

Predation capacity and prey preference of *Chrysoperla carnea* on *Pieris brassicae*

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Abstract The predation capacity and prey preference of larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on eggs or larvae of *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae) in the absence and presence of cabbage aphids as an alternative prey were evaluated in laboratory experiments at 25°C. Both instars preyed upon butterfly eggs and larvae as well as on cabbage aphids with the third instar being the most voracious. The lacewings had a strong preference for caterpillars to butterfly eggs. In the presence of the aphids the predation on *P. brassicae* eggs or larvae was either completely abandoned or reduced by about 70%, respectively, by second instar lacewings and either reduced by about 80% or maintained, respectively, by third instar lacewings. Both instars thus had a clear preference for aphids compared to eggs of *P. brassicae*. However, second instar lacewings preferred aphids to caterpillars whereas the opposite was the case for

third instar lacewings. The results indicate that 3rd instar *C. carnea* has a potential as biocontrol agent against *P. brassicae*.

Keywords Chrysopidae · Lacewings · Lepidoptera · Biological control · *Brevicoryne brassicae* · Aphididae

Introduction

Green lacewing larvae (Neuroptera: Chrysopidae) are known as voracious predators within the aphidophagous guild and several species are exploited for biological control of aphids (Principi and Canard 1984). The biological control strategy may be based on enhancing the effect of naturally occurring lacewings through habitat manipulations (Principi and Canard 1984; Landis et al. 2000; Tauber et al. 2000) or on inundative releases of mass produced lacewings, as is the case for the common green lacewing *Chrysoperla carnea* (Stephens) (Principi and Canard 1984; Tauber et al. 2000) and *Chrysoperla rufilabris* (Burmeister) (Richman et al. 1980; Wang and Nordlund 1994). Both species are commercially available in North America (Hunter 1997; Tauber et al. 2000) and the former in Europe (e.g. Biobest 2009; Bioplanet 2009). Both have been used for a number of years for aphid biocontrol in various greenhouse and field crops (e.g. Biobest 2009; Bioplanet 2009; Carillo et al. 2009).

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However, lacewings are able to prey on a wide range of other pest species such as scale insects, leafhoppers, whiteflies, psyllids, thrips, psocids, lepidopterans and mites (Principi and Canard 1984). Several studies have been undertaken to investigate the ability of lacewings to control other pests besides aphids, e.g. leaf miners (Chen et al. 1989; Urbaneja et al. 2001; Ecole et al. 2002; Rao et al. 2003) and lepidopteran pests (e.g. El-Dakrouy et al. 1977; Ding and Chen 1986; Kabissa et al. 1996; Parajulee et al. 2006; Rajabaskar 2007). Among the lacewing species studied for control of lepidopteran pests, *C. carnea* has been found to be an important predator against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Bakthavatsalam et al. 2000). In addition, this lacewing species in some cases seems to be more effective in controlling lepidopteran pests such as *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae) compared to other predators such as *Orius insidiosus* (Say) (Heteroptera: Anthoridae) and *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) (Eigenbrode et al. 1995). In a field cage study examining biological control of *Heliothis zea* (Boddie) and *H. virescens* (Fabricius) (Lepidoptera: Noctuidae) Ridgway and Jones (1968) found indications that inundative releases of *C. carnea* offered considerable potential in cotton. Supportive evidence of the potential of *C. carnea* against lepidopteran pests comes from observations that this species could complete juvenile development when feeding on eggs of *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) or of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Şengonca and Grooterhorst 1985). Similarly, Klingen et al. (1996) found that most *C. carnea* in their experiments could develop to adulthood by feeding on eggs or larvae of *M. brassicae*.

No information is, however, available on the predation capacity of *C. carnea* on the large white butterfly, *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae), an important pest on cabbage (Feltwell 1978). Preliminary tests (N. Huang, pers. obs.) revealed that *C. carnea* larvae were able to prey upon both *P. brassicae* eggs and larvae. To examine the potential of *C. carnea* as a biocontrol agent of this pest, we therefore further quantified the predation capacity and prey preference of lacewing larvae on eggs or young larvae of *P. brassicae* in the absence and presence of cabbage aphids, *Brevicoryne brassicae*

(Linnaeus) (Hemiptera: Aphididae), as an alternative prey. Second and third instar larvae of *C. carnea* were used in the experiments. Second instars were selected to get information on the instant control potential of releases of *C. carnea* directly from commercial shipments, which are usually based on this developmental stage. The third instar was chosen to obtain information on the maximum predation capacity of this species since the third instar larval stage is the most voracious, consuming more than 3/4 of the total prey intake during larval development (Principi and Canard 1984; Klingen et al. 1996; Silva et al. 2002).

