vial containing the scent of a given concentration was blown out with a rubber bulb toward each test individual on host plant in a glass jar. Number of individuals dropped from the host plant or those began to creep away was the criterion of the biological activity of the samples.

**Gas liquid chromatography and mass spectrometry.** The extracted scent from the whole bodies of the three species was analyzed with a gas chromatograph, F & M Model 402, equipped with a hydrogen flame ionization detector. The column used was 1.8 m × 3 mm glass tube packed with 15% PEGA on chromosorb W AW. Operating temperatures were 80°, 150° and 200°C for column oven, injection port and detector, respectively. The flow rates of the carrier gas (nitrogen), hydrogen and air were 30 ml/min, 30 ml/min and 250 ml/min, respectively. Mass spectra were obtained by Shimadzu LKB 9000 Gas chromatograph-mass spectrometer by using a column packed with 5% PEGA on chromosorb W AW at the temperature of 75°C. Each compound was identified by comparing with the gas chromatograms and the mass spectra of authentic compounds.

**RESULTS**

*Existence of alarm pheromone*

About 30 individuals of the 2nd and 3rd instar larvae of *Eurydema rugosa* were

<table>
<thead>
<tr>
<th>Instar</th>
<th>Scent</th>
<th>Air</th>
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<tr>
<td>1st</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
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<td>3rd</td>
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Fig. 1. Percent reaction of the each instar of *E. rugosa* to the air with or without the scent discharged from young instar larvae. Hollow area, no moving; dotted area, creeping away; solid area, falling.
confined in a glass vial. When they were shaken and disturbed, they discharged a scent which was considered to be a defensive secretion. The air containing the scent was blown out with a rubber bulb from the vial toward a test larva of *E. rugosa* on a host plant. The test larvae reacting to this scent fell from the host plant or crept away from the initial position. Fig. 1 shows percent reaction of each instar larva. About 80% individuals reacted by falling or creeping regardless of their instar. The number of individuals fallen to the ground decreased from the 2nd to the 5th instar.

*Reaction to the extracted scent of different concentrations*

A hundred ml dichloromethane containing the scent extracted from 200 larvae of *E. rugosa* was serially diluted with the same solvent. The amount of the scent in 0.5 ml of each solution was 1/1, 1/10, 1/100, 1/1,000 or 1/10,000 individual equivalent. The results of the blow-tests by using these scent sources are given in Fig. 2. The vertical bar shows the percentage of the total number of the 1st to 3rd instar larvae which fell and began to creep. Reaction of more than 80% was obtained by using the scent source of one tenth individual equivalent.

![Graph showing percent reaction of larvae to different concentrations of scent](image)

Fig. 2. Relationship between percent reaction of larvae (1st to 3rd instar) and the different concentrations of the scent extracted from the young instar larvae of *E. rugosa*.

*Major components of the extracted scent*

Fig. 3 shows gas chromatograms of the body extracts of the 2nd and 3rd instar larvae of the three species. Retention time of peak 1 corresponded with that of trans-2-hexenal. Peak 2 was identified as *n*-tridecane by gas chromatograms and mass spectra. Chromatograms of the extracts from *E. rugosa* and *E. pulchra* showed similar pattern but no peak of trans-2-hexenal was found in the chromatogram of *N. viridula*.

*Reactions to trans-2-hexenal*

A commercial trans-2-hexenal was diluted with dichloromethane to obtain 1, 0.1, 0.01, 0.001 and 0.0001% (w/v) solutions: the compound in 0.5 ml of each solution was 5,000, 500, 50, 5 and 0.5 µg, respectively. These scent sources
Fig. 3. Gas chromatographic separation of the scent components extracted from the whole bodies of the 2nd and 3rd instar larvae of *E. rugosa*, *E. pulchra* and *N. viridula*. Retention time of peak 1 corresponded with that of trans-2-hexenal. Peak 2 was identified as *n*-tridecane. 15% PEGA, 1.8 m, 80°C.

were tested by the same methods as previously mentioned after evaporating the solvent. Fig. 4 shows the reaction of the 1st to 3rd instar larvae of *E. rugosa* to trans-2-hexenal of different concentrations. More than 80% of the reaction was obtained only in the case in which the scent source contained 5,000 µg of trans-2-hexenal. On those of 500 and 50 µg solution, however, each percent reaction was 76 and 70%, respectively. In the lower concentrations, it was the same level as the reaction with acetic acid used for control.

