Effects of the Calyx and Venom Fluids of Apanteles kariyai WATANABE (Hymenoptera: Braconidae) on the Fat Body and Hemolymph Protein Contents of Its Host Pseudoletia separata (WALKER) (Lepidoptera: Noctuidae)\(^1\)

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Apanteles kariyai WATANABE (Hymenoptera, Braconidae) is successfully able to parasitize Pseudoletia separata (WALKER) (Lepidoptera: Noctuidae) until 4 days before pupation. The function of the substances (calyx fluid and venom fluid) injected at the time of oviposition seems to be important. The fat body functions as an important energy source for the host larvae to pupate as well as for the parasitoid. The fat body in Pseudoletia separata parasitized by Apanteles kariyai sequestered no protein granules, while the control larvae in the same day stored many protein granules in trophocytes. The protein concentration in hemolymph of the parasitized host showed low level compared with that of the control larvae. These phenomena were reproduced on the host in case of simultaneous injection of calyx and venom fluids. Both calyx and venom fluids seem to regulate the physiological phase of host in early stage of the parasitization.

INTRODUCTION

Apanteles kariyai WATANABE is a gregarious parasitoid of the armyworm, Pseudoletia separata (WALKER). It was observed from a laboratory colony that the armyworm did not pupate when parasitized by A. kariyai. Interestingly, successful parasitism occurred until day 2 of the sixth (last) instar and on day 3 decreased rapidly. As the unparasitized larvae became pupae on day 6, the parasitoid seemed rapidly to block the process of pupation of its host in some way (TANAKA et al, in preparation). The fat body functions as an important energy source for the parasitoid as well as being an important organ for the host at the last larval stage in the process of pupation. SAJAP et al. (1978) reported that the only vital organ directly affected by parasitism was the fat body, which did not accumulate protein granules. LOCKE and COLLINS (1968) reported the change of the fat body with the process of pupation as follows: the time when the storage granule accumulated in trophocytes was after molting (M) + 156 hr in the three phases. Before this time the amino acids incorporated into trophocytes and the blood proteins

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increases in concentration at the same time. The formation of the protein granules in trophocytes was caused by the function of ecdysone (Collins, 1969). Iwantsch and Smilowitz (1976) suggested that the absence of protein granules in the trophocytes was caused by the failure of the prothoracic glands to become activated for the pupal molt in parasitized T. ni.

In this study, it showed that the inhibition of the accumulation of protein granules in trophocytes and the decrease of blood proteins resulted from the existence of the calyx fluid and venom fluid injected with the eggs at the time of oviposition.

MATERIALS AND METHODS

Synchronously developing P. separata larvae were taken from the stock culture and parasitized or injected with various materials when they were in day 2 of the last instar. Prior to injection host larvae were narcotized with CO₂ to prevent loss of hemolymph. Injection was made by means of a glass micropipette inserted just in the proleg of the sixth abdominal segment. The calyx fluid and the venom fluid were separately collected by dissection of female wasps. The ovaries were taken out of female wasps, rinsed in physiological saline (9.993 g NaCl, 0.3 g KCl, 0.2 g MgSO₄·7H₂O, 0.2 g CaCl₂·2H₂O, 0.003 M phosphate buffer pH 7.0 in 1 L H₂O), and placed in a hole slideglass. Firstly, the venom apparatus (gland and reservoir) was taken apart with forceps to protect it from contamination by the calyx fluid. The other parts of ovary were broken with two tungsten needles and then the calyx fluid was collected by micropipette. Care was taken to ensure that no tissues or cells were collected. These collected fluids were cooled to protect them from degeneration. Larvae stung by one female wasp were replaced individually on the artificial diet and incubated at 25°C under a 16-hr photoperiod. The larvae were dissected in Carnoy's fixative. The fat body 1 hr after immersion in the fixative were dehydrated, embedded in paraffin, and sectioned at 5 μm in thickness. The protein granules in the fat body were stained by the azo-coupling reaction of amino radicals in proteins for aldehyde radicals of hydroxy-naphthaldehyde (Sano, 1965). Furthermore, a fat body in a parasitized host was stained for melanin by the iron ferricyanide method (Sano, 1965).

The hemolymph was collected separately from a single larva by amputating the hind leg and storing it at −20°C until use. The contents of hemolymph protein was determined by the Bio-Rad protein assay (BIO-RAD laboratories). Coloration intensity was read in a spectrophotometer at 595 nm wavelength and samples were calibrated against a bovine gamma globulin standard curve (Bradford, 1976; Spector, 1978).

RESULTS

The trophocytes of day 4 control larvae injected with saline had many protein granules (Fig. 1A, B), while that of the parasitized host had no granules (Fig. 2). When the host larvae were injected either with the calyx fluid or with the venom fluid, a few hosts sequestered the stored granules in the fat body (Table 1, Figs. 3, 4). On the other hand, both the level of protein contents of the hemolymph and the degree of formation of the protein granules in trophocytes of the host injected with the calyx and venom fluids are similar to those in the parasitized host (Fig. 5).

