Larval interactions in aphidophagous predators: effectiveness of wax cover as defence shield of *Scymnus* larvae against predation from syrphids

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Abstract

Small-sized predators in the aphidophagous guild of *Aphis gossypii* Glover colonies on hibiscus trees in Japan exploit aphids at low prey abundance. *Scymnus* (*Pullus*) *posticalis* Sicard beetles were the first predatory species to attack aphids in the spring, and their larvae co-occurred with larvae of *Eupeodes freguens* (Matsumura) syrphids in aphid-infested leaves of hibiscus for 3 weeks in absence of large-sized coccinellid predators. Larval interaction between *Scymnus* and syrphid predators was examined in relation to effectiveness of wax cover of *Scymnus* against predation from syrphids. Waxless first instar larvae were not protected but wax-covered larvae of second, third and fourth instars were protected from predation by syrphid larvae. The protection was lower in the second instar which has a thin wax cover and significantly higher in the third and fourth instars having a thick wax cover. In addition, larvae from which the wax was removed were significantly more vulnerable to predation. Vulnerability of *Scymnus* larvae to predation from syrphids was directly related to the thickness of wax cover. Results suggest that the wax cover of *Scymnus* larvae act as an effective defence mechanism against predation from syrphid larvae.

Introduction

Many insects in the orders Coleoptera, Homoptera and Hymenoptera produce wax to cover their bodies. Several functions have been attributed to wax coverings in insects, viz. reflection of UV radiation, prevention of water transpiration and protection from natural enemies (Barlett, 1961; Eisner, 1970; Bradley, 1973; Pope, 1979; Takabayashi & Takahashi, 1993). Wax-producing coccinellids include a number of aphid- and coccid-feeding predators (Pope, 1979; Richards, 1985; Hodek & Honek, 1996). Wax covers in larvae of some of these predators defend them against ant predation (Barlett, 1961; Richards, 1985; Völkl & Vohland, 1996) and are also helpful in foraging among the wax-producing prey without eliciting any response (Richards, 1985; Arakaki, 1992).

Wax coverings in Coccinellidae have several patterns including regularly arranged wax tubes in discrete blocks on thoracic and abdominal tergites of several species of Scymnini, tufts of long and short waxy hairs in some members of Hyperaspini and Noviini, a powdery cover in some members of Coccidulini (Pope, 1979), and a woolly sac in one Rodatus species (Richards, 1985). Sticky nature of wax makes it difficult to bite into it and serves as an entangling agent (Eisner, 1994). Wax-covered larvae of the two aphidophagous *Scymnus* beetles, *S. nigrinus* Kugelann and *S. interruptus* (Goeze), were found to be protected from attacks by the ants that attended aphid colonies on conifers (Völkl & Vohland, 1996).

*Aphis gossypii* Glover colonies on hibiscus trees, *Hibiscus syriacus* L., in Japan are not attended by ants (Inaizumi, 1980), but they attract a number of aphidophagous insects like small-sized predators (2-day-old fourth instar larvae: 4.05–7.33 mm in length) such as *Scymnus* (*Pullus*) *posticalis* Sicard and a syrphid, *Eupeodes freguens* (Matsumura), and relatively large-sized coccinellid predators (2-day-old fourth instar larvae: 10.35–14.26 mm in length) including *Harmonia axyridis* Pallas and *Coccinella septempunctata* L. (H. Yasuda, unpublished). Coexistence of diverse
predators sharing the same food in patchy habitats depended on a number of factors, the most important being the degree of aggregation in relation to prey density (Taylor et al., 1979), the intensity of interspecific competitions (Lawton, 1987) and net larval productivity of aggregating species (Begon et al., 1996). Species with lower competitive abilities and slower reproductive modes tend to avoid coexistence with species having higher competitive abilities and higher reproductive modes (Tokeshi, 1999). Thus the response of predators to their food patches often has temporal components, that is, depending on their competitive abilities and rate of increase, a species’ presence in a prey resource could be limited to a short time in order to avoid interaction with predators and parasites. There are some well-documented cases of such phenomena in insects (Huffaker et al., 1963; Murdoch & Stewart-Oaten, 1975), but in aphidophagous guilds there is a lack of data regarding temporal effect of the aggregation of species. Furthermore, intraguild predation may be frequent among aphidophagous insects foraging in patchy habitats (Rosenheim et al., 1995; Lucas et al., 1997; Obrycki et al., 1998; Agarwala et al., 1998; Yasuda & Ohnuma, 1999).

