Bulletin of Insectology 68 (1): 23-29, 2015
ISSN 1721-8861

Effect of two temperatures on biological traits and susceptibility to a pyrethroid insecticide in an exotic and native coccinellid species

Maurizio Benelli[1], Simon R. Leather[2], Santolo Francati[1], Elisa Marchetti[1], Maria Luisa Dindo[1]
[1]*Dipartimento di Scienze Agrarie - Entomologia, Università di Bologna, Italy*
[2]*Department of Crop and Environment Science, Harper Adams University, United Kingdom*

Abstract

The Asian ladybird *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae), an active predator of aphids and other insect pests, has long been used as a biocontrol agent and deliberately or accidentally introduced into many countries. Over the last few decades concerns have been raised about its invasiveness in those areas where it has been introduced. A test was carried out to investigate the ability of *H. axyridis* to survive, grow and reproduce at higher temperatures (30 °C) than the recorded optimum of 25 °C. In a preliminary experiment, the temperature of 35 °C was also tested, but was subsequently excluded, because all eggs were dehydrated. The eggs placed at 30 °C hatched, but at a considerably lower rate compared with the control eggs. This temperature resulted in faster larval and overall development compared with the control temperature. Adult weight and reproductive parameters were adversely affected at 30 °C. In particular, fecundity and fertility were dramatically lower for the females reared and maintained at 30 °C. The higher temperature tested had thus an overall negative impact on *H. axyridis* development and performance. A second experiment was designed to test the effects of a pyrethroid insecticide (λ-Cyhalothrin) on *H. axyridis* in comparison with *Adalia bipunctata* (L.), a species commonly found in Europe. Larval mortality suggested that *H. axyridis* was less susceptible than *A. bipunctata* to λ-Cyhalothrin. Further studies are needed to better evaluate the impact of this and other pesticide treatments on the exotic *vs*. native ladybirds, also under field conditions, where other factors may play a key role.

Key words: Coleoptera, Coccinellidae, fecundity, development, mortality, exotic insects, temperature, λ-Cyhalothrin.

Introduction

Over the last few decades interest in the multicoloured Asian ladybird, *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae), has grown greatly. This ladybird, native to Eastern Asia, is a generalist predator of aphids and other soft-bodied arthropods (Koch, 2003).

H. axyridis voracity and ability to prey different key pest insects made it a potentially useful biocontrol agent (Pervez and Omkar, 2006). The species, the eggs of which are laid in clusters of approximately 20 to 30 eggs (Takahashi, 1987), can also be easily mass reared on aphids, *Ephestia kuehniella* Zeller eggs and artificial diets (Soares *et al.*, 2004; Sighinolfi *et al.*, 2013).

H. axyridis was intentionally released as a biocontrol agent in various locations around the world, but also accidentally introduced via international trade, leading to colonization of areas outside its native range (Roy *et al.*, 2005). Nowadays, *H. axyridis* is established in many areas of different continents, such as North America, South America, Europe and a few regions of Africa (Brown *et al.*, 2011). In some cases, such as the Azores Islands (Soares and Serpa, 2007) releases did not lead to establishment.

The current spread and distribution of *H. axyridis* in Europe is reported in PQR - EPPO Plant Quarantine data Retrieval (EPPO, 2014). In Italy, evidence of establishment was provided in Northern regions (Burgio *et al.*, 2008; Cornacchia and Nardi, 2012). The species has also been sporadically recorded in Central Italy (Canovai and Lucchi, 2011; Olivieri, 2011), whereas no records are presently known for Southern Italy, Sicily or Sardinia (EPPO, 2014). In the UK, *H. axyridis* has been reported as established and its rapid spread is clear (Brown *et al.*, 2007).

Adverse effects of this coccinellid on other arthropods, crops and humans have been reported (Burgio *et al.*, 2005; Roy *et al.*, 2012; Nedvěd *et al.*, 2013; Lombaert *et al.*, 2014). Over the last decades, the consequences associated with the deliberate introduction of exotic biocontrol agents have received increasing attention. *H. axyridis* is the best known example of a beneficial insect currently considered an invasive species which itself now requires control (Roy *et al.*, 2006; Kenis *et al.*, 2008).

The spread of *H. axyridis* can be limited by several factors, including parasitoids, predators, fungi (Santi and Maini, 2006; Berkvens *et al.*, 2010; Riddick and Cottrell, 2010; Roy *et al.*, 2011) and even egg cannibalism (Santi and Maini, 2007). Climate, in particular temperature, is also important. The limiting low temperature for this ladybird ranges from 9.3 °C (Soares *et al.*, 2003) to 13.3 °C (Hodek and Honěk, 1996) while the highest limiting temperature reported in the literature varied between 30 °C and 35 °C (Michaud, 2002a). The thermal optimum is 20-25 °C (Soares *et al.*, 2003), 15-25 °C (Acar *et al.*, 2001) and 23-25 °C (Yuan *et al.*, 1994). The data found in the literature are therefore not fully consistent, although the maximum thermal optimum is 25 °C for all the above mentioned authors. Several biological parameters of *H. axyridis* have been shown to be affected by temperature, including adult weight. Larvae reared at higher temperatures produced smaller adults than at lower temperatures (Kawauchi, 1979). The temperature to which pupae are exposed may also influence elytra colouration and maculation (Sakai *et al.*, 1974). Recently Zhang *et al.* (2014) showed that exposure of eggs to high temperature negatively affects the subsequent development, survival and reproduction of *H. axyridis*.

