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# On intra-guild predation and cannibalism in *Harmonia axyridis* (Pallas) and *Adalia bipunctata* L. (Coleoptera: Coccinellidae)

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#### Abstract

Interspecific competition between the exotic coccinellid *Harmonia axyridis* (Pallas) and the native species *Adalia bipunctata* L. was investigated in the laboratory by determining the consumption of interspecific eggs by fourth instar larvae and adult females. Larvae and adult ladybirds were individually put into petri dishes at 25 °C with 20 eggs of the selected target species. The tests included three treatments: (a) only coccinellid eggs, (b) coccinellid eggs and 5 aphids, and (c) coccinellid eggs and 40 aphids. Intraguild predation (IGP) by *H. axyridis* of eggs of the native species, *A. bipunctata*, was lower in comparison with egg cannibalism (CANN) by *H. axyridis*, for both adults and larvae. No differences were detected for larvae at the density of 5 aphids. IGP of exotic against the native species was never higher than CANN of native species. IGP of native species was never higher in comparison with CANN. *A. bipunctata* adult CANN was significantly higher than IGP at densities of 0 and 40 aphids. In the set of experiments on CANN and IGP an inverse correlation was observed between egg consumption by adults and larvae and aphid density. Our 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. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Coccinellidae; Harmonia axyridis; Adalia bipunctata; Intra-guild predation; Cannibalism

## 1. Introduction

Several reviews have addressed the issues of nontarget impacts of biocontrol (Howarth, 1997; Pimentel et al., 1994; Samways, 1994; Simberloff and Stiling, 1996; Wajnberg et al., 2001). Negative impacts of introduced exotics include competitive suppression or displacement of native natural enemies and suppression or extinction of non-target prey species, some of which may be beneficial (Elliott et al., 1996). Lynch et al. (2001) recently collected data relating to introductions of both insect and microbial agents against insect pests, including classical and augmentative biocontrol. Conversion of the descriptions of non-target effects into a more objective severity scale would allow a quantitative summary of recorded non-target effects to be made which will provide a useful basis for future research into side effects.

Several species of predatory Coccinellidae are used in biological control against introduced prey and the efficiency of these beneficials has often been demonstrated in crops (Hodek and Honek, 1996; Ferran et al., 1996). Because of spatial and temporal co-occurrence and polyphagous habits of exotic species, 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). Such predator-predator interactions are thought to decrease the efficacy of biological control (Rosenheim et al., 1995).

The Asiatic polyphagous ladybird *Harmonia axyridis* (Pallas) was introduced to France by the I.N.R.A. (Ferran et al., 1996), United States (Brown, 1999; Brown and Miller, 1998; Colunga-Garcia and Gage, 1998; La-Mana and Miller, 1996) and Canada (Coderre et al., 1995). Field surveys in West Virginia (USA) by Brown

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and Miller (1998) found that this exotic had become the dominant species in the tribe Coccinellini in 1995 and it continues to dominate the Coccinellinae guild on apple. H. axyridis has displaced the exotic Coccinella septempunctata (L.) that had been present in the region since 1983. H. axyridis is providing better biological control of Aphis spiraecola Patch on apple (Brown and Miller, 1998). Native coccinellids have been rare in apple orchards since the arrival of C. septempunctata but the presence of *H. axyridis* permitted the return of some natives (Brown, 1999). In addition to affecting the coccinellid guild, the arrival of H. axyridis has negatively impacted at least one aphid predator, Aphidoletes aphidimyza Rondani (Diptera: Cecidomyiidae) (Brown, 1999). Elliott et al. (1996) report that the structure of native coccinellid communities in South Dakota (USA) differed significantly for years prior to, compared with years after, establishment of C. septempunctata in fields of some crops. Differences in community structure were accounted for by reduced abundance of two species, C. transversoguttata richardsoni Brown and Adalia bipunctata L. Therefore, introduction of a new species, may not increase total coccinellid abundance, and may not have a clear effect on biological control of aphid pests (Elliott et al., 1996). H. axyridis became a dominant coccinellid species in the agricultural landscape of Southwestern Michigan. Adults of this species were found in all habitats monitored, including early secondary succession, poplar plantation, alfalfa, soybean, corn, and winter wheat (Colunga-Garcia and Gage, 1998).

