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Overwintering, phenology and fecundity of *Harmonia axyridis* in comparison with native coccinellid species in Italy

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Abstract. Classical biological control is generally understood as an environmentally safe practice of insect pest management. However, questions have been raised about possible negative effects for native species. As part of a risk assessment study a semi-field trial was carried out in northern Italy in order to compare the overwintering of the native species *Propylea quatuordecimpunctata* (L.) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) with the exotic *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). All the experiments were carried out from April 1998 to April 2001. *Harmonia axyridis* was able to overwinter successfully in northern Italy. Fecundity, oviposition rate, longevity and rate of increase of overwintering females were calculated and the phenology of the three species was studied. *Harmonia axyridis* overwintering mortality (31.9%) was lower in comparison with mortality of native species (68.9% for *P. quatuordecimpunctata* and 61.3% for *A. bipunctata*). As a consequence, post-overwintering rate of increase of the exotic species was higher. Longevity of overwintered females was similar among the species. Mean fecundity of *H. axyridis* (783.8 eggs per female) was slightly higher than *A. bipunctata* (720.2 eggs per female), and much higher than *P. quatuordecimpunctata* (193.7 eggs per female). *H. axyridis* and *A. bipunctata* completed four generations and *P. quatuordecimpunctata* three generations in a year. The results show that *H. axyridis* appears to posses a high potential for establishment in Italy.

Key words: *Adalia bipunctata*, biological control, Coccinellidae, Coleoptera, *Harmonia axyridis*, fecundity, overwintering, phenology, *Propylea quatuordecimpunctata*, risk assessment

Introduction

The Asian multicoloured ladybeetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is a Palearctic species originating from the Far East. This beetle occurs in Korea, Japan, Bonin Islands, China, Himalayas, Formosa, and Siberia (Iablokoff-Khnozorian, 1982), and has been introduced into the

United States (LaMana and Miller, 1996; Brown and Miller, 1998; Colunga-Garcia and Gage, 1998; Brown, 1999), Canada (Coderre et al., 1995) and France (Ferran et al., 1996).

This arboreal ladybird occurs in orchard and forest habitats and preys mostly on various aphid species, but it also accepts scales and two species of chrysomelids (Tedders and Schaefer, 1994). *H. axyridis* shows two types of diapause: hibernation (true diapause) and aestivation that is a facultative dormancy (Sakurai et al., 1988).

H. axyridis is an effective biological control agent against aphid pests. In France, this exotic species was released against *Macrosiphum rosae* L. on rose bushes (Ferran et al., 1996) and against *Phorodon humuli* (Schrank), the most important pest of hop (Trouve et al., 1997). In Italy, this coccinellid has been released for some years to control *Aphis gossypii* Glover in greenhouses. At present there are no data about its establishment in Italy.

The EU-funded ERBIC (Evaluating Environmental Risks of Biological Control Introduction Into Europe) research project aims at designing the 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 evaluating the consequences of the introduction of exotic natural enemies (van Lenteren et al., 2003). In this view, predictive methods that will permit us to evaluate the chances of establishment of an exotic, are required. The evaluation of the overwintering capacity in a new environment is the first step to consider in the risk assessment of the introduction of an exotic.

The specific aim of our experiments was to develop rapid and reliable methods to assess the overwintering, the phenology and some post-overwintering biological traits of the exotic *H. axyridis* in semi-field conditions in northern Italy (∼45°N). It is reported that the exotic *H. axyridis* can exhibit a strong impact on the native species, affecting the coccinellid guild. Field surveys in West Virginia (USA) by Brown and Miller (1998) found that this species has become the dominant species in the tribe of Coccinellini in 1995 and it continues to dominate the Coccinellinae guild on apple. *H. axyridis* became a dominant coccinellid species in the agricultural landscape of Michigan (Colunga-Garcia and Gage, 1998): adults of this species were found in all habitats monitored, including early secondary succession, poplar plantations, alfalfa, soybean, corn and winter wheat. For these reasons, considering that winter mortality can closely affect the population dynamics of a species in the landscape, the study of this factor plays a major role in determining the potential impact of this exotic species on the coccinellid guild that shares the same habitats. Two indigenous species of coccinellids, *Propylea quatuordecimpunctata* (L.) and *Adalia bipunctata* (L.) (Coleoptera:

Coccinellidae), common in hedgerows and orchard trees (Hodek and Honěk, 1996) and abundant in the agroecosystems of Northern Italy (Boriani et al., 1998; Ferrari et al., 1998; Burgio et al., 1999), were chosen as a comparison with the exotic *H. axyridis*.