Scymnus beetles and syrphid predators in A. gossypii colonies on hibiscus trees share features including solitary egg-laying, relatively low mobility of their larvae, and relatively small size in comparison to large-sized coccinellid predators, including Harmonia, Coccinella and Exochomus. No previous study has examined the role of Scymnus larvae against predation from syrphid larvae, therefore, we examined larval interactions between the two predators. Our prediction is that as both predators are attracted to aphid colonies at low prey abundance where large-sized predators do not come yet, they might interact for the common food resource. We first examined seasonal changes in the number of these aphidophagous insects in relation to aphid abundance on hibiscus trees, and then did experiments to understand the effectiveness of wax cover as a possible defence mechanism of small-sized larvae of different developmental stages of S. posticalis against predation from relatively larger and aggressive larvae of the syrphid species, E. freguens.

Materials and methods

Incidence of aphids and predatory larvae on trees. Aphids and larvae of E. freguens, S. posticalis and H. axyridis were sampled at 7-day intervals from two perennial trees of H. syriacus, of about 2 m height, located on the campus of the Yamagata University, Tsuruoka, Japan (38°43’ N, 139°49’ E). We counted all aphids and different larval stages of the three predatory species from shoots of six randomly selected branches, each measuring 30 cm from the apical end that was cut and brought to the laboratory. Sampling was started on 1 May 2000 when fundatrices of A. gossypii began to hatch from overwintering eggs at bud bursts and continued until 19 June 2000 when no more aphids or larvae of the two predators, E. freguens and S. posticalis, were observed. No other aphid species formed colonies on hibiscus trees during the period of this study. As the study progressed, unfurled leaves of sprouting buds stretched and developed into shoots, each shoot consisted of a whorl of 3–7 leaves of various lengths. Number of shoots in the six branches from the weekly samplings varied in the range of 47–59. Ants did not attend aphid colonies in this study.

Laboratory experiments. Larvae of S. posticalis were obtained from the laboratory stock culture established from paired adults collected on hibiscus trees. Additional larvae of this predator and all larvae of E. freguens were obtained from 15 hibiscus trees that were planted at the University farm, Tsuruoka, Yamagata, and infested with A. gossypii. Aphid requirements of the stock culture of S. posticalis were also met from this source. Scymnus larvae were kept on live aphids of A. gossypii in 9 cm Petri dishes, three larvae in each pair of dishes, and observed at 6 h intervals for any molting. Paired adults of S. posticalis were also kept in 9 cm Petri dishes on A. gossypii aphids. A cotton wad and folded corrugated papers were provided as substrates for laying eggs.

In experiments of larval interactions, larvae of the two predatory species were held in 5 cm Petri dishes that were lined with filter paper. Ten replicates were used in each interaction. Larvae of the potential syrphid predator, E. freguens, were starved for 16 h prior to experiments in order to induce the same level of hunger. All experiments were carried out at 22 ± 1.0 °C, 60% r.h. and a L16: D8 photoperiod. To obtain Scymnus larvae without waxes in the experiments, we followed the procedure of Vökl & Vohland (1996), and also used their terminology of ‘waxy’ and ‘waxless’ to refer to larvae with natural wax cover and to larvae with wax removed, respectively.
the second, third and fourth instar S. posticalis wax cover on the dorsum of thorax and abdomen in instars of larvae also experienced periods either waxless or without full wax cover immediately following ecdysis. Time taken to develop complete wax cover in second, third and fourth instars was recorded to evaluate the relative risks of predation in their waxless durations. This was done by monitoring the development of S. posticalis larvae from the first instar to the pupal stage at 2 h intervals from 7 a.m. to 9 p.m. each day during their developmental time. Larvae that moulted outside this time were not included in these observations.

