BioControl (2008) 53:277–287
DOI 10.1007/s10526-007-9133-x

Estimation of mortality by entomophages on exotic *Harmonia axyridis* versus native *Adalia bipunctata* in semi-field conditions in northern Italy

Giovanni Burgio · Alberto Lanzoni · Gianumberto Accinelli · Stefano Maini

Received: 6 August 2007 / Accepted: 23 October 2007 / Published online: 8 November 2007

Abstract A semi-field experiment was carried out in two peach orchards in northern Italy to assess mortality due to predators and parasitoids on the exotic coccinellid *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in comparison with the native coccinellid *Adalia bipunctata* L. (Coleoptera: Coccinellidae). The experiments were conducted in cages to avoid the possible escape of the exotic ladybird (not yet established in Italy). Two kinds of cage experiments were included: 'exclusion cages' (access by walking predators impeded) and 'free cages' (walking predators free to enter). The cages, containing all the stages of the two ladybird species, were placed in two localities and left for 24 h. All ladybird stages used for the semi-field experiments came from a laboratory rearing. The eggs of *H. axyridis* experienced less mortality than those of *A. bipunctata.* The ant workers were the most frequent predators in 'free cages' but *A. bipunctata* cannibalism on eggs was also detected. Larvae of both coccinellid species were predated equally but larval predation of L1 and L2 was higher in comparison to predation of L3 and L4. Pupae and adults of both exotic and native ladybirds were never attacked by predators. Predation on younger larval stages was higher in the 'free cages' in comparison with 'exclusion cages'. No ladybird parasitisation was observed. The 'free cage' technique seems to provide a standardised and realistic estimation of predation impact but more studies are needed to evaluate ladybird parasitisation in semi-field conditions.

G. Burgio (✉) · A. Lanzoni · G. Accinelli · S. Maini
Dipartimento di Scienze e Tecnologie Agroambientali – Entomologia, Alma Mater Studiorum,
Università di Bologna, viale G. Fanin, 42-40127 Bologna, Italy
e-mail: giovanni.burgio@unibo.it

A. Lanzoni
e-mail: alberto.lanzoni2@unibo.it

G. Accinelli
e-mail: gaccinelli@entom.agrsci.unibo.it

S. Maini
e-mail: stefano.maini@unibo.it

Keywords *Adalia bipunctata* · Field cage experiments · *Harmonia axyridis* · Intraguild predation · Mortality factors · Parasitoids · Predators

Introduction

Biotic factors, including intraguild predation (IGP), can play an important role in the establishment of exotic coccinellids, like *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). For this reason quantifying the effects of natural enemies in such a system could contribute to explaining and understanding the relationships between introduced species and native ones. Increased understanding of the dynamics of natural enemies of coccinellids could also contribute to an evaluation of the potential efficacy of biological control agents against an invasive alien coccinellid that has established in a new area.

Although the knowledge of the natural enemies of coccinellids has progressed in recent years, the real impact of the biotic factors on the population changes of coccinellids has been poorly investigated (Hodek and Honek 1996; Lucas 2005). This scenario seems particularly true for the predators of coccinellids, including vertebrates and invertebrates, while parasitoids have received a little more attention (Iperti 1964; Cartwright et al. 1982; Obrycki et al. 1985; Obrycki 1989) and a quantification of their impact was assessed in various part of the world (Iperti 1964, Cartwright et al. 1982; Obrycki et al. 1985; Obrycki 1989; Hodek and Honek 1996; Geoghegan et al. 1998; Okuda and Ceryngier 2000). Furthermore, the role of microorganisms as mortality factors of coccinellids has been poorly investigated with limited information on entomopathogenic protozoans and fungi (Hodek and Honek 1996). Fungal disease is considered to be one of the main biotic factors causing winter mortality of coccinellids (see papers cited in Hodek and Honek 1996) and recently the susceptibility of some species of coccinellids, including *H. axyridis* to infection by *Beauveria bassiana* (Balsamo) Vuillemin has been studied by Roy et al. (2007). Further data on the natural enemies of *H. axyridis* are included in the paper by Kenis et al. (2007).

There is considerable interest in the role and prevalence of IGP among aphidophagous predators, partly because such interactions among predators are thought to influence the efficacy of biological control (Rosenheim et al. 1995). Declines in populations of native aphidophages in North America have been linked to IGP by the exotic *H. axyridis* (Brown and Miller 1998; Colunga-Garcia and Gage 1998). In a study measuring the impact of IGP by *H. axyridis* on the control of the newly established soybean aphid, *Aphis glycines* Matsumura in Michigan, Gardiner and Landis (2007) showed that while the exotic *H. axyridis* does act as an intraguild predator and may contribute to local declines in *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecyiidomidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). In European countries, the impact of *H. axyridis* on native coccinellids is uncertain but IGP interactions are thought to occur and to have an influence on aphidophagous guilds. In general, in Europe *H. axyridis* is considered to be an invasive alien species (Brown et al. 2007) and in the UK the IGP of immature stages of British and Japanese coccinellids by the invasive ladybird *H. axyridis* has been demonstrated (Ware and Majerus 2007).

