The importance of honeydew as food for larvae of *Chrysoperla carnea* in the presence of aphids

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Abstract

Larvae of the common green lacewing *Chrysoperla carnea* are predacious and feed on a wide range of small, soft-bodied arthropods. In addition to their feeding on prey arthropods to cover their nutritional requirements for growth and development, the consumption of non-prey foods such as honeydew has been reported. It is commonly believed that these food supplements are primarily exploited by the larvae when prey is scarce or of low nutritional quality. Here, we assess whether *C. carnea* larvae also use honeydew when high-quality aphid prey are readily available. In a choice experiment, the feeding behaviour of *C. carnea* larvae was observed in the presence of both aphids and honeydew. The larvae were starved, aphid-fed, or honeydew-fed prior to the experiment. The time spent feeding on honeydew compared with feeding on aphids was highest for starved larvae and lowest for honeydew-fed larvae. Among the three treatments, the aphid-fed larvae spent the most time resting and the least time searching. In an additional experiment food intake was assessed in terms of weight change when larvae were provided with an *ad libitum* supply of either aphids or honeydew. Larvae yielded a significant lower relative weight increase on honeydew compared with aphids. The reduced weight increase on honeydew was compensated when larvae were subsequently provided with aphids, but not when honeydew was provided again. This study showed that (i) prior honeydew feeding reduces overall aphid consumption, and (ii) larvae do consume honeydew even after they have been given *ad libitum* access to aphids. The fact that larvae of *C. carnea* still use honeydew as a food source in the presence of suitable prey underlines the importance of carbohydrates as foods.
ity and prolong foraging activity (Sunby 1966; McEwen et al. 1993; Limburg and Rosenheim 2001; Patt et al. 2003). A study by Patt et al. (2003) demonstrated that the inclusion of pollen and sucrose in a diet of low-quality prey (larvae of Drosophila melanogaster Meigen), decreased development time and generated larger adults of C. carnea. Limburg and Rosenheim (2001) showed that extra-floral nectar was an important food source for neonate lacewings on cotton and that its consumption increased strongly as the availability of aphid prey declined. Furthermore, McEwen et al. (1993) showed that the applications of artificial honeydew in the field could allow C. carnea to complete its development at lower prey densities. These examples show that C. carnea utilizes non-prey foods at times when high-quality prey are scarce. However, the question remains whether larvae of C. carnea consume non-prey food such as honeydew in the presence of high-quality prey like aphids.

Honeydew is an excretion product of sap-feeding Sternorrhyncha, which is dominated by sugars. In addition to plant-derived fructose, glucose, and sucrose it commonly also contains aphid-synthesized oligosaccharides (Wäckers 2000, 2005; Hogervorst et al. 2007), as well as lower levels of amino acids (Sandstrom and Moran 2001; Fischer et al. 2002) and other compounds, including some vitamins (Hagen 1986). When honeydew is available in the field, the honeydew producers are usually not far away. Aphids are generally considered a relatively high-quality (Principi and Canard 1984; Obrycki et al. 1989; Dutton et al. 2002) and common (Canard 2001) prey for larvae of C. carnea.

In the first (no-choice) experiment, it was studied how previous honeydew- or aphid consumption affects subsequent uptake of either food source. In the second (choice) experiment, the feeding behaviour of C. carnea larvae was observed in the presence of both aphids and honeydew.

Materials and Methods
Insects and honeydew

Eggs of C. carnea were collected from our permanent laboratory colony as described by Romeis et al. (2004), and were kept separately in a climatic chamber (23±1°C, 85±5% RH, 16:8 L:D) until they hatched. Larvae were individually reared in plexiglass containers (2x2x1.5 cm) on an ad libitum supply of Ephesia kuehniella Zeller (Lepidoptera: Pyralidae) eggs, which were provided by Biotop (Valbonne, France). Larvae that had moulted into the second instar (L2) overnight were removed from the food in the morning and placed individually into new plexiglass containers. In addition, larvae that had moulted into L2 in the morning were used for a second batch of experiments in the afternoon. Larvae were subsequently starved for 24h before the start of the experiment.