Materials and methods

Coccinellids had been reared in the Entomological laboratories of DiSTA (Dipartimento di Scienze e Tecnologie Agroambientali), University of Bologna, for six months before the experiments. Cultures of *A. bipunctata* and *P. quatuordecimpunctata* were established from field-collected specimens; *H. axyridis* was supplied by the biofactory Koppert (The Netherlands). Larvae of the three coccinellid species were fed with *Ephestia kuehniella* (Zeller) frozen eggs. Adults of *H. axyridis* and *A. bipunctata* were fed with *Myzus persicae* (Sulzer) reared on "Primizia" green pea (*Pisum sativum* L.) sprouts, and *P. quatuordecimpunctata* on *Aphis gossypii* Glover reared on summer squash (*Cucurbita pepo* L.).

The trials were conducted under an outdoor shelter (essentially two shelves covered by a roof) located in a shaded area surrounded by trees and bushes, in the middle of a garden near the laboratory. Temperature was continuously monitored. All the experiments were carried out from April 1998 to April 2001.

Phenology

The experiments were carried out from April 2000 to April 2001. Egg-batches (up to 120 eggs), laid by overwintering females, were collected weekly and isolated in cylindrical cages (diameter = 9 cm, h = 27 cm). Afterwards they were put under the outdoor shelter. As soon as the eggs hatched, the larvae were fed with *E. kuehniella* eggs and their development was checked three times a week. When the post-embryonic development was completed the adults were put in a Plexiglas cage (27 cm by 37 cm by 20 cm) and kept under the outdoor shelter. *H. axyridis* and *A. bipunctata* adults were fed with *E. kuehniella* frozen eggs. Adults of *P. quatuordecimpunctata* were provided with *A. gossypii*, since laboratory trials showed the incapability of this species to lay eggs when fed on *E. kuehniella* eggs. The following generations were separated from one another by collecting the eggs weekly and by putting the newly-hatched larvae in new cages. The adults of each generation were kept separate. At the beginning of spring 2001 winter mortality and fertility of the overwintering females were checked.

Overwintering

Egg-batches (for a total of at least 400 eggs) of *H. axyridis* and *P. quatuordecimpunctata* (first year), and *H. axyridis*, *P. quatuordecimpunctata* and *A. bipunctata* (second year), were collected and placed in separate white semi-transparent plastic cages (27 cm by 37 cm by 20 cm) under the outdoor shelter four times at 10-day intervals. The first egg placement was made on August 16 in 1998 and on July 27 in 1999. Further groups (5th group the first year, 5th, 6th and 7th the second year) set up from egg-batches oviposited by the 1st group (first year) and by the 1st, 2nd and 3rd groups (second year) respectively, were also monitored.

Larvae were reared together and, in order to limit cannibalism, increased hiding space was provided by adding a layer of curled paper towel. As soon as adults emerged two roofing tiles were put inside each cage in order to simulate an appropriate dormancy site [based on the requirements reported by Hodek and Honěk (1996)].

Larvae of the three species and adults of *H. axyridis* and *A. bipunctata* were fed with *E. kuehniella* frozen eggs. Adults of *P. quatuordecimpunctata* were provided with *A. gossypii*. Food was given and replaced every 2–3 days and the ladybird eggs laid were collected.

The number of dead adults was recorded weekly until the beginning of diapause. The number of adults entering diapause was recorded. Overwintering capacity and winter mortality were assessed later in the spring. For each group a sample of eggs laid by overwintering females was collected and the percentage of eggs hatched was calculated.

Fecundity and longevity of overwintering females

An experiment on fecundity and longevity of the overwintering females was carried out during the spring of 2000. In April, as soon as mating was observed, 12 pairs of *H. axyridis*, 10 of *A. bipunctata* and 9 of *P. quatuordecimpunctata* from the overwintering specimens were isolated in cylindrical cages (diameter = 9 cm, h = 27 cm) and placed under the outdoor shelter. The pairs of *H. axyridis* and *A. bipunctata* were fed with *E. kuehniella* eggs, while the pairs of *P. quatuordecimpunctata* were fed with *A. gossypii*. When males died in the course of the experiment, they were substituted with other males from the overwintering experiment. Three times a week the eggs laid were counted and removed until the females' death. Fecundity, oviposition rate, longevity and rate of increase (r) (see Statistical analyses) of overwintering females were calculated.