IGP in coccinellid guilds was recently reviewed by Dixon (2000) and a review by Pervez and Omkar (2006) focuses more specifically on the predator–predator interaction involving *H. axyridis*. The problem of IGP in aphidophagous systems was critically commented on by Lucas (2005); by an analysis of the literature this author states that IGP, like cannibalism, seems difficult to observe and quantify in the field and this explains the scarcity of data within this topic. For example Cottrell and Yeargan (1998a, b) report the amount of egg cannibalism by a coccinellid and direct observation of other predators feeding on that coccinellid's eggs.

In conclusion, although some data are available on the impact of natural enemies of coccinellids, the degree of their influence has been poorly investigated. This is particularly true for *H. axyridis*, an exotic species for which no field data on the natural enemies are available in Italy.

The objective of this study was to assess the impact of natural enemies on exotic *H. axyridis*, an East Palearctic species, compared with the native West Palearctic species *Adalia bipunctata* L., using field cages studies. The native species was selected because it is common in hedgerows and orchard trees (Hodek and Honek 1996) and is abundant in northern Italy agroecosystems (Burgio et al. 2004, 2006). Furthermore *A. bipunctata*, as *H. axyridis*, is a cosmopolitan species with strong colonisation potential and is expected to establish in Japan (Kajita et al. 2000; Toda and Sakuratani 2006). A specific objective of the research was to quantify the incidence of the entomophages on all life stages of the two selected coccinellid species, in order to understand if the exotic species display a differential degree of protection against predation or parasitism in comparison with the native one.

Materials and methods

Insect rearing

Coccinellids were reared in the entomological laboratories of Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), Alma Mater Studiorum, University of Bologna. A culture of *A. bipunctata* was initiated from field-collected specimens; *H. axyridis* was supplied by the commercial insectary Koppert (The Netherlands). The biology and overwintering capacity of this biofactory-reared strain of *H. axyridis* were previously studied in northern Italy (Bazzocchi et al. 2004; Lanzoni et al. 2004). Larvae of the two coccinellid species were fed with frozen eggs of *Ephestia kuehniella* (Zeller). Adults were fed with *Aphis gossypii* Glover that had been reared on potted summer squash (*Cucurbita pepo* L.) plants. Adult coccinellids were maintained in Plexiglas cages (40 × 30 × 45 cm) and larvae in plastic cylinders (Kartell©, Milano, Italy) (diameter = 18 cm, h = 18 cm). Both adults and larvae were kept at 20–25°C with a relative humidity of 60–80% and photoperiod 16L: 8D.

Field cage experiments

Field cage experiments that assessed the impact of natural enemies on the exotic species *H. axyridis* in comparison with the native *A. bipunctata*, were conducted in the summer of 2000 in two localities in Emilia-Romagna region (northern Italy). This period was chosen because the occurrence of aphids and their antagonists are at the maximum population level (Burgio et al. 2004, 2006) in this area. Field cage experiments were chosen to avoid the possible escape of the exotic ladybird, not yet established in Italy (only recently this species was recorded in Turin, Brown et al. (2007), but not yet in Emilia-Romagna region). Two treatments (*H. axyridis* versus *A. bipunctata*) were compared and each composed of all the ontogenic stages (eggs, L1, L2, L3, and L4 larvae, pupae and adults, each stage isolated in a separate cage). In addition to split predation by walking predators from predation by all the predators, two kinds of field exposure were set up for each treatment: ‘exclusion cages’, where access by walking predators was impeded, and ‘free cages’, that allowed access to all the predators.

All cages used consisted of a PVC container that varied in size according to the ontogenic stage added: 25 × 18 × 11 cm for each of the preimaginal stages and 33 × 22 × 12.5 cm for the adult stage. Twenty-five eggs in clusters, 10 L1, L2, L3 and L4 larvae, pupae and adults were placed separately into the cages. Some summer squash leaves infested with *A. gossypii* were added in each of the larval and adult cages. To avoid specimens escaping, the inner surface of the cages was covered with PTFE (polytetrafluoroethylene, Algoflon®), an inert substance preventing the insects climbing up but not falling down into the cage. This attribute was previously demonstrated in laboratory tests (unpublished data). Moreover, the adult’s hind wings were distally cut. All the cages were stored on open metallic shelves covered by a roof (100 × 40 cm, 100 cm above ground level), in two organic peach orchards in the Bologna province (Imola, 41°18′57″ N, 11°39′36″ E and Mercatale, 44°23′7″ N, 11°25′37″ E). Each shelf was wrapped in garden netting of 1 cm^2 mesh to prevent bird and mammalian predation. The exclusion of walking predators was achieved by covering the base of the shelves containing the ‘exclusion cages’ with insect glue (The Tanglefoot Company, MI, USA) and placing each cage in a small basin full of water plus a surfactant (liquid soap). In each site, all the cages were randomly arranged on four shelves (two for the exclusion system and two for the free system) every 3 weeks, and a total of seven replicates, from June to September, in the site of Mercatale, and five replicates, from July to September, in the site of Imola were completed. For each replicate cages were left in the fields for 24 h, and then returned to the laboratory. Temperature and relative humidity were continuously monitored in the site of Mercatale.