Fig. 4. Percent reaction of the 1st to 3rd instar larvae of *E. rugosa* to the different concentrations of the commercial trans-2-hexenal (black column) and acetic acid (oblique line column.)
Alarm Pheromone of Stink Bugs

Reaction to the scent extracted from two other species of Pentatomid bugs

Each extracted scent of three species, Eurydema rugosa, E. pulchra and Nezara viridula was tested to the 2nd and 3rd instar larvae of each species. Percent reactions of the test insects to the scent of different concentrations are shown in Table 1. From these results, it was found the reactions to the scent of different species were hardly distinguished from that of the same species. Among these three species, the activity of the scent as an alarm pheromone was not species-specific.

Table 1. Percent Reaction of the First to Third Instar Larvae to Different Concentrations of the Scents Extracted from the Three Species of Pentatomidae

<table>
<thead>
<tr>
<th>Scent source</th>
<th>Insect tested</th>
<th>Scent equivalent</th>
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<tr>
<td></td>
<td></td>
<td>1/1</td>
</tr>
<tr>
<td>E. rugosa</td>
<td>E. rugosa</td>
<td>90%</td>
</tr>
<tr>
<td>E. pulchra</td>
<td>E. rugosa</td>
<td>89</td>
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<tr>
<td>N. viridula</td>
<td>E. rugosa</td>
<td>68</td>
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<tr>
<td>E. rugosa</td>
<td>E. pulchra</td>
<td>64</td>
</tr>
<tr>
<td>E. pulchra</td>
<td>E. pulchra</td>
<td>57</td>
</tr>
<tr>
<td>N. viridula</td>
<td>E. pulchra</td>
<td>55</td>
</tr>
<tr>
<td>E. rugosa</td>
<td>N. viridula</td>
<td>79</td>
</tr>
<tr>
<td>E. pulchra</td>
<td>N. viridula</td>
<td>79</td>
</tr>
<tr>
<td>N. viridula</td>
<td>N. viridula</td>
<td>98</td>
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DISCUSSION

The scent discharged from stink bugs has been considered as a defensive secretion because many kinds of ants were obviously repelled by the bugs and paralyzed by their scent (Waterhouse, 1961; Remold, 1962). On the other hand, some predacious animals and insects, e.g., hens, rats, lizards, mantis, spiders and carabid beetles, eat certain species of bugs without hesitation in the laboratory. There is, in these facts, a possibility that the scent has some other biological functions.

Most chemical defensive secretions discharged from insects cannot be considered as a pheromone because they do not affect the behaviour of any other individuals of their own species (Butler, 1967). However, some defensive secretions, e.g., formic acid, released from the worker ants of Formica fusca against their enemies make the other individuals of the same colony alert. Therefore, they are regarded as pheromone (Gabra and Pavan, 1970).

Present results shown in Fig. 2 indicate that one or more components of the scent extracted from the larvae of Eurydema rugosa are apparently “alarm pheromone”. trans-2-Hexenal, major component of the scent, has an alarm effect, though the percent reaction of the bug on this compound is obviously lower than on the scent extracted from the whole bodies of E. rugosa. This phenomenon indicates that the scent extracted with dichloromethane contains some additional components of alarm pheromone or a synergist of trans-2-hexenal.

Caliam and Youdeowei (1968) showed that trans-2-hexenal and some other aldehydes, scent components of 5th instar larvae of Dysdercus intermedius, had alarm effect to both larvae and adults. The present experiments also indicate that the same unsaturated aldehyde produces a non species-specific alarm effect against the larvae of
at least three species of Pentatomidae. Bower et al. (1972) reported that trans-β-farnesene, an alarm pheromone, was broadly interspecific in aphids. Similar examples were also in sex pheromone. cis-11-Tetradecen-1-ol acetate was reported as a female sex pheromone in common with 5 species of Tortricidae and Noctuidae (Jacobson, 1972; Tamaki, 1972; Comeau and Roelofs, 1973).

Insects are elaborately differentiating species and are generally considered as one of the most prosperous creatures on earth. It is the undisputed fact, however, that most of them have been eaten by many carnivorous animals. They had evolved the numerous means for survival. Among them, the defensive mechanisms against their enemies must have been indispensable. Though the scent of stink bugs is discharged for the purpose of defense against their predators, the effect is imperfect (Ishiwatari, unpublished data). However, the same scent, mainly unsaturated aldehydes, makes the other members of the same species near the predators disperse. Therefore, the scent discharged from stink bugs is considered to be an alarm pheromone for other members of the same species rather than a defensive secretion against their enemies.

ACKNOWLEDGEMENTS

The author wishes to express his thanks to Associated Professor T. Ichinose of Tokyo University of Agriculture and Technology for valuable criticism and encouragement. Thanks are also due to Dr. Y. Tamaki of National Institute of Agricultural Sciences and Dr. C. Hirano of Kochi University for the discussion of the problems.

REFERENCES