Statistical analyses

Differences in post-overwintering oviposition and longevity among the three coccinellid species were analysed by Kruskal-Wallis ANOVA test followed by distribution-free multiple comparisons based on rank sums ($P < 0.05$) (Zar, 1984). A post-overwintering rate of increase (r) was calculated by means of the Birch (1948) method, based on the equation of Lotka; r can be determined by iteratively solving the equation:

$$\int_0^n \exp(-rt) * l_x m_x = 1,$$

where m_x is the age specific fecundity and l_x is the survival rate (Dent, 1997). Immature stage mortality and pre-winter adult mortality and fecundity were assumed to be 0. *Sex-ratio* was assumed to be 1:1. The STATISTICA software for Windows StatSoft^{TM} (1994) was used for statistical analysis.

Results and discussion

Phenology

In 2000–2001, *H. axyridis* and *A. bipunctata* completed four generations and *P. quatuordecimpunctata* three generations (Figure 1). Sakurai et al. (1988) reported that *H. axyridis* has a bivoltine cycle in central Japan, while in Greece this species completed four annual generations (Katsoyannos et al., 1997). *A. bipunctata* is generally reported as a multivoltine species (Hodek and Honěk, 1996). In particular Obrycki et al. (1983) reported that this species completes two to three generations per year in the Ithaca, N.Y. area (∼42°N). Even *P. quatuordecimpunctata* is a multivoltine species and has at least three generations annually in France (Iperti, 1966).

The overwintered *H. axyridis* and *A. bipunctata* females started laying eggs in the second half of April. *P. quatuordecimpunctata* started later, at the beginning of May. This observation confirms that *A. bipunctata* disperses earlier after the winter in comparison to *P. quatuordecimpunctata* (Hodek and Honěk, 1996). Lamana and Miller (1996) observed oviposition by *H. axyridis* from the beginning of April in the open field in Oregon. The adults of the 1st generation of the 3 species of coccinellids emerged almost at the same time (second half of May). Adults of each generation of *H. axyridis* and *A. bipunctata* and of 2nd and 3rd generations of *P. quatuordecimpunctata* were able to overwinter, giving rise to the following year's 1st generation. Hence some adults of the 1st generation of *H. axyridis* and *A. bipunctata* lived up to 10 months. *P. quatuordecimpunctata* adults of the

