Host Specificity of the Egg-Larval Parasitoid, *Ascogaster reticulatus* WATANABE (Hymenoptera: Braconidae) among Five Torticid and One Noctuid Species

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*Ascogaster reticulatus* WATANABE (Braconidae) is an egg-larval parasitoid of certain tortricids, e.g., *Adoxophyes* sp. (Ad) and *Adoxophyes orana fasciata* WALSINGHAM (Ao). In this study, the host preference by *A. reticulatus* was tested among two habitual hosts, Ad and Ao, and three unusual host species, *Pandemis hoparana* DENIS et SCHIFFERMÜLLER (Ph), *Homona magnanima* DIAKONOFF (Hm), and *Hoshinoa longicellana* WALSINGHAM (Hl). The two-choice tests showed that the order of oviposition preference among the five tortricids was Ad = Ao > Hm > Hl > Ph. Female parasitoids deposited eggs in all of the tortricid egg masses. Among parasitized egg masses of the unusual host species (Ph, Hm and Hl) artificially reared, parasitoid larvae emerged only from Ph larvae, made cocoons and then emerged. Parasitoid eggs hatched in Hm and Hl eggs and became 1st-instar larvae, then grew after the host eggs hatched. However, the parasitoid 1st-instar larvae were encapsulated by the hemocytes of these host larvae of later instars. The Hm egg mass coated with the Ad egg mass extract containing the kairomone increased oviposition time and percent parasitism by *A. reticulatus*. To the noctuid moth, *Leucania separata* (Walker) (Ls), the parasitoid showed no response to the egg mass, but coating of the kairomone on the egg mass stimulated oviposition. The parasitoid eggs hatched in Ls eggs, but the 1st-instar larvae were soon encapsulated with melanin on their surface.

INTRODUCTION

The braconid parasitoid, *Ascogaster reticulatus* WATANABE is a solitary, egg-larval endoparasitoid of the smaller tea tortrix (STT) moth, *Adoxophyes* sp. (Torticidae). The 3rd-instar parasitoid larva egresses from the 4th-instar host larva, feeds on it externally, and makes a cocoon (KAWAKAMI, 1985).

On contact with the STT egg mass, a female *A. reticulatus* responds to a chemical(s) on the egg mass, and the antennal-host-searching and ovipositor-host-searching behavior is sequentially provoked (KAINOH et al., 1982). The chemical(s) (kairomone) is watersoluble, heat stable and consists of at least two active compounds of different molecular weight (KAINOH et al., 1982).

Seven tortricid species were described for the host range of *A. reticulatus* (WATANABE,
1967; Kamiyo, 1973; Takagi, 1974). However, three tortricid species used in this study, *Pandemis heparana* Denis et Schiffermüller, *Homona magnanima* Diakonoff, and *Hoshinoa longicellana* Walsingham have not yet been recorded as habitual hosts.

In this study, both the host specificity of *A. reticulatus* among five tortricid and one noctuid species and the role of the kairomone in host selection were analyzed with much attention given to the following steps: host acceptance, host suitability, that is, the latter part of the four steps in the host selection process (Doutt, 1959).

**MATERIALS AND METHODS**

*Insects.* *A. reticulatus* adults were collected at Kanaya in Shizuoka Prefecture and reared by the method of Kainoh and Tamaki (1982) and Kawakami (1983).

Five tortricid species, i.e., *Adoxophyes* sp. (Ad), *Adoxophyes orana* fasciata Walsingham (Ao), *Pandemis heparana* (Ph), *Homona magnanima* (Hm) and *Hoshinoa longicellana* (Hl), were obtained from the stock culture maintained in the National Institute of Agro-Environmental Sciences and reared on common rearing medium by the method of Tamaki (1966); the egg masses were collected on wax paper on each oviposition day to standardize their age. The noctuid moth, *Leucania separata* (Walker) (Ls), was obtained from the stock culture in the Tokyo University of Agriculture and Technology and the egg masses were collected.

The rearing and experiments were all done under 24±1°C, 16L–8D photoperiodic conditions.

