

Intra-guild predation and cannibalism of *Harmonia axyridis* and *Adalia bipunctata* in choice conditions

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Abstract

A laboratory experiment was carried out to examine intra-guild predation and cannibalism of exotic *Harmonia axyridis* (Pallas) and the native species *Adalia bipunctata* L. (Coleoptera Coccinellidae) in choice condition. Experiments were carried out in glass petri dishes at 25°C, 70% RH, using a 5x4 grid with 10 eggs of exotic and 10 of natives arranged alternatively. One larva or adult of coccinellid was put in the arena and was observed for one hour. Each experiment was replicated 15 times. *H. axyridis* larvae and adults, and *A. bipunctata* adults, showed a preference to prey and eat their own eggs rather than interspecific eggs; these differences were detected for both naive and experienced females and larvae. For *A. bipunctata* larvae no differences were observed between IGP and CANN in choice conditions. The results indicate a tendency for both species to attack and eat their own eggs rather than interspecific eggs. The risk of introduction of exotic generalist predators is discussed, with particular attention to coccinellids. Laboratory experiments on intra-guild predation could give a preliminary indication of the potential for competition between an exotic ladybird and a native one.

Key words: *Adalia bipunctata*, cannibalism, choice test, *Harmonia axyridis*, intra-guild predation, biological control.

Introduction

The Asiatic polyphagous ladybird *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae) is a Palearctic species originating from the far East; the beetle occurs in Korea, Japan, Bonin Islands, China, Himalayas, Formosa, Siberia (Iablokoff-Khnzorian, 1982). This species was introduced and established in United States (La-Mana and Miller, 1996; Brown and Miller, 1998; Colunga-Garcia and Gage, 1998; Brown, 1999), Canada (Coderre *et al.*, 1995) and released in European countries like France (Ferran *et al.*, 1996) and Italy mainly to control aphids damaging protected crops.

Because of polyphagous habits of these species of coccinellids, there is potential for intra-guild predation (IGP), defined as “the killing and eating of species that use similar, often limiting, resources and are thus potential competitors” (Polis *et al.*, 1989). Predator-predator interactions, as reported by Rosenheim *et al.* (1993), may reduce the biocontrol. Classic biocontrol, that lies on introduction of exotic beneficial insect, in these cases may not increase Coccinellidae species and population abundance and may therefore not have a net effect on biological control of aphid pests (Elliott *et al.*, 1996). Lynch *et al.* (2001) collected data relating to introductions of both insect and microbial agents against insect pests, including classical and augmentative biocontrol. Recently a methodology for risk assessment has been developed by van Lenteren *et al.* (2003) as a basis for regulation of import and release of exotic natural enemies used in inundative forms of biocontrol; the paper proposes a general framework of a risk assessment methodology for biological control agents, integrating information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets.

In a previous paper, IGP and cannibalism (CANN) of *H. axyridis* and *Adalia bipunctata* L. (Coleoptera Coccinellidae) were examined in laboratory experiments in

no choice condition on eggs and on larvae (Burgio *et al.*, 2002; Burgio *et al.*, submitted for publication). IGP competition was investigated by determining the consumption of interspecific and CANN eggs by fourth instar larvae and adult females, because prey suitability may be an indicator of the likelihood that the prey would be attacked in the field (Phoofolo and Obrycki, 1998); and could give a preliminary indication of the potential for competition between an exotic ladybird and a native one. The bioassays indicate that *H. axyridis* does not demonstrate IGP vs. native species at a higher level than CANN of native species. It appears that *H. axyridis* does show a high potential for CANN and therefore seems unlikely this species will to have a negative impact on native species by IGP of eggs (Burgio *et al.*, 2002).

The aim of this laboratory study was to examine IGP and CANN of exotic *H. axyridis* and the native species *A. bipunctata* in choice condition and *viceversa*. Further data regarding the possibility that IGP and CANN depend by the preconditioning of the individuals were also taken into account. CANN is a quite common behaviour in ladybirds and we try to evince a difference between naive or experienced individuals (larvae and adults) on their own species prey or IGP.