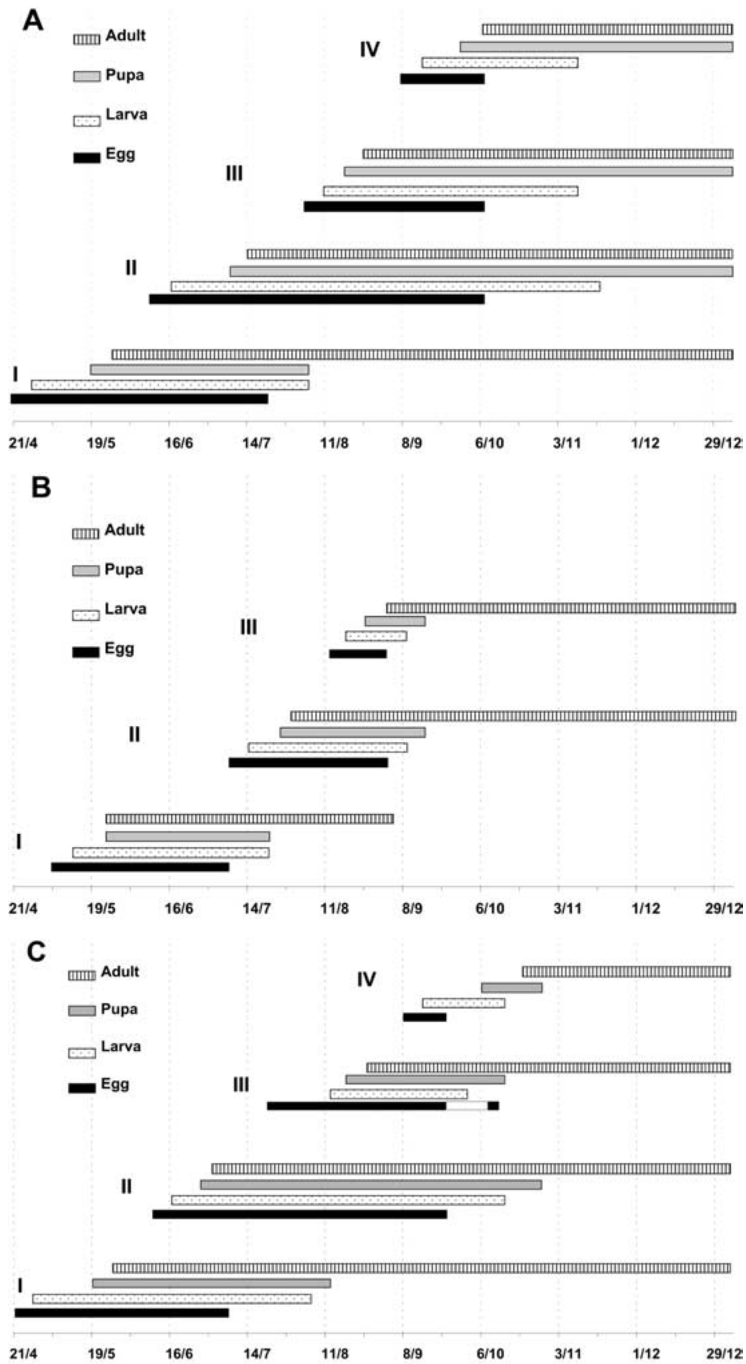

Figure 1. Phenology of *H. axyridis* (A), *P. quatuordecimpunctata* (B) and *A. bipunctata* (C) reared in outdoor cages in 2000–2001. I–IV denotes successive generations.

1st generation survived until the end of August. In contrast Katsoyannos et al. (1997) reported that only the *H. axyridis* 4th generation adults gave rise to the following's year 1st generation. This discrepancy in the behaviour of *H. axyridis* could be due to the different mass-reared strains used in the experiments.

The three coccinellid species had an egg-laying activity throughout the warm season and there was no aestivation diapause. For *H. axyridis*, Katsoyannos et al. (1997) did not find an interruption of activity in summer, while Sakurai et al. (1988) reported a bivoltine cycle interrupted twice by aestivation (facultative dormancy) and hibernation (true diapause) in central Japan. However the same authors reported that the aestivation might be a mere quiescence. In our experiment the continuous availability of food could be one of the reasons for the lack of an aestivation diapause.

In each species, except for adults of the last generation that did not oviposit before the diapause, females of each generation stopped ovipositing at a definite time of the year. The deadline was the last week of August (LD 13:11, Bologna Italy: ~45°N) for *P. quatuordecimpunctata*, the third week of September (LD 12:12) for *A. bipunctata* and the first week of October (LD 11.5:12.5) for *H. axyridis* (Figure 1). Only in the case of the 3rd generation of *A. bipunctata* did the oviposition continue for about 15 days, but these eggs were not able to hatch. Lamana and Miller (1996) reported that oviposition by *H. axyridis* in the open field in Oregon was observed until October. Obrycki et al. (1983) reported that the critical photoperiod for diapause induction in *A. bipunctata* lies between LD 14:10 and LD 13:11 in laboratory conditions at 23 °C. In agreement with our finding, *A. bipunctata* did not reproduce at LD 12:12 in southeastern France (Iperti and Prudent, 1986). The synchronisation of the last oviposition is likely to be regulated by environmental conditions such as photoperiod and temperature, and may be an adaptation to permit the ladybird species to reach the winter only in the adult stage. In fact, in our experiment, *H. axyridis* pupae of 2nd, 3rd and 4th generation tried to overwinter but died: this phenomenon may be a consequence of a partial adaptation of this exotic species to conditions in Northern Italy.

Overwintering

In 1998 (Table 1) adults of *H. axyridis* from the first four groups of egg batches collected were able to overwinter. The adults, from each of these groups, were able to oviposit, but only the eggs from the 1st and 2nd groups hatched. The 5th egg group, oviposited by the adults from the 1st group, could not complete development before the winter. Winter mortality, ranging from 10 up to 75%, increased from the 1st group to the last one and the pooled mortality was 31.9%. For the native species *P. quatuordecimpunctata*, adults

Table 1. Capacity to overwinter and winter mortality of *Harmonia axyridis* and *Propylea quatuordecimpunctata* reared in outdoor cages in 1998–1999