A colony of the bird cherry-oat aphid Rhopalosiphum padi (L.) (Hemiptera: Aphidae) was kept as a continuous culture on 4-6-weeks old wheat plants in the glasshouse (24±2°C). Rhopalosiphum padi is a good quality prey for larvae of C. carnea (Dutton et al. 2002). In preparation of the bio-assays, aphids were collected from the colony and placed in an empty Petri dish (5 cm Ø, 1 cm high) in a climatic chamber (23±1°C, 85±2% RH) for 3h to prevent honeydew production during the bio-assays.

Honeydew from R. padi was collected in the aphid culture by placing Petri dish lids under infested leaves during 24h. Subsequently, a water-satiated piece of cotton wool was placed in the bottom part of the Petri-dish for 2h, with the honeydew-sprinkled lid on top in order to reduce the viscosity. Honeydew droplets were accumulated using a plastic spatula and then slightly diluted with 2–5μl water (depending on the amount of honeydew). Diluted honeydew was collected with a 5μl micropipette (Blaubrand® intramark, Brand GmbH+Co KG, Wetzlar, Germany) and stored at −20°C until further use.

No-choice experiment

In this experiment, the weight increase of larvae with access to aphids or honeydew during 1h was determined, as well as the weight increase with access to either one of these food sources for a second hour. Second instar larvae were weighed individually on a microbalance (Mettler Toledo MX5, d=1μg; ±2μg; Mettler Toledo, Greifensee, Switzerland). Subsequently, larvae were placed back into their plexiglass containers in a climatic chamber (23±1°C, 85±5% RH) and provided with either 10 aphids of different developmental stages (ad libitum) or a 2μl drop of honeydew (ad libitum). After 1h, larvae were re-weighed and provided either aphids or honeydew for another hour. After this second hour, larvae were weighed once more. The four treatments were (i) aphids in first and second hour; (ii) aphids in first hour; honeydew in second hour; (iii) honeydew in first hour, aphids in second hour; and (iv) honeydew in first and second hour. As a control
treatment, larvae were left without food during the first and second hour. A total of 37–38 replications were conducted for each treatment. The relative weight increase after 1 or 2h of feeding was calculated as percentage of the initial weight at the start of the experiment (t=0h). This was done to show the weight increase in the second hour independently of differences in weight increase in the first hour. Data on the relative weight increase were separately analysed for the two feeding periods using one-way ANOVA. Means were subsequently separated using Tukey’s honestly significant difference (HSD) test. All statistical analyses were computed in STATISTICA (version 6, Statsoft Inc., Tulsa, OK).

Choice experiment

As a pre-treatment, the second instar larvae were randomly divided into three groups. During 1h before the start of the observations, the first group of larvae was provided with wet cotton wool, the second group was provided with three aphids (standardized in size) in addition to wet cotton wool, and the third group was given access to a 3µl drop of honeydew and wet cotton wool.

Subsequently, individuals were released into an experimental arena containing a wet filter paper (5cm Ø) with a wheat leaf disc (1cm Ø) placed in the middle of the filter paper. The leaf disc contained four food sources distributed in the corners of a square. These food sources consisted of two diagonally opposed droplets of 1µl honeydew and two diagonally opposed R. padi (fourth instar). Aphids were allowed 15min of settling time before the experiment was started and the observations were aborted when an aphid would leave its feeding spot. The C. carnea larva was released in close vicinity of the leaf disc. The observation started once the larva had contacted the leaf disc. Larvae that did not find the leaf disc within 15min in the middle of the filter paper. The observation was terminated when (i) the larva left the experimental arena; (ii) one of the two food source was depleted; or (iii) after 1h. This maximum observation period was chosen as feeding to repletion can occur within the first 30min of exposure to food (Bond 1978). In total, 16 replications were conducted for each of the three treatments.

