
I. Presence of Arresting Stimulants Produced by the Host Larvae

Junji TAKABAYASHI, Takashi NODA1 and Shozo TAKAHASHI2

*Pesticide Research Institute, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan*

(Received May 21, 1985)

General sequence of the host finding behavior of female *Apanteles kariyai* WATANABE was observed. When a female *A. kariyai* found either feeding traces, exuviae or frass of a host larvae on a corn leaf, she exhibited an intense antennal contact behavior toward these materials. Chemical properties of the arresting stimuli for *A. kariyai* produced by the host larvae were investigated. Bioassay using a paper disc impregnated with extract from the exuviae or frass showed that the arresting stimuli was present in the material derived from the host larvae. Secretion left on the feeding traces was also tested for its arresting activity. The extracts from three different sources were separately purified by column chromatography under the same condition and the arrestance appeared to have the same chemical properties. Presence of the same arrestance in the feeding trace, frass and exuviae of *P. separata* larvae is of particular interest.

INTRODUCTION

The braconid wasp *Apanteles kariyai* WATANABE is a gregarious parasitoid of the common armyworm *Pseudaletia separata* WALKER. Although the ecology of this wasp is not yet clear, it seems to have an active flight behavior in the daytime during which it searches for and attacks a host (SATO et al., 1983). *A. kariyai* showed a sequential ovipositional behavior in the laboratory. During random walking in a test tube, she found host larvae by antennal contact; then she immediately inserted her ovipositor and deposited eggs inside the host. When she encountered a feeding trace left by a host larva on a plant, or exuviae or frass, her walking was immediately arrested.

The presence of kairomonal stimuli in the host habitat is a critical factor in the parasitoid’s host-finding behavior and many kairomones are recognized. JONES et al. (1971) reported that *Microplitis croceipes* GREEN, a braconid parasitoid of *Heliothis zea* BODDIE responded to frass of the host and identified 13-methylhentriacontane as a kairo-

---

1 Present address: Division of Entomology, National Institute of Agro-Environmental Sciences, Yatabe, Tsukuba, Ibaraki 305, Japan
2 Please address reprint requests to S. T.
mone from the frass extract. A novel class of compounds in the larval mandibular gland of the phycitid moth was identified as a kairomone eliciting ovipositional response in the ichneumonid parasitoid Venturia cannesens (Grav.) (Mudd and Corbet, 1973, 1982; Mudd, 1978, 1981). The kairomone components were also identified in the frass of Plodia interpunctella (Hubner), a phycitid moth (Kuwahara et al., 1983).

In this paper, we report the arresting stimuli for A. kariyai which are present in the buccal secretion, exuviae and frass of the host larvae. The active compounds were purified by monitoring the behavior of A. kariyai at the time of arrest.

MATERIALS AND METHODS

Insect. P. separata used in this study was supplied from stock cultures kept at Take-da Chemical Industries, Ltd., and reared successively in our laboratory on either corn leaves or artificial diet under the condition of 26±2°C and 17L-7D. A. kariyai were descendants of those collected in Kanoya, Kagoshima and reared in the Department of Zoology, Faculty of Science, Kyoto University. The 4th- to 5th-instar larvae of P. separata were offered to A. kariyai for their oviposition and emerged wasps were kept in a test tube (22 mm diameter, 200 mm long) with a small piece of paper towel moistened with a 50% aqueous honey solution as food. One day after emergence, females were separated from males and kept at 10°C until bioassay.

Bioassay. Female A. kariyai at least 2 days old were kept at 26°C for more than 1 hr before bioassay and confined one to a petri dish (50 mm diameter) with the test material. A filter paper disc (6 mm diameter) was impregnated with a test solution and offered to each female. The number of arresting responses following antennal contact with the paper disc was recorded. A blank test was carried out with a paper disc soaked in solvent.

Extraction of arrestants. For extraction of the arrestants, exuviae and frass were separately collected and soaked in hexane. P. separata larvae often bit the paper towels covering the artificial diet in the rearing containers, leaving a buccal secretion. This secretion was extracted with hexane from the paper towels.