Materials and Methods

Predatory species utilised in the experiment were *H. axyridis* (starting material from a biofactory and reared for more than 10 generations in laboratory) and the native species *A. bipunctata* collected in the field and then reared in laboratory at least for 4 generations. Ladybird larvae of both species were feed with frozen eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera Crambidae). The adults were fed on aphids [*Myzus persicae* (Sulzer)

(Rhynchota Aphididae) on beans plants].

Experiments were carried out in glass petri dishes (12cm diameter) at 25°C, 70% RH, using a 5x4 grid with 10 eggs of exotic and 10 of natives arranged alternately. One larva or adult of coccinellid was put in the arena and was observed for one hour. Each experiment was replicated 15 times. The sets of experiments included: 1) naive females and larvae; 2) experienced females and larvae. To obtain experienced larvae and females we put the specimens in a petri dish for 4 hours with eggs of both species. Only the individuals that were observed to eat at least 4 eggs of exotic and 4 eggs of native were considered as experienced. As "eaten" were considered the eggs partially or completely wolfed down. The "rejected" were eggs that after a contact by mouthparts and antennae were drastically refused.

For each species and stage data were analysed in order to:

- compare the mean number of intraspecific eggs eaten vs mean number of interspecific;
- compare the mean number of intraspecific eggs rejected vs mean number of interspecific.

Data were analysed by Wilcoxon matched pairs test ($P < 0.05$) to compare, for a species, egg consumption and egg rejection of intraspecific vs interspecific prey. The comparison, for a species, of IGP and CANN in naive vs experienced females was analysed by Mann-Whitney U test (Zar, 1984).

Results and discussion

H. axyridis larvae and adults, and *A. bipunctata* adults showed a preference to prey and eat their own eggs rather than interspecific eggs (table 1); these differences were detected for both naive and experienced females and larvae. For *A. bipunctata* larvae no differences were observed between IGP and CANN in choice conditions (table 1). For *H. axyridis* larvae, both IGP and CANN were significantly higher in experienced vs naive (table 1). Naive adults of the exotic species showed an IGP not statistically different in comparison with experienced

adults, and the CANN data were near to reach the significance level ($P = 0.053$). For *A. bipunctata* adults, no differences were detected for IGP and CANN, between naive and experienced females. For *A. bipunctata* larvae, on the contrary of what was detected on *H. axyridis*, the IGP ($P = 0.056$) and CANN ($P = 0.044$) were higher in naive rather in experienced females. Anyway the level of egg consumption in these cases was very low and the P values were very close to the level of 5%; moreover the high standard deviations demonstrate a consistent variability in these latter parameters.

In general, in the experiments with larvae there was clearly a low level of egg CANN and IGP for both exotic and native species. Moreover for indigenous ladybird adults and larvae, the consumption of interspecific eggs was lowest in comparison with the consumption of interspecific eggs by the exotic species.

For *H. axyridis* experienced adults and larvae, rejections of interspecific eggs were highest in comparison with rejections of intraspecific eggs (table 2). The same observation for adults and larvae of the native ladybird, i.e. the rejections in case of IGP were highest in comparison of rejections of CANN both for naive or experienced individuals. The differences were not significant for *A. bipunctata* experienced larvae, also if P values were slightly higher to the level of 5% (table 2).

The results indicate a tendency for both species to attack and eat their own eggs rather than interspecific eggs confirming the results in no choice condition experiments (Burgio *et al.*, 2002). For *A. bipunctata* larvae no differences were detected between IGP and cannibalism. The predation of eggs was dependent by the preconditioning of the individuals: for larvae of exotic species both IGP and CANN were significantly higher in experienced vs naive. Naive adults of the exotic species showed an IGP not statistically different in comparison with experienced adults, but CANN in experienced adult females was higher in comparison with naive females. For indigenous species adults and larvae the consumption of interspecific eggs was in general lowest in comparison with the consumption of interspecific eggs by exotic.

