Chrysoperla rufulabris (Neuroptera: Chrysopidae) females do not avoid ovipositing in the presence of conspecific eggs

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Abstract

Previous experiments have demonstrated that green lacewing (Neuroptera: Chrysopidae) adults could be attracted to field crops using artificial honeydew. To be effective as a biological control method, such a technique would require that the increase in female abundance translate in an increase egg deposition. An experiment was conducted to evaluate whether the honeydew-feeding females of the green lacewing Chrysoperla rufulabris (Burmeister) avoid laying eggs in the presence of conspecific eggs. The potential risk associated with oviposition in a site already occupied by conspecific eggs was also studied. The preference of C. rufulabris larvae for kin and non-kin eggs and the susceptibility of C. rufulabris eggs to cannibalism relative to their age was determined. The results demonstrate that females are not reluctant to oviposit in the presence of conspecific eggs. Larvae show no preference for kin or non-kin eggs, and lacewing eggs become less susceptible to cannibalism as they age. This indicates that the risk of egg cannibalism by neonate in the field may be low. The results are discussed from ecological and biological control points of view.

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1. Introduction

The green lacewing Chrysoperla rufulabris (Burmeister) (Neuroptera: Chrysopidae) is a native North-American species (Garland, 1985; Greve, 1984; Putman, 1937) whose larvae are generalist predators that seem to reach higher larval performance when fed on some aphid species (Hydorn and Whitcomb, 1979). Due to their high larval voracity (Principi and Canard, 1984), as well as their relative tolerance to many insecticides (Bigler, 1984; Mizell and Schnathauer, 1990; Pree et al., 1989; but see Grafton-Cardwell and Hoy, 1985), green lacewings are considered potentially useful biological control agents of aphids and other soft-bodied insects (Breene et al., 1992; Daane and Yokota, 1997; Hagley, 1989; Hassan et al., 1985; Heinz and Parrella, 1990; Nordlund et al., 1991; Ridgway and Jones, 1969; Ridgway and Murphy, 1984; Scopes, 1969; Tulisalo, 1984). The adults of C. rufulabris are not predators and feed mainly on honeydew, nectar and pollen (Garland, 1985). Since the non-predaceous adults cannot act as biological control agents per se, and the larvae are relatively limited in their capacity to disperse over long distances (Daane and Yokota, 1997), the aggregative response of this species to a patchily distributed aphid prey would be mainly determined by the oviposition behaviour of the females. It has been demonstrated that a single C. rufulabris female can lay about 22–23 single eggs per night (over 15–20 nights) under laboratory conditions (Ru et al., 1976). However, egg production is likely to be lower in real situations as it has been demonstrated that fecundity is decreased by sub-optimal diets and harsh conditions, which is what lacewings are likely to encounter in the field (Rousset, 1984; Tauber and Tauber, 1983). Moreover, Chrysoperla spp. females are highly mobile and usually no more than a few eggs are laid

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in each oviposition site (B. Fréchette, personal observation). The oviposition behaviour of a single *C. rufilabris* female could thus be insufficient to control severe aphid infestations.

Honeydew-feeding lacewing females are attracted to aphid honeydew (e.g. Dean and Satasook, 1983; Duelli, 1980, 1984; van Emden and Hagen, 1976), as well as to some plant volatiles (e.g. Ballal and Singh, 1999; Flint et al., 1979; Reddy, 2002; Zhu et al., 1999). Some authors have successfully attracted several honeydew-feeding green lacewing adults by spraying artificial honeydew in field crops (Hagen et al., 1976). Adding an artificial food source to the attractant, Tassan et al. (1979) have also enhance the short term reproductive capacity of females. However, to effectively control pests, such a strategy would require that the aggregative response of the adults to the attractant would also elicit a concentrated oviposition response.

Many lacewing species avoid laying eggs in sites where they detect the presence of conspecific or heterospecific larvae (Ruzicka, 1994, 1996, 1997a). This behaviour is considered adaptive since laying larvae have a strong propensity to cannibalism (Afzal and Khan, 1978; Arzet, 1973; Canard and Duelli, 1984; Fleschner, 1950), and young eggs are particularly vulnerable to cannibalism (Canard, 1970).

The presence of conspecific eggs in a potential oviposition site may also constitute a risk for a female’s progeny. The eggs already present are more advanced in their development than eggs laid later, and thus the later eggs could run the risk of being cannibalised. *C. rufilabris* egg development time takes between 3 and 7 days in the field at temperatures fluctuating between 18.2 and 32.2 °C (Burke and Martin, 1956; Putman, 1937). However, whether or not *C. rufilabris* females are reluctant to oviposit in the presence of conspecific eggs is still unknown.

According to optimal oviposition theory (Jaenike, 1978; Mangel, 1987) females should avoid to oviposit in a site already occupied by conspecific eggs when high quality sites are readily available.

