

# Predation upon *Adalia bipunctata* and *Harmonia axyridis* eggs by *Chrysoperla carnea* larvae and *Orius laevigatus* adults

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## Abstract

Intra-guild predation (IGP) between sucking predators in relation to eggs of the exotic ladybird *Harmonia axyridis* (Pallas) and native *Adalia bipunctata* (L.) (Coleoptera Coccinellidae) was studied in laboratory. The green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera Chrysopidae) and the pirate bug *Orius laevigatus* (Fieber) (Rhynchota Anthocoridae) may share the same niche as the two Coccinellidae species. Intra-guild predation may occur in glasshouses and crops when several predator species are used in integrated releases for the biocontrol of arthropod pests. In experimental arenas, the two sucking predators were individually offered the choice between eggs of the two ladybird species. Adult *O. laevigatus* reared on frozen *Ephestia kuehniella* Zeller eggs were not seen to feed on ladybird eggs, and immediately rejected this kind of food. Conversely, *C. carnea* larvae attacked the ladybird eggs without showing a preference for either species, although we observed that the *A. bipunctata* eggs were either completely eaten or more shrivelled than those of *H. axyridis*. In prey suitability experiments, *C. carnea* larvae that were allowed to develop by being fed an *ad libitum* supply of *A. bipunctata* eggs took longer to develop and had a lower survivorship compared to data (from literature) on *E. kuehniella* frozen eggs. *H. axyridis* eggs were not suitable for the larval development of *C. carnea*. We discuss the relevance of these results and intra-guild predation to the use of these species as biocontrol agents.

**Key words:** green lacewing, multicoloured Asian ladybird, two spotted ladybird, pirate bug, intra-guild predation, IGP, *Ephestia kuehniella*.

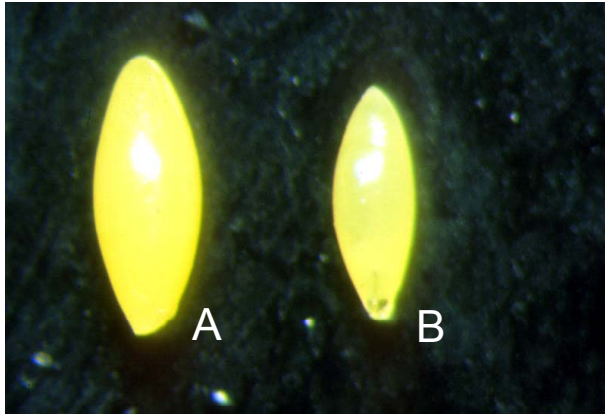
## Introduction

Intra-guild predation (IGP) represents an extreme form of competition between species (Polis *et al.*, 1989). When an insect predator from a specific trophic level attacks another entomophagous arthropod from the same trophic level, and both species eat the same prey or fight each other, we can define this interaction as intra-guild predation (Polis and Holt, 1992). At low densities, polyphagous predators may eat common prey such as aphids, lepidopteran eggs, and larvae, but when prey and predator densities increase, the predators may also show negative intraspecific interactions such as cannibalism (Coderre *et al.*, 1987; Majerus, 1994; Lucas *et al.*, 1997; Hironori and Katsuhiko, 1997; Burgio *et al.*, 2002; Santi *et al.*, 2003; Burgio *et al.*, 2005). Within the community, predation risk of individuals depends upon a large range of factors: the risk experienced may be reduced with an increase in body size as observed in *Orius* spp. by Tommasini *et al.* (2002), but this risk is also dependent upon factors such as their mobility and the semiochemicals (allomones) that they produce as demonstrated in Coccinellidae (Felix and Soares, 2004; Omkar *et al.*, 2004). The egg masses of ladybirds are generally not protected externally, and therefore can be easily preyed upon (Polis *et al.*, 1989). However, Hemptinne *et al.* (2000) found that some alkaloids are present in *Adalia bipunctata* (L.) and *Coccinella septempunctata* L. eggs, which may contribute to reducing intra-guild predation as in other ladybird species (Omkar *et al.*, 2004).