The number of larvae that had fed on aphids, on honeydew, and that had fed in general were compared between the three pre-treatments (starved, honeydew-, or aphid-fed) using chi-square contingency tables (3x2 tables). Post hoc calculations were performed as three pair-wise chi-squared tests with Bonferroni correction resulting in α=0.017. Furthermore, it was investigated using chi-squared test whether the pre-treatment affected the choice of the first food source (honeydew or aphids). Differences in the number of aphids fed on by the larvae in the three pre-treatments were analysed using Kruskal-Wallis ANOVA.

Results

No-choice experiment

The relative weight change of the C. carnea larvae after the first and second hour expressed as percentage of the initial weight are summarized for the five treatments in fig. 1. The no-food treatment (control) showed a small but steady weight decrease during the 2h. For the other four treatments, one-way ANOVA of relative weight increase during the first hour showed significant differences among the treatments (F=8.46; d.f.=3, 147; P<0.0001). Subsequent comparison of the means showed a significant lower relative weight increase (Tukey’s HSD test, P<0.01) after an hour of honeydew feeding compared with aphid feeding (except for one out of four comparisons that showed a P-value of 0.053). ANOVA results of the second hour again showed significant differences among the treatments (F=22.35; d.f.=3, 147; P<0.0001). Tukey’s HSD test revealed that the relative weight increase during the second hour of feeding was highest when feeding on aphids following honeydew feeding during the first hour (P<0.0001), even though the relative weight increase was smaller than for C. carnea larvae feeding on aphids during the first hour. After having fed on aphids during the first hour, there was no significant difference in relative weight increase between honeydew and aphid feeding in the second hour.
There was also no significant difference between the relative weight increase when larvae fed on honeydew during the second hour (P=0.83), independent of the food source consumed during the first hour (fig. 1).

The total relative weight increase in 2h was 74% for larvae that had fed aphids during both hours, 42% for those that had only fed honeydew, 63% when aphids were consumed during the first hour and honeydew during the second hour, and 75% when honeydew was consumed during the first hour and aphids during the second hour.

**Choice experiment**

When the *C. carnea* larvae were starved (but water satiated) prior to the experiment, 15 out of 16 larvae managed to find and feed on honeydew and/or aphids during the experiment. This number was lower (however, not significantly; $\chi^2=4.48$, P>0.1) for larvae that had *ad libitum* access to aphids or honeydew in the hour before the experiment (table 1).

No difficulties in prey handling were observed, thus unsuccessful attacks on *R. padi* have not played an important role in the choice experiment. Both starved and aphid-fed larvae accepted honeydew and aphids equally. For the honeydew-fed larvae, on the other hand, more larvae fed on aphids while only a few continued feeding on honeydew during the experiment (table 1). Overall, there was no significant difference in the number of larvae that fed on aphids among the three pre-treatments ($\chi^2=5.25$, P>0.05); however, for honeydew feeding a significant difference among the treatments was found ($\chi^2=12.76$, P<0.05). Pair-wise comparison of the pre-

![Fig. 1](image)

**Fig. 1** Relative weight change (to initial weight, t=0) of second instar *Chrysoperla carnea* larvae (±SE), when fed on either aphids or honeydew for 1h and subsequently on one of the food sources for a second hour (n=37–38). Different letters above the bars indicate significant differences [one-way ANOVA, followed by Tukey’s honestly significant difference test, P<0.05; capital letters: feeding in the first hour (assessed at t=1); small letters: feeding in the second hour (assessed at t=2)].