The number of eggs, larvae of each instar, pupae and adults predated or missing was recorded. Missing specimens were considered as totally preyed, due to the impossibility for predators trapped in the cages to escape. In order to avoid cannibalism events, aphid colonies were introduced in the cages. The alive specimens were reared individually in cylindrical cages (8 cm diameter, 8 cm height) in a climatic chamber at 25 ± 1°C, 75 ± 10% RH and a photoperiod of 16L: 8D to assess parasitization of the coccinellid. Adults were reared on *A. gossypii* for 30 days, and larvae were reared on *E. kuehniella* frozen eggs until adult emergence. Egg-batches were kept at the same conditions until eclosion.

Field sampling of coccinellids

In each site, four field samplings by means of mechanical knock down were carried out in order to collect coccinellids within peach orchards, hedgerows and shrubs. This sampling was employed in order to estimate the coccinellid assemblages and to assess the natural parasitization in the study areas. Four field collections were carried out from July to October. Mechanical knock down consisted of beating branches with a stick and collecting the dislodged insects in a 90 cm diameter funnel for examination in the laboratory. Plants were beaten 100 times on each of five branches of a tree or shrub. Plants and branches were randomly selected at each sampling date. The collected coccinellids were identified to species and reared at the same conditions as described for the field cage experiments.

A χ^2 test in 2 × 2 contingency tables was carried out in order to compare the predation ratio between the treatments (Zar 1984).

Results

Different arthropods entered the field cages and the insects found in both treatments are listed in Table 1. The predators included: ant workers, *C. carnea* larvae and adults of the coccinellids *Hippodamia variegata* (Goeze) and *A. bipunctata*. The wild specimens of *A. bipunctata* were easily detected since they were characterised by uncut hind wings.

The predation on *A. bipunctata* eggs by predators was significantly higher in comparison with the predation on *H. axyridis* eggs, in both sampling sites (Fig. 1). No predation on pupae and adults occurred for either the exotic or native ladybirds. It is likely that the larvae of the native and exotic species were preyed on by generalist predators. For both coccinellid species, L1 and L2 instars were more likely to be eaten in comparison with L3 and L4 (Fig. 1). The percentage predation for all the instars of *A. bipunctata* was not different in comparison with predation on *H. axyridis* ($P > 0.05$, χ^2 test).

In general, predation was higher in the 'free cages' in comparison with the 'exclusion cages', for both coccinellid species and localities (Figs. 2, 3). In both sites, ants were the most abundant generalist predator found in the 'free cages' (Table 1) and we suppose that they play an important role in the predation of coccinellids, as reported also by Hodek and Honek (1996).

In the site of Mercatale, one third instar of *C. carnea* was directly observed in the 'free cage' system and this could have been responsible for the predation of all of the *A. bipunctata* eggs. Also, in two collections in the site of Imola, *A. bipunctata* adults were trapped in the 'free cages'; in these two samplings the predation of eggs was again 100% and we suggest that cannibalism strongly contributed to the egg mortality.

No parasitoids emerged from our exposed coccinellids in spite of the regular presence of parasitoid wasps (Hymenoptera, Apocrita, Parasitica) in the cages, which indicates that these wasps was not impeded from entering the cages. These parasitoids were not identified and they were probably just attracted by aphid honeydew.

Among the naturally occurred species sampled in the field by beating, *H. variegata* was the most abundant coccinellid in both localities (relative abundances were 52.3% ($n = 57$) in Mercatale and 70.9% ($n = 66$) in Imola), followed by *A. bipunctata* (32.1% ($n = 35$) and 1.1% ($n = 1$), respectively) and *Chilocorus* spp. (11.0% ($n = 12$) and 24.7% ($n = 23$), respectively). From the samples of native species collected in field and reared in laboratory, only one parasitoid emerged from one pupa of *A. bipunctata*. The species was identified as *Aprostocetus neglectus* (Domenichini) (Hymenoptera: Eulophidae). The rate of parasitism calculated on native species obtained by field samplings was very low (0.5%) within the period of the experiment.