The spread of *H. axyridis* may also be influenced by pesticide treatments performed against aphids or other target insect pests. The impact of pesticides on this ladybird has been tested in laboratory and field experiments (Buntin and Bouton, 1997; Cho *et al.*, 1997; Jansen and Hautier, 2006). The majority of the studies used mortality as an indicator of susceptibility, but some also examined sub-lethal effects (Weissenberger *et al.*, 1997) or behavioural effects (Vincent *et al.*, 2000). The toxic effects of a number of insecticides (e.g. indoxacarb, pyriproxifen and spinosad) to *H. axyridis* were minimal (Michaud, 2002b; 2003; Musser and Shelton, 2003).

The aim of this work was to increase knowledge about *H. axyridis* capacity to survive, grow and reproduce at higher temperatures than the optimum of 25 °C. In detail, the effects on *H. axyridis* development (from egg to adult) and quality parameters (adult weight, longevity, fecundity and fertility) were investigated. Experiments were also designed to test the effects of a pyrethroid insecticide (λ-Cyhalothrin) on *H. axyridis* in comparison with the native ladybird *Adalia bipunctata* (L.). This species was selected as an indicator for other ladybirds, because it generally shows greater sensitivity to pesticides than larger species (Jansen and Hautier, 2006; Jansen *et al.*, 2011).

Materials and methods

The effect of temperature and susceptibility to pyrethroid insecticide were respectively investigated at DipSA (Department of Agricultural Sciences) of the University of Bologna (Italy) and at CERC (Crop and Environment Research Centre) of Harper Adams University (UK).

Insect culture

A colony of *H. axyridis* was maintained in the laboratory at DipSA in a rearing chamber at 25 ± 1 °C, 65 ± 5% RH and L16:D8 photoperiod. The colony was established in 2010 with adults collected from Crevalcore (Northern Italy, 44°43'0"N 11°9'0"E) and augmented in the following years with individuals captured in the Bologna area (Northern Italy, 44°29'38"N 11°20'34"E). Adults and larvae were fed with green peach aphids (*Myzus persicae* Sulzer) *ad libitum*. At CERC, laboratory colonies of *H. axyridis* and *A. bipunctata* were started in June 2013, originating from adults collected on the University campus (Shropshire, England, 52°46'45.1"N 2°25'55.1"W). They were maintained in a greenhouse at approximately 22 °C, 40% RH and a photoperiod of L16:D8. All ladybirds were fed *ad libitum* with green peach aphids on Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*).

All adult ladybirds were kept in cages, separated from the preimaginal stages which were maintained in plastic boxes with lids having holes covered by a steel mesh net or tulle for aeration.

Effect of temperature on *H. axyridis* biological parameters

Egg hatching at 35 °C was preliminarily tested. Since the eggs were dehydrated and none hatched, this temperature was excluded from the experiment. Two temperatures (= treatments), namely 25 °C and 30 °C were tested.

Egg clusters were collected from the laboratory colony of *H. axyridis* and placed individually in 5.5 cm diameter plastic Petri dishes. The dishes with clusters (= 42) were divided randomly between the two temperatures. Namely, half of them (21 clusters with 546 eggs in total) were kept under the standard rearing conditions (25 ± 1 °C, 65 ± 5% RH and L16:D8 photoperiod), whereas the others (21 clusters with 564 eggs in total) were placed at 30 ± 1 °C, 65 ± 5% RH and L16:D8 photoperiod.

Newly-hatched larvae (= 40 per treatment) were collected and placed in plastic cylinders (5.5 cm diameter, 7 cm height) with lids having holes covered by a steel mesh net. Each larva was reared individually until adult eclosion. Excess larvae were transferred to the laboratory colony.

For each temperature, the newly-emerged adults were counted, weighed and sexed. Couples (= 12 at 25 °C and 11 at 30 °C) were created and maintained in cylinders of the above mentioned type until death (1 couple/cylinder). In each cylinder a layer of bubble wrap was inserted to facilitate the collection of the egg clusters laid. Each cluster was individually placed in 2.5 cm diameter Petri dishes until hatching. The newly-hatched larvae were counted.

Throughout the experiment, the ladybird larvae and adults were fed *ad libitum* on pea seedlings infested with *M. persicae*.

The parameters considered were: 1) hatched eggs (number and percentage, calculated on the original egg number minus cannibalized eggs); 2) cannibalized eggs (number and percentage, calculated on the original egg number); 3) duration (in days) of the larval, pupal and overall preimaginal development (= time elapsed from egg hatching until pupation, from pupation until adult eclosion and from egg hatching until adult eclosion, respectively); 4) number and proportion of individuals reaching the adult stage calculated on the larvae (= 40 per treatment) selected for the experiment; 5) sex-ratio; 6) newly-emerged adult weight (g); 7) pre-oviposition time (days) = time from adult emergence to first oviposition; 8) oviposition time (days) = time from the first to the last oviposition; 9) number of eggs laid by each female over a period of 10 days starting from the first oviposition (E_{10}) to represent fecundity; 10) percentage of first instar larvae obtained from eggs to represent fertility; 11) adult longevity from emergence (days).