Group of egg-batches	Date of exposure	No. of eggs	Adult emergence		Oviposition		No. adults entering in diapause	Biological traits of overwintering			
			from	to	from	to		Survival		Oviposition	Hatching
								No.	%		%
Exotic species: *Harmonia axyridis*											
1	17 Aug	498	07 Sep	09 Sep	05 Oct	02 Nov	190	171	90	Yes	41
2	27 Aug	439	30 Sep	08 Oct	no oviposition		147	99	67	Yes	45
3	06 Sep	433	15 Oct	26 Oct	no oviposition		158	93	59	Yes	0
4	16 Sep	417	06 Nov	25 Nov	no oviposition		60	15	25	Yes	0
5	08 Oct	54	no emergence								
Native species: *Propylea quatuordecimpunctata*											
1	17 Aug	291	04 Sep	09 Sep	12 Oct	26 Oct	61	48	79	Yes	25
2	27 Aug	402	17 Sep	02 Oct	no oviposition		72	32	44	No	—
3	06 Sep	386	08 Oct	19 Oct	no oviposition		56	21	38	Yes	29
4	16 Sep	404	06 Nov	10 Nov	no oviposition		50	0	0		
5	15 Oct	23	eggs collapsed								

from 1st to 3rd groups were able to overwinter. 93% of the overwintering adults from the 2nd group died in a short time, before oviposition assessment (probably because of a disease) and did not oviposit, while the other groups produced offspring. The adults from the 4th group did not overwinter. The 5th egg group, oviposited by the adults from the 1st group, did not hatch. The winter mortality ranged from 21 up to 100% and the pooled mortality was 57.7%. The monthly average temperatures are shown in Figure 2.

In 1999 (Table 2) the 1st to 6th groups of *H. axyridis* produced overwintering adults, instead the 7th did not complete development. Each group of overwintering females oviposited viable eggs except the 6th one. The winter mortality ranged from 17 up to 85% with a pooled mortality of 37.7%. For *P. quatuordecimpunctata* the 1st, 2nd and 4th groups produced overwintering adults able to reproduce even if the winter mortality was high (from 40 up to 100%; pooled mortality 68.9%). All the adults from the 5th group died before the winter. The first three groups of *A. bipunctata* produced adults, which were able to overwinter, but the two adults from the 3rd group did not oviposit. All the specimens from the 4th group died as larvae. Winter mortality of *A. bipunctata* ranged from 0 to 100% with a pooled mortality of 61.3%. The monthly average temperatures are shown in Figure 2.

Fecundity and longevity of overwintering females

Data on biological traits of overwintering specimens are shown in Table 3. *H. axyridis* overwintering mortality was significantly lower in comparison with mortality of native species *A. bipunctata* (χ^2 = 15.19; df = 1; P = 0.0001) and *P. quatuordecimpunctata* (χ^2 = 25.73; df = 1; P = 0.00001). Fecundity of overwintering females was significantly different among the three species, with the highest found in *H. axyridis* followed by *A. bipunctata* and *P. quatuordecimpunctata* (Kruskal-Wallis test; H = 14.30; df = 2, 31; P = 0.0008). The post-overwintering oviposition rate was significantly higher in *A. bipunctata* than *H. axyridis* and *P. quatuordecimpunctata* (Kruskal-Wallis test; H = 18.89; df = 2, 31; P = 0.0001). Moreover, *H. axyridis* showed more intense oviposition activity in the early post-overwintering period than native species (Figure 3). Longevity of overwintering females did not show significant differences among the three coccinellid species (Kruskal-Wallis test; H = 3.43; df = 2, 31; P = 0.1799) (Table 3); however, the native species showed a quicker decline of the survival curves after the winter (Figure 4). The longest survival of an adult was 211, 176 and 197 days for *H. axyridis*, *A. bipunctata* and *P. quatuordecimpunctata*, respectively.

H. axyridis showed the highest "post-overwintering rate of increase" (r) in comparison with the other coccinellid species (Table 3). The ratio between r and the rate of population increase calculated at 25 °C (r_m) (A. Lanzoni,