**Table 1** Summary of the feeding behaviour observations in the choice experiment of second instar *Chrysoperla carnea* larvae

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Feeding</th>
<th>Feeding on aphids</th>
<th>Feeding on honeydew</th>
<th>First food source</th>
<th>Mean number of aphids fed on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aphids</td>
<td>Honeydew</td>
</tr>
<tr>
<td>Starved</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Aphid fed</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Honeydew fed</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

The larvae had been provided either with wet cotton wool (starved), or three aphids, or with a 3µl drop of honeydew, both in addition to wet cotton wool (n=16). In the first four columns, the number of individuals that showed the listed behaviour is recorded. The last column shows the average amount of aphids fed from the two aphids available.
treatments by chi-squared test showed a significant difference in honeydew feeding between starved and honeydew-fed larvae ($\chi^2=12.70, P<0.001$), but no significant differences among the other two comparisons (starved vs. aphid fed: $\chi^2=1.40, P>0.1$; aphid fed vs. honeydew fed: $\chi^2=2.13, P>0.1$). It was further investigated whether the larvae showed a preference for aphids or honeydew, expressed in the first food source accepted. Although starved larvae showed a tendency to feed more frequently on honeydew as a first food source, and honeydew-fed larvae more frequently started feeding on an aphid (table 1), chi-squared tests revealed no significant differences ($P>0.05$). Aphid-fed larvae showed the lowest tendency to attack aphids during the experiment, whereas starved larvae consumed the highest aphid numbers (table 1). However, these patterns again only approached significance (Kruskal–Wallis ANOVA, $H_2=5.14, P=0.077$).

The larval behaviour during the time spent on the leaf disc is shown in fig. 2. Overall, C. carnea larvae spent more time on the leaf disc when they have been starved prior to the experiment. This additional time is mostly spent feeding. The time of honeydew consumption compared with aphid consumption (ratio of time spent honeydew feeding divided by the time spent aphid feeding), is highest for starved lacewing larvae (0.31), lower for aphid-fed larvae (0.13), and lowest for honeydew-fed larvae (0.04). The aphid-fed larvae spent most of their time resting and the least time searching of the three pre-treatments.

**Discussion**

Our results demonstrate that larvae of C. carnea use honeydew as a food source, even in the presence of high-quality prey. Under experimental conditions, carbohydrate sources are not only used to extend longevity in the absence of prey, but form an integral part of the diet of this omnivorous predator. Because the observation period only lasted several hours under experimental conditions, these results do not account for possible long-term changes in food choice or differences in behaviour under field conditions.

In the case of lacewings, non-prey foods are not sufficient for larval development and the omnivorous predator remains dependent on prey consumption. Limburg and Rosenheim (2001) showed that lacewing larvae were able to utilize nutrients in extra-floral nectar, but were unable to moult to the next instar. The consumption of a sucrose solution increases longevity, but does not allow larval development (Romeis et al. 2004). Both (extra-) floral nectar and honeydew are carbohydrate-dominated food sources. Even though honeydew often contains higher levels of amino acids compared to (extra-) floral nectar, these are often non-essential and poorly balanced (Wäckers 2005). The contribution of honeydew in terms of amino acids for growth and development of predator larvae is therefore likely to be very limited.

In both the experiments described here, it was seen that previous feeding reduced absolute and relative
consumption of honeydew. The reduced likelihood of further honeydew feeding is most pronounced after previous honeydew feeding. This could be because of satiation or, in the case of previous aphid feeding, caused by the fact that aphids also contain sugars and the larvae may therefore cover (part of) their carbohydrate requirements through aphid feeding. With one exception, the total relative larval weight increase in 2 h is more or less equal for all treatments in the no-choice experiment (fig. 1). Only when honeydew was consumed in both hours, total relative weight increase was significantly lower. This implies that larvae are not completely satiated after honeydew consumption alone. Rather, larvae appear to restrict honeydew consumption to allow for subsequent feeding on more nutritionally balanced prey. Limburg and Rosenheim (2001) suggested that lacewing larvae do not reject opportunities to feed on extra-floral nectar, but reduce their nectar foraging time when prey are abundant. This reduced foraging time would lead to a lower encounter rate and therefore lower consumption of extra-floral nectar. Here, we see that the inhibition can also work in the other direction as our experiments show that prior honeydew feeding does reduce aphid consumption. Both laboratory studies have shown that regulation of food uptake takes place when honeydew as well as prey are available in excess, because both aphids and honeydew droplets were abandoned on several occasions before they were completely consumed. Robinson et al. (2008) demonstrated that feeding on floral resources reduced consumption of aphids by adult brown lacewings. Similar reduction in prey consumption or cannibalism as a result of the consumption of plant resources has been found in other omnivores (e.g. Nomikou et al. 2004: Leon-Beck and Coll 2007). Our study indicates that aphid honeydew can mediate omnivore–prey relationships in similar ways as reported for plant resources. The complex interactions between omnivores, prey and non-prey food will be influenced by life history parameters of both prey and predator, as well as numerous factors in the field (Robinson et al. 2007). It is therefore too early to speculate how honeydew consumption will influence the efficiency of lacewings as biological control agents.