Rates of encounters and attacks. Third instar syrphids were individually caged with a waxless first instar or a combination of similar-aged waxy and waxless larvae, one each, of second, third or fourth instar of Scymnus. Number of encounters between larvae of the two predators and number of Scymnus larvae fatly attacked during interactions in one h were recorded. This was repeated 10 times for each instar of Scymnus. Observations were discontinued on attacked Scymnus larvae suffering mortality. Non-mortal attacks on waxy and waxless larvae were counted as encounters.

Effects of wax cover on predation from syrphid larvae. Third instar syrphid larvae were individually caged with a waxy second, third or fourth instar Scymnus larva. Number of Scymnus larvae been preyed upon was recorded after 1, 3, 6, 18 and 24 hours.

Thickness of wax cover in Scymnus larvae and their relative defence against predation. Earlier studies suggested that thickness of wax cover in coccinellid larvae varied between species (Pope, 1979; Takabayashi & Takahashi, 1993) and within species (Richards, 1985). In our study we recorded differences in the thickness of wax tubes that made up the wax cover on the dorsum of thorax and abdomen in the second, third and fourth instar S. posticalis larvae. Thickness of wax tubes was measured by gently removing the longest wax tubes from their bases from the spinal region of abdomen and the pronota with the help of a pair of fine needles. As wax tubes are weakly held on raised tubercles (Pope, 1979), with practice they easily come off their bases without damage. Separated wax tubes were transferred to a glass slide and their lengths measured in a binocular microscope (at a magnification of 40 ×) using an ocular micrometer. Length and weight of different instars were also recorded in order to understand their possible relationship with thickness of wax tubes. Lengths of CO2-anæsthetised larvae were measured from the tip of the head to the posterior tip of abdomen. Body weights were measured with wax cover using a microbalance.

<table>
<thead>
<tr>
<th>Larval instars</th>
<th>Ratio of waxy and waxless larvae</th>
<th>No. of encounters made with larvae</th>
<th>No. of larvae attacked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Waxy</td>
<td>Waxless</td>
</tr>
<tr>
<td>First</td>
<td>0:1</td>
<td>–</td>
<td>1.20 ± 0.21</td>
</tr>
<tr>
<td>Second</td>
<td>1:1</td>
<td>5.20 ± 0.35a</td>
<td>1.50 ± 0.27b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.90 ± 0.18b</td>
<td>0.0 ± 0.0a</td>
</tr>
<tr>
<td>Third</td>
<td>1:1</td>
<td>6.20 ± 0.37a</td>
<td>1.60 ± 0.29b</td>
</tr>
<tr>
<td>Fourth</td>
<td>1:1</td>
<td>6.80 ± 0.36a</td>
<td>2.50 ± 0.31b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.50 ± 0.23b</td>
</tr>
</tbody>
</table>

Figures in the same row followed by different letters differ significantly at P < 0.05: χ²-test.

Results

Incidence of aphids and predatory larvae on hibiscus trees. Fundatrices from overwintering eggs of A. gossypii started to hatch in the first week of May and colonised buds that were sprouting on branches. Examination of samples did not reveal any predatory larvae in the first week of sampling, but paired adults of S. posticalis were observed. Number of aphids per shoot of hibiscus progressively increased in the following weeks until it declined in the seventh week of observation (Figure 1A). Scymnus larvae were first to attack A. gossypii colonies in the second week (8 May) at bud burst, and the first incidence of syrphid larvae was recorded one week later (15 May) when bud leaves began to unfurl. From 15 May to 5 June, larvae of both predators were active in aphid colonies, mostly
restricted to inner and smaller leaves of whorls. Their incidences increased in 3 successive weeks beginning 15 May, and then declined in the 6th week beginning 5 June when \( H. \ axyridis \) larvae became active and dominated the predatory complex (Figure 1B). Within one week of attacks by \( H. \ axyridis \) larvae, \( Scymnus \) and syrphid larvae were absent from \( A. \ gossypii \) colonies, and in the 7th week (12 June) only fourth instar larvae of \( H. \ axyridis \) and few aphids were present in the sample. Sampling in the 8th week (19 June) did not reveal any aphid or predatory larva.

The difference of one week between the first attacks by larvae of \( Scymnus \) and syrphid species gave the former an initial advantage. Waxy second, third and fourth instars were active in aphid colonies by the time the third or fourth instar syrphids appeared. Analysis of sampling data indicates that \( Scymnus \) and syrphid larvae co-occurred on hibiscus trees for at least 3 weeks, and both predators shared aphid colonies with \( H. \ axyridis \) larvae for one week.