For the parameters 3 and 6-11, the replicates corresponded to the number of individuals (parameters 3, 6 and 11) or couples (parameters 7-10) (Lanzoni *et al.*, 2004; Sighinolfi *et al.*, 2013).

Effects of a pyrethroid insecticide on *H. axyridis* vs. *A. bipunctata* mortality

The experiment investigated the effects of Hallmark with Zeon Technology® (Syngenta), a microcapsule suspension containing 100 g/l λ-Cyhalothrin and 1,2-Benzisothiazolin-3-one. This product was selected, because it is commonly used in the agricultural sector of the United Kingdom (against aphids, beetles, caterpillars, moths, suckers and weevils) on a wide range of crops, including cabbage (Springate and Colvin, 2012).

Egg clusters of *H. axyridis* or *A. bipunctata* were collected from stock cultures, transferred into 5 cm diameter plastic Petri dishes (1 cluster/dish), and placed under controlled conditions at 20 ± 1 °C, 65 ± 5% RH and L16:D8 photoperiod. This temperature was selected because it more closely represents UK summer temperatures (Oliver *et al.*, 2006; Aqueel and Leather, 2012).

Newly hatched larvae were individually placed in Petri dishes of the same type (to avoid cannibalism) and fed *ad libitum* on *M. persicae* until reaching the third instar.

Following the label instructions, the dose used was 0.033 ml of insecticide/100 ml of water. The suspension was sprayed on three weeks old Chinese cabbage plants using an automatic pot-sprayer.

Prior to the experiments, preliminary tests were carried out to ascertain the appropriate waiting time (days between insecticide spraying and release of coccinellid larvae), so as to avoid an excessive mortality rate of coccinellid larvae. This parameter takes into account the natural degradation of the insecticide on plant surfaces. After those tests the waiting time for the experiment was set as 7 days.

For each coccinellid species, 5 replicates were carried out, each consisting of 1 treated and 1 untreated (control) cabbage plant. On each plant, 10 *H. axyridis* or *A. bipunctata* larvae were released 7 days after insecticide (treated) or distilled water (untreated) spraying. In order to prevent larval escape, each plant was covered with a transparent breathable bread bag (Cater4you, High Wcombe, UK), secured by a rubber band around the pot. Green peach aphids were added daily to provide food. The dead coccinellids were removed and counted 3 days and 7 days after release onto plants.

The results were evaluated in terms of number and percentage of dead larvae (mortality) calculated on the original number of larvae released.

Statistical analysis

In the first experiment hatched eggs, cannibalized eggs, adults and sex-ratio were analyzed by 2 × 2 contingency tables with Yates' correction for small numbers (< 100) when necessary. The one-way analysis of variance or the Kruskal-Wallis non-parametric procedure (when heteroscedasticity occurred) were used to analyze the other parameters.

In the second experiment the data were analysed using 2 × 2 contingency tables with Yates' correction for small numbers (< 100).

The percentage values were transformed prior to analysis using an arcsine transformation (Zar, 1984). All statistical tests were done with Statistica™ 10.0 (StatSoft, 2010).

Results

Effect of temperature on *H. axyridis* biological parameters

There was a significant effect of temperature on *H. axyridis* egg hatching (χ^2 = 48.64; df = 1; *P* = 0.00001) and cannibalism (χ^2 = 18.46; df = 1; *P* = 0.00001). The percentages of hatched eggs and cannibalized eggs were higher at 25 °C than at 30 °C (figure 1). Overall preimaginal development of the ladybird was significantly faster at the higher temperature compared with the control temperature, as a result of the faster larval development that occurred at 30 °C. Conversely, pupal development was not significantly affected by temperature (table 1). The percentage of adults obtained (92.5 ± 4.2 at 25 °C and 87.5 ± 5.3 at 30 °C) was not significantly different between the two temperatures (χ^2 = 0.14; df = 1; *P* = 0.71) as well as the sex ratio (male percentages 45.9 ± 5.3 at 25 °C and 68.6 ± 7.9 at 30 °C; χ^2 = 2.89; df = 1; *P* = 0.09). At 30 °C, the sex-ratio was male-biased, although not significantly so.

All adult life history parameters were affected by temperature except for pre-oviposition period and longevity (table 2). At 30 °C adults attained a significantly lower weight, had a significantly shorter oviposition period and dramatically lower fecundity and fertility compared with the control temperature.