Materials and methods

Insects

One- to two-day-old 2nd instar *C. carnea* were supplied by EWH BioProduction, Tappernøje, Denmark and reared individually to the 3rd instar on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae; supplied by the same producer) eggs in Petri dishes (diameter: 5.5 cm) in a climate cabinet at $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ rh, and a 16:8 L:D photoperiod. One-day old 3rd instar *C. carnea* were used in the experiments. The two-day-old 2nd instar *C. carnea* used for experimentation were collected directly from the supplied material and allowed a brief feeding period (approx. 2 h) on *E. kuehniella* eggs offered ad libitum. All larvae used were starved for 24 h prior to experimentation by keeping them individually in Petri dishes (diameter 5.5 cm) in a climate cabinet at the same conditions as above.

Pieris brassicae and *B. brassicae* were reared separately on curly kale [*Brassica oleracea* Linneus var. *acephala* (Brassicales: Brassicaceae)] in cages ($68 \times 75 \times 82$ cm) in a climate-controlled greenhouse compartment at $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ rh, and a 16:8 L:D photoperiod (supplemented with light in the dark season). Aphid nymphs (2nd–3rd instar), caterpillars and butterfly eggs laid within two days (clusters on small leaf pieces) used for experimentation were collected directly from the rearings.

Experimental set-up

All the experiments were done in plastic Petri dishes (diameter 5.5 cm) with ventilation secured by built-in

notches on the inside edge of the lid. Each Petri dish contained a curly kale leaf fitted onto a moistened cotton sheet. The dishes were placed in Plexiglas boxes (30.5 × 22.0 × 5.5 cm) with a saturated salt water solution at the bottom to achieve a 70–75% rh. The boxes were kept in a climate cabinet at 25 ± 1°C, 70 ± 5% rh, and a 16:8 L:D photoperiod.

Experiment 1. Lacewings vs. *P. brassicae* eggs and *B. brassicae* nymphs

The larvae of *C. carnea* were provided with the following prey combinations: (1) *P. brassicae* eggs; (2) *P. brassicae* eggs and *B. brassicae* nymphs; (3) *B. brassicae* nymphs. Each treatment had 30 replicates and five controls without predators. Per replicate, the second instar *C. carnea* was given 50 eggs (1–2 clusters) of *P. brassicae* and (or) 20 nymphs of *B. brassicae* while the third instar lacewings were given 150 eggs (3–5 clusters) of *P. brassicae* and (or) 50 nymphs of *B. brassicae*. The eggs were harvested from the rearing by cutting pieces of leaf with egg clusters from the rearing plant. The leaf pieces were subsequently added to the Petri dish with the side with the eggs facing upwards. After 24 h the number of the eggs and aphids killed by *C. carnea* were counted under a stereomicroscope. Based on preliminary trials, the experimental period was set to 24 h to ensure sufficient predation on *P. brassicae* eggs.

Experiment 2. Lacewings vs. *P. brassicae* larvae and *B. brassicae* nymphs

The larvae of *C. carnea* were provided with the following prey combinations: (1) *P. brassicae* larvae; (2) *P. brassicae* larvae and *B. brassicae* nymphs; (3) *B. brassicae* nymphs. Each treatment had 30 replicates and five controls without predators. Per replicate, the second instar *C. carnea* was given 10 *P. brassicae* 1st instar caterpillars and (or) 20 nymphs of *B. brassicae* while the third instar lacewings were given 10 *P. brassicae* 2nd instar caterpillars (2-day old) and (or) 20 nymphs of *B. brassicae*. After 2 h the numbers of the preys killed by *C. carnea* were counted under a stereomicroscope. Based on preliminary trials, the experimental period was set to 2 h to minimize the size increase of *P. brassicae* larvae and to limit the accumulation of lepidopteran feces in the set-ups.