*Oviposition preference test.* Oviposition preference of *A. reticulatus* was tested among the egg masses of *Adoxophyes* sp. and four other tortricid species. The 3-day-old egg masses of two different species were arranged in a 25-ml sample vial and a 2- to 4-day-old female parasitoid was introduced. The response of the parasitoid, i.e., ovipositor-host-searching, to each of these egg masses was observed for less than 30 sec.

*Dissection of parasitized tortricid eggs.* Each 1-day-old egg mass of five tortricids was presented to a 2- to 4-day-old parasitoid in a 15-ml sample vial and it was allowed to oviposit for 1 to 2 hr. The egg mass was separated when a parasitoid became indifferent to it, and each egg was dissected under a dark-field stereomicroscope (10×–50×) when the egg mass darkened. Percent parasitism (P) of an egg mass was determined as:

\[
P(\%) = \frac{\text{No. of parasitized eggs}}{\text{No. of eggs in an egg mass}} \times 100
\]

*Dissection of parasitized unusual host larvae.* The parasitized egg masses of Ph, Hm and Hl were reared, and the parasitoid development and egression were observed at arbitrary stages of unusual host larvae.

*Extraction of kairomone.* Crude extract of the STT (Ad) egg masses was obtained by the method of Kainoh et al. (1982). The egg masses were immersed in 70% ethanol for about a month at 24°C, and the extract was filtrated and concentrated.

*Effect of kairomone coating on unusual host egg masses.* Five microliters of concentrated egg mass extract [1.48×10⁻¹ or 1.72×10⁻¹ EME (egg mass equivalent)], which is effective in eliciting almost 100% response of ovipositor-host-searching behavior (Kainoh et al., 1982), was applied on the egg masses of Hm and Ls and air-dried at room temperature. A female parasitoid was introduced into a 15-ml sample vial containing the
egg mass and was allowed to oviposit. In the case of Hm, the oviposition time, i.e., from the beginning of ovipositor-host-searching to the moment when the parasitoid left the egg mass, was recorded. Then, those parasitized egg masses (Hm and Ls) were dissected, and the parasitoid development was observed under a dark-field stereomicroscope (10×–50×).

RESULTS

Oviposition preference test

The parasitoid responded to the egg masses of tortricid species, i.e., Ph, Hm and HL, by showing both the antennal-host-searching and ovipositor-host-searching behavior observed in the case of Ad and Ao. In selection tests of two egg masses, those of Ad and Ao were the most preferred among four tortricids (Table 1). The Hm egg mass was preferred to HL, but 24% of the parasitoids responded only by antennal-host-searching and no ovipositor-host-searching was observed on either of these egg masses (Table 1).

Parasitism among five tortricids

Parasitoid eggs were found in the egg masses of all tortricid species, i.e., Ad, Ao, Ph, Hm and HL. Percent parasitism is shown in Table 2. While the number of host eggs in each species of egg mass differed, the highest parasitism (≥80%) was obtained in the habitual host egg masses (Ad and Ao). Among other tortricid species, parasitism in HL was the lowest (6.3%).

Parasitoid development in three tortricids

Among parasitized egg masses of the unusual host species (Ph, Hm and HL), parasitoid larvae egressed only from Ph larvae, made cocoons and emerged. These female parasitoids could normally oviposit on Ad egg masses. No parasitoids egressed from Hm or HL larvae. Parasitoid eggs hatched in Hm eggs, and the 1st-instar parasitoid larvae grew after hatching of Hm eggs; however, this growth was retarded compared

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>No. parasitoids used</th>
<th>Percent ovipositional response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ad</td>
</tr>
<tr>
<td>I</td>
<td>76</td>
<td>50.0</td>
</tr>
<tr>
<td>II</td>
<td>98</td>
<td>99.0</td>
</tr>
<tr>
<td>III</td>
<td>87</td>
<td>86.2</td>
</tr>
<tr>
<td>IV</td>
<td>90</td>
<td>92.2</td>
</tr>
<tr>
<td>V</td>
<td>74</td>
<td>—</td>
</tr>
</tbody>
</table>

a Three-day-old egg masses of two different species were presented to a 2- to 4-day-old female parasitoid in a 25-ml sample vial.

b Ad: Adoxophyes sp., Ao: Adoxophyes orana fasciata, Ph: Pandemis heparana, Hm: Homona magnanima, HL: Hoshinoa longicellana.

