Rearing *Theretra oldenlandiae* (Lepidoptera: Sphingidae) Larvae on an Artificial Diet

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**Abstract**

The hawk moth *Theretra oldenlandiae* (Fabricius) is an important insect pest because in the larval stage it feeds on agricultural crops and ornamental plants such as the eddoe and garden balsam. In this study, we established methods for rearing *T. oldenlandiae* in the laboratory using an artificial diet containing dry powder of a wild grass *Cayratia japonica* (Thunb.) Gagnep. Several artificial diets were tested with different ratios of a commercial diet, *Insecta* LFM, and the dry leaf powder, and including different antibiotics, and the composition of the standard diet on which larvae performed best was determined. The standard diet contains 20 g of *Insecta* LFM, 4 g of leaf powder, 100 ml of water, 75 mg of chloramphenicol, and 200 μl of propionic acid. Larvae reared on the standard diet became larger pupae than those reared on *C. japonica* leaves. This result suggests that the larvae have growth potential that is masked on *C. japonica* leaves, and that *C. japonica* may not be the most suitable host species for *T. oldenlandiae* larvae in terms of nutrient level.

**Key words:** artificial diet, larval growth, larval development, host plant suitability, mass rearing

The hawk moth *Theretra oldenlandiae* (Fabricius) is distributed in southern to eastern Asia, including Japan (Sambath 2011, Rafi et al. 2014) and Oceania (Rougerie et al. 2014), and is an important insect pest that feeds on agricultural and ornamental plants such as eddoe *Colocasia esculenta* (L.) Schott, grape *Vitis* spp, and garden balsam *Impatiens balsamina* (L.) in the larval stage (Okada 2003, Japanese Society of Applied Entomology and Zoology 2006). As in some other hawk moths, larvae of this species consume large amounts of host leaves and grow to a body weight of 7 g. In particular, eddoe is one of the main agricultural products in southern Kyushu, and thus effective methods for control of *T. oldenlandiae* larvae are needed. However, there is little ecophysiological information on this moth. The aim of this study was to establish methods for rearing the moth on an artificial diet.

Larvae of *T. oldenlandiae* can be reared on fresh host leaves in the laboratory, but this method requires a large number of host leaves: Even if host leaves are stuffed into a 430-ml plastic cup for one larva, the food resources are exhausted within 2 d in the midst of the final (fifth) instar when the larval body weight approaches the maximum. Thus, rearing on fresh leaves also needs much space and labor. Furthermore, we observed that rearing larvae with field-collected leaves sometimes results in parasitism by a fly *Sturmiella bella* (Meigen), because female adults of this fly lay very small eggs on plant leaves, and the eggs may be swallowed by *T. oldenlandiae* larvae through feeding (Hirai and Ishii 1995). For these reasons, it is not feasible to mass-rear *T. oldenlandiae* larvae on host leaves.

In Sphingidae, rearing methods for larvae of *Manduca sexta* (L.) (Yamamoto 1969) and *Agrius convolvuli* (L.) (Kiguchi and Shimoda 1994) have been established to utilize them as common experimental insects. In the method for *A. convolvuli*, dry leaf powder of the host (the sweet potato *Ipomoea batatas* (L.) Lam.) is used as an ingredient of the artificial diet. Thus, rearing *T. oldenlandiae* larvae on an artificial diet may also be possible.

Larvae of *T. oldenlandiae* also feed on the bushkiller *Cayratia japonica* (Thunb.) Gagnep. This wild grass grows thickly from spring to autumn on vacant land, roadsides, margins of forests, and other places where land management is neglected. In this study, we established a method for rearing *T. oldenlandiae* larvae on an artificial diet, including dry powder of this easily available host plant.

**Materials and Methods**

**Rearing of Insects**

A laboratory population of *T. oldenlandiae* was established from ca. 100 eggs collected from *C. japonica* grown around Miyakonojo Campus of Minami Kyushu University, Miyakonojo (31°44′N, 131°06′E, ca. 160 m altitude), Miyazaki Prefecture, Japan, in August 2017, and was kept for successive generations in the laboratory. The larvae of the laboratory first generation were reared on fresh leaves of *C. japonica*. Larvae of following generations were reared on standard diet (as described below). The resulting pupae were kept at 15°C to retard pupal development until use. Newly eclosed adults...
of *T. oldenlandiae* were confined in a cardboard box (45-cm width × 35-cm depth × 150-cm height) with windows of transparent plastic boards. The box was kept at 23 ± 2°C under an artificial light condition that was not precisely controlled. Pupae were transferred to the box from the 15°C stock every 3 d to keep the number of adults in the box at ca. 10–20. For provision of food, a small Petri dish (6 cm in diameter, 1.5 cm in depth) filled with honey water (ca. 20% v/v) was suspended from the ceiling of the box with a wire at a height of 130 cm. To allow the female adults to lay eggs, foliage of *C. japonica* arranged in a bottle filled with water was placed on the bottom of the box. Eggs laid on the leaves were collected carefully, placed on a moist paper towel in a large Petri dish (9 cm in diameter, 2 cm in depth), and kept at 25 ± 1°C under a photo regime of 16:8 (L:D) h until hatching. More than 200 eggs were obtained per day.