Table 1. Summary of the results of the choice test. Numbers indicate the mean of eggs eaten per female \pm standard deviations. Line comparisons analyzed by Mann-Whitney U test; row comparisons analyzed by Wilcoxon's signed-Rank test.

Species	Stage	Behaviour	Naive	Experienced	P
<i>H. axyridis</i>	Adult	CANN	3.8 \pm 2.3	5.5 \pm 2.7	0.053
<i>H. axyridis</i>	Adult	IGP	1.9 \pm 2.4	2.1 \pm 2.2	0.851
		P	0.025	0.004	
<i>H. axyridis</i>	Larval	CANN	2.2 \pm 2.0	4.9 \pm 2.2	0.0079
<i>H. axyridis</i>	Larval	IGP	0.7 \pm 1.2	3.2 \pm 2.2	0.0012
		P	0.011	0.005	
<i>A. bipunctata</i>	Adult	CANN	2.1 \pm 2.1	3 \pm 2.6	0.319
<i>A. bipunctata</i>	Adult	IGP	0.8 \pm 1	0.6 \pm 1	0.724
		P	0.016	0.003	
<i>A. bipunctata</i>	Larval	CANN	1.7 \pm 1.4	0.8 \pm 1.33	0.044
<i>A. bipunctata</i>	Larval	IGP	1.6 \pm 1.6	0.5 \pm 0.8	0.056
		P	0.812	0.177	

Table 2. Summary of the results of the choice test. Numbers indicate the mean of rejected eggs per female \pm standard deviations. Line comparisons analyzed by Mann-Whitney U test; row comparisons analyzed by Wilcoxon's signed-Rank test.

Species	Stage	Behaviour	Naive	Experienced	P
<i>H. axyridis</i>	Adult	CANN	3.3 \pm 6	1.1 \pm 3.4	0.271
<i>H. axyridis</i>	Adult	IGP	4.4 \pm 4	5.3 \pm 2.4	0.589
		P	0.258	0.002	
<i>H. axyridis</i>	Larval	CANN	4.6 \pm 7.4	1.1 \pm 2.3	0.395
<i>H. axyridis</i>	Larval	IGP	4.3 \pm 5.1	2.8 \pm 3.2	0.467
		P	0.813	0.048	
<i>A. bipunctata</i>	Adult	CANN	10 \pm 9.2	3.7 \pm 3.8	0.032
<i>A. bipunctata</i>	Adult	IGP	17.3 \pm 11.7	8.2 \pm 4.2	0.015
		P	0.001	0.001	
<i>A. bipunctata</i>	Larval	CANN	1.3 \pm 2.5	4.9 \pm 5.7	0.085
<i>A. bipunctata</i>	Larval	IGP	2.7 \pm 2.5	7.1 \pm 6.3	0.031
		P	0.054	0.061	