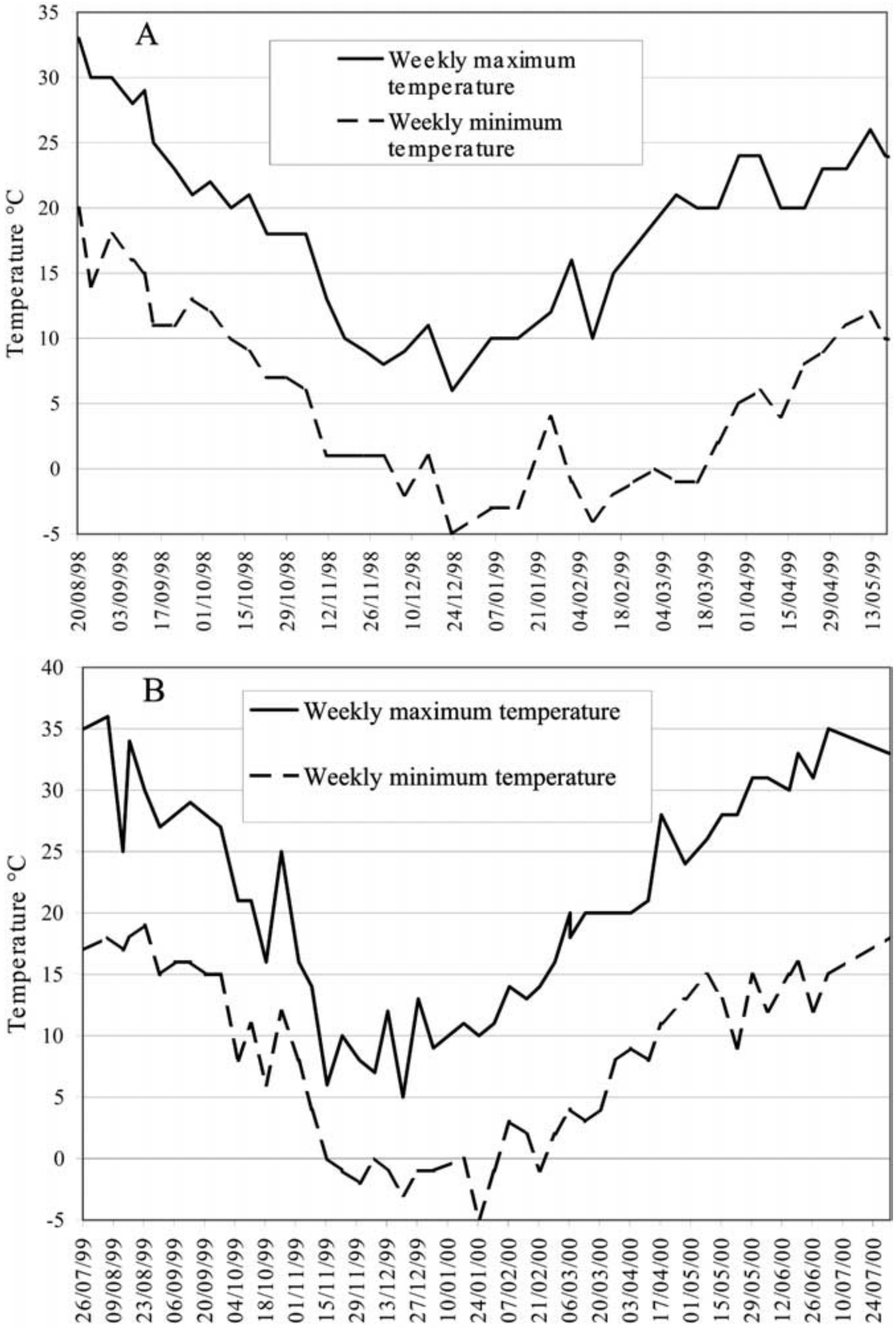

Figure 2. Weekly maximum and minimum temperatures in 1998–1999 (A) and in 1999–2000 (B).

Table 2. Capacity to overwinter and winter mortality of *Harmonia axyridis, Propylea quatuordecimpunctata* and *Adalia bipunctata* reared in outdoor cages in 1999–2000