Experiment 3. Preference between *P. brassicae* eggs and larvae

Second or third instar *C. carnea* were provided with *P. brassicae* eggs (50 eggs (1–2 clusters) for 2nd instar lacewings; 150 eggs (3–5 clusters) for 3rd instar lacewings) and *P. brassicae* larvae (10 1st instar caterpillars for 2nd instar lacewings; 10 2nd instar caterpillars (2-day-old) for 3rd instar lacewings). Each treatment had 30 replicates and five controls without predators. After 2 h the number of eggs and larvae of *P. brassicae* killed by *C. carnea* were recorded.

Statistical analysis

For each experiment the mean number of prey killed was corrected for the mortality in the controls (Abbott 1925) and compared with one-way ANOVA by SPSS 13.0 (SPSS 2004). Preference was analyzed by calculating Manly's preference index (Manly 1974) considered appropriate in the present case, since it applies to data originating from experiments where killed prey items were not replaced (Cock 1978; Sherratt and Harvey 1993). Manly's preference index was calculated as

$$\beta_1 = \frac{\text{Log}(e_1/A_1)}{\text{Log}(e_1/A_1) + \text{Log}(e_2/A_2)}$$

where β_1 was the preference to prey type 1, e_1 and e_2 were the number of prey of type 1 and type 2 remaining after the experiment, A_1 and A_2 were the number of prey of type 1 and type 2 presented to the predator. Prey type 1 was chosen as follows: experiment 1: *B. brassicae* nymphs; experiment 2: *B. brassicae* nymphs for 2nd instar *C. carnea* and *P. brassicae* larvae for 3rd instar *C. carnea*; experiment 3: *P. brassicae* larvae. If the preference index is close to 1, the predator prefers prey type 1 and if close to 0 the prey type 2 was preferred. An index value close to 0.5 indicated no preference. Each preference index was tested for significance (i.e. difference from a value of 0.5) by one-sample *t*-test by SPSS 13.0 (SPSS 2004). Replicates in which neither of the two prey types offered had been predated were excluded from the analysis of prey preference (i.e. Manly's preference index) (one replicate was excluded for 2nd instar *C. carnea* in experiment 2; 2 replicates were

excluded for 2nd instar *C. carnea* in experiment 3; 1 replicate was excluded for 3rd instar *C. carnea* in experiment 3).

Results

Predation capacity on *P. brassicae* eggs and aphids

Both second and third instar lacewings were able to prey upon eggs of *P. brassicae* (experiment 1) (Table 1). The third instar was significantly ($F = 187.78$, $df = 1, 59$, $P < 0.001$) more voracious than the second instar, consuming more than 30 times as much as the second instar during the 24 h experimental period. Likewise both instars of *C. carnea* were capable of preying on cabbage aphids, again with the third instar being the most voracious ($F = 286.26$, $df = 1, 59$, $P < 0.001$).

In the presence of cabbage aphids the predation by both lacewing instars on *P. brassicae* eggs was significantly reduced ($F = 20.81$, $df = 1, 59$, $P < 0.001$ and $F = 97.21$, $df = 1, 59$, $P < 0.001$, respectively). For the second instar lacewings the predation on eggs was completely absent in the presence of aphids—predation on aphids, however, remained unchanged ($F = 0.60$, $df = 1, 59$, $P = 0.444$) compared to the situation where only aphids were provided. By contrast, predation of third instar lacewings on the lepidopteran eggs in the presence of aphids was reduced by approx. 80%. For this

instar, aphid predation was significantly reduced ($F = 6.82$, $df = 1, 59$, $P = 0.011$) when both prey types were available with about 15% of the value attained when aphids were the sole prey.

Predation capacity on *P. brassicae* larvae and aphids

Regarding predation on *P. brassicae* larvae (experiment 2), again both second and third lacewing instars were able to prey upon this prey type (Table 1). Although the two instars were presented with different stages of caterpillars thus precluding direct comparisons, the results still indicated that the third instars were the most voracious with a consumption of second instar caterpillars more than twice the consumption of first instar caterpillars achieved by the second instar lacewings.

In the presence of cabbage aphids the predation by second instar lacewings on larvae of *P. brassicae* was significantly reduced ($F = 19.11$, $df = 1, 59$, $P < 0.001$). This was, however, not the case for third instar lacewings ($F = 0.20$, $df = 1, 59$, $P = 0.659$), which maintained their predation unchanged.