We suppose that eggs of the exotic ladybird contains an allomone that protected from interspecific predation in comparison with the indigenous ladybird eggs. Ladybird beetles defend their own with bitter-tasting alkaloids and volatile pyrazine and quinolones. The alkaloids "coccinelline" and "adaline" occur in *Coccinella septempunctata* (L.) and *A. bipunctata* respectively (Pasteels *et al.*, 1973). Agarwala and Dixon (1992) demonstrated that larvae and adults of both coccinellid species were reluctant to cannibalize eggs treated with a water extract of the other species' eggs. Larvae of *C. septempunctata* were more likely to die after eating a few eggs of *A. bipunctata* than vice versa. Agarwala and Dixon (1992) concluded that eggs of *A. bipunctata* and *C. septempunctata* have some protection against interspecific predation and the eggs of the former appear to be better defended by *C. septempunctata* than vice versa. Results of Agarwala and Dixon (1992) indicate that cannibalism occurs mainly when aphid prey is scarce and is adaptive in that it improves the chances of survival, and coccinellid, to varying degrees, are defended against IGP. Burgio *et al.* (2002) found in an experiment on CANN and IGP an inverse correlation between egg consumption by adult and larvae, and aphid density. The occurrence of sibling cannibalism in coccinellids has mainly been considered as a result of two factors: asynchrony of hatching among eggs and the existence of infertile eggs in an egg batch (Kawai, 1978). Osawa (1992) found that coccinellids cannibalise both infertile and fertile eggs thereby increasing in body length and survival rate with the intensity of sibling cannibalism. The cannibalistic trait was clearly beneficial to the cannibal when aphid density was low, but it could be detrimental when aphid density was high and the victims were full siblings. At high aphid densities a female's fitness decreased with the intensity of sibling cannibalism, indicating that sibling cannibalism is maladaptive for the females when larval food availability is high (Osawa, 1992). Interspecific interactions, favouring *C. septempunctata* over *Coleomegilla maculata* (DeGeer), occurred at a low prey density (one aphid per day); no interspecific interactions were observed between larvae of these two species at high prey density

(>20 aphids per predator per day) (Obrycki *et al.*, 1998). In choice tests, where different proportions of conspecific eggs and aphids were offered, *Coleomegilla maculata lengi* Thimberlake neonates always showed a consistent preference for conspecific eggs; when aphids were painted with extract of crushed eggs and eggs with crushed aphids to determine whether neonates found eggs by chemical cues, neonates preferred aphids painted with egg extract to eggs painted with aphid extract (Gagné *et al.*, 2002).

Brown and Miller (1998) reported after field surveys in West Virginia that *H. axyridis* became the dominant species in the tribe Coccinellini in 1995 and continues to dominate the Coccinelline guild on apple. *H. axyridis* has displaced the exotic *C. septempunctata* that had been present in the region since 1983; *H. axyridis* is providing better biological control of *Aphis spiraecola* Pagenstecher on apple (Brown and Miller, 1998). Native coccinellids have been rare in apple orchard since the arrival of *C. septempunctata* but the presence of *H. axyridis* allowed a return of some natives (Brown, 1999). In addition to affecting the coccinellid guild, the arrival of *H. axyridis* has negatively impacted at least one other aphid predator, *Aphidoletes aphidimyza* Rondani (Diptera Cecidomyiidae) (Brown, 1999). Elliott *et al.* (1996) report that structure of native coccinellid communities in South Dakota field crops differed significantly for years prior to, compared with years after *C. septempunctata* establishment. Differences in community structure were accounted for by reduced abundance of two species, *C. transversoguttata richardsoni* Brown and *A. bipunctata*. IGP, of course, is not the only way by which an exotic can compete with a native species and other factors are involved, like polyphagy, predatory potential, resource competition and other biological traits. These factors determine the predatory potential of a coccinellid in an agroecosystem and for these reasons it is difficult to predict the effect of an introduced species on biological control and on populations of native coccinellid species, conditioning biocontrol and natural control.

Our choice tests on IGP and CANN in a small arena simplifies the field situation but we can conclude that

both exotic and native species prefer to prey and eat their own eggs rather interspecific eggs, confirming the results in no choice conditions (Burgio *et al.*, 2002). At the moment we have not data on *H. axyridis* establishment in Italy, so we undertaken simple basic experiments. We assume that if in such extreme situation the IGP of eggs is not relevant, negligible effects could be predicted also in the field. Our simple tests could give a preliminary indication of the potential for competition between an exotic ladybird and a native one, within a general framework of a risk assessment methodology for biological control agents (van Lenteren, 2002). Further experiments are in progress to complete the studies on competition between *H. axyridis* and *A. bipunctata*, including IGP and CANN of larvae, life-table studies and analysis of mortality factors under field conditions.

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