In the no-choice experiment, it was seen that relative weight increase of lacewing larvae during the first hour was only about one-third (32%) lower when provided with honeydew when compared to the aphid treatment. This shows that honeydew is consumed in rather high amounts when larvae have been starved for a long period (24 h). In the second hour, after 1 h of aphid feeding, the relative weight gain on honeydew feeding was 39% lower when compared with the aphid treatment. Although the total amounts consumed in the second hour are much lower than in the first hour, the ratio between aphid and honeydew consumption is very similar. The multidimensional geometric approach to insect feeding and nutrition, described by Raubenheimer and Simpson (1993, 1999) and Simpson et al. (1995), could provide an explanation for the reduced relative consumption of honeydew and the relative stable intake-ratio between aphids and honeydew. They define the intake of nutrients optimal for the insect’s fitness as ‘intake target’ and when the intake target cannot be reached because of nutritionally imbalanced food, the organism seeks to achieve the ‘point of best compromise’ (Simpson et al. 1995). Lacewing larvae require both carbohydrates and proteins at a given ratio. Animals given two or more imbalanced but complementary foods have been found to be able to reach the intake target by ‘compensatory feeding’ (Raubenheimer and Simpson 1993; Simpson et al. 1995). As honeydew provides almost exclusively carbohydrates, it represents a nutritionally imbalanced food. Compensatory feeding can explain the high relative weight increase of lacewing larvae when given access to aphids (a source of both proteins and carbohydrates) after honeydew feeding during the previous hour (fig. 1).

The choice experiment showed that larvae spent much less time feeding on honeydew than on aphids. This could reflect a preference by the larvae for prey over honeydew. However, it could also (in part) reflect a difference in food handling time. Liquid honeydew may be more easily imbibed (Downes 1974), whereas feeding on aphids requires prey identification, overcoming prey defence and possibly pre-digestion (Canard 2001). The hypothesis that honeydew is a quick and easy source of energy (and water) may also explain the observation in the choice experiment that most starved lacewing larvae fed on honeydew as a first food source, before attacking an aphid (table 1).

Honeydew-fed larvae spent 7.7% of the time on the leaf disc actively searching, whereas this number was 5.6% for starved larvae, and 5.5% for aphid fed larvae (fig. 2). Limburg and Rosenheim (2001) showed that extra-floral nectar feeding as well prolongs foraging activity of lacewing larvae. In addition, aphid honeydew is not only used as a food source by lacewing larvae, but also appears to serve as a kairomone, by increasing the intensity of searching behaviour (Kawecki 1932; Canard and...
Duelli 1984) and by guiding the larvae to their prey (Downes 1974). As honeydew is used as a kairomone, exploitation of this food source will save the larvae time and energy.

The results presented here give a further indication of the importance of non-prey foods, such as honeydew, for omnivorous green lacewing larvae. These non-prey foods are not only important during a period of prey scarcity (Limburg and Rosenheim 2001) or in the absence of high-quality prey (Patt et al. 2003), but may also form part of the diet when suitable prey are available.

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