Effects of Venom from an External Parasitoid, *Euplectrus kuwanae* (Hymenoptera: Eulophidae) on Larval Ecdysis of *Argyrogramma albostriata* (Lepidoptera: Noctuidae)

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The venom apparatus in female *Euplectrus kuwanae* consists of two spheroidal venom reservoirs and a gland. A number of crystals which are rhomb in shape are observed in the venom reservoir. Although venom reservoirs of newly emerged females are small and contain no venom fluid, they develop rapidly until the fourth day of adulthood. When the hosts, *Argyrogramma albostriata* larvae, were injected with a venom equivalent to 1/2-1/10 of the volume of the venom reservoir of one female, they failed to molt and died. This indicates that the venom of *E. kuwanae* has a specific effect on the host’s endocrine system.

INTRODUCTION

The venom of parasitic Hymenoptera has an important role in the parasitoid-host relationship. The venom of parasitoids is well known to produce temporary or permanent paralysis of the hosts attacked; recently other functions have also been noted. For instance, BOCCHINO and SULLIVAN (1981) found that the venom of a hyperparasitoid, *Dendrocerus carpenteri* did not immediately paralyze the host but inhibited host pupation in the prepupal stage. The venom of *Eulophus larvarum* prevents apolysis in its host after a depressed feeding period, and the venom employed by *Clinocentrus graciipes* altered the host to a pharate pupal stage irrespective of its larval instar (SHAW, 1981). KITANO (1982) reported that venom apparatus material of *Apanteles glomeratus* (L.) was an important factor in suppressing the encapsulation of the eggs in the host. Also, UEMATSU (1986) showed that host larvae were not able to molt or pupate when they were attacked by preovipositional stinging of the *Euplectrus kuwanae*.

This study was initiated to clarify the morphology of the venom apparatus of *E. kuwanae* and to determine the gross effects of venom on the development of the host larvae. *E. kuwanae* is an external gregarious parasitoid of the free living lepidopterous larvae, *Argyrogramma albostriata* (UEMATU, 1980, 1981).

MATERIALS AND METHODS

*E. kuwanae* used in this study were offspring of wasps propagated in a previous study (UEMATU, 1986). Adult wasps were reared in a test tube (20 cm long with an inner diameter of 3 cm) with a cotton plug at about 25°C and a photoperiod of 14L–10D. Honey streaked across the inner surface of the test tube and a droplet of water served as food for the wasps.
Host larvae, *A. albostrata* were reared on the leaves of goldenrod, *Solidago altissima* in a plastic cage (7 x 12 x 20 cm). The insects were obtained in Miyazaki.

*Morphology of the venom gland and crystals in venom reservoirs.* Living materials were anaesthetized with carbon dioxide and dissected in a physiological saline solution [NaCl 750, KCl 35, CaCl₂ 21 (mg/100 ml)]. The morphological structure of the venom gland was examined in intact female reproductive organs immersed in the saline solution. In order to observe the crystals in venom fluid, venom reservoirs of 5–8 day old wasps were torn on the slide glass and their contents were allowed to disperse into a small amount of saline solution. A cover glass was placed on it and crystals were observed by a differential-interference microscope.

*Development of the venom reservoirs.* Seventy newly emerged female wasps were divided into 7 groups of 10 individuals each. Wasps of each group were dissected in saline solution to examine the size of the venom reservoirs at the age of 0, 1, 2, 4, 6, 8 and 10 days. As the shape of the venom reservoirs was more or less spheroidal, their volume was calculated by the equation:

$$V = \pi \left( \frac{D}{2} \right)^2 H + \pi \left( \frac{d}{2} \right)^2 h$$

where *V* is volume of venom reservoirs, *D* and *H* are diameter and length of the main reservoir, and *d* and *h* those of the sub-reservoir, respectively.

*Venom injection test.* Venom apparatus was resected from the female reproductive organ in the saline solution, and was transported into a penicillin-streptomycin solution (PS solution) (Grand Island Biological Co., Grand Island, NY 14072, U.S.A.), where a small section of the reservoir was cut out. Venom fluid and crystals flowing into the PS solution, which were visible under a dissecting microscope, were collected in a pointed tip glass capillary tube with a small amount of the solution. The venom was diluted with 2, 5, 10, 20 and 40 μl of PS solution to make various concentrations. One microliter of these venom solutions was injected into the body cavity through the integument between the first and second abdominal legs of young fourth instar larvae. Control larvae received an equivalent amount of PS solution. Treated larvae were singly placed in the test tube with the goldenrod leaves. They were held in an incubator controlled at 25°C to examine the effect of the venom on the larval ecdysis.

**RESULTS**

*Morphology of the venom gland and crystals in venom reservoirs*

The venom apparatus in female *E. kuwanae* consists of venom reservoirs and a gland (Fig. 1). The venom reservoirs are composed of two pouches, i.e., main and sub-reservoir. They are united with each other at their basal regions. The gland originating from the apex of the main reservoir is 2–3 mm in length, 0.07–0.09 mm in width and terminates in a globular enlargement. There is one large duct running longitudinally in the center of the gland.