Effects of a pyrethroid insecticide on *H. axyridis* vs. *A. bipunctata* mortality

On the control plants no *H. axyridis* or *A. bipunctata* mortality occurred, either 3 or 7 days after releasing the larvae. The difference in larval mortality between the treated and untreated plants was highly significant (*P* = 0.00001; df = 1) for both *H. axyridis* (χ^2 = 30.72 [3 days]; 38.08 [7 days]) and *A. bipunctata* (χ^2 = 48.53 [3 days]; 66.14 [7 days]). At both exposure times, λ-Cyhalothrin induced higher mortality in *A. bipunctata* than in *H. axyridis* (figure 2). Mortality was, however, not significantly affected by the coccinellid species at 3 days after releasing the larvae on the treated plants (χ^2 = 2.65; df = 1; *P* = 0.103), whereas a significant effect was found at 7 days (χ^2 = 5.76; df = 1; *P* = 0.016).

Discussion and conclusions

Temperature is a key factor for the spread of insects (Messenger, 1959) and plays a very important role in the survival and fitness of *H. axyridis*, thus ultimately influencing its distribution (Poutsma *et al.*, 2008). The experiment aimed at evaluating the effects of temperature on *H. axyridis* biological parameters confirmed its role. In particular, the constant temperature of 35 °C proved too high for egg hatchability and thus unsuitable for *H. axyridis* development. This result is in line with the finding of previous studies which showed that most *H. axyridis* instars tolerate high temperature only for a short time, namely 48 hrs at 33 °C (Knapp and Nedvěd, 2013) and two hours at 35 °C and 40 °C (Acar *et al.*, 2004). In addition, Wang *et al.* (2009) reported that 35 °C was too high for the survival of this coccinellid species.

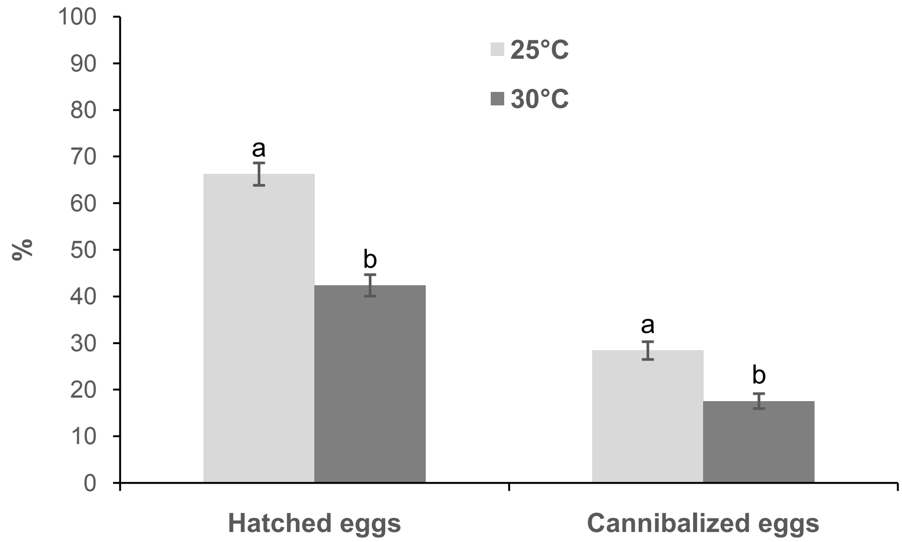

Figure 1. Effect of temperature on *H. axyridis* egg hatching and egg cannibalism. Columns indicate the percentage (± SE) of hatched eggs (calculated on the original egg number minus cannibalized eggs = 391 [25°C] and 465 [30°C]) and cannibalized eggs (calculated on the original egg number = 546 [25°C] and 564 [30°C]). For each parameter, different letters above the columns indicate the differences in the percentages of eggs as determined by 2 × 2 contingency tables.

Table 1. Effect of temperature on *H. axyridis* duration of larval, pupal and overall preimaginal development (from egg hatching until adult eclosion). Number of replicates is given in parentheses above the means (± SE). The replicates correspond to the number of individuals. Values followed by different letters in a column are significantly different, using the Kruskal-Wallis test (overall preimaginal development) or the one-way analysis of variance (other parameters) ($P < 0.05$).

	Larval development (days)	Pupal development (days)	Overall preimaginal development (days)
25 °C	(n = 37) 8.76 ± 0.17a	(n = 37) 5.1 ± 0.16a	(n = 37) 13.84 ± 0.09a
30 °C	(n = 35) 7.46 ± 0.24b	(n = 35) 5.34 ± 0.19a	(n = 35) 12.8 ± 0.19b
F (df)	19.78 (1, 70)	1.07 (1,70)	
H (N)			21.25 (72)
P	0.000032*	0.3	0.00001*

Table 2. Effect of temperature on *H. axyridis* newly-emerged adult weight, pre-oviposition and oviposition duration, fecundity, fertility and adult longevity from emergence. Number of replicates is given in parentheses above the means (± SE). For each temperature, the replicates correspond to the number of newly-emerged adults (weight), or couples (other parameters). Values followed by different letters in a column are significantly different, using the Kruskal-Wallis test (fertility) or the one-way analysis of variance (other parameters) ($P < 0.05$).