Discussion

Our cage system for ladybird seems to provide a standardised and realistic estimation of mortality by predation, including IGP predation. These events are difficult to observe and quantify in the field for aphidophagous predators (Lucas 2005) and probably for this reason most of the observations on ladybirds are confined to the laboratory while only a few experiments are conducted in semi-field or field conditions (Obrycki et al. 1998a, b;

Table 1 Number of insect adults trapped in the field cages (*A.b.* = *Adalia bipunctata*, *H.v.* = *Hippodamia variegata*, *C.c.* = *Chrysoperla carnea*)

	Mercatale					Imola				
	Ant workers	Hym. parasitoids	*A.b.*	*H.v.*	*C.c.*	Ant workers	Hym. parasitoids	*A.b.*	*H.v.*	*C.c.*
Harmonia axyridis										
Free egg cages										
Free larval cages (L1–L2)	2	1				2				
Free larval cages (L3–L4)	3					3	1			
Free pupal cages	2									
Free adult cages										
Exclusion egg cages		1								
Exclusion larval cages (L1–L2)				1			1			
Exclusion larval cages (L3–L4)										
Exclusion pupal cages	3									
Exlcusion adult cages			1	1						
Adalia bipunctata										
Free egg cages			1					2		1 larvae
Free larval cages (L1–L2)	2	1				2				
Free larval cages (L3–L4)	2			1		2				
Free pupal cages						2	1			
Free adult cages	2		1	1						
Exclusion egg cages			1	1						
Exclusion larval cages (L1–L2)										
Exclusion larval cages (L3–L4)		1								
Exclusion pupal cages										
Exclusion adult cages	1	1								

Gardiner and Landis 2007). In particular, life tables for two predatory ladybirds, *C. septempunctata* and *H. axyridis*, were constructed through a field experiment (Hironori and Katsuhino 1997) and provided quantification of cannibalism and IGP in relation to these coccinellids. However, many factors concerning larval mortality were not known and so it is difficult to be assured that the most important factors structuring the ladybird guild were cannibalism and IGP (Hironori and Katsuhino 1997). Furthermore, in areas where an

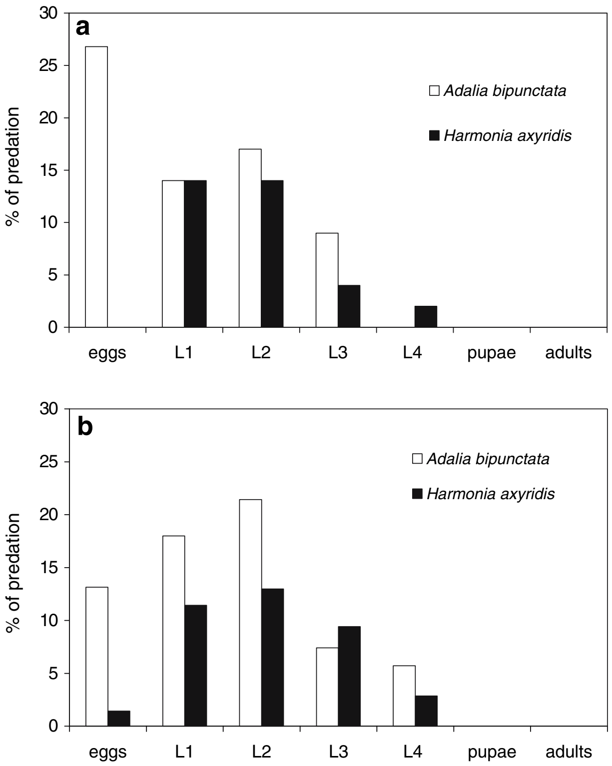

Fig. 1 Total percentage of predation on different life stages (eggs, L1–L4, pupae and adults) of native (*Adalia bipunctata*, *A.b.*) and exotic ladybird (*Harmonia axyridis*, *H.a.*) in field cages at the Imola (**a**) (eggs $\chi^2 = 77.37$; df = 1; $P = 0.00001$; *A.b.* $N = 250$; *H.a.* $N = 250$; L1 $\chi^2 = 0$; df = 1; P = 0; *A.b.* $N = 100$; *H.a.* $N = 100$; L2 $\chi^2 = 0.34$; df = 1; $P = 0.55$; *A.b.* $N = 100$; *H.a.* $N = 100$; L3 $\chi^2 = 2.76$; df = 1; $P = 0.096$; *A.b.* $N = 100$; *H.a.* $N = 100$; L4 $\chi^2 = 2.02$; df = 1; $P = 0.155$; *A.b.* $N = 100$; *H.a.* $N = 100$; pupae *A.b.* $N = 94$; *H.a.* $N = 97$; adults *A.b.* $N = 100$; *H.a.* $N = 100$) and Mercatale (**b**) (eggs $\chi^2 = 35.5$; df = 1; $P = 0.00001$; *A.b.* $N = 350$; *H.a.* $N = 350$; L1 $\chi^2 = 2.39$; df = 1; $P = 0.12$; *A.b.* $N = 139$; *H.a.* $N = 140$; L2 $\chi^2 = 3.37$; df = 1; $P = 0.0663$; *A.b.* $N = 140$; *H.a.* $N = 131$; L3 $\chi^2 = 0.36$; df = 1; $P = 0.54$; *A.b.* $N = 135$; *H.a.* $N = 138$; L4 $\chi^2 = 1.39$; df = 1; $P = 0.23$; *A.b.* $N = 140$; *H.a.* $N = 140$; pupae *A.b.* $N = 138$; *H.a.* $N = 134$; adults *A.b.* $N = 120$; *H.a.* $N = 140$) field sites. Data pooled for collection times