When beneficial insects are released as biocontrol agents into agroecosystems and habitats such as protected crops, it is important to evaluate the possible in-

teractions between polyphagous predators. Inoculative or augmentative releases and the side effects of introducing exotic generalist predators are other issues that require accurate analyses (van Lenteren *et al.*, 2003). In Italian agroecosystems, the biological and IPM control techniques recommend the use of the larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera Chrysopidae) and several species of ladybirds (Coleoptera Coccinellidae) as a means of controlling aphids, combined with the pirate bug *Orius laevigatus* (Fieber) (Rhynchota Anthocoridae) as a means of controlling thrips. These beneficial species are commercially available from European biofactories. The exotic Asian ladybird *Harmonia axyridis* (Pallas) is a potential competitor of native Coccinellidae, but is not yet established in Italy (as far as we know), which may in part be because releases of this species in greenhouses and the open field have been stopped. Recently, the flightless strain (Gil *et al.*, 2004) has been reared and commercially produced for the biological control of aphids in French greenhouses. The two spotted ladybird *A. bipunctata* is another candidate for augmentative releases in Italy and other regions of Europe (Kehrli and Wyss, 2001). The mouthparts of *C. carnea* larvae and *O. laevigatus* nymphs and adults allow these species to perforate the egg chorion, and then suck the contents from the egg. In commercial insectaries, *C. carnea* larvae and *O. laevigatus* are usually reared on the same artificial prey, i.e. the frozen eggs of *Ephestia kuehniella* Zeller. The intra-guild predation between *C. carnea* and *O. laevigatus* has not been investigated previously because they rarely interact in the environment, due to their differing modes of egg deposition (i.e. inside the vegetal

Class 1



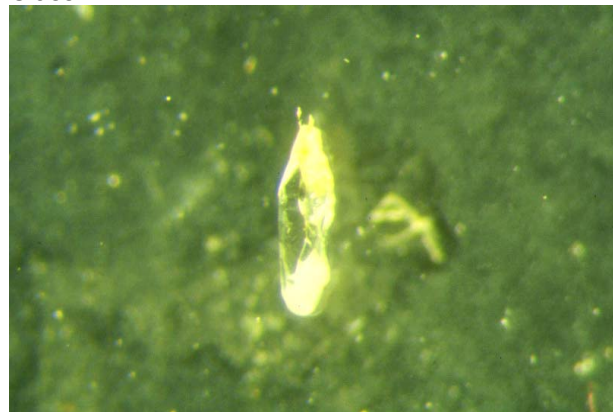
Class 2



Class 3



Class 4



**Figure 1.** Classes of feeding effects by *C. carnea* larvae on ladybird eggs (A: *H. axyridis*; B: *A. bipunctata*).

tissues the pirate bugs and on stalk the green lacewing), but these species may be competitors for opportunities to feed upon eggs. In the open field, *O. laevigatus*, *C. carnea* and *A. bipunctata* are found in the same weeds and shrubs nearest to the crops, and therefore these predators are the major contributors to the natural control of insect pests in Italian agroecosystems (Burgio *et al.*, 2004; Tommasini, 2004).

The aim of this study was to investigate in the laboratory the intra-guild predation between sucking generalist predators (*C. carnea* and *O. laevigatus*) in relation to the exotic species *H. axyridis* and the indigenous species *A. bipunctata*.

### Materials and methods

The two ladybird species were reared in the laboratory under the conditions described by Burgio *et al.* (2002) and Santi *et al.* (2003). The predators *C. carnea* and *O. laevigatus* were supplied by a commercial producer (Bioplanet, Cesena, Italy).

### Choice tests of *C. carnea* larvae and ladybird egg species

Experiments were performed as choice tests in a rearing room at temperature of  $25 \text{ }^{\circ}\text{C} \pm 2$ , 60-70 RH, and light 300-400 lux.