The 2-h consumption of aphids by second instar lacewings when aphids were the sole prey was significant reduced ($F = 9.44$, $df = 1, 59$, $P = 0.003$) in the presence of caterpillars. Similarly, the 2-h aphid predation by third instar lacewings seen in situations where only aphids were present was significantly reduced ($F = 165.17$, $df = 1, 59$, $P < 0.001$) when both prey types were offered as

Table 1 Mean number ($\pm$ SE) of cabbage aphids, eggs and caterpillars of *Pieris brassicae* consumed by 2nd and 3rd instar *Chrysoperla carnea* in the two experiments

Predator stage	<i>Pieris brassicae</i> eggs vs. cabbage aphids (24 h experiment)			
	Eggs	Eggs and aphids		Aphids
	# Eggs consumed	# Eggs consumed	# Aphids consumed	# Aphids consumed
2nd instar	2.6 $\pm$ 0.56 a	0 b	8.3 $\pm$ 0.49 A	8.9 $\pm$ 0.49 A
3rd instar	84.1 $\pm$ 5.92 a	17.1 $\pm$ 3.33 b	30.8 $\pm$ 1.46 A	36.4 $\pm$ 1.6 B
	<i>Pieris brassicae</i> caterpillars vs. cabbage aphids (2 h experiment)			
	Caterpillars	Caterpillars and aphids		Aphids
	# Caterpillars consumed	# Caterpillars consumed	# Aphids consumed	# Aphids consumed
2nd instar	1.2 $\pm$ 0.15 a	0.4 $\pm$ 0.11 b	1.6 $\pm$ 0.27 A	2.8 $\pm$ 0.30 B
3rd instar	3.0 $\pm$ 0.35 a	3.2 $\pm$ 0.28 a	1.4 $\pm$ 0.27 A	11.5 $\pm$ 0.75 B

Means followed by different lower or upper case letters in the same row were significantly different at $P = 0.05$

prey. Compared with the 24-h predation rates above, it is indicated that starved lacewing larvae consume almost 1/3 of their daily intake of aphids within the first 2 h.

Predation capacity on *P. brassicae* eggs and larvae

As revealed in experiment 1 and 2 both lacewing instars were able to prey upon both butterfly eggs and larvae. When both these prey items were presented to the predators (experiment 3) the predation of first instar caterpillars by 2nd instar lacewings was not significantly different (1.3 ± 0.17 mean $\pm$ SE) ($F = 0.207$, $df = 1$, 59 , $P = 0.651$) compared to that when caterpillars were presented alone (1.2 ± 0.1). In contrast the predation of second instar caterpillars by 3rd instar lacewings was significantly decreased to 1.6 ± 0.27 ($F = 10.011$, $df = 1$, 59 , $P = 0.002$) compared to that when caterpillars were presented alone (3.0 ± 0.35). Similar comparisons between the consumption of butterfly eggs presented alone or in combination with caterpillars are not possible due to the difference in the experimental period between experiment 1 and 3.

Prey preference

Both second and third instar of *C. carnea* preferred caterpillars to *P. brassicae* eggs (experiment 3) ($\beta_1 = 0.90 \pm 0.05$ and $\beta_1 = 0.92 \pm 0.05$, respectively, both values being significantly different from 0.5 ($t = 8.17$, $df = 27$, $P < 0.001$ and $t = 8.81$, $df = 28$, $P < 0.001$, respectively)). When the lacewings were given a choice between aphids and *P. brassicae* larvae (experiment 2), the third instars had a significant preference for caterpillars to aphids ($\beta_1 = 0.85 \pm 0.03$, the value being significantly different from 0.5 ($t = 10.69$, $df = 29$, $P < 0.001$)) whereas the second instars had a significant preference for aphids ($\beta_1 = 0.69 \pm 0.09$, the value being significantly different from 0.5 ($t = 2.17$, $df = 28$, $P = 0.039$)). However, both instars of the lacewing clearly preferred aphids to the lepidopteran eggs (experiment 1) ($\beta_1 = 1.00 \pm 0.00$ and 0.88 ± 0.028 for second and third instar, respectively, both values being significantly different from 0.5 (t -test not possible for 2nd instars; $t = 13.59$, $df = 29$, $P < 0.001$ for 3rd instars)).