The protein contents in the hemolymph increased steadily from day 2, 24.6 ± 1.3,
Fig. 1. Sections of *Pseudaletia separata* fat body were stained by the azo-coupling reaction of hydroxy-naphthaldehyde. Day 4-stage of the control last instar. Many protein granules (pg) accumulated into trophocytes. A: \( \times 640 \), B: \( \times 1,280 \).

Fig. 2. Parasitized host, day 4-stage, 2 days after parasitization. No formation of protein granules. \( \times 640 \).
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Fig. 3. Trophocytes when the venom fluid was injected. Most of the injected hosts presented no stored granules. N: nucleus. ×640.

Fig. 4. (A) Most of the hosts injected with calyx fluid showed no granules in the trophocytes as the parasitized host, but (B) a few of the injected host stored protein granules (pg) as normal. N: nucleus. ×640.
Table 1. Effects of calyx fluid and venom fluid of the parasitoid, *Apaneles karjai*, on the fat body of the host, *Pseudaleria separata*

<table>
<thead>
<tr>
<th>Injected materials</th>
<th>No. of host with stored protein granules in trophocytes</th>
<th>No. of host with melanized cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological saline</td>
<td>6/6</td>
<td>0/30</td>
</tr>
<tr>
<td>Calyx fluid</td>
<td>3/11</td>
<td>5/31</td>
</tr>
<tr>
<td>Venom fluid</td>
<td>4/12</td>
<td>0/32</td>
</tr>
<tr>
<td>Calyx and venom fluids</td>
<td>0/7</td>
<td>23/23</td>
</tr>
<tr>
<td>Parasitized host</td>
<td>0/7</td>
<td>11/11</td>
</tr>
</tbody>
</table>

Denominators mean the number of host larvae used.

to day 4, 55.7 ± 2.6 mg/ml in the control hosts injected with physiological saline (Fig. 7). Interestingly, in day 4, both the parasitized host and the host injected with the calyx and venom fluids presented the same low level of protein contents, 28.1 ± 1.5 and 28.9 ± 1.1 mg/ml, respectively (Fig. 7). In day 4 the normal host larvae wandered about to search for a suitable site for pupation and showed that the activation of prothoracic glands had occurred (TANAKA, unpublished data).

A lot of melanized cells in the fat body of the parasitized host and the host injected with calyx and venom fluids were observed with no exception (Table 1, Fig. 6).

**DISCUSSION**

There have been many reports that the parasitoid reforms the host to make it suitable for itself. If the pupation of the host is unsuitable for the parasitoid, the parasitoid controls the host's pupation. When the parasitization occurs successfully within a short time previous to pupation, the process of pupation of the host must be blocked immediately after parasitization. The effective substances functioning shortly after parasitization seems to be the calyx fluid and/or the venom fluid injected simultaneously at the time of oviposition. The host injected with calyx and venom fluids showed a low level of protein contents and no formation of protein granules in trophocytes just as in the case of an actually parasitized host. This suggested the possibility that the calyx and venom fluids manipulate the hormonal level during the process of pupation. COLLINS (1969) reported, in the pupation of the normal host, the protein concentration increases rapidly following the critical period for action of the brain hormone, and the sequestration of blood proteins by the fat body to make granules was caused by the ecdysone, the action of the prothoracic glands. IWANTSCH and SMILOWITZ (1975 a) reported that *Hyposoter exiguae* is successfully able to parasitize two days before pupation and cause a low level of protein contents of hemolymph after oviposition. Furthermore, they confirmed that the parasitoid prevented host larvae from pupating by preventing activation of host prothoracic glands in the fifth instar (IWANTSCH and SMILOWITZ, 1975 b) and suggested that this would appear to occur either through inhibition of brain hormone secretion or by nullifying its action on the prothoracic glands (SMILOWITZ, 1974). The low level of protein contents in hemolymph and no formation of protein granules in the fat body may suggest the possibility that the action of the brain hormone is inhibited, which is caused by the calyx and venom fluids.
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Fig. 5. No formation of protein granules and a lot of melanized cells (MC) were observed in the fat body of all host injected with calyx and venom fluids. ×640.

Fig. 6. (A) Fat body in unparasitized host, day 4 of last instar. (B) Parasitized host in the same stage, 2 days after parasitization. Many melanized cells (MC) are observed. ×64.
Fig. 7. Change of the protein contents by calyx fluid and venom fluid or by parasitization. The calyx fluid and venom fluid were mixed 1:1. The volume injected in all cases was two microliter. Parasitization and injection were done on day 2 of the last instar host, as shown by the arrow (↑). Vertical bars indicates SD. Means followed by the same letter (a, e, f) were not significantly different at the p<0.05 level, by DUNCAN's multiple range test. Numbers in parenthesis indicates the number of host examined.

Whether the melanized cells in trophocytes, which were observed in both parasitized hosts and hosts injected with calyx and venom fluids, have any relation to calyx virus or not is being studied.

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REFERENCES


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