The experimental arena was a 12 cm diameter glass Petri dish. The border of the dish was treated with 5 mm of Teflon to stop the predators escaping. Inside the dish, 10 fresh eggs of *A. bipunctata* and 10 of *H. axyridis* were placed alternately on a filter paper grid of 20 points (5 x 4 points separated at 16 x 14 mm distance). The eggs were gently removed from a fresh egg cluster using a smooth brush.

25 second instar and 25 third instar *C. carnea* larvae were individually released into the arena after being starved for 24 hours. After introduction, the behaviour of the larvae was observed for two hours under a binocular microscope. The behaviour of the larvae to the eggs was designated using the following categories (figure 1):

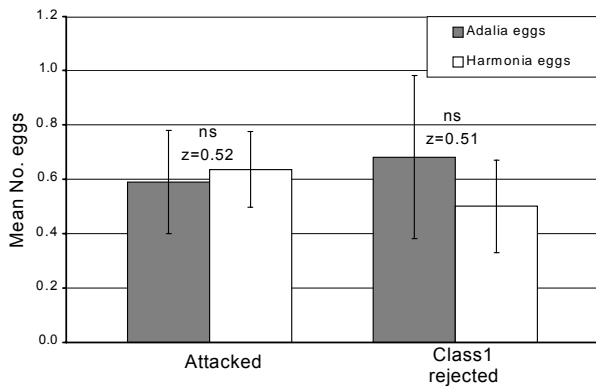
**Class 1:** the egg was rejected without mouth parts being inserted;

**Class 2:** mouth parts were inserted, but the egg was immediately rejected;

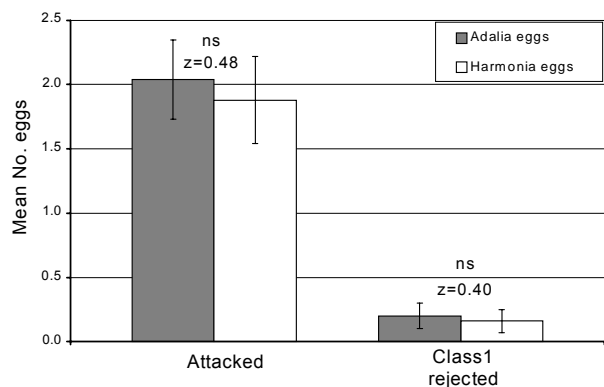
**Class 3:** mouth parts were inserted, with some of the egg contents eaten;

**Class 4:** mouth parts were inserted, with most or all of the egg contents eaten, or the egg ended up totally shrivelled.

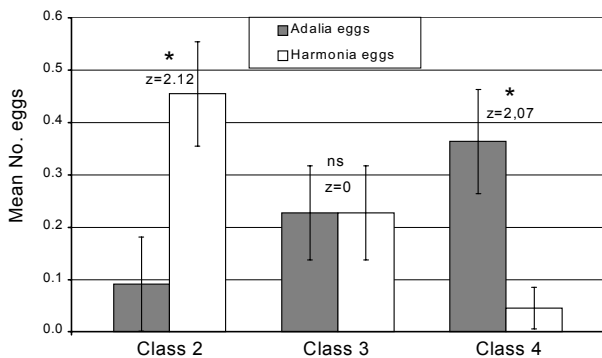
The number of rejected and attacked eggs (including the eggs partially or totally eaten) according to the above mentioned classes, was analysed using a signed rank Wilcoxon non-parametric test.



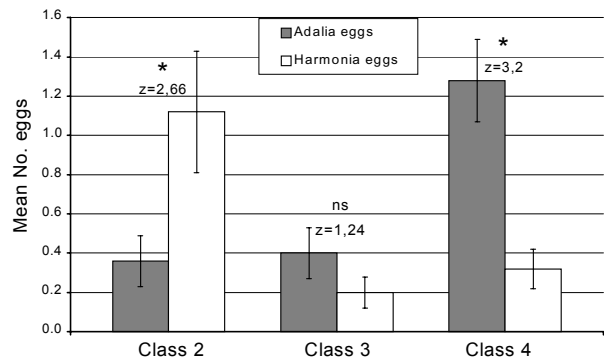
**Figure 2a.** Choice test between the two species of ladybird eggs (*A. bipunctata* and *H. axyridis*) and second instar larvae of *C. carnea*.  
n.s.:  $P > 0.05$  (Wilcoxon signed rank non-parametric test, data given as mean  $\pm$  S.E.).