**Diet Ingredients**

Insecta LFM (dry powder type, Nosan Co., Yokohama, Japan) and dry leaf powder were the major solid components of the diet (Table 1). Insecta is a commercial diet used for a wide range of phytophagous insects (e.g., common cutworm, legume pod borer, carpenter moth, and yellow-spotted longicorn beetle) without admixture (Kudo et al. 2014; Nagamine et al. 2016, 2017; Nakanishi et al. 2017; Nakano et al. 2018), and is also used for oligophagous insects (e.g., fungivorous moth, swallowtail butterfly, and chrysanthemum longicorn beetle) with host powder (Kitajima 2013, Nishikawa et al. 2013, Shintani 2011). To prepare leaf powder of *C. japonica*, foliage was cut from around the campus and the leaves were dried naturally in the laboratory for at least 5 d before being pulverized with an electric blender. Chloramphenicol and propionic acid (Nacalai Tesque, Kyoto, Japan) were added as antibacterial and antifungal agents, respectively.

**Preparation of a Standard Diet**

A ‘standard diet’ (Table 1) was prepared from Insecta LFM, dry leaf powder, and water that were mixed thoroughly in a stainless steel container (20 × 14 × 7.5 cm) and steamed for 20 min. After the temperature of the mixture dropped to below 60°C, 75 mg of chloramphenicol dissolved in 750 μl of 100% ethanol, and 200 μl of propionic acid were added to the mixture. This standard diet could be stored for 30 d at 10°C.

**Larval Culture**

Individuals in the second and third generations of the laboratory population were used for this study. In all experiments, larvae were reared at 25 ± 1°C under 16:8 (L:D) h until pupation and all manipulations were conducted within 1–3 h after light on. Larvae that hatched within 24 h were assigned to either of two food groups: host leaf or standard diet. Leaves of the host plant (*C. japonica*) or standard diet were supplied throughout the larval stage. Thirty neonate larvae were placed in a large Petri dish and were provided with 5 g of young host leaves or 10 g of standard diet. In the standard diet group, sliced pieces of standard diet (5 g in total) were attached to the underside of lids and the bottom of Petri dishes (Supp. Fig. S1). In *T. oldenlandiae*, ecdisis to the next instar and head capsule slippage (HCS) occur about 4–10 h after light on and about 12 h before ecdisis, respectively. Larvae that attained second head capsule slippage (2HCS) by day 6 from hatching were counted as surviving individuals. At 2HCS, larvae were weighed and transferred to new small Petri dishes individually. Larvae that did not attain 2HCS by day 6 were regarded as developmentally abnormal because almost all larvae reared on host leaves reached 2HCS by day 6. Third-instar larvae were supplied with approximately 3 g of mature host leaves or 5 g of standard diet. Each larva was individually transferred to a plastic cup (430 ml) at 3HCS and reared in this cup until pupation. A larva was supplied with 20 g of mature host leaves or 15 g of standard diet on the first day of the fourth instar, and on the first and fifth days of the fifth instar. Larvae spun cocoons in the leftover food and then pupated in the cocoons. Larval development was observed daily to record the dates and body weights at 2HCS, 3HCS, 4HCS, and pupation. The details on dietary manipulation were described in Results and Discussion.

**Pupal Culture**

Pupae were sexed by the morphology of the abdomen tip and transferred individually to small Petri dishes in which a moist paper towel was placed. Pupae were kept at 25 ± 1°C under 16:8 (L:D) h for observation of pupal development. The dates of adult eclosion were recorded.

**Statistical Analysis**

R was used for statistical analyses, mostly using R commander (Fox 2005, R core team 2017) and the means and proportions were compared by t-test and Fisher exact test, respectively.

**Results and Discussion**

**Composition of the Standard Diet**

The major solid components of the standard diet were Insecta LFM and leaf powder of *C. japonica* (Table 1), with the leaf powder probably providing the feeding stimulant. We tested other artificial diets with several different ratios of Insecta LFM and leaf powder, and different antibiotics. The selected standard diet gave the highest survival rate and growth increment among these test diets (Supp. Fig. S2).

**Table 2. Effects of food resources on the performance of early-instar larvae of *Theretra oldenlandiae***

<table>
<thead>
<tr>
<th>Food resources</th>
<th>Survival rate (%)&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Weight at 2HCS (mg)&lt;sup&gt;c,d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host leaf</td>
<td>70.0 (n = 90)</td>
<td>45.4 ± 1.2 (n = 58)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard diet</td>
<td>56.7 (n = 90)</td>
<td>55.9 ± 1.6&lt;sup&gt;***&lt;/sup&gt; (n = 51)</td>
</tr>
</tbody>
</table>

Larvae were reared on *Cayratia japonica* leaves or standard diet from hatching.