The "EC" funded Evaluating Environmental Risks of Biological Control Introductions Into Europe (ER-BIC) program aims to design European guidelines to ensure that biological control agents which are to be introduced are environmentally safe. To achieve these objectives, specific case studies of different systems have been started to obtain information for evaluation of the consequences of introductions of exotic natural enemies.

Our specific aim of this laboratory study was to examine IGP and egg cannibalism (CANN) of exotic H. axyridis and the native species A. bipunctata to develop rapid and reliable methods to assess the potential risk of importation and release of exotic coccinellids. We selected A. bipunctata, as the native species for this study, because it is common in hedgerows and orchard trees (Hodek and Honek, 1996) and is abundant in Northern Italy agroecosystems (Boriani et al., 1998; Burgio et al., 1999). We investigated interspecific competition by determining the consumption of interspecific and intraspecific eggs by fourth instar larvae and adult females. 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.

#### 2. Material and methods

#### 2.1. Insect rearing

Aphids and coccinellids used in experiments were reared in the Entomology laboratories of the Department DiSTA, University of Bologna. Larvae of the coccinellid species were fed with *Ephestia kuehniella* Keller eggs and adults were fed with *Myzus persicae* Sulz reared on green pea. Adult coccinellids were maintained in transparent methyl methacrylate cages ( $40 \times 30 \times 45$  cm) and larvae in plastic cylinders (Kartell) ( $18 \times 18$  cm diameter) with a ventilation hole on the top lid. Both adults and larvae were kept at  $27 \pm 2$  °C with relative humidity of 60–80% and L:D (16:8) h.

#### 2.2. Experiments of intra-guild predation and cannibalism

Laboratory experiments were carried out on intraguild predation (IGP) and cannibalism (CANN) of eggs by adult females and fourth instars of *H. axyridis* and *A. bipunctata*. The studies included: (a) cannibalism in the exotic (CANN<sub>ex</sub>) and native species (CANN<sub>nat</sub>); (b) IGP of the *H. axyridis* vs. *A. bipunctata*; and (c) IGP of the *A. bipunctata* vs. *H. axyridis*.

Trials using the methyl methacrylate rearing cages to assess IGP were attempted but in such large cages interactions were rare during the observation period. Thus, similar to the method of Agarwala and Dixon (1992), all our experiments were conducted in glass petri dishes (12 cm diameter) at  $25 \pm 2$  °C, RH =  $70 \pm 10\%$  and L:D (24:0) h. The predators used in the tests were fed for 24 h with aphids and starved for the next 24 h to induce the same level of hunger (Agarwala and Dixon, 1992). Individuals were placed in petri dishes with 20 coccinellidae eggs of the selected target species and, eventually, with 5 or 40 aphids. After 1 h, the remaining number of coccinellid eggs and live aphids were counted. Each experiment was replicated 30 times. The laboratory trials included three treatments: (a) with only eggs of coccinellids; (b) with eggs of coccinellids and 5 aphids; and (c) with eggs of coccinellids and 40 aphids.

Results of the experiments using females and fourth instars were combined to compare: (i) intra-guild predation and cannibalism of *H. axyridis* (IGP<sub>ex</sub> vs. CANN<sub>ex</sub>); (ii) cannibalism of *A. bipunctata* and intraguild predation of *H. axyridis* (CANN<sub>nat</sub> vs. IGP<sub>ex</sub>); and (iii) intra-guild predation of *A. bipunctata* and cannibalism of *A. bipunctata* (IGP<sub>nat</sub> vs. CANN<sub>nat</sub>).

Data were analysed by non-parametric analysis of variance (Kruskal–Wallis test). A non-parametric approach was used because of heteroscedasticity and departures from normality (Zar (1984)). The relationship between egg consumption and aphid density was analysed by Spearman rank correlation.