Group of egg-batches	Date of exposure	No. of eggs	Adult emergence		Oviposition		No. adults entering in diapause	Biological traits of overwintering			
			from	to	from	to		Survival		Oviposition	Hatching
								No.	%		%
Exotic species: *Harmonia axyridis*											
1	26 Jul	412	10 Aug	12 Aug	26 Aug	18 Oct	71	51	72	Yes	26
2	04 Aug	453	23 Aug	01 Sep	14 Sep	18 Oct	66	46	70	Yes	52
3	16 Aug	417	17 Sep	17 Sep	29 Sep	29 Sep	12	10	83	Yes	82
4	26 Aug	448	23 Sep	29 Sep	no oviposition		43	21	49	Yes	67
5	09 Sep	167	01 Oct		no oviposition		32	21	66	Yes	50
6	21 Sep	152	05 Nov		no oviposition		20	3	15	No	—
7	01 Oct	21	no pupation								
Native species: *Propylea quatuordecimpunctata*											
1	26 Jul	329	09 Aug	12 Aug	26 Aug	05 Nov	24	8	33	Yes	44
2	04 Aug	277	16 Aug	26 Aug	01 Sep	01 Sep	30	18	60	Yes	40
3	16 Aug	410	01 Sep	13 Sep	no oviposition		6	0	0		
4	25 Aug	277	17 Sep	24 Sep	no oviposition		30	2	7	Yes	88
5	21 Sep	14	05 Oct	05 Oct	no oviposition		0	—	—		
Native species: *Adalia bipunctata*											
1	26 Jul	420	10 Aug	12 Aug	26 Aug	05 Nov	40	21	53	Yes	87
2	04 Aug	408	23 Aug	26 Aug	05 Oct	05 Oct	25	13	52	Yes	49
3	16 Aug	435	07 Sep	07 Sep	no oviposition		2	2	100	No	—
4	25 Aug	415	All died as larvae								
5	21 Sep	153	26 Oct	05 Nov	no oviposition		26	0	0		

Table 3. Biological traits of overwintering specimens

Species	r_m (25 °C)[a]	Post-overwintering rate of increase r[b]	r/r_m	Overwintering mortality X%	Fecundity (No. eggs laid/female)[c]	No. eggs female/day[c]	Post-overwintering adult longevity (days)[c]
Harmonia axyridis	0.120	0.150	1.250	37.7	783.8 ± 242.6 a	15.4 ± 3.9 b	52.6 ± 16.4 a
Propylea quatuordecimpunctata	/	0.069	/	68.9	193.7 ± 148.6 c	4.2 ± 2.8 c	36.4 ± 22.2 a
Adalia bipunctata	0.113	0.129	1.142	61.3	720.2 ± 479.2 b	17.3 ± 6.4 a	37.8 ± 16.6 a

[a] A. Lanzoni, G. Accinelli, G.G. Bazzocchi and G. Burgio, in preparation.
[b] Immature stage mortality, adult mortality and fecundity before winter were assumed to be 0.
[c] Means followed by different letters indicate significant differences among the three species ($P < 0.05$; Kruskal-Wallis ANOVA test).