	Newly-emerged adult weight (g)	Pre-oviposition (days)	Oviposition (days)	Eggs laid in 10 days (= E_{10})	Fertility (1^{st} instar larvae/eggs) %	Adult longevity (days)
25 °C	(n = 37) 0.026 ± 0.0005a	(n = 12) 8.33 ± 1.37a	(n = 12) 18.08 ± 2.41a	(n = 12) 228.33 ± 30.7a	(n = 12) 21.63 ± 5.12a	(n = 24) 28.75 ± 2.04a
30 °C	(n = 35) 0.024 ± 0.0006b	(n = 11) 6.37 ± 1.16a	(n = 11) 11.09 ± 2.19b	(n = 11) 56 ± 17.96b	(n = 11) 1.15 ± 1.01b	(n = 22) 22.77 ± 2.66a
F (df)	11.36 (1, 70)	1.17 (1, 21)	4.53 (1, 21)	22.03 (1, 21)		3.24 (1, 44)
H (N)					12.25 (23)	
P	0.0012*	0.29	0.045*	0.0001*	0.0005*	0.08

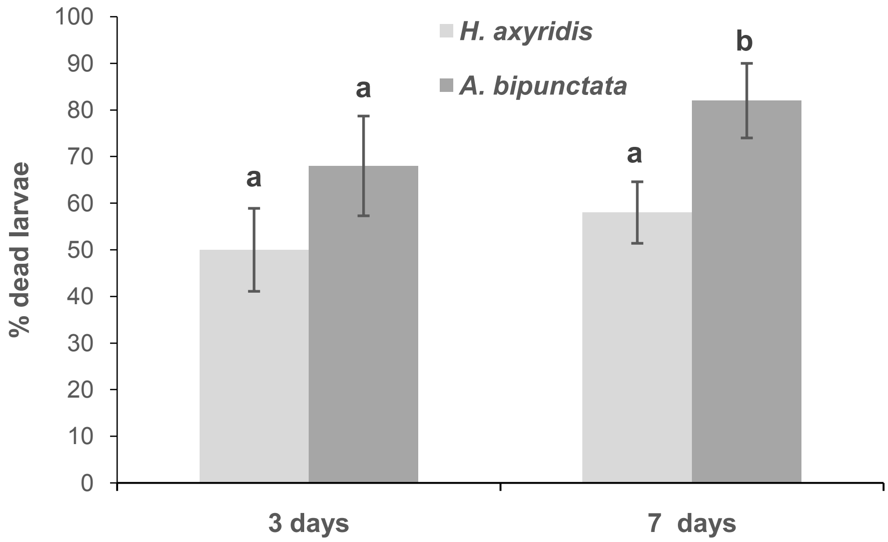

Figure 2. Effect of λ-Cyhalothrin on *H. axyridis vs A. bipunctata* larval mortality occurred 3 or 7 days after releasing the larvae (3rd instar) on treated plants. The waiting time (= days between insecticide spraying and release of the larvae) was 7 days. Columns indicate the percentages of dead larvae (± SE) calculated on the original number of larvae released. For each time, different letters above the columns indicate the differences in the percentages of dead larvae as determined by 2 × 2 contingency tables.

The 30 °C had an overall negative effect, as the eggs hatched at a considerably lower rate compared with the control eggs, maintained at 25 °C. At 30 °C most unhatched, non-cannibalized eggs appeared dehydrated. The lower level of egg cannibalism observed at 30 °C was possibly related to the lower number of newly hatched first instars that occurred at this temperature compared with the control one. It cannot, however, be excluded that the young larvae were less active and ravenous at 30 °C than at 25 °C. The high temperature resulted in faster larval and overall preimaginal development compared with the control temperature. Adult weight and reproductive parameters showed that the temperature of 30 °C negatively affected the fitness of *H. axyridis*. In particular, fecundity and fertility (often correlated with weight in entomophagous insects, Dindo *et al.*, 2003; 2007) were dramatically lower for the females grown and maintained at 30 °C compared with the control females. Moreover, in spite of lack of significance, at 30 °C the sex ratio was male-biased (something considered as negative for insect species, Quezada-García *et al.*, 2014). The percentage of individuals reaching the adult stage was not affected by temperature. Adult longevity was also not affected, although at 30 °C the adults survived about one week less than the controls.

The overall results obtained suggested that a constant temperature of 30 °C is not optimal for *H. axyridis.* In Central Japan (an area of origin) aestivation was reported to occur for this species (Sakurai *et al.*, 1988), whereas in the areas of introduction characterized by warm summers (including Northern Italy), the ladybird showed continuous reproductive activity throughout the warm season (Bazzocchi *et al.*, 2004; Uliana, 2008). Our data, however, showed that at 30 °C reproductive activity decreased. More research is needed to investigate the effects of fluctuating temperatures, a situation more likely to occur in field conditions.