#### 3. Results and discussion

A lower level of IGP of *H. axyridis* vs. eggs of the native species *A. bipunctata* was observed, in comparison with cannibalism by *H. axyridis* (Figs. 1 and 2), for both adults (H = 13.50, df = 1, N = 60, P < 0.05; H = 11.90, df = 1, N = 60, P < 0.05; H = 10.87, df = 1, N = 60, P < 0.05; Kruskal–Wallis test) and larvae (H = 14.69, df = 1, N = 60, P < 0.05; Kruskal–Wallis test). At the density of 5 aphids, no differences were detected for fourth instars.

Intra-guild predation of exotic against the native species (IGPex) was never higher than cannibalism of native species (CANN<sub>nat</sub>) (Figs. 3 and 4) (P > 0.05, Kruskal-Wallis test). The results of the comparison between intra-guild predation of natives (IGP<sub>nat</sub>) against cannibalism of A. bipunctata (CANNnat) are shown in Figs. 5 and 6: for the native species, IGP was never higher than CANN (P > 0.05). For adult A. bi*punctata* the CANN was significantly higher than IGP at densities of 0 (H = 3.89, df = 1, N = 60, P < 0.05, Fig. 5) and 40 aphids (H = 10.1, df = 1, N = 60, P < 0.05, Fig. 5). For larvae of A. bipunctata the CANN was significantly higher in comparison with IGP at densities of 0 (H = 26.25, df = 1, N = 60, P < 0.05, Fig. 6) and 5 aphids (H = 6.91, df = 1, N = 60, P < 0.05, Fig. 6). Furthermore, for A. bipunctata, there were no differences between CANN and IGP at density of 5 aphids for adults (P > 0.05, Fig. 5) and at density of 40 aphids for larvae (P > 0.05, Fig. 6).

In the set of experiments on CANN and IGP, it was generally found that an inverse correlation existed between the number of eggs consumed by coccinellid adults and larvae vs. aphid densities (Tables 1 and 2). This relationship possibly resulted from a decline in the probability of encountering eggs as aphid density increased, as found by Agarwala and Dixon (1992) for *A. bipunctata*. The Spearman correlation coefficient in our set of experiments was in some cases low and this was due to the variability of the egg consumption rate among individuals. Agarwala and Dixon (1992) found an inverse relationship between the proportion of the clutches of egg and larvae eaten vs. aphid abundance.

Our laboratory studies indicate that the exotic species *H. axyridis* does not demonstrate IGP vs. *A. bipunctata* at levels intrinsically higher than *A. bipunctata* cannibalism. It appears that the exotic *H. axyridis* does show a high potential for cannibalism, but seems unlikely to potentially have a negative impact on native species by direct means such as IGP.

Ladybird beetles defend themselves with bitter-tasting alkaloids and volatile pyrazine and quinolenes. The alkaloids "coccinelline" and "adaline" occur in *C. septempunctata* and *A. bipunctata*, respectively (Pasteels et al., 1973). Agarwala and Dixon (1992) demonstrated that larvae and adults of both coccinellid species were reluctant to eat conspecific eggs treated with a water extract of the other species' eggs. *C. septempunctata* larvae were more likely to die after eating a few *A. bipunctata* eggs 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. It is adaptive because it improves the chances of survival, and to varying degrees, coccinellids are defended against interspecific predation. The occurrence of sibling can-



Fig. 1. Adults of *H. axyridis* comparisons between intra-guild predation (IGP<sub>ex</sub>) and cannibalism (CANN<sub>ex</sub>); experiments focus on *H. axyridis* eggs or *A. bipunctata* eggs. \*: P < 0.05 (Kruskal–Wallis test).

Cann exotic IGP exotic





Fig. 2. Fourth instar larvae of *H. axyridis*: comparisons between intra-guild predation (IGP<sub>ex</sub>) and cannibalism (CANN<sub>ex</sub>); experiments focus on *H. axyridis* eggs or *A. bipunctata* eggs. \*: P > 0.05; ns: P > 0.05 (Kruskal–Wallis test).