The aim of this experiment was to determine whether the green lacewing *C. rufilabris* is reluctant to lay eggs in the presence of conspecific eggs. The risk associated with oviposition in such a site was also evaluated by determining the preference of newly hatched larvae for kin and non-kin eggs, as well as their propensity to cannibalise eggs of different ages.

**2. Materials and methods**

*Chrysoperla rufilabris* adults were obtained from the insect supplier Natural Insect Control, Stevensville, Ontario, Canada, and were about 10 days old on reception. Prior to shipping, lacewings had been fed with frozen eggs of *Ephesia kuehniella* Zeller as larvae and an undisclosed artificial diet as adults. After arrival, adults were given water. Experiments were done using this generation of adults.

**2.1. Oviposition response to conspecific eggs**

Experiments were conducted in a cardboard tube (4 cm × 10.6 cm long) with ends covered with muslin. A gravid female was placed in each tube for 1 h 30 in a lighted chamber at 28°C, in the afternoon. A female was considered gravid when its abdomen was swelled with eggs. Three experimental treatments were compared: (1) cardboard tubes containing 15 conspecific eggs (*n* = 24), (2) cardboard tubes where previously deposited conspecific eggs were removed (*n* = 25), and (3) cardboard tubes that had never contained conspecific eggs (*n* = 25). A Likelihood Ratio test was used to compared the proportions of tubes where oviposition occurred for each treatment, and the numbers of eggs laid per tube in each treatment were compared with a Wilcoxon test due to non-normal distribution of data.

To obtain tubes with conspecific eggs, one female was placed in each tube at 28°C, 16 L: 8 D. After 24 h females were removed and tube containing eggs were kept for the experiments. For treatment 1, each tube was standardised to 15 eggs by manually removing eggs exceeding 15. For treatment 2, all the eggs laid were removed.

**2.2. Preference for kin and non-kin eggs**

An experiment was conducted to determine whether newly hatched *C. rufilabris* larvae cannibalised kin or non-kin eggs preferentially. Newly hatched (less than 24-h old) lacewing larvae were put in the presence of two kin and two non-kin eggs in a 50-mm Petri dish. The genetic relatedness of the first egg consumed (kin or non-kin) was noted. Ruzicka (1997b) suggested that the green lacewing *Chrysopa oculata* Say may be able to determine that an egg is a conspecific or not using cues present on the eggs stalk. This experiment was thus conducted in a first time with stalked eggs (*n* = 22), and in a second time with eggs on which stalk had been removed (*n* = 23).

To obtain kin and non-kin eggs, a female was placed in a cardboard tube (4 cm × 10.6 cm long) at 28°C, 16 L:8 D. After 24 h the eggs laid in the tube were removed. For each replicate, one egg was kept at 24°C to allow it to hatch, and two eggs from the same female (kin) and two eggs from another female (non-kin) were kept at 7°C to delay their development. Upon hatching, the neonate larvae were placed in the presence of two kin and two non-kin eggs. Stalked eggs were glued (with non-toxic glue, LePage, Brampton, Ontario, Canada) on the underside of the Petri dish lid while unstalked eggs were deposited on the bottom. The proportions of kin and non-kin eggs chosen by larvae were compared with the theoretical value of 50–50% with a Likelihood Ratio test.

**2.3. Eggs age and susceptibility**

The potential influence of age on the susceptibility to cannibalism of *C. rufilabris* eggs was tested. Newly hatched lacewing larvae were put in a 50-mm Petri dish in the
3. Results

3.1. Oviposition response to conspecific eggs

There were no significant differences in the proportions of females that laid eggs (Table 1, Likelihood Ratio: \( \chi^2 = 1.79, df = 2, P = 0.41 \)), or in the number of eggs laid (Table 1, Wilcoxon: \( \chi^2 = 1.82, df = 2, P = 0.40 \)), in the presence of conspecific eggs, in the control treatment, or in the treatment where conspecific eggs have been removed.

3.2. Preference for kin and non-kin eggs

Larvae did not show a significant preference for kin or non-kin eggs. Kin eggs were chosen in 57% of the cases when eggs were stalked (Likelihood Ratio: \( \chi^2 = 0.39, df = 1, P = 0.53 \)), and in 55% of the cases when eggs were not stalked (Likelihood Ratio: \( \chi^2 = 0.18, df = 1, P = 0.67 \)).

Table 1

<table>
<thead>
<tr>
<th>Ovipositing females (%)</th>
<th>Number of eggs</th>
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<tr>
<td>Conspecific eggs present 25</td>
<td>0.42 ± 0.19</td>
</tr>
<tr>
<td>Conspecific eggs removed 32</td>
<td>0.80 ± 0.30</td>
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<tr>
<td>No conspecific eggs 16</td>
<td>0.40 ± 0.23</td>
</tr>
</tbody>
</table>

3.3. Eggs age and susceptibility

The number of eggs eaten was significantly affected by their age (Fig. 1, Wilcoxon: \( \chi^2 = 14.27, df = 3, P = 0.003 \)). Egg susceptibility to cannibalism significantly decreased with age.