As shown by Michaud (2002b; 2002c) *H. axyridis* invasiveness may also be related to its lower susceptibility to insecticide treatments, when compared with indigenous coccinellid species. In general, beneficial coccinellids can encounter the insecticides used against target insect species either by direct contact during their application, or by contact with their residues on plants, or even through ingestion of poisoned prey (Katsarou *et al.*, 2009). Laboratory experiments aimed at assessing the LR_{50} of five pesticides, for *H. axyridis* and three native ladybird species in Belgium, were carried out by Jansen and Hautier (2006). The authors showed that, as a general trend, *H. axyridis* was more sensitive than *Coccinella septempunctata* (L.) but less susceptible than the other coccinellids, including *A. bipunctata*. The results of our experiment aimed at testing the effects of λ-Cyhalothrin on *H. axyridis vs*. *A. bipunctata* suggested that the exotic coccinellid was less susceptible than the native to this active ingredient. Further studies are needed to better evaluate the impact of pesticide treatments on the exotic *vs*. native ladybirds, also under field conditions, where other factors may play a key role. For instance, as suggested by Jansen and Hautier (2008), in conditions of low prey availability following an insecticide treatment, *H. axyridis* may have more probability to survive compared to *A. bipunctata*, or other native ladybirds, through phenomena of intra guild predation or cannibalism.

Acknowledgements

This research was funded by the Italian Ministry of Education and Research (PRIN Project 2010-2011: "Insects and globalization: sustainable control of exotic species in agro-forestry ecosystems" GEISCA). The *Alma Mater Studiorum* University of Bologna supported Maurizio Benelli's stage at CERC (Crop and Environment Research Centre) of Harper Adams University.

References

ACAR E. B., SMITH B. N., HANSEN L. D., BOOTH G. M., 2001.- Use of calorespirometry to determine the effects of temperature on metabolic efficiency of an insect.- *Environmental Entomology*, 30: 811-816.

ACAR E. B., MILL D. D., SMITH B. N., HANSEN L. D., BOOTH G. M., 2004.- Calorespirometric determination of the effects of temperature on metabolism of *Harmonia axyridis* (Col: Coccinellidae) from second instars to adults.- *Environmental Entomology*, 33: 832-838.

AQUEEL M. A., LEATHER S. R., 2012.- Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids.- *Annals of Applied Biology*, 160: 6-15.

BAZZOCCHI G. G., 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.

BERKVENS N., MOENS J., BERKVENS D., SAMIH M. A., TIRRY L., DECLERCQ P., 2010.- *Dinocampus coccinellae* as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe.- *Biological Control*, 53: 92-99.

BROWN P. M., ROY H. E., ROTHERY P., ROY D. B., 2007.- *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid.- *BioControl*, 53: 55-57.

BROWN P. M., THOMAS C. E., LOMBAERT E., JEFFRIES D. L., ESTOUP A., LAWSON HANDLEY L.-J., 2011.- The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion.- *BioControl*, 56: 623-641.

BUNTIN G. D., BOUTON J. H., 1997.- Aphid (Homoptera: Aphididae) management in alfalfa by spring grazing cattle.- *Journal of Entomological Science*, 32: 332-342.

BURGIO G., SANTI F., MAINI S., 2005.- Intra-guild predation and cannibalism between *Harmonia axyridis* and *Adalia bipunctata* adults and larvae: laboratory experiments.- *Bulletin of Insectology*, 58 (2): 135-140.

BURGIO G., SANTI F., LANZONI A., MASETTI A., DE LUIGI V., MELANDRI M., REGGIANI A., RICCI C., LOOMANS A. J. M., MAINI S., 2008.- *Harmonia axyridis* recordings in northern Italy.- *Bulletin of Insectology*, 61 (2): 361-364.

CANOVAI R., LUCCHI A., 2011.- La coccinella arlecchino in vigneti della Toscana.- *L'Informatore Agrario*, 67 (11): 65-66.

CHO J. R., HONG K. J., YOO J. K., BANG J. R., LEE J. O., 1997.- Comparative toxicity of selected insecticides to *Aphis citricola*, *Myzus malisuctus* (Homoptera: Aphididae), and the predator *Harmonia axyridis* (Coleoptera: Coccinellidae).- *Journal of Economical Entomology*, 90: 11-14.

CORNACCHIA P., NARDI G., 2012.- Nuovi dati su *Harmonia axyridis* in Italia.- *Bollettino dell'Associazione Romana di Entomologia*, 67 (1-4): 51-68.

DINDO M. L., MARCHETTI E., GALVAGNI G., BARONIO P., 2003.- Rearing of *Exorista larvarum* (Diptera Tachinidae): simplification of the *in vitro* technique.- *Bulletin of Insectology*, 56 (2): 253-257.

DINDO M. L., MARCHETTI E., BARONIO P., 2007.- *In vitro* rearing of the parasitoid *Exorista larvarum* (Diptera: Tachinidae) from eggs laid out of host.- *Journal of Economic Entomology*, 100 (1): 26-30.

EPPO, 2014.- PQR - EPPO database on quarantine pests.- [online] URL: http://www.eppo.int (accessed September 16, 2014).

HODEK I., HONĚK A., 1996.- *Ecology of Coccinellidae*.- Kluwer Academic Publishers, Dordrecht, The Netherlands.

JANSEN J-P., HAUTIER H., 2006.- Comparative sensitivity of four ladybird species to five pesticides.- *IOBC/wprs Bulletin*, 29 (10): 95-103.

JANSEN J-P., HAUTIER H., 2008.- Ladybird population dynamics in potato: comparison of native species with an invasive species, *Harmonia axyridis*.- *BioControl*, 53: 223-233.