**Rates of encounters and attacks.** All first instar \( Scymnus \) larvae (\( n = 10 \)) were encountered and attacked by the third instar syrphids within one hour of exposure (Table 1). During the same time, syrphid larvae made significantly higher numbers of encounters with the waxy second, third and fourth instar \( Scymnus \) compared to waxless larvae (\( n = 10 \), for waxy and waxless larvae of each instar offered in pair) but the numbers that were fatally attacked varied among instars depending on their stage of development and presence or absence of wax covers. Only two out of ten second instar and none of the third and fourth instars of waxy larvae were attacked. In comparison, nine, seven and five waxless larvae of second, third and fourth instars, respectively, were attacked. Thus, more surviving second, third and fourth instar waxy \( Scymnus \) larvae met with higher encounter rates by syrphids compared to fewer surviving waxless larvae.

**Effect of wax cover on predation by syrphid larvae.** First and second instar larvae were significantly more vulnerable to predation than the third and fourth instar larvae (Figure 2). After 3 h, 40% of the second instar waxy larvae were attacked compared to none of the third and fourth instar. However, after 6 h, increasingly higher proportions of larvae of the three instars were attacked and, after 24 h, 90%, 50%, and 20% of the second, third and fourth instars, respectively, were attacked by syrphids. \( Scymnus \) larvae were never observed to attack syrphid larvae in this study.

**Thickness of wax cover in Scymnus larvae and their relative defence against predation.** First instars were without an apparent wax cover although their bodies appeared minutely dusted with wax under a binocular microscope. In older instars, wax covers assumed definite segmental pattern that consisted of distinct wax tubes, six on each segments and more on pronota. Although the head did not bear wax tubes, those on the pronota were long enough to project in front of the head, thus effectively concealing it within wax cover. Interssegment areas of thorax and abdomen were nearly waxless that provided flexibility to larval body in movements and at ecdysis. Thickness of wax tubes increased from the first instar to the fourth instar larvae (Table 2). As predicted, linear regressions showed strong inverse correlations between thickness of wax tubes (\( X \)), both on abdomen and pronota, and vulnerability (\( Y \)) of \( Scymnus \) larvae to predation by syrphids (on abdomen: \( Y = 1.18 - 3.00X, r = 0.99 \); on pronota: \( Y = 1.13 - 2.29X, r = 0.99 \)). Heavy and longer larvae (third and fourth instars \( Scymnus \)) having thick wax covers were significantly less vulnerable in terms of exposure to the danger of predation.
Durations of *Scymnus* wax covers. Smaller larvae (second instar *Scymnus* species) are more aggressive to predators of aphids than those with full wax covers, the wax- or honeydew-producing coccinellids give them an advantage to exploit ant-attended aphid colonies. However, this study shows that in absence of ants *Scymnus* species can also exploit aphid colonies. In this situation, *Scymnus* species acts as a pioneer predator by establishing first in aphid colonies at low prey abundance. This is possible because of their small size and low voracity. In the present study, *Scymnus* larvae were active on hibiscus trees within one week after fundatrices hatched from hibernating eggs at hibiscus bud burst. Syrphids were next to become active when aphid colonies were 2 weeks older and leaves in the buds began to unfurl and were infested with aphids. Early appearance of larvae of *Scymnus* gave them the initial advantage of founding a population of wax-covered larvae that were defended in interactions with syrphid larvae. Despite high aphid abundance on hibiscus trees, both these predators suffered from sharp decline in abundance within one week of attacks by *Harmonia* larvae. This is mainly attributed to potential competition from larger coccinellid predators, and might also indicate their pupation. These conclusions are drawn on the basis of a limited sample size from which abundance of aphids and predators were measured, nonetheless the results seem to supplement what is known of the natural history of these predators. It is expected that more results obtained from larger sample sizes would strengthen these conclusions.