exotic is not yet recorded, only cage studies are recommended to avoid the establishment of the species.

Our ‘exclusion cages’ did not completely prevent access by walking predators but in general it was discriminated between the predation by walking predators like ants from groups of flying predators. The predators collected, including ant workers, could have been also attracted by aphids within the cages, thus resulting in an overestimation of their importance as coccinellid predators. Among polyphagous predators, ant workers were the most abundant insects found in the cages in our experiment and we speculate that this group plays a fundamental role in the predation of eggs. Ants can affect IGP in aphidophagous systems and this complex topic was reviewed by Lucas (2005). For example ants can prey on aphids, or ants may attend aphids and protect them against predators and parasitoids. Ants most often associated with aphids and coccinellids include *Lasius* spp. and *Formica* spp. (Hodek and Honek 1996) and many observations confirm that ants are

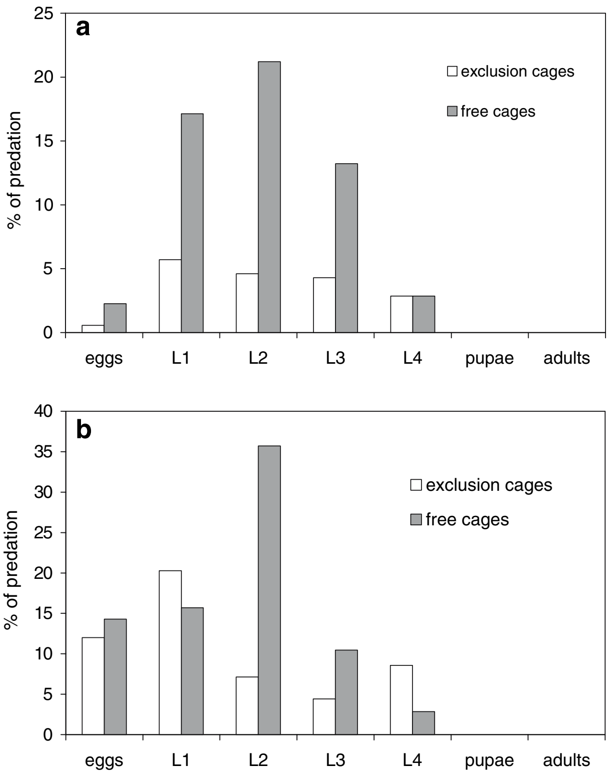

Fig. 2 Percentage of predation on *Harmonia axyridis* (*H.a.*) (eggs, L1–L4, pupae and adults) in exclusion (e.c.) versus free cages (f.c.) in the site of Mercatale (**a**) (eggs $\chi^2 = 1.83$; df = 1; $P = 0.17$; e.c. *H.a.* $N = 175$; f.c. *H.a.* $N = 175$; L1 $\chi^2 = 26.04$; df = 1; $P = 0.00001$; e.c. *H.a.* $N = 70$; f.c. *H.a.* $N = 70$; L2 $\chi^2 = 26.01$; df = 1; $P = 0.00001$; e.c. *H.a.* $N = 65$; f.c. *H.a.* $N = 66$; L3 $\chi^2 = 2.29$; df = 1; $P = 0.13$; e.c. *H.a.* $N = 70$; f.c. *H.a.* $N = 68$; L4 $\chi^2 = 0$; df = 1; $P = 0$; e.c. *H.a.* $N = 70$; f.c. *H.a.* $N = 70$; pupae e.c. *H.a.* $N = 67$; f.c. *H.a.* $N = 67$; adults e.c. *H.a.* $N = 175$; f.c. *H.a.* $N = 175$) and on *Adalia bipunctata* (*A.b.*) in exclusion (e.c.) versus free cages (f.c.) in the site of Mercatale (**b**) (eggs $\chi^2 = 0.40$; df = 1; $P = 0.52$; e.c. *A.b.* $N = 175$; f.c. *A.b.* $N = 175$; L1 $\chi^2 = 0.49$; df = 1; $P = 0.48$; e.c. *A.b.* $N = 69$; f.c. *A.b.* $N = 70$; L2 $\chi^2 = 15.48$; df = 1; $P = 0.0001$; e.c. *A.b.* $N = 70$; f.c. *A.b.* $N = 70$; L3 $\chi^2 = 1.79$; df = 1; $P = 0.18$; e.c. *A.b.* $N = 68$; f.c. *A.b.* $N = 67$; L4 $\chi^2 = 2.12$; df = 1; $P = 0.14$; e.c. *A.b.* $N = 70$; f.c. *A.b.* $N = 70$; pupae e.c. *A.b.* $N = 69$; f.c. *A.b.* $N = 69$; adults e.c. *A.b.* $N = 70$; f.c. *A.b.* $N = 50$)