JANSEN J-P., DEFRANCE T., WARNIER A. M., 2011.- Side effects of flonicamide and pymetrozine on five aphid natural enemy species.- *BioControl*, 56: 759-770.

KATSAROU I., MARTINOU A., PAPACHRISTOS D., ZOAKI D., 2009.- Toxic effects of insecticide residues on three aphidophagous coccinellid species.- *Hellenic Plant Protection Journal*, 2: 101-106.

KAWAUCHI S., 1979.- Effects of temperatures on the aphidophagous coccinellids.- *Kurume University Journal*, 28: 47-52.

KENIS M., ROY H. E., MAJERUS M. E., 2008.- Current and potential management strategies against *Harmonia axyridis*.- *BioControl*, 53: 235-252.

KNAPP M., NEDVĚD O., 2013.- Gender and timing during ontogeny matter: effects of a temporary high temperature on survival, body size and colouration in *Harmonia axyridis*.- *Plos ONE*, 8 (9): e74984.

KOCH R. L., 2003.- The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts.- *Journal of Insect Science*, 3: 1-16.

LANZONI A., ACCINELLI G., BAZZOCCHI G. G., BURGIO, G., 2004.- Biological traits and life table of the exotic *Harmonia axyridis* compared with *Hippodamia variegata*, and *Adalia bipunctata* (Col.: Coccinellidae).- *Journal of Applied Entomology*, 128: 298-306.

LOMBAERT E., ESTOUP A., FACON B., JOUBARD B., GREGOIRE J. C., JANNIN A., BLIN A., GUILLEMAUD T., 2014.- Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*.- *Journal of Evolutionary Biology*, 27 (3): 508-517.

MESSENGER P. S., 1959.- Bioclimatic studies with insects.- *Annual Review of Entomology*, 4: 183-206.

MICHAUD J. P., 2002a.- Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*.- *Environmental Entomology*, 31: 827-835.

MICHAUD J. P., 2002b.- Relative toxicity of six insecticides to *Cycloneda sanguinea* and *Harmonia axyridis* (Coleoptera: Coccinellidae).- *Journal of Entomological Science*, 37: 83-93.

MICHAUD J. P., 2002c.- Non-target impacts of acaricides on ladybeetles in citrus: a laboratory study.- *Florida Entomologist*, 85 (1): 191-196.

MICHAUD J. P., 2003.- Toxicity of fruit fly baits to beneficial insects in citrus.- *Journal of Insect Science*, 3: 1-9.

MUSSER F. R., SHELTON A. M., 2003.- Bt sweet corn and selective insecticides: impacts on pests and predators.- *Journal of Economical Entomology*, 96: 71-80.

NEDVĚD O., FOIS X., UNGEROVÁ D., KALUSHKOV P., 2013.- Alien vs. Predator - the native lacewing *Chrysoperla carnea* is the superior intraguild predator in trials against the invasive ladybird *Harmonia axyridis*.- *Bulletin of Insectology*, 66: 73-78.

OLIVER T. H., TIMMS J. E. L., TAYLOR A., LEATHER S. R., 2006.- Oviposition responses to patch quality in the larch ladybird *Aphidecta obliterata* (Coleoptera: Coccinellidae): effects of aphid density, and con- and heterospecific tracks.- *Bulletin of Entomological Research*, 96: 25-34.

OLIVIERI N., 2011.- Segnalazioni faunistiche italiane.- *Bollettino della Società Entomologica Italiana*, 143 (3): 138.

PERVEZ A., OMKAR, 2006.- Ecology and biological control application of multicoloured Asian ladybird, *Harmonia axyridis*: a review.- *Biocontrol Science and Technology*, 16: 111-128.

POUTSMA J., LOOMANS A. J. M., AUKEMA B., HEIJERMAN T., 2008.- Predicting the potential geographical distribution of the harlequin ladybird, *Harmonia axyridis*, using the CLIMEX model.- *BioControl*, 53: 103-125.

QUEZADA-GARCÍA R., PURESWARAN D., BAUCE É., 2014.- Nutritional stress causes male-biased sex ratios in eastern spruce budworm (Lepidoptera: Tortricidae).- *Canadian Entomologist*, 146: 219-223.

RIDDICK E. W., COTTRELL T. E., 2010.- Is the prevalence and intensity of the ectoparasitic fungus *Hesperomyces virescens* related to the abundance of entomophagous coccinellids?- *Bulletin of Insectology*, 63: 71-78.

ROY H. E., ROWLAND F., BROWN P. M., MAJERUS M. E., 2005.- Ecology of the harlequin ladybird: a new invasive species.- *British Wildlife*, 16: 403-407.

ROY H. E., BROWN P., MAJERUS M. E., 2006.- *Harmonia axyridis*: a successful biocontrol agent or an invasive threat?, pp. 295-309. In: *An ecological and societal approach to biological control* (EILENBERG J., HOKKANEM H., Eds).- Springer, Dordrecht, The Netherlands.