Discussion

The results have demonstrated that third instar *C. carnea* has a high predation capacity on eggs and larvae of *P. brassicae*, being able to consume more than 80 eggs in 24 h or about three-second instar caterpillars in 2 h. The third instars are even capable of killing early stages of the large 3rd instar *P. brassicae* larvae (N. Huang, pers. obs.). The results confirmed the more voracious nature of third instar lacewings compared to second instars as reported by others (Principi and Canard 1984; Klingen et al. 1996; Silva et al. 2002). Both instars of *C. carnea* showed a clear preference for *P. brassicae* caterpillars over eggs when both prey types were presented together. A possible explanation could be that moving prey are more attractive to lacewing larvae.

Chrysoperla carnea also showed a clear preference for cabbage aphids over *P. brassicae* eggs when both prey types were presented together. This is in accordance with results reported by others for the same (e.g. Ables et al. 1978) or other species of lacewings [e.g. Ding and Chen 1986 (*Chrysoperla sinica* (Tjeder)); Nordlund and Morrison 1990 (*C. rufilabris*)]. Ables et al. (1978) showed that *C. carnea* larvae displayed a 30.6% reduction in their consumption of *H. virescens* eggs when alternative prey in the form of cotton aphids (*Aphis gossypii* Glover (Hemiptera: Aphididae)) was present.

The reason for the observed preference of larvae of *C. carnea* for aphids over eggs of *P. brassicae* might be speculated to be a reflection of the nutritional quality of the two prey types. Firstly, however, ample consumption of a certain prey does not necessarily indicate that the prey is nutritionally adequate for *C. carnea* (Osman and Selman 1996) and, secondly, in terms of food quality effects on the larval development of chrysopids, lepidopteran eggs have generally been considered equal to or better than aphids (Principi and Canard 1984; Lopez-Arroyo et al. 1999; Pappas et al. 2007). However, generalizations across chrysopid species may not be appropriate as shown by El-Arnaouty et al. (1996), who found that *E. kuehniella* was of better quality to *C. carnea* compared to peach-potato aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), whereas the opposite was the case for *Chrysoperla nipponensis* (Okamoto). Besides Kabissa et al. (1996) considered both *H. armigera* eggs and *A. gossypii* nymphs

to be unsuitable prey for larvae of *Mallada desjardinsi* (Navas) and *Chrysoperla congrua* (Walker) as evidenced by high mortality of 3rd instar before pupation.

The second instar of *C. carnea* not only had a preference for aphids when *P. brassicae* was present in the form of eggs, but also when caterpillars were present. The preference of both instars of the lacewing for aphids compared to lepidopteran eggs as well as the similar preference of second instar lacewings for aphids compared to caterpillars might at first seem to reduce the potential for utilizing lacewings for biological control against lepidopteran pests. However, Ridgway and Jones (1968) indicated that although the efficiency of *C. carnea* in suppressing populations of *Heliothis* spp., at least in field cages, appeared to be influenced by the amount of alternative prey. *C. carnea* larvae were still able to kill a relatively high percentage of eggs and larvae of *Heliothis* spp. in the presence of large quantities of other prey types.

Further in favor of the utility of *C. carnea* for use in biological control of *P. brassicae* is the fact that the much more voracious third instar showed a clear preference for larvae of *P. brassicae* over aphids. This is similar to results obtained for *C. rufilabris*, which has been shown to have a significant preference for larvae of *H. virescens* over both eggs and aphids (Nordlund and Morrison 1990). Again the preference of *C. carnea* might be explained in terms of differences in nutritional qualities between lepidopteran larvae and aphids although some research results have suggested that the former prey is not optimal for larval lacewing development (El-Dakrouy et al. 1977; Obrycki et al. 1989; Osman and Selman 1996; Auad et al. 2003; Pappas et al. 2007). However, Bortoli et al. (2005) reported that butterfly larvae and eggs were both good quality food for lacewing larval development.

The present results indicate that 3rd instar *C. carnea* has potential as a biocontrol agent against *P. brassicae* although additional aspects relating to the control efficiency in the field of course need to be examined. Thus, further studies on e.g. the functional response and searching efficiency of third instar larvae of *C. carnea* to *P. brassicae* eggs and larvae, its prey preference at different ratios of butterflies and aphids, as well as the influence of host plant species on the activity and predation are of relevance for

elucidation of the control potential of the lacewing against the large white butterfly, as are larger-scaled experiments in more realistic three-dimensional set-ups.

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