hostile to enemies of attended aphids. Predatory ants may attack any insects within their foraging territories (Hodek and Honek 1996) and our data seems to confirm these observations.

In our semi-field study, *C. carnea* was likely responsible for predation on *A. bipunctata* eggs. In fact, *C. carnea* completed preimaginal development when fed *A. bipunctata* eggs but it did not complete preimaginal development on *H. axyridis* eggs (Santi and Maini 2006).

Our data demonstrate that eggs of *H. axyridis* are protected against intraguild predation more than eggs of *A. bipunctata*, because the egg-predation was significantly lower than that of the native species. This evidence confirms the results of laboratory experiments on IGP and cannibalism on *H. axyridis* and *A. bipunctata* (Burgio et al. 2002). In a laboratory study Cottrell (2005) reported that the *H. axyridis* eggs were predated on to a lesser extend

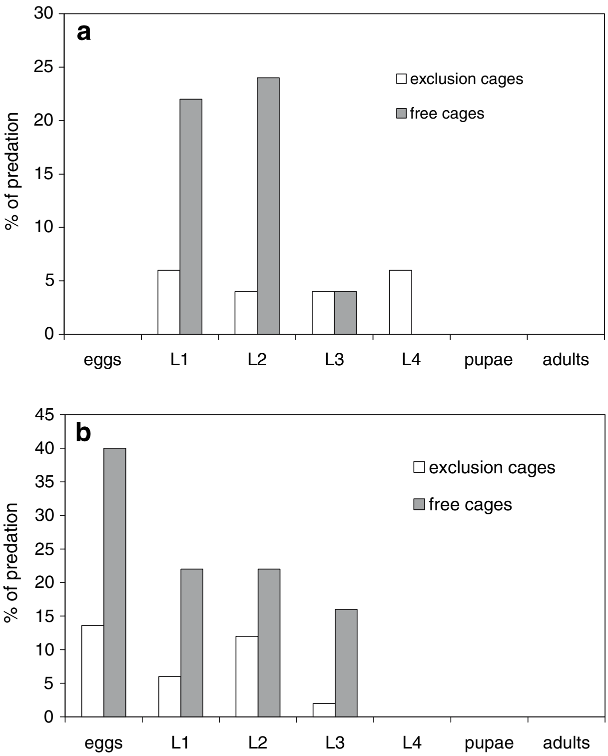

Fig. 3 Percentage of predation on *Harmonia axyridis* (*H.a.*) (eggs, L1–L4, pupae and adults) in exclusion (e.c) versus free cages (f.c.) in the site of Imola (**a**) (eggs e.c. *H.a.* *N* = 125; eggs f.c. *H.a.* *N* = 125; L1 $\chi^2 = 5.32$; df = 1; *P* = 0.021; e.c. *H.a.* *N* = 50; f.c. *H.a.* *N* = 50; L2 $\chi^2 = 8.31$; df = 1; *P* = 0.004; e.c. *H.a.* *N* = 50; f.c. *H.a.* *N* = 50; L3 $\chi^2 = 0$; df = 1; *P* = 0; L4 $\chi^2 = 2.04$; df = 1; *P* = 0.15; e.c. *H.a.* *N* = 50; f.c. *H.a.* *N* = 50; pupae e.c. *H.a.* *N* = 47; f.c. *H.a.* *N* = 50; adults e.c. *H.a.* *N* = 50; f.c. *H.a.* *N* = 50) and on *Adalia bipunctata* (*A.b.*) in exclusion (e.c.) versus free cages (f.c.) in the site of Imola (**b**) (eggs $\chi^2 = 35.2$; df = 1; *P* = 0.00001; e.c. *A.b.* *N* = 125; f.c. *A.b.* *N* = 125; L1 $\chi^2 = 5.32$; df = 1; *P* = 0.021; e.c. *A.b.* *N* = 50; f.c. *A.b.* *N* = 50; L2 $\chi^2 = 1.77$; df = 1; *P* = 0.183; e.c. *A.b.* *N* = 50; f.c. *A.b.* *N* = 50; L3 $\chi^2 = 4.0$; df = 1; *P* = 0.045; e.c. *A.b.* *N* = 50; f.c. *A.b.* *N* = 50; L4 e.c. *A.b.* *N* = 50; f.c. *A.b.* *N* = 50; pupae e.c. *A.b.* *N* = 47; f.c. *A.b.* *N* = 47; adults e.c. *A.b.* *N* = 50; f.c. *A.b.* *N* = 50)