Isolation of arrestants. Isolation of the arrestants from the extracts of the various sources was monitored by the antennal contact response. Exuviae (22.4 g) of the host larvae were soaked in hexane for more than 1 day. The hexane extract (180 mg) was chromatographed on a silicic acid column (Poligosil, 40 g) and eluted with 1,200 ml each of hexane, benzene, ether and methanol. The active eluate was chromatographed on a gel permeation column (TSK G1000H₈+TSK G2000H₈+TSK G2000H₁₀) with chloroform. Retention time of the active peak was about 33 min (flow rate 1 ml/min) and the fraction was further purified using high performance liquid chromatography (HPLC: Nucleosil 8 mm φ × 300 mm long) with 0.2% ether in hexane. A peak appearing at 10 min (flow rate 2 ml/min) was active on the bioassay. The isolated arrestant (5 mg) was analyzed by gas liquid chromatography on OV-1. The extracts from the buccal secretion and frass were purified under the same chromatographic conditions as above.
RESULTS AND DISCUSSION

Host finding behavior

The general sequence of the host finding behavior of *A. kariyai* was described by Sato et al. (1983) and we confirmed their observations. *A. kariyai* recognized a host habitat through antennal perception of chemical cues present in the feeding traces on host plants, exuviae and frass. When *A. kariyai* found a feeding trace left by a host larvae, she showed intensive antennal contact to the fed-on edge (Fig. 1-a). This characteristic behavior was also elicited when she found exuvium and frass (Fig. 1-b,c). After the antennal contact, she walked around the site and began host searching. This arresting behavior was an orthokinetic response involving stopping, slowed walking and probing with her antennae. When she found the host larvae, she immediately oviposited (Fig. 1-d). Arresting behavior was also elicited by a filter paper disc impregnated with crude extract of buccal secretion, exuviae and frass, indicating the presence of arrestant in these materials.

![Fig. 1. Host searching behavior of *A. kariyai*. a: antennal contact to the feeding trace by a host larva. b: antennal contact to the exuvium. c: antennal contact to the frass. d: insertion of ovipositor.](image)

![Fig. 2. Antennal contact to a paper disc impregnated with extract.](image)
Bioassay of arrestance

The antennal contact behavior of *A. kariyai* to the paper disc impregnated with the arrestance is shown in Fig. 2; this characteristic was used as a criterion of the bioassay.

The crude extract from exuviae was dissolved in hexane to make a standard solution

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>µg/disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10²</td>
</tr>
<tr>
<td>Exuvium</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Buccal secretion</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Frass</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>µg/disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Exuvium</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Buccal secretion</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Frass</td>
<td>20</td>
<td>70</td>
</tr>
</tbody>
</table>

Fig. 3. GLC (OV-1, 250–300°C, 4°C/min) pattern of each extract after purification by HPLC (Nucleosil).
of 100 μg/ml. One milliliter of the solution contained about one insect equivalent extract. Solutions of the same concentration as above were prepared from the crude extract of buccal secretion and frass. Bioassay results of the crude extracts from the three different sources are shown in Table 1. The difference in activity was due mainly to the quantity of arrenant in a crude extract.

Isolation of arrenants

The extracts from buccal secretion, exuviae and frass were successively purified by column chromatography under the same condition and showed the same chromatographic properties. The arrenants obtained from three crude extracts of buccal secretion (2 g), exuviae (0.18 g) and frass (5 g) were 50 mg, 5 mg and 20 mg respectively. Bioassay results of the arrenant isolated from the three extracts (Table 2) showed similar activity. Impregnation of 0.01 μg of each arrenant on to a paper disc elicited 75–80% response of A. kariyai. Above that amount per disc, the response was not increased accordingly. The arrenant isolated by HPLC (Nucleosil) showed a single spot on TLC (Rf 0.43) developed with 1% ether in hexane. Gas liquid chromatography (GLC) of the arrenant from all these solutions showed a similar pattern on OV-1 (Fig. 3). The number of major components was the same but differed in composition according to the extraction source. Peaks of the arrenant appearing on GLC suggest that it consists of a series of homologs.

It is very interesting that the same arrenant was present in the buccal secretion, exuviae and frass of P. separata larvae regardless of whether the larvae had fed on corn leaves or artificial diet. Although the presence of kairomones in the larval mandibular gland of the phycitid moth was reported (Corbet, 1971; Mudd, 1981; Mudd and Corbet, 1973, 1982), the glands of buccal secretion of P. separata larvae have not yet been identified. Buccal secretion obtained at seizure of the larval body with forceps was probably reflex vomit from the larval gut and was shown to be inactive at the bioassay.

Chemical cues are important in the host finding behavior of A. kariyai. When a female A. kariyai perceived a feeding trace by a host larvae through antennal contact, her movement was arrested. The arrenant is also present in cuticle of the host larvae and frass and keeps A. kariyai in the host habitat. A. kariyai responded to the arrenant on a paper disc by characteristic antennal contact behavior. Since ovipositor probing was elicited with the fresh exuvium, there must be other stimulant(s) on the cuticle of the host larvae. Upon recognition of an intact host larva, A. kariyai immediately ovi-posed.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. Yasuo Sato, Takeda Chemical Industry, Ltd., for supplying the P. separata. They also wish to express their gratitude to Dr. Yoshibumi Sato and Mr. T. Tanaka, Department of Zoology, Faculty of Science, Kyoto University who supplied the A. kariyai.

REFERENCES