4. Discussion

The results of this study indicated that C. rufilabris females are not reluctant to oviposit in the presence of conspecific eggs. Even though this absence of response to conspecific eggs may seem counter-intuitive it could be the consequence of weak selection pressure for such a behaviour. For example, this experiment demonstrated that C. rufilabris neonate larvae do not feed preferentially on non-kin eggs (heterocannibalism) than on kin eggs (sibling cannibalism). That is, from a genetic relatedness point of view, the eggs laid by a female in a site already occupied are not more susceptible to be the victim of cannibalism than the eggs already present. Moreover, this experiment demonstrated that, as C. rufilabris eggs get older, they are less susceptible to be preyed upon. A similar situation was observed by Canard (1970) with the species Chrysoperla perla (L.); this author observed that the eggs are highly susceptible to cannibalism when they are freshly laid, or just before hatching, but are less susceptible in between these two events. The difference in the susceptibility to egg cannibalism was attributed to the mechanical resistance provided by the development of embryonic sheets (Canard, 1970). We did not observe the susceptibility of C. rufilabris eggs about to burst, but we would expect similar results than what was observed with C. perla. Thus, C. rufilabris eggs would be highly vulnerable to cannibalism by neonates for only a small proportion of their development time (Fig. 2).

Finally, Duelli and Johnson (1992) and Ruzicka (1997b) have demonstrated that the stalk of lacewing eggs lower the...
incidence of cannibalism. It is thus proposed that, in natural settings, the risk of non-kin egg cannibalism associated with oviposition in the presence of conspecific eggs is probably low for *C. rufilabris*.

The results presented here are in contradiction with those obtained by Ruzicka (1994) with the species *C. oculata*. This author reported that this species was reluctant to oviposit in the presence of conspecific eggs. However, in contrast to *C. rufilabris*, *C. oculata* adults are aphidophagous. Duelli (1987) observed that the oviposition site selection of honeydew-feeding lacewings seems to be less associated with aphid colonies than that of species with predaceous (aphidophagous) adults. Boo et al. (1998) have demonstrated that the adults of *Chrysopa cognata* Wesmael, a species with predaceous adults, are attracted to aphids alarm pheromones. On the other hand, the results obtained by Fréchette and Coderre (2000) indicate that *C. rufilabris* females are not more reluctant to lay eggs in the absence than in the presence of aphids over an 8 h period. The selection pressures on lacewings with honeydew-feeding adults and their resultant oviposition behaviour are thus likely to be different from those of lacewings with predaceous adults. The stronger association between oviposition site selection and the patchily distributed aphid colonies in lacewings with aphidophagous adults probably makes it more likely that different females would lay their eggs in the same oviposition sites. In such a situation, the probability of egg cannibalism may be thought to be higher than for species with honeydew-feeding adults, for whom oviposition site selection seem to be less associated with patchily distributed oviposition cues. For that reason, the selection pressure on females that avoid ovipositing in the presence of conspecific eggs may be weak.

A possible bias in this experiment is that the experimental females came from a stock culture of about 100 individuals. Females were thus held in an environment with constant contact with conspecific eggs. Fréchette et al. (2004) have demonstrated that the ladybird *Adalia bipunctata* (L.) can become less reluctant to lay eggs in the presence of an oviposition-deterring pheromone following constant exposure. The existence of such a mechanism is not known for chrysopids. However, the results presented here, even though not significantly different, indicated that more females laid eggs in the presence of eggs or where eggs were previously present than in control treatments. That is, no sign of reluctance to oviposition in the presence of conspecific eggs were observed. Nevertheless, the capacity of lacewings females to learn or to acclimatise to the presence of potential oviposition-deterring stimuli should be studied.

It can also be objected that females were held in a close environment that did not allowed females to leave in search of a better place to lay eggs. However, since experiments were of short duration (1 h 30), it can be assumed that reluctant females would have withheld oviposition. This was observed with ladybirds in a similar experimental unit: females held in Petri dishes in the presence of an oviposition-deterring pheromone withhold oviposition for a longer time than females in control Petri dishes (Doumbia et al., 1998; Fréchette et al., 2004).

The results presented here suggest that a biological control program based on the use of artificial lacewing attractants could not only result in a significant augmentation of adults within the targeted crop (Hagen et al., 1976), but also in a potential high density egg deposition. Such a response is crucial for programs using honeydew-feeding green lacewings, since the adults per se have no potential for pest control.

The potential of lacewings as biological control agents in field and greenhouse cultures has been well studied. However, they have seldom been used with success in the field, and many aspects of lacewings biology are still understood. The oviposition behaviour, as demonstrated here, is still largely misunderstood, particularly for honeydew-feeding females. Further studies are thus needed to understand the egg distribution of Chrysopidae.

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