than those of the native species *Coleomegilla maculata* DeGeer and *Olla v-nigrum* (Mulsant). For these reasons *H. axyridis* seems to display a competitive advantage in colonizing new areas, that could be partially responsible for high competitiveness of this species compared to native species in many countries (Elliott et al. 1996; Brown and Miller 1998; Colunga-Garcia and Gage 1998; Gardiner and Landis 2007). The results of the laboratory studies of Cottrell (2005) agree with those of our semi-field trials. Ladybirds defend themselves with bitter-tasting alkaloids, volatile pyrazine and quinolenes (Pasteels et al. 1973; Hodek and Honek 1996; Ware et al. (2007), and surface alkanes (Hemptinne et al. 2000). Agarwala and Dixon (1992) demonstrated that larvae and adults of *Coccinella septempunctata* L. and *A. bipunctata* were reluctant to eat conspecific eggs treated with a water extract of the other species' eggs.

The most important result that emerged from our experiments is that the eggs of the exotic *H. axyridis* are more protected against predation in comparison with those of the native ones. This evidence could explain the success of *H. axyridis* in niches already exploited by other coccinellids; however our findings showed that predation by generalist predators on *H. axyridis* larvae was similar to that observed on *A. bipunctata* larvae.

Other experiments and sampling techniques that allow us to study IGP and cannibalism in the field need to be developed particularly to estimate the impact of parasitoids and general parasitism in the field. We suppose that the exposure time of 24 h in our experiment might be insufficient to estimate the rate of parasitism in field. The parasitism of coccinellids is influenced by the period of the year and by many factors and variables (climatic and biotic, the latter including the relative densities of ladybirds) (Hodek and Honek 1996) and it is probably a local-scale phenomenon. Collection of coccinellid specimens, by manual or beating samplings, should be employed to integrate with cage systems and to avoid an under-estimation of the parasitism due to the consumption of parasitized coccinellids.

Acknowledgements We are grateful to Michele Ruzza for technical assistance during the experiments. This research was funded by ERBIC (Evaluating Risks of Biological Control Introductions into Europe) FAIR5-CT97-3489. A special thank to Helen Roy for the English revision of the manuscript and for useful suggestions.

References

Agarwala BK, Dixon AFG (1992) Laboratory study of cannibalism and interspecific predation in ladybirds. Ecol Entomol 17:303–309

Bazzocchi GG, Lanzoni A, Accinelli G, Burgio G (2004) Overwintering, phenology and fecundity of *Harmonia axyridis* in comparison with native coccinellid species in Italy. BioControl 49:245–260

Brown MW, Miller SS (1998) Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and their impact of invasion by *Harmonia axyridis*. Ent News 109(2):136–142

Brown PMJ, Adriaens T, Bathon H, Cuppen J, Goldarazena A, Hägg T, Kenis M, Klausnitzer BEM, Kovář I, Loomans AJM, Majerus MEN, Nedved O, Pedersen J, Rabitsch W, Roy HE, Ternois V, Zakharov IA, Roy DB (2007) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. BioControl (this issuse). doi:10.1007/s10526-007-9132-y

Burgio G, Santi F, Maini S (2002) On intraguild predation and cannibalism in *Harmonia axyridis* Pallas and *Adalia bipunctata* L. (Coleoptera: Coccinellidae). Biol Control 24:110–116

Burgio G, Ferrari R, Pozzati M, Boriani L (2004) The role of ecological compensation areas on predator populations: an analysis on biodiversity and phenology of Coccinellidae (Coleoptera) on non-crop plants within hedgerows in Northern Italy. Bull Insectology 57:1–10

Burgio G, Ferrari R, Boriani L, Pozzati M, van Lenteren J (2006) The role of ecological infrastructures on Coccinellidae (Coleoptera) and other predators in weedy field margins within northern Italy agroecosystems. Bull Insectology 59:5–67

Cartwright B, Eikenbary RD, Angalet GW (1982) Parasitism by *Perilitus coccinellae* (Hym.: Braconidae) of indigenous coccinellids hosts and the introduced *Coccinella septempunctata* (Col.: Coccinellidae), with notes on winter mortality. Entomophaga 27:237–244

Colunga-Garcia M, Gage SH (1998) Arrival, establishment, and habitat use of the multicolor Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. Environ Entomol 27:1574–1580

Cottrell TE (2005) Predation and cannibalism of lady beetle eggs by adult lady beetles. Biol Control 34: 159–164

Cottrell TE, Yeargan KV (1998a) Influence of a native weed, *Acalypha ostryaefolia* (Euphorbiaceae), on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. Environ Entomol 27:1375–1385