c Only antennal-host-searching behavior was observed.
Host Specificity of *Ascogaster reticulatus*

Table 2. Comparison of the rate of parasitism among 5 tortricid egg massesa

<table>
<thead>
<tr>
<th>Egg mass species</th>
<th>No. egg masses used</th>
<th>Percent parasitism in an egg massb</th>
<th>Mean no. eggmass/egg mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad</td>
<td>13</td>
<td>88.6±0.9 a</td>
<td>88.0</td>
</tr>
<tr>
<td>Ao</td>
<td>12</td>
<td>83.7±0.7 a</td>
<td>111.6</td>
</tr>
<tr>
<td>Ph</td>
<td>9</td>
<td>59.1±4.2 c</td>
<td>70.7</td>
</tr>
<tr>
<td>Hm</td>
<td>10</td>
<td>43.5±3.2 d</td>
<td>124.4</td>
</tr>
<tr>
<td>HI</td>
<td>8</td>
<td>6.3±1.5 e</td>
<td>111.5</td>
</tr>
<tr>
<td>Hm + kairomonec</td>
<td>10</td>
<td>66.8±2.8 b</td>
<td>124.3</td>
</tr>
</tbody>
</table>

*a* Each 1-day-old egg mass was presented to a 2- to 4-day-old female parasitoid for 1 to 2 hr.

*b* Mean±S.E. Values followed by the same letters are not significantly different from each other at the 5% level by Duncan's new multiple range test.

c Crude Ad egg mass extract: $1.48 \times 10^{-1}$ or $1.72 \times 10^{-1}$ EME/egg mass.

![200 μm](image1)

Fig. 1. First-instar larva of *A. reticulatus* partially encapsulated by the hemocytes of the 3rd-instar larva of *H. magnanima*.

![2 mm](image2)

Fig. 2. Oviposition of *A. reticulatus* on *L. separata* egg mass coated with the crude extract of *Adoxophyes* sp. egg masses.

Table 3. Oviposition time of *A. reticulatus* on *H. magnanima* egg mass coated with *Adoxophyes* sp. kairomonea

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. parasitoids used</th>
<th>Oviposition timeb</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>With kairomonec</td>
<td>22</td>
<td>39.5±1.9</td>
<td></td>
</tr>
<tr>
<td>Without kairomone</td>
<td>21</td>
<td>21.5±1.8</td>
<td>$t=6.81$</td>
</tr>
</tbody>
</table>

*a* Each 1-day-old egg mass was presented to a 2-day-old female parasitoid.

*b* Mean±S.E. Recorded from the start of ovipositor-host-searching until an egg mass was left.

c Crude Ad egg mass extract: $1.48 \times 10^{-1}$ or $1.72 \times 10^{-1}$ EME/Hm egg mass.

with Ad. In the late 2nd-instar or early 3rd-instar larvae of Hm, the 1st-instar parasitoid larvae were encapsulated by host hemocytes (Fig. 1). No parasitoid larvae were found in Hm larvae of later instars. Also in HI larvae, several observations showed the parasitoid larvae to be encapsulated. These results are summarized in Table 4.
Table 4. Comparison of ovipositional responses and development of *A. reticulatus* among host and unusual host species

<table>
<thead>
<tr>
<th>Species</th>
<th>Antennal- and ovipositor-host-searching</th>
<th>Egg deposition</th>
<th>Parasitoid development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Ao</td>
<td>+</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Pa</td>
<td>+</td>
<td>+</td>
<td>+ a</td>
</tr>
<tr>
<td>Hm</td>
<td>+</td>
<td>+</td>
<td>+ a</td>
</tr>
<tr>
<td>Hi</td>
<td>+</td>
<td>+</td>
<td>+ a</td>
</tr>
<tr>
<td>Ls</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ls</td>
<td>+</td>
<td>+</td>
<td>+ e</td>
</tr>
</tbody>
</table>

* a Parasitoid larval development was retarded and the 1st-instar parasitoid larvae were encapsulated before the molting from the 3rd- to 4th-instar turgidic larvae.
* b Crude Ad egg mass extract: \(1.72 \times 10^{-1}\) EME/egg mass.
* c Larvae were encapsulated immediately after hatching.