<sup>a</sup>Fisher exact test.

<sup>b</sup>Survival rate = [(number of larvae that attained 2HCS by day 6 from hatching) / (number of larvae that hatched)] × 100.

<sup>c</sup>t-test.

<sup>d</sup>Mean ± SE.

<sup>1</sup>Data were not available for five individuals.

<sup>***</sup>P < 0.001: significant difference between food resources.
Survival Rate and Growth in Early-Instar Larvae

To examine the suitability of the standard diet as food for early-instar (first- and second-instar) larvae, the survival rate and growth were compared between the host leaf and standard diet groups (Table 2). The survival rate in early-instar larvae did not differ significantly between the food groups (Fisher exact test, $P > 0.05$). The larval weight at 2HCS was significantly higher in the standard diet group than in the host leaf group ($t$-test, $P < 0.001$). These results indicate that the standard diet provided the nutrients, water, and feeding stimulant required for normal development.

Survival Rate in Late-Instar Larvae

To examine the suitability of the standard diet as food for late-instar (third- to fifth-instar) larvae, survival rates were compared between the food groups (Table 3). In this experiment, larvae were fed host leaves until 2HCS, and then assigned to either of the two food groups. The survival rate through late instars was lower in the standard diet group than in the host leaf group (Fisher exact test, $P < 0.01$). There was a significant difference in the survival rate between the food groups for the third instar (Fisher exact test, $P < 0.001$), but not for the fourth or fifth instar. These results suggest that some third-instar larvae are not able to adjust to the food change from host leaves to standard diet. Thus, rearing on a standard diet throughout the larval stage may improve the survival rate in late instars.

Growth in Late-Instar Larvae

Comparison of larval growth trajectories between food groups revealed different patterns of growth (Fig. 1). The weight at 3HCS was significantly heavier in the host leaf group than in the standard diet group ($t$-test, $P < 0.001$), while there was no significant difference in weight at 2HCS ($t$-test, $P = 0.53$). The duration of the third instar (days from 2HCS to 3HCS) was significantly prolonged in the standard diet group ($t$-test, $P < 0.001$), even though the weight at 4HCS was significantly lower in the standard diet group ($t$-test, $P < 0.01$). In contrast, the duration of the fifth instar did not differ significantly between the two groups ($t$-test, $P = 0.84$).

In *M. sexta*, larvae grown on an artificial diet (‘naive larvae’) are less sensitive to host-plant preference because of increased sensitivity to certain chemicals in non-host plants (Schoonhoven 1967). Moreover, naive larvae become less sensitive to deterrent chemicals through exposure to the chemical during larval development (Städler and Hanson 1978, Glendinning et al. 2001). In *T. oldenlandiae*, the growth in the third instar suggested that larvae transferred from the host leaf to a standard diet need time to increase sensitivity to certain chemicals and/or decrease sensitivity to deterrents. The growth trajectories in the fourth-instar larvae of *T. oldenlandiae* suggest probable loss of the food preference in the third instar. The fifth instar had a markedly higher performance with the standard diet, with a higher growth rate rather than longer growth period. The data distribution of pupal weight with the standard diet (3.0–4.1 g) did not

**Table 3.** Survival rates (%) in late instars of *Theretra oldenlandiae* larvae

<table>
<thead>
<tr>
<th>Food resources</th>
<th>From third to fifth instars</th>
<th>Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Third</td>
<td>Fourth</td>
</tr>
<tr>
<td>Host leaf</td>
<td>78.1 (n = 64)</td>
<td>93.8 (n = 64)</td>
</tr>
<tr>
<td>Standard diet</td>
<td>52.7*** (n = 55)</td>
<td>70.9*** (n = 55)</td>
</tr>
</tbody>
</table>

**P < 0.01, ***P < 0.001: significant difference between food groups (Fisher exact test).
Table 4. Effect of food resources in the larval stage on pupal development in *Theretra oldenlandiae*

<table>
<thead>
<tr>
<th>Food resources</th>
<th>Pupal duration (days) $^{ab}$</th>
<th>Eclosion rate (%$^{b,c,d}$)</th>
<th>Female rate (%$^d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host leaf</td>
<td>15.0 ± 0.4 (n = 45)</td>
<td>88.2 (n = 51)</td>
<td>58.8 (n = 51)</td>
</tr>
<tr>
<td>Standard diet</td>
<td>14.5 ± 0.2 (n = 26)</td>
<td>89.7 (n = 29)</td>
<td>58.6 (n = 29)</td>
</tr>
</tbody>
</table>

No significant difference between food groups in any column at $P > 0.05$ ($^a$ t-test, $^b$ Fisher exact test).

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

References Cited