Repeated encounters between the larvae of the two predators were predicted as they were foraging in low prey densities. There does not seem to be any aposematic influence of wax cover on starving syrphids as their larvae encountered *Scymnus* larvae without any avoidance response. Rather wax cover served as an effective defensive shield against attacks from syrphid larvae. Attacking syrphid larvae had their mouth parts

### Discussion

Compared to other insect predators of aphids, wax-producing coccinellid predators are smaller in size (Pope, 1979; Hodek & Honek, 1996), have lower voracity (Agarwala & Saha, 1986; Kawauchi, 1990), and forage in areas of low prey density. Wax-producing *Scymnus* species do not possess alkaloids (Pasteels et al., 1973) and, thus, lack chemical defence against predators. Waxy covering of their larvae is the effective means of defence from natural enemies and makes them competitive in a guild of diverse predators that share a similar food resource in patchy habitats. Available studies also suggest that wax-covered larvae of coccinellids are generally found foraging in wax- or honeydew-producing prey and/or ant-attended prey colonies (Barlett, 1961; Richards, 1985; Arakaki, 1992; Eisner, 1994; Völkl & Vohland, 1996). Ants attending wax- or honeydew-producing aphids are often aggressive to predators of aphids. Wax covers in

### Table 3. Waxy and waxless durations (mean ± SE) in the development of different larval instars of *S. posticalis* by feeding on *A. gossypii* (n = 10)

<table>
<thead>
<tr>
<th>Larval instar</th>
<th>Waxy period</th>
<th>Waxless period</th>
<th>% of developmental waxless stage</th>
<th>Total period</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>–</td>
<td>2.35 ± 0.21</td>
<td>100.00</td>
<td>2.35 ± 0.21</td>
</tr>
<tr>
<td>Second</td>
<td>1.73 ± 0.06a</td>
<td>0.27 ± 0.06b</td>
<td>13.50</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Third</td>
<td>1.67 ± 0.19a</td>
<td>0.33 ± 0.06b</td>
<td>16.50</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Fourth</td>
<td>2.11 ± 0.19a</td>
<td>0.44 ± 0.06b</td>
<td>17.25</td>
<td>2.55 ± 0.19</td>
</tr>
<tr>
<td>Total</td>
<td>5.52 ± 0.19a</td>
<td>3.39 ± 0.20b</td>
<td>38.09</td>
<td>8.90 ± 0.23</td>
</tr>
</tbody>
</table>

Figures in a row followed by different letters differ significantly at P < 0.05: Mann–Whitney U-test.
and delicate sense organs smeared with sticky wax. It took syrphid larvae some time to clear them off before mounting a fresh search for food. This cleansing behaviour gave attacked-Scymnus larvae time to move from the site of attack. Thickness of wax cover seems to be crucial in interactions between the larvae of the two predators. A thin layer of wax in the second instar made them more vulnerable to attacks than the thicker layers of wax in the third and the fourth instar larvae.

Proportions of wax-covered larvae that were attacked by starving syrphid larvae increased with time. This indicated that wax cover did not provide Scymnus larvae absolute protection from syrphid predators. In addition to differences in thickness of wax covers, larvae were waxless for a period following each ecdysis and also in intersegmental areas of their thorax and abdomen. Thus success of attacking a wax-covered larva by a syrphid predator could be dependent on encountering it during a waxless period or attacking waxless areas of the larval body. Considering that syrphids have piercing and sucking mouth parts that are stretchable, and they often attack their prey in an angular manner, there is probability that they will attack waxless areas of Scymnus larvae.

Results also indicate that wax cover of Scymnus larvae is not effective against larvae of large-sized ladybirds like Coccinella, Harmonia and Exochomus (Völkl & Vohlund, 1996). These predators aggregate in patches with high prey density and possess strong competitive abilities in terms of higher voracity, faster mobility, higher rate of increase and chemical defence of their eggs and larvae (Agarwala & Dixon, 1992; Hodek & Honek, 1996; Dixon, 2000). On a comparative scale, both Scymnus beetles and syrphids may be considered to have evolved adaptations for survival at lower prey densities compared to larger coccinellid species, which may be ‘inferior’ at low prey densities. Risks of competition for limited food from bigger coccinellid predators might have caused constraints on
smaller beetles and syrphids to limit their foraging in low prey densities when bigger predators were not active. Coexistence of superior and inferior consumers in temporary resources is usually short and their aggregation is separated in space and/or time (Shorrocks & Rosewell, 1987; Tokeshi, 1999).

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