Fig. 3. Encapsulated 1st-instar larva of *A. reticulatus* soon after hatching in *L. separata* egg.

**Effect of kairomone coating on unusual host egg masses**

Kairomone coating on an Hm egg mass increased oviposition time (Table 3) as well as percent parasitism (Table 2) by *A. reticulatus*. Moreover, kairomone coating on an Ls egg mass stimulated the antennal-host-searching and ovipositor-host-searching behavior (Fig. 2; Table 4), while no response was shown to an untreated Ls egg mass. The parasitoid eggs were found in those eggs by dissection of the treated Ls egg mass. The parasitoid eggs hatched in Ls eggs, but the 1st-instar larvae of the parasitoid were encapsulated with melanin (Fig. 3).

**DISCUSSION**

The oviposition preference test among five turgidic species (Table 1) and the oviposition experiment on each egg mass (Table 2) suggested that a common chemical(s) (kairomone) might exist on those turgidic egg masses, or that *A. reticulatus* might respond...
to a certain group of chemicals variously existing on those egg masses. If a common active chemical(s) does exist on these egg masses, the amount of the chemical(s) on Ph, Hm and HI is rather less than on Ad and Ao. Otherwise, a certain repellent or masking agent(s) may occur in the unusual host species. From the results of the oviposition preference (Table 1), percent parasitism (Table 2), and also the taxonomic viewpoint (Honma, 1972, 1973), Ad and Ao may possess a common kairomone for *A. reticulatus*. The kairomone in Ad egg masses is water-soluble and consists of at least two active compounds (Kainoh et al., 1982). Its chemical properties must be further analyzed in relation to active material(s) contained in other tortricid egg masses.

From the comparison of percent parasitism among the egg masses of five tortricids (Table 2) and the number of parasitized eggs in each egg mass, the order of oviposition intensity was Ad = Ao > Ph ≈ Hm > HI, and the order of oviposition preference (Table 1) was Ad = Ao > Hm > HI > Ph. These orders coincided except for Ph. The results suggested that egg deposition was elicited not only by stimuli on the surface of the egg mass, but also by chemical and/or physical stimuli inside the eggs. The stimuli for the oviposition by *A. reticulatus* may be more defined in Ph eggs than in HI eggs. For the Ls egg mass coated with the kairomone, deposition of the parasitoid eggs may indicate the presence of suitable stimuli in these eggs. A similar effect of kairomone on the oviposition behavior of *Cardiochiles nigriceps* was also reported by Vinson (1975); the treatment of potential host larvae with parasite-searching stimulant activated the ovipositional response and egg deposition of *C. nigriceps*. Moreover, several amino acids and MgCl₂ in the pupal hemolymph of *Galleria mellonella* are reported to stimulate oviposition of *Iproctis conquisitor* (Arthur et al., 1972; Hegedekar and Arthur, 1973).

Also, in *Trichogramma pretiosum*, K⁺, Mg²⁺, Cl⁻ and SO₄²⁻ in artificial eggs stimulate oviposition (Nettles et al., 1982, 1983). In the case of *A. reticulatus*, common oviposition stimulant(s) inside the eggs may exist among both habitual and unusual hosts.

From the oviposition experiments and rearing of the parasitized Ph egg mass, it is possible that this species could become a host under at least laboratory conditions. However, it is unknown whether or not Ph is an actual host for *A. reticulatus* in the field. Other egg-larval parasitoids such as *Chelonus* spp. show a specific oviposition preference for the major host species compared with other host species (Broodryk, 1969; Hafez et al., 1977; Rechav, 1978). In this sense, the egg-larval parasitoids, as in *A. reticulatus* and *Chelonus* spp., may have a limited host range and may also show specific preference among those hosts.

Although the parasitoid eggs hatched and grew in Hm and HI larvae, they were soon encapsulated (Fig. 1; Table 4). In contrast, they were encapsulated in Ls eggs soon after hatching (Fig. 3; Table 4). The different degree of encapsulation among the unusual hosts may explain the host specificity at the final level of the host selection process in *A. reticulatus*. Thus, a study of interactions between *A. reticulatus* and these tortricid and noctuid species could further elucidate host-suitalbility, immune response of hosts, and the mechanism(s) of inhibition of the host response by the parasitoid.

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