**Figure 3a.** Choice test between the two species of ladybird eggs (*A. bipunctata* and *H. axyridis*) and third instar larvae of *C. carnea*.  
n.s.:  $P > 0.05$  (Wilcoxon signed rank non-parametric test, data given as mean  $\pm$  S.E.).



**Figure 2b.** Choice test between the two species of ladybird eggs attacked (*A. bipunctata* and *H. axyridis*) and second instar larvae of *C. carnea*.  
\*:  $P < 0.05$ ; n.s.:  $P > 0.05$  (Wilcoxon signed rank non-parametric test, data given as mean  $\pm$  S.E.).



**Figure 3b.** Choice test between the two species of ladybird eggs attacked (*A. bipunctata* and *H. axyridis*) and third instar larvae of *C. carnea*.  
\*:  $P < 0.05$ ; n.s.:  $P > 0.05$  (Wilcoxon signed rank non-parametric test, data given as mean  $\pm$  S.E.).

### Choice tests of *O. laevigatus* adults and ladybird eggs species

Choice tests using 20 starved *O. laevigatus* were conducted using an identical protocol and under identical conditions to the previous experiment. The data were not analysed due to the difficulty of direct observations and the pirate bugs' scarce propensity to feed on eggs.

### *O. laevigatus* adult behavioural studies on *E. kuehniella* and *H. axyridis* eggs

Bioassays were conducted in the arena (using the previous protocol) using one *O. laevigatus* adult (starved for 24 hours) and 5 eggs of *E. kuehniella* (frozen eggs that are usually used as an artificial prey for industrially rearing pirate bugs). The same bioassay was performed offering 5 *H. axyridis* eggs to one *O. laevigatus* adult. Each of these bioassays was replicated 15 times and lasted for one hour of direct observation. The time in seconds of contact with the eggs was recorded.

Data (no. of seconds feeding on *E. kuehniella* eggs vs *H. axyridis* eggs) were analysed using a signed rank Wilcoxon non-parametric test.

### *C. carnea* development on ladybird eggs

To test the suitability of ladybird eggs as a food for green lacewings, we fed 20 *C. carnea* second instar larvae solely with *A. bipunctata* eggs, and 20 larvae solely with *H. axyridis* eggs. During the feeding period, each larva was isolated in a 12 cm Petri dish, and offered ladybird eggs *ad libitum* (initially, larvae were offered 5 *A. bipunctata* eggs per day, until the third instar, when larvae were furnished more than 20 eggs per day until pupation). The rearing and observation rooms were kept at  $25\text{ }^{\circ}\text{C} \pm 4$  temperature and 70-80 RH, 16:8 LL:DD. Every day, fresh eggs were supplied, the condition of the *C. carnea* larvae was recorded.

## Results

### Choice tests

Green lacewing larvae, as shown in figure 2a and 3a, did not show a preference for either attacking or rejecting the eggs of *A. bipunctata* or *H. axyridis*. However, if we only consider the attacked eggs (Classes 2, 3 and 4), there was a significant difference, with a significant

proportion of the *A. bipunctata* eggs being eaten. This demonstrated that when a *C. carnea* larva found a ladybird egg, its first behaviour was to “taste” it (Class 1, figure 1). The second step was potential rejection, with a different reaction between exotic and native ladybird eggs. The results indicate that when the larvae “continued to eat” (eggs of Class 2, 3 and 4) (figure 1) they fed preferentially on the eggs of the native two spotted ladybird species instead of those of the exotic Asian species (figure 2b and 3b). If the chorion of a ladybird egg was broken by the mouthparts of the lacewing larva, this stopped the development of the embryo, and so we can assume that the predatory activity by *C. carnea* larvae was similar between the two species of ladybird studied. Furthermore, if we consider the fact that ladybird eggs are laid in a cluster, we can hypothesise that if a lacewing encounters an *A. bipunctata* egg mass, it is likely that more than one egg will be destroyed. Conversely, the low palatability of *H. axyridis* eggs may cause the lacewing larvae to stop feeding earlier, and therefore offer increased protection for the egg masses of the exotic species, when compared to the native species.