Fig. 3. Adults of *A. bipunctata* and *H. axyridis*: comparisons between cannibalism of native (CANN<sub>nat</sub>) and intra-guild predation of exotic (IGP<sub>ex</sub>); experiments focus on *A. bipunctata* eggs. ns: P > 0.05 (Kruskal–Wallis test).

nibalism 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 cannibalize both infertile and fertile eggs, thereby increasing in body length and survival rate with the intensity of sibling cannibalism.

This cannibalistic trait was clearly beneficial to the organism when aphid density was low, but it was not always beneficial 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 maladaptative 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 densities (>20 aphids per predator per day) (Obrycki et al., 1998).

Of course, IGP is not the only way by which an exotic natural enemy can compete with a native species and other factors are involved, like polyphagy, predatory potential, resource competition, and other biological traits. Coccinellids exhibit many interactions in agricultural landscapes that are related to spatial patterns of habitat use (Kieckhefer et al., 1992). The seasonal timing of occurrence and reproduction in various habitats (Coderre et al., 1995; Elliott and Kieckhefer, 1990a,b) and the extent of overlap in the distributions of cocc-



Fig. 4. Fourth instar larvae of *A. bipunctata* and *H. axyridis*: comparisons between cannibalism of native (CANN<sub>nat</sub>) and intra-guild predation of exotic (IGP<sub>ex</sub>); experiments focus on *A. bipunctata* eggs. ns: P > 0.05 (Kruskal–Wallis test).



Fig. 5. Adults of *A. bipunctata*: comparisons between cannibalism (CANN<sub>nat</sub>) and intra-guild predation (IGP<sub>nat</sub>); experiments focus on *A. bipunctata* eggs or *H. axyridis* eggs. \*: P < 0.05; ns: P > 0.05 (Kruskal–Wallis test).



Fig. 6. Fourth instar larvae of *A. bipunctata*: comparisons between cannibalism (CANN<sub>nat</sub>) and intra-guild predation (IGP<sub>nat</sub>); experiments focus on *A. bipunctata* eggs or *H. axyridis* eggs. \*: P < 0.05; ns: P > 0.05 (Kruskal–Wallis test).

Fable 1
Summary of the correlation analysis of egg consumption by adults vs. aphid density ( $n = 60$ for all experiments)

Predatory species	Target eggs	Behavior	R	df	Р
H. axyridis	H. axyridis	H. axyridis CANN	-0.55	58	< 0.001
A. bipunctata	A. bipunctata	A. bipunctata CANN	-0.19	58	0.067
H. axyridis	A. bipunctata	H. axyridis IGP	-0.42	58	0.001
A. bipunctata	H. axyridis	A. bipunctata IGP	-0.35	58	< 0.001

Table 2

Summary of the correlation analysis of egg consumption by larvae vs. aphid density (n = 60 for all experiments)

Predatory species	Target eggs	Behavior	R	df	Р	
H. axyridis A. bipunctata	H. axyridis A. bipunctata	H. axyridis CANN A. bipunctata CANN	-0.52 -0.50	58 58	<0.001 <0.001	
H. axyridis A. bipunctata	A. bipunctata H. axyridis	H. axyridis IGP A. bipunctata IGP	-0.53 -0.34	58 58	<0.001 <0.005	

inellid and aphid species on a particular plant species (Coderre and Tourneur, 1986) are factors to be considered. 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. Our bioassays on IGP and CANN in a small arena drastically simplifies the field situation. Presently, we have no data on H. axyridis establishment in Italy, so we have undertaken simple basic experiments. In our cage observations, the coccinellids showed no evidence of IGP on eggs when plants and aphids were present. Thus, to obtain sufficient data in a short time we choose an "extreme" condition (i.e., small arenas). We assume that if IGP is not relevant in such extreme situations, then negligible effects could also be predicted in the field.

Further experiments are in progress to complete the studies on competition between *H. axyridis* and *A. bipunctata*, including IGP and CANN of eggs in choice tests, IGP and CANN of larvae, life-table studies and analysis of mortality factors under field conditions.

In conclusion, field and laboratory post-release studies, in combination with predictive methods, will permit the design of a conceptual, theoretical, and ecological framework for examining, understanding, and predicting population dynamics in terms of risks of biological control, in particular its effects on non-target species (Loomans and van Lenteren, 1999).

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