ROY H. E., RHULE E., HARDING S., LAWSON HANDLEY L.-J., POLAND R. L., RIDDICK E. W., STEENBERG T., 2011.- Living with the enemy: parasites and pathogens of the ladybird *Harmonia axyridis*.- *BioControl*, 56: 663-679.

ROY H. E., DE CLERCQ P., LAWSON HANDLEY L.-J., SLOGGETT J. J., POLAND R. L., WAJNBERG E., 2012.- *Invasive alien arthropod predators and parasitoids: an ecological approach*.- Springer, Dordrecht, The Netherlands.

SAKAI T., UEHARA Y., MATSUKA M., 1974.- The effect of temperature and other factors on the expression of elytral pattern in lady beetle, *Harmonia axyridis* Pallas.- *Bulletin of the Faculty of Agriculture, Tamagawa University*, 14: 33-39.

SAKURAI H., TAKEDA S., KAWAI T., 1988.- Diapause regulation in the lady beetle, *Harmonia axyridis*, pp. 67-70. In: *Ecology and effectiveness of aphidophaga* (NIEMCZYK E., DIXON A. F. G., Eds).- S.P.B. Academic Publishing, The Hague, The Netherlands.

SANTI F., MAINI S., 2006.- Predation upon *Adalia bipunctata* and *Harmonia axyridis* eggs by *Chrysoperla carnea* larvae and *Orius laevigatus* adults.- *Bulletin of Insectology*, 59 (1): 53-58.

SANTI F., MAINI S., 2007.- Ladybirds mothers eating their eggs: is it cannibalism?.- *Bulletin of Insectology*, 60 (1): 89-91.

SIGHINOLFI L., FEBVAY G., DINDO M. L., REY M., PAGEAUX J. F., GRENIER S., 2013.- Biochemical content in fatty acids and biological parameters of *Harmonia axyridis* reared on artificial diet.- *Bulletin of Insectology*, 66 (2): 283-290.

SOARES A. O., SERPA A., 2007.- Interference competition between ladybird beetle adults (Coleoptera: Coccinellidae): effects on growth and reproductive capacity.- *Population Ecology*, 49: 37-43.

SOARES A. O., CODERRE D., SCHANDERL H., 2003.- Effect of temperature and intraspecific allometry on predation by two phenotypes of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae).- *Environmental Entomology*, 32: 939-944.

SOARES A. O., CODERRE D., SCHANDERL H., 2004.- Dietary self-selection behaviour by the adults of the aphidophagous ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae).- *Journal of Animal Ecology*, 73: 478-486.

SPRINGATE S., COLVIN J., 2012.- Pyrethroid insecticide resistance in British populations of the cabbage whitefly, *Aleyrodes proletella*.- *Pest Management Science*, 68 (2): 260-267.

STATSOFT, 2010.- *STATISTICA data analysis software system, version 10*.- StatSoft Inc., Tulsa, USA.

TAKAHASHI K., 1987.- Differences in oviposition initiation and sites of lady beetle, *Coccinella septempunctata bruckii* Mulsant and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the field.- *Japanese Journal of Applied Entomology and Zoology*, 31: 253-254.

ULIANA M., 2008.- Nuove segnalazioni di *Harmonia axyridis* (Pallas, 1773) in Italia settentrionale (Coleoptera, Coccinellidae).- *Bollettino del Museo Civico di Storia Naturale di Venezia*, 59: 51-53.

VINCENT C., FERRAN A., GUIGE L., GAMBIER J., BRUN J., 2000.- Effects of imidacloprid on *Harmonia axyridis* (Coleoptera: Coccinellidae) larval biology and locomotory behavior.- *European Journal of Entomology*, 97: 501-506.

WANG H., JI S., ZHAI W., 2009.- Effect of temperature on survival, development and fecundity of *Harmonia axyridis*.- *Chinese Bulletin of Entomology*, 46: 449-452.

WEISSENBERGER A., BRUN J., HEROLD D., 1997.- Essais de toxicite d'un aphicide (Confidor) sur la Coccinelle *Harmonia axyridis* Pallas.- *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent*, 62: 589-593.

YUAN R. C., ZHANG F. M., WEN G. Z., 1994.- Study on control of soybean aphid by *Harmonia* (*Leis*) *axyridis*.- *Journal of Jilin Agricultural Sciences*, 44: 30-57.

ZAR J. H., 1984.- *Biostatistical analysis*.- Prentice Hall, Englewood Cliffs, NJ, USA.

ZHANG S., CAO Z., WANG Q., ZHANG F., LIU T-X., 2014.- Exposing eggs to high temperatures affects the development, survival and reproduction of *Harmonia axyridis*.- *Journal of Thermal Biology*, 39 (1): 40-44.

Authors' addresses: Maurizio BENELLI, Santolo FRANCATI, Elisa MARCHETTI, Maria Luisa DINDO (corresponding author: marialuisa.dindo@unibo.it), Dipartimento di Scienze Agrarie - Entomologia, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin 42, 40127 Bologna, Italy; Simon R. LEATHER, Department of Crop and Environment Science, Harper Adams University, Edgmond Newport, Shropshire TF10 8NB, United Kingdom.

Received October 1, 2014. Accepted February 6, 2015.