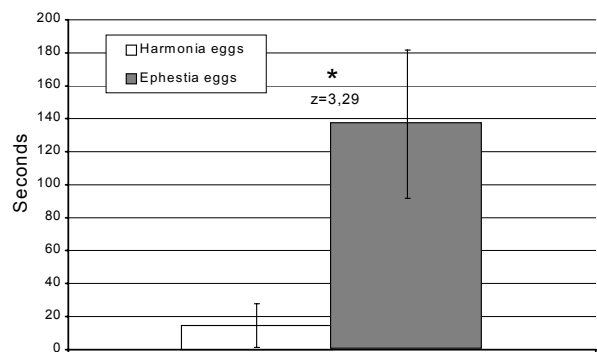
The adult pirate bugs rejected the eggs of both species of ladybird. When the starved *O. laevigatus* “tasted” the egg of a ladybird, after about 15 seconds they moved off in another direction; only in a two cases (one in a *H. axyridis* and one in *A. bipunctata* egg) were the bugs observed to insert their mouth parts into the chorion of the eggs (figure 4). The high mobility of *O. laevigatus* adults did not permit us to directly observe the feeding behaviour of this species, and so we consequently performed the bioassays reported below.

#### *O. laevigatus* adult behavioural studies on *E. kuehniella* and *H. axyridis* eggs

It took an adult *O. laevigatus* more than two minutes to completely consume a frozen *E. kuehniella* egg (figure 4). Compared to the few seconds that was measured for ladybird eggs, this clearly indicates that the pirate bug was just probing, and then avoiding feeding on the eggs. Because we recorded no difference between the rejection likelihood’s of both species of ladybird egg in our earlier experiments, we suppose that the behaviour of the pirate bugs on *A. bipunctata* eggs is similar to the data obtained using the exotic ladybird eggs. Therefore, Coccinellidae eggs were unsuitable as a food source for adult *O. laevigatus*.

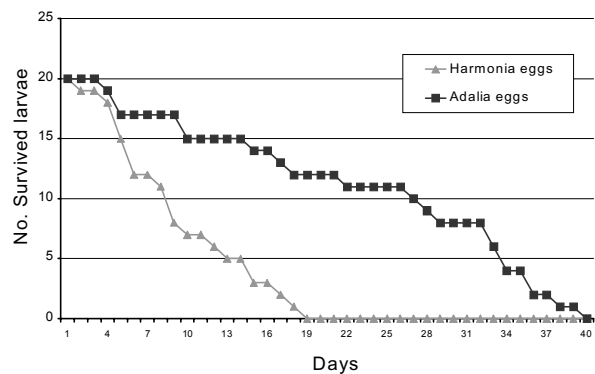
#### *C. carnea* development on ladybird eggs

Our results suggest that *A. bipunctata* eggs are suitable food for green lacewings, as it was found that the eggs of the native two spotted lady bird were less toxic than those of the exotic *H. axyridis*. As is demonstrated in figure 5, the survivorship of *C. carnea* larvae was limited, where larvae feeding only on *A. bipunctata* eggs developed through to the pupal stage, but those feeding only on *H. axyridis* eggs did not. The average developmental time of the Italian strain of green lacewing from second instar larvae until pupation when feeding on artificial prey (such as frozen eggs of *E. kuehniella*) was 8 days (Nicoli *et al.*, 1991). This clearly indicates that if



**Figure 4.** Time spent in seconds by *O. laevigatus* adults on *H. axyridis* eggs vs *E. kuehniella* eggs.

\*:  $P < 0.05$  (Wilcoxon signed rank non-parametric test, data given as mean  $\pm$  S.E.).



**Figure 5.** *C. carnea* survival (starting from second instar larvae), where larvae were only fed with *H. axyridis* or *A. bipunctata* eggs.

lacewing larvae eat ladybird eggs, they grow much slower (taking around 39 days) when compared to larvae feeding only on frozen *E. kuehniella* eggs. Only two out of the 20 larvae studied reached the pupal stage, and neither of these pupae produced adults. Green lacewing larva fed with *H. axyridis* eggs were not able to survive, where all individuals were dead by day 19.

## Discussion and conclusions

The pirate bug *O. laevigatus* was not able to feed on the eggs of either the two spotted or Asian ladybird species. Mallampalli *et al.* (2002) and De Clercq *et al.* (2003) observed the attacks and prey feasibility of the spined soldier bug *Podisus maculiventris* (Say) (Rhynchota Pentatomidae), when presented with the eggs of the ladybirds *Coleomegilla maculata* De Geer and *H. axyridis*. The rostrum of these polyphagous bugs may be an efficient apparatus to kill eggs, but in the case of *O. laevigatus*, we only observed rejection behaviour when they were presented with ladybird eggs. Independently of egg size, it appears that when *O. laevigatus* adults extend their mouthparts to Coccinellid eggs, they may detect an allomone that subsequently deters feeding.

This is probably not the case in *P. maculiventris* nymphs and adults as reported by the above mentioned authors. Alternatively, we found that the green lacewing larvae attacked the ladybird eggs without an immediate preference between the *A. bipunctata* or *H. axyridis* eggs.

If pirate bugs and green lacewings are used for biological control (simultaneous or integrated, i.e. more inoculative or inundative releases at different timing) in greenhouses, these polyphagous predators will only contribute to reducing prey. It is probable that harmful interactions between the predator species during a combined release are unlikely to happen, but we should nonetheless consider whether intra-guild predation occurs when green lacewing larvae and ladybird species are released together. In particular, the eggs of *A. bipunctata* will be attacked more intensively than the eggs of *H. axyridis*. Further intra-guild predation studies between green lacewing larvae and ladybird larvae and pupae may be necessary.

Lucas *et al.* (1997) found that *Chrysoperla rufilabris* (Burmeister) can predate the larvae of the coccinellid *Coleomegilla maculata lengi* Timberlake. Intra-guild predation on the eggs of these species was not reported. Phoofolo and Obrycki (1998) studied the intra-guild predation between *C. carnea* and three ladybird species (*C. maculata*, *C. septempunctata* and *H. axyridis*). *C. maculata* and *H. axyridis* were able to complete their development if they fed only on *C. carnea* eggs, but *C. septempunctata* could not. Furthermore, *C. carnea* was able to eat *C. maculata* eggs and pupate, but the *H. axyridis* eggs were not suitable for *C. carnea* development. Our data concerning the survival of *C. carnea* larvae feeding on Asian ladybird eggs therefore confirms the results of Phoofolo and Obrycki (1998). As with our observation that the development of *C. carnea* was delayed when eating only the eggs of *A. bipunctata*, this was also the case when *C. carnea* larvae were fed with *C. maculata* eggs (Phoofolo and Obrycki, 1998).

If we conduct intra-guild predation experiments in the laboratory using simple behavioural observations, we may then be able to avoid any possible negative interaction that could limit the efficacy of integrated releases of polyphagous predators on protected crops. We suggest, as is also indicated by van Lenteren *et al.* (2003), that it is sensible to perform preventive bioassays examining possible intra-guild predation effects. These tests are particularly necessary when exotic insects are planned for release as a biological control agent in agroecosystems.

Competition for food, differences in biological traits, intra-guild predation, and other many factors may complicate our predictions of the fate and establishment of the polyphagous predator, as well as its potential effects upon the native fauna (Koch, 2003, reports the side-effects of a population of *H. axyridis* establishing itself in a new environment). Intra-guild predation data collected in the laboratory may represent the first step for further studies of intra-guild predation and population dynamics in open field environments, as well as at the landscape scale.

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