Role of kairomones from host accessory gland secretion in host recognition by *Telenomus remus* and *Trichogramma pretiosum*, with partial characterization

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Abstract

Accessory gland materials from *Spodoptera frugiperda* (J. E. Smith) and *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) contained kairomones that influenced the host recognition or acceptance behavior of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), respectively. Material from *S. frugiperda* accessory glands stimulated ovipositor probing and drilling by female *T. remus* but not *T. pretiosum*. Accessory gland material from *H. zea* stimulated ovipositor probing and drilling by female *T. pretiosum* but not *T. remus*. An active material for *T. remus* is found in the 700 K protein fraction of accessory gland material from *S. frugiperda*. The oviposition behavior of the parasitoids is also discussed.

Introduction

Involvement of kairomones in various stages of the host selection process of parasitoids is well documented (Nordlund et al., 1981; Vinson, 1976). Furthermore, parasitoids often use different kairomones at various stages of the host selection sequence (Lewis et al., 1976; Nordlund et al., 1977; Noldus & van Lenteren, 1985a, b) such that these chemicals may serve as long distance attractants, searching stimulants, or in stimulating host recognition and host acceptance.

*Telenomus remus*, a scelionid egg parasitoid indigenous to Indonesia, was introduced into Israel for control of *Spodoptera littoralis* Boisd. (Gerling, 1972). It has also been successfully established in Barbados and Montserrat on several *Spodoptera* species (Wojcik et al., 1976). Females respond to extracts of the abdominal tips and sex pheromone components of *Spodoptera frugiperda* with increased host searching behavior (Nordlund et al., 1983).

Wojcik et al., (1976) conducted laboratory studies on eggs of 39 species of Lepidoptera and found that eleven noctuid species and one pyralid species were suitable hosts for *T. remus*. Based on parasitoid emergence rates, species of *Spodoptera* were more suitable as hosts than the other species.
Heliothis zea, Feltia subteranea (F.) and two Elaphria spp. also had high parasitoid emergence rates, but lower than Spodoptera. Despite the fact that Wojcik et al. (1976) found that H. zea eggs are suitable hosts for T. remus and that they have been used for rearing this parasitoid, H. zea is not a host that would normally be attacked in nature. We found that the T. remus females would stand on one egg while ovipositing in an adjacent egg in an egg mass (Fig. 1). Single eggs, such as those deposited by H. zea, elicited little or no response from the parasitoid.

Trichogramma pretiosum is one of the most common native species of Trichogramma in agro-ecosystems in the southern United States (Pinto et al., 1978; Lopez et al., 1982; and Hung et al., 1985). Like other species of Trichogramma, it is polyphagous and has been reared from eggs of seven genera of Lepidoptera (Hung et al., 1985 and references therein). Components of the sex pheromone and moth scales of H. zea have been shown to increase rates of parasitism by T. pretiosum (Lewis et al., 1982; Lewis et al., 1979). Nordlund et al. (1977) found that stimuli from several sources were involved in the differential response of T. pretiosum to the eggs of H. zea and Trichoplusia ni (Hb.), and suggested that the accessory gland secretion of the moth was involved. T. pretiosum generally attacks individual eggs and, although it attacks a large number of host species, S. frugiperda egg masses are not normally attacked (Ashley, 1979). T. pretiosum females stand on the egg into which they are ovipositing.

Strand & Vinson (1982, 1983a) reported that the accessory gland secretion of Heliothis virescens (F.) serves as an egg adhesive, and that components of this secretion are responsible for host recognition by Telenomus heliothidis Ashmead. In addition, Strand & Vinson (1983a) reported that the accessory gland proteins of H. virescens and H. zea, another host of T. heliothidis, are electrophoretically similar where as the accessory gland proteins of the nonhost S. frugiperda are different. They suggested that these differences may be partially responsible for the inability of T. heliothidis to recognize the eggs of S. frugiperda and that accessory gland secretions may influence host recognition and preferences by other egg parasitoids (Strand & Vinson, 1983a, c).

The purpose of this study was to examine the responses of T. remus and T. pretiosum to the accessory gland secretions of S. frugiperda and H. zea and to partially characterize any active material.

**Materials and methods**

*T. remus* and *T. pretiosum* were reared in eggs of *H. zea*, at ca. 70% r. h. and 26°C, using the method described by Lewis & Redlinger (1969). The *H. zea* eggs used for rearing were processed with a sodium hypochlorite wash, as described by Burton (1969) and irradiated with ca. 25 Krad (60Co source) when 8–36 h old. The parasitoids were used in the bioassays when 1–2 days old. Prior to their use, the female *T. remus* were exposed to fresh egg masses of *S. frugiperda* and observed to drill. Female *T. pretiosum* were exposed to *H. zea* eggs, which had been processed as described above and also observed to drill.

The *H. zea* used here were obtained from a
laboratory culture maintained according to the procedures of Burton (1969) where as the S. frugiperda were maintained according to the procedures described by Perkins (1979).