A number of crystals are observed at the anterior half region of the main venom reservoir through the transparent membrane (Fig. 2-A); these are not observed in the sub-reservoir. The crystals are thin and rhomb in shape and their sizes are not uniform.
Fig. 1. Venom apparatus of Euscelis kusanei. G: gland, mVr: main venom reservoir, sVr: venom sub-reservoir.

Fig. 2. Venom reservoir and crystals. (A) main venom reservoir, a number of crystals are observed through the transparent membrane; (B) crystals flowing out of the main venom reservoir; (C) crystals being broken down in the saline solution.

(Fig. 2-B). Crystals begin to break down several minutes after being put in the saline solution (Fig. 2-C).
Development of venom reservoirs

Venom reservoirs of the newly emerged female are small and contain no venom fluid. They begin to grow from the first day after emergence and venom fluid gradually accumulates in them. Size of the reservoirs increases rapidly during the four days after emergence. Thereafter, the rate of increase gradually diminishes, and the reservoirs reach maximum size at about the eighth day of adulthood (Fig. 3).

Figure 4 shows the relationship between size of the venom reservoirs and the number of mature eggs in the ovaries. In younger wasps (0–6 days old), there is a correlation between them. This indicates that the venom reservoirs and the ovarian eggs develop parallel with each other in young wasps; however, this relationship is not observed in older wasps (8 and 10 days old). Although the number of ovarian eggs varies from 3 to 24, volume of the venom reservoirs maintains a constant level of about 0.015 mm³.

![Fig. 3. Development of venom reservoirs in the female of Euplectrus kuwanae after emergence.](image1)

![Fig. 4. Relationship between the number of ovarian eggs and size of venom reservoirs. Open circles: young materials (0 to 6 days after emergence), solid circles: old materials (8 to 10 days after emergence). Broken line is a regression for open circles.](image2)

Table 1. Effect of the venom on the ecdysis of fourth instar larvae of A. albostriata. Twenty individuals were used in each treatment. Numbers in parentheses indicate the duration (days) until the insects died or exuviated.

<table>
<thead>
<tr>
<th>Amount of venom fluid injecteda</th>
<th>Ecdysis inhibited</th>
<th>Ecdysis occurred</th>
<th>Died as result of other factors</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Completely</td>
<td>Incompletelyb</td>
<td></td>
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<tr>
<td>1/2</td>
<td>18 (8–10)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>1/5</td>
<td>19 (7–11)</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>1/10</td>
<td>14 (5–8)</td>
<td>3 (3–4)</td>
<td>2 (3–4)</td>
</tr>
<tr>
<td>1/20</td>
<td>0</td>
<td>6 (3–5)</td>
<td>12 (2–4)</td>
</tr>
<tr>
<td>1/40</td>
<td>0</td>
<td>0</td>
<td>20 (1–3)</td>
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<tr>
<td>Controlc</td>
<td>0</td>
<td>0</td>
<td>20 (2–3)</td>
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</table>

a Ratio to the total volume of venom fluid stored in the venom reservoirs of one female.
b Apolysis occurred but not ecdysis.
c Injected with the penicillin-streptomycin solution.
This suggests that regression of mature eggs has advanced in some of the older wasp individuals.

**Injection tests**

When the larvae were injected with a solution of the venom or PS solution, they reacted by wriggling violently and regurgitating a green fluid. Also, when the cillings were extracted from the larvae, a large quantity of the body fluid flowed from the site of the puncture. Therefore, a small quantity of the venom might flow out with the body fluid. The insects recovered within several hours and began feeding behaviour again. After 1 or 2 days they completed feeding on the instar.

The effect of venom on ecdysis of the host larvae is summarized in Table 1. Most of the larvae injected with a venom equivalent to 1/2, 1/5, or 1/10 of the volume of the venom reservoirs of one female were not able to molt. These larvae then entered a state of repose, though activity of the heart persisted for about a week. On the other hand, all of the insects injected with a venom equivalent to 1/40 of the volume of the venom reservoirs of one female succeeded in molting and reached the next instar.

**DISCUSSION**

The venom apparatus of parasitic wasps has been studied in many species of Ichneumonidae and Braconidae (Bender, 1943; Togashi, 1963; Robertson, 1968; Edson and Vinson, 1979). Edson and Vinson (1979) examined the venom apparatus in 160 species of female Braconidae, and showed that there were two types.

Venom apparatus in *E. kuwanae* has several points similar to those of Braconidae in appearance. However, it apparently differs from that species which has glands that are branched and attached at the base or along the median of the reservoir.

The most interesting discovery in the venom apparatus of *E. kuwanae* is probably a number of crystals which are recognized in the main reservoir. As these crystals are concentrated in the anterior part of the reservoir where a gland extends, glandular secretion seems to be crystallized when it flows down into the venom reservoir from the duct.

Uematsu (1986) showed that host larvae attacked by preovipositional stinging failed in larval ecdysis and pupation. In this study, we obtained the same results by artificial injection of fluid from venom reservoirs. This indicates that the venom of *E. kuwanae* has a specific effect on the host's endocrine system directly or indirectly.

Although the critical quantity of venom for the inhibition of ecdysis in the fourth instar larvae has not been precisely determined, it is presumably about 1/10 of the volume of the venom reservoirs of one female (Table 1). Activity of *E. kuwanae* venom does not seem as strong as that of Braconidae which is known to be very effective in paralyzing host insects.

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REFERENCES