Cottrell TE, Yeargan KV (1998b) Effect of pollen on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. Environ Entomol 27:1402–1410

Dixon AFG (2000) Insect predator–prey dynamic: ladybird, beetles and biological control. Cambridge University Press, Cambridge, UK

Elliott N, Kieckhefer R, Kauffman W (1996) Effects on an invading coccinellid on native coccinellids in an agricultural landscape. Oecologia 105:537–544

Gardiner MM, Landis DA (2007) Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptea: Aphididae) biological control in cage studies. Biol Control 40:386–395

Geoghegan IE, Majerus TMO, Majerus MEN (1998) Differential parasitization of adult and pre-imaginal *Coccinella septempunctata* (Coleoptera: Coccinellidae) by *Dinocampus coccinellae* (Hymenoptera: Braconidae). Eur J Entomol 95:571–579

Hemptinne JL, Lognay G, Gauthier C, Dixon FG (2000) Role of surface chemical signals in egg cannibalism and intraguild predation in ladybirds (Coleoptera: Coccinellidae). Chemoecology 10:123–128

Hironori Y, Katsuhino S (1997) Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. Entomophaga 42:153–163

Hodek I, Honék A (1996) Ecology of coccinellidae. Kluwer Academic Publishers, Dordrecht, The Netherlands

Iperti G (1964) Les parasites des coccinelles aphidiphages dans les Alpes-Maritimes et les Basses-Alpes. Entomophaga 9:153–180

Kajita H, Takano F, Yasuda H, Agarwala BK (2000) Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on survival of an exotic species in relation to prey abundance. Appl Entomol Zool 35(4):473–479

Kenis M, Roy HE, Zindel R, Majerus MEN (2007) Current and potential management strategies against *Harmonia axyridis*. BioControl (this issuse). doi:10.1007/s10526-007-9136-7

Lanzoni A, Accinelli G, Bazzocchi GG, Burgio G (2004) Biological traits and life table of the exotic *Harmonia axyridis* compared with *Hippodamia variegata*, and *Adalia bipunctata* (Col., Coccinellidae). J Appl Entomol 128:298–306

Lucas E (2005) Intraguild predation among aphidophagous predators. Eur J Entomol 102:351–364

Obrycki JJ (1989) Parasitization of native and exotic Coccinellids by *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae). J Kans Entomol Soc 32:211–218

Obrycki JJ, Tauber MJ, Tauber CA (1985) *Perilitus coccinellae* (Hymenoptera: Braconidae): parasitization and development in relation to host-stage attacked. Ann Entomol Soc Am 78:852–854

Obrycki JJ, Giles KL, Ormord AM (1998a) Experimental assessment of interactions between larval *Coleomegilla maculata* and *Coccinella septempunctata* (Coleoptera: Coccinellidae) in field cages. Environ Entomol 27:1280–1288

Obrycki JJ, Giles KL, Ormord AM (1998b) Interactions between an introduced and indigenous coccinellid species at different prey densities. Oecologia 117:279–285

Okuda T, Ceryngier P (2000) Host discrimination in *Dinocampus coccinellae* (Hymenoptera: Braconidae). Appl Entomol Zool 35:595–539

Pasteels JM, Deroe C, Tursch B, Braekman JC, Daloze D, Hootele C (1973) Distribution et activites des alcaloides defensifs des Coccinellidae. J Insect Physiol 19:1771–1784

Pervez A, Omkar GM (2006) Ecology and biological control application of multicoloured Asian ladybird, *Harmonia axyridis*: a review. Biocontrol Sci Technol 16:111–128

Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BAJ (1995) Intraguild predation among biological-control agents: theory and evidence. Biol Control 5:303–335

Roy HE, Brown PMJ, Rothery P, Ware RL, Majerus MEN (2007) Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. BioControl (this issuse). doi:10.1007/s10526-007-9122-0

Santi F, Maini S (2006) Predation upon *Adalia bipunctata* and *Harmonia axyridis* eggs by *Chrysoperla carnea* larvae and *Orius laevigatus* adults. Bull Insectology 59:53–58

Toda Y, Sakuratani Y (2006) Expansion of the geographical distribution of an exotic ladybird beetle, *Adalia bipunctata* (Coleoptera: Coccinellidae), and its interspecific relationships with native ladybird beetles in Japan. Ecol Res 21:292–300

Ware RL, Majerus MEN (2007) Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. BioControl (this issuse). doi:10.1007/s10526-007-9135-8

Ware RL, Ramon-Portugal F, Magro A, Ducamp C, Hemptinne J-L, Majerus MEN (2007) Chemical protection of *Calvia quatuordecimguttata* eggs against intraguild predation by the invasive ladybird *Harmonia axyridis*. BioControl (this issuse). doi:10.1007/s10526-007-9129-6

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs, NJ