Accessory gland materials (AGM) were prepared by dissecting the glands and the accessory gland reservoir from 48–96 h old moths, under saline. The glands and reservoirs from 100 females were placed in 1 ml of distilled water and sonified for 3 min at 50% pulsed with a Bronson W-350 sonifier. The AGM was stored at -10°C when not in use.

Bioassays were conducted in depression slides. Observations were made with the aid of a stereo microscope and a stop watch. In bioassays involving T. remus, four glass beads (0.45–0.50 mm in diameter) were attached to the bottom of the depression with a small amount of Plantgard® (Polymetrics International, New York, N. Y.). Treated bead masses were prepared by applying one female equivalent AGM to the beads with a 10 μl pipet and allowing it to dry before presentation to the parasitoid. For bioassays involving T. pretiosum, a single 0.45–0.50 mm diameter glass bead was attached to the bottom of the depression with a small amount of Plantgard. Beads were treated with 0.2 female equivalents of AGM with a 2 μl pipet. Untreated bead masses or individual beads were used as controls.

One female parasitoid was released into the depression and her first contact with a bead mass or a bead, depending on the species, was scored using a method similar to that of Strand & Vinson (1982), as follows:

Rejection – touched bead mass or bead with antennae but remained in contact for less than 5 s; Examination – remained in contact for more than 5 s and conducted antennal examination; Recognition – conducted antennal examination and attempted to drill with ovipositor.

A total of 40 female parasitoids were exposed to treated bead masses or beads and 40 additional females, from the same rearing tube and at approximately the same time, were exposed to untreated control bead masses or individual beads for each of the test combinations.

Previous study (Strand & Vinson, 1983a) had shown that accessory gland proteins from H. zea had molecular weights of ca. 1100 K and 50 K, and the accessory gland proteins from S. frugiperda had molecular weights of ca. 700 K and 30 K. To determine whether any of these proteins induced host recognition behavior by T. remus, the following tests were performed. The proteins were separated as described by Strand & Vinson (1983a) and suspended in distilled water at a concentration of 1.5 μg/μl. Glass beads were arranged in a depression slide, as previously described, and were coated with 10–15 μg/bead of the protein fractions, allowed to dry, and bioassayed.

For statistical analysis, data for ‘examination’ and ‘recognition’ were combined into one group and compared to the ‘rejection’ group by use of a Chi-square test or Chi-square test of homogeneity of proportions (Marascuilia & McSweeney, 1977). Such an arrangement was used because the ‘examination’ and ‘recognition’ both indicated a response to the treatment while ‘rejection’ indicated no response.

**Results**

Female T. remus responded to significantly more glass bead masses coated with AGM from S. frugiperda than to uncoated controls (χ² = 75.6; p < 0.001) and T. pretiosum responded to significantly more glass beads coated with AGM from H. zea than to uncoated controls (χ² = 14.3; p < 0.001) (Table 1). Relative to controls however, female T. remus did not respond to bead masses coated with AGM from H. zea (χ² = 2.8; p > 0.075) and female T. pretiosum did not respond to beads coated with AGM from S. frugiperda (χ² = 0.4; p > 0.25). Both T. remus and T. pretiosum initiated their response by contacting the properly treated beads with their antenne. After briefly antennating the beads, the parasitoid would mount, continue the antennal examination, and then attempt to drill. After failing to penetrate the glass bead, females would often reexamine the bead and attempt to drill again, if sufficient time were allowed during the observation.

Female T. remus responded to significantly more bead masses coated with the 700 K accessory gland
Table 1. The percentage of Telenomus remus or Trichogramma pretiosum females which reject, examine, or recognize glass beads treated with accessory gland material (AGM) from Spodoptera frugiperda or Heliothis zea.

<table>
<thead>
<tr>
<th>Host AGM</th>
<th>N</th>
<th>Reject</th>
<th>Examine</th>
<th>Recognize</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. remus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. zea</td>
<td>40</td>
<td>83</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>S. frugiperda</td>
<td>40</td>
<td>12.5</td>
<td>12.5</td>
<td>75</td>
</tr>
<tr>
<td>Control</td>
<td>80</td>
<td>93</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>T. pretiosum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. zea</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>S. frugiperda</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Control</td>
<td>80</td>
<td>56</td>
<td>26</td>
<td>18</td>
</tr>
</tbody>
</table>

fraction from S. frugiperda then masses coated with the 30 K fraction from S. frugiperda, the two fractions from H. zea, or uncoated controls (α = 0.05; Chi Square Test of Homogeneity of Proportions) (Table 2). However, bead masses coated with the 1100 K accessory gland protein fraction from H. zea also elicited a significant (α = 0.05) response from female T. remus when compared to the 50 K protein fraction or control bead masses, despite the fact that T. remus did not respond to bead masses coated with AGM from H. zea.

Discussion

Until recently, very little work had been done on the mechanisms involved in host recognition by egg parasitoids. Early research by workers, such as Salt (1935), implicated host size and shape as the primary factor involved in host recognition. However, the similarity in egg shapes, especially among related insects, and the tendency of some egg parasitoids to show decided preferences for one host species over another suggest that other stimuli play a role in host recognition.

The data from this study demonstrate that the accessory gland secretion of S. frugiperda and H. zea contain kairomones that have a pronounced effect on host recognition by the egg parasitoids T. remus and T. pretiosum, respectively. It is unlikely that they are solely responsible for host recognition, however, and certainly not for acceptance since several studies have shown that host shape, size, color, and other kairomones are also important (Salt, 1935; Nordlund et al., 1977; Strand & Vinson, 1983b; de Jong & Pak, 1984, Nettles et al., 1982; Schmidt & Smith, 1985). In all likelihood, a number of factors work in tandem to determine whether or not the host is actually accepted and oviposited in. Data from this study suggest that this is the case. Female T. remus did not attempt to drill any beads in the absence of S. frugiperda AGM, and responded poorly to H. zea AGM. The S. frugiperda AGM material appears to play an important role in recognition of this host species by T. remus. While T. remus does show some response to AGM fractions from H. zea, the stimulus may not be sufficient to initiate consistent host recognition. T. pretiosum females, on the other hand, recognized more beads coated with AGM from H. zea than uncoated beads or beads coated with S. frugiperda AGM. Nonetheless, considerable num-

Table 2. The percentage of Telenomus remus females which reject, examine, or recognize glass beads masses treated with separated fractions of the accessory gland material (AGM) of Heliothis zea and Spodoptera frugiperda.

<table>
<thead>
<tr>
<th>Host</th>
<th>Fraction</th>
<th>N</th>
<th>Reject</th>
<th>Examine</th>
<th>Recognize</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. frugiperda</td>
<td>700 K</td>
<td>38</td>
<td>11</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>30 K</td>
<td>30</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>H. zea</td>
<td>1100 K</td>
<td>31</td>
<td>52</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>50 K</td>
<td>33</td>
<td>86</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>90</td>
<td>7</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
number of female *T. pretiosum* examined and, in a few cases, attempted to drill into beads in the absence of kairomones. Thus, while our data indicate that recognition is influenced strongly by AGM associated kairomones, general host shape and size are also important factors in recognition by *T. pretiosum* (Salt, 1935).

The female accessory gland secretions of numerous insects, especially in many holometabolous insects, are used as adhesives for attachment of eggs to oviposition sites (Hinton, 1981). The ubiquitous nature of these substances suggests that they may also have other important functions. For example, Rothschild & Schoonhoven (1977) found that *Pieris brassicae* L. females deposit an oviposition-deterring pheromone on and around eggs during oviposition. This pheromone is probably in the accessory gland secretion (Behan & Schoonhoven, 1978) and can be collected by washing eggs with methanol or water. Noldus & van Lenteren (1985b) report that the host searching behavior of *Trichogramma evanescens* females is stimulated by methanol and water washes of *P. brassicae* eggs. *Trichogramma maidis* Pintureau & Voegele females also respond to glass beads coated with *P. brassicae* accessory gland secretion (de Jong & Pak, 1984) and, as mentioned previously, *T. heliothidis* females respond to *H. virescens* AGM.

The chemical composition of the secretions used as adhesives for attachment of eggs to oviposition sites is poorly characterized for the most part, but for at least a few Lepidoptera the secretions appear to be produced in the accessory glands and are composed of neutral and acidic glycoproteins (Strand & Vinson, 1983a; Strand, 1985). In the case of the pentatomid *Nezara viridula*, the adhesive is also composed of glycoproteins, and also serves as a kairomone for the scelionid *Trisoleus basalis* (F. Bin, M. R. Strand & S. B. Vinson, unpublished).

Efforts to understand the nature of host recognition by parasitoids remains in its infancy (Nordlund et al., 1981). Most of what is known involves host recognition by larval parasitoids with very little known about parasitoids of other host stages.

Résumé

Influence (et caractérisation partielle) des kairomones contenues dans les sécrétions des glandes annexes des hôtes sur la sélection de ces derniers *par Telenomus remus et Trichogramma pretiosum*.

Cette étude a utilisé une technique voisine de celle employée par Strand & Vinson (1982), pour examiner les réactions de parasitoïdes à des billes de verre enduites de substances, afin de déterminer l'influence des sécrétions des glandes annexes (AGM) de *Spodoptera frugiperda* et *Heliothis zea* dans le repérage des hôtes par *Telenomus remus* et *Trichogramma pretiosum*.

Les femelles de *T. remus* réagissaient plus aux billes enduites d'AGM de *S. frugiperda* qu'aux billes témoins non enduites; celles de *T. pretiosum* réagissaient plus aux billes enduites d'AGM de *H. zea* qu'aux témoins. *T. remus* n'a pas réagi aux billes enduites d'AGM de *H. zea*, ni *T. pretiosum* à celles enduites d'AGM de *S. frugiperda*.


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References