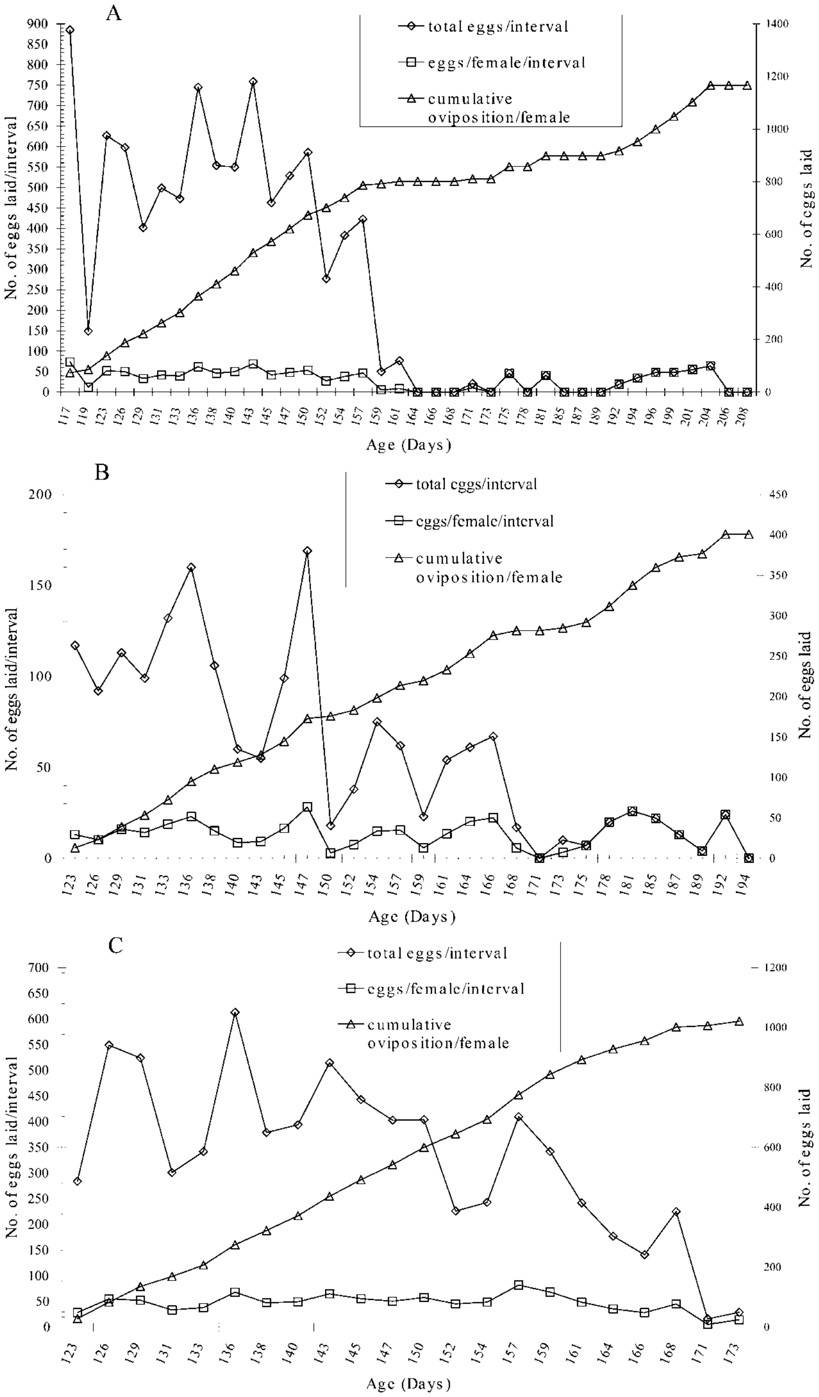

Figure 3. Age-specific reproduction of overwintered females of *H. axyridis* (A), *P. quatuordecimpunctata* (B) and *A. bipunctata* (C).

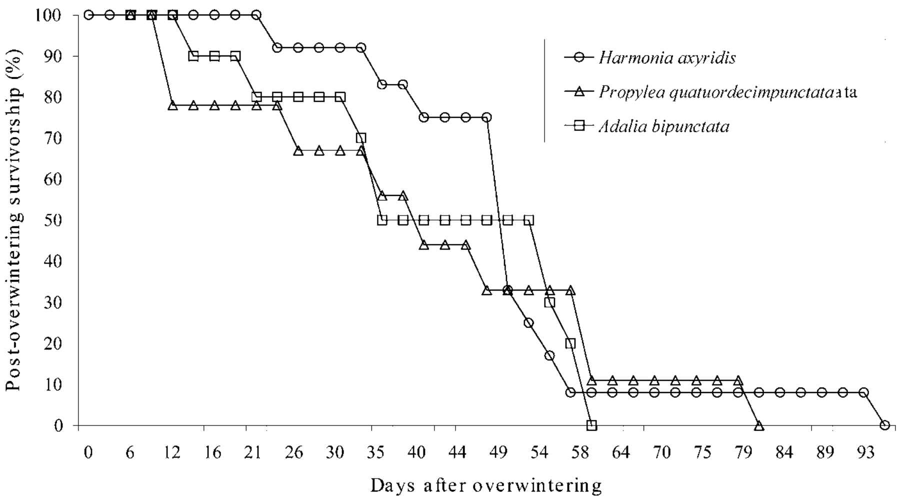

Figure 4. Post-overwintering survivorship of females of three coccinellid species.

G. Accinelli, G.G. Bazzocchi and G. Burgio, in preparation), was very similar for *H. axyridis* and *A. bipunctata* (1.250 and 1.142 respectively). This finding suggests that the life-history characteristics of the two species are similarly influenced by winter. The post-overwintering rate of increase can quantify the biotic potential of a species after diapause and give an estimation of the capacity to colonise the environment and should be useful for comparison among species. However, this parameter does not take into account the winter mortality due to biotic factors.

Our findings indicate that *H. axyridis* appears to posses a high potential for establishment in Northern Italy. Indeed, adults of the exotic ladybird are not only able to overwinter, but their post-overwintering performance is even better than that of the two native coccinellids. In addition, the exotic showed continuous reproductive activity during the warm season, without aestivation. More laboratory pre-release studies to determine interspecific competition between exotic ladybirds and native ones (Burgio et al., 2002, 2003), life-table studies, and investigations on mortality factors under field conditions are valuable within a general framework of a risk assessment methodology for exotic biological control agents (van Lenteren et al., 2003).

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