

# Laboratory Investigations Reveal that *Harmonia axyridis* (Coleoptera: Coccinellidae) Is a Poor Host for *Dinocampus coccinellae* (Hymenoptera: Braconidae) in Brazil

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Subject Editor: Paul Ode

Received 17 November 2015; Accepted 15 May 2016

## Abstract

*Harmonia axyridis* (Pallas, 1773) is an Asian coccinellid released in several places to act as a biological control agent of aphids. *Dinocampus coccinellae* (Schrank, 1802) is an endoparasite that uses more than 40 coccinellid species as hosts. Thus, the aim of this study was to investigate the interactions between *D. coccinellae* and *H. axyridis* and to determine the impact of the parasitoid on the establishment capacity of *H. axyridis*. It was also investigate the influence of host on the development of *D. coccinellae* using other Coccinellidae species as hosts: *Cycloneda sanguinea*, (L., 1763) *Cycloneda pulchella* (Klug, 1829), *Eriopis connexa* (Germar, 1824), and *Olla v-nigrum* (Mulsant, 1866). In no-choice tests, pupa was the least attacked stage, and the fourth instar and adults the most attacked. In choice tests, the pupa was less attacked when combined with all the other stages, and the fourth instar and adults the most attacked. There was statistical difference only for fecundity, fertility, and number of eggs/day, with higher values found in the non-parasitized control group. Due to the low rate of parasitism it is believed that *D. coccinellae* has little impact on the populations of this coccinellid in Brazil. However, it is noteworthy that an increase in *H. axyridis* coverage areas can affect the populations of *D. coccinellae*, as in some places of occurrence, *H. axyridis* has become the predominant species of Coccinellidae. The result can be a decrease in populations of this species of parasitoid or its better adaptation to the new host.

**Key words:** Exotic specie; host preference; invasion success; natural enemy

*Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) is an Asian species released in various places in the world to act as a biological control agent of aphids (Castro et al. 2011, Ukrainsky and Orlova-Bienkowskaja 2014).

In many places where *H. axyridis* has been introduced, its populations increased quickly, expanding its coverage area, and it is therefore considered an exotic invasive species that is very successful (Brown et al. 2008, 2011; Koyama and Majerus 2008, Comont et al. 2013). However, it has had some negative effects, such as an impact on non-target organisms (Adrianes et al. (2008), Majerus and Roy 2005, Koyama and Majerus 2008), intraguild predation and displacement of native species (Brown et al. 2011, Roy et al. 2012, Katsanis et al. 2013), and contamination in fruit production as well (Guedes and Almeida 2013).

One of the most intriguing questions and not yet fully answered is why does *H. axyridis* coexist with other species of Coccinellidae in its native region, but it has a tendency to displace native species in areas where it has been introduced (Brown et al. 2011). Some of the hypotheses suggest that this is due to its larger size, better attack

capabilities, jaw shape, polyphagy, more efficient chemical and physical defenses of immature forms, phenotypic plasticity, high reproductive potential, multivoltinism, high fecundity and low susceptibility to parasites, pathogens and parasitoids, in areas where it has been introduced (Brown et al. 2011).

One of the key factors for establishment success for an invasive species is the absence of natural enemies (Torchin et al. 2003). According to the literature, little is known about the potential impact of natural enemies of *H. axyridis*. Some authors include the pathogens *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycotina: Hyphomycetes), *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes), and *Hesperomyces virescens* Thaxter (Laboulbeniales: Ascomycetes) as its most important natural enemies (Kenis et al. 2008).

Besides these are mentioned some birds species, such as *Picus canus* (Gmelin, 1788) (Piciformes: Picidae) and *Sitta europaea* (L. 1758) (Passeriformes: Sittidae), as well as the parasitoids *Dinocampus coccinellae* (Schrank, 1802) (Hymenoptera: Braconidae), *Strongygaster triangulifera* (Loew, 1863) (Diptera: Tachinidae), *Medina luctuosa*

(Meigen, 1824) (Diptera: Tachinidae), *Medina separata* (Meigen, 1824) (Diptera: Tachinidae) (Kenis et al. 2008), *Oomyzus scaposus* (Thomson, 1878) (Hymenoptera: Eulophidae) (Riddick et al. 2009) and *Phalacrotophora philaxyridis* (Disney, 1997) (Diptera: Phoridae) (Comont et al. 2013). Other predators of *H. axyridis* are the nematodes *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) which occur in laboratory (Kenis et al. 2008) and the parasitic mite *Coccipolipus hippodamiae* (McDaniel and Moril, 1969) (Actinedida: Podapolipidae) (Riddick et al. 2009).

Parasitoids are probably the most important natural enemies of coccinellids, and the main species belong to Eulophidae, Encyrtidae, and Braconidae (Hymenoptera). The Euphorinae (Braconidae) include endoparasitoids of Coleoptera adults, where the majority of species belong to the genus *Dinocampus* and parasitize mainly species of Coccinellidae and Curculionidae (Hodek 1973).

*Dinocampus coccinellae* (Schrank, 1802) is a cosmopolitan endoparasitic Euphorinae, and it has been studied because it uses more than 40 coccinellid species as hosts, especially Coccinellinae adults. It can parasitize both immatures and adults, and it has been observed parasitizing *H. axyridis* (Berkvens et al. 2010, Ware et al. 2010) Balduf (1926).

*Harmonia axyridis* and *D. coccinellae* populations coexist in Japan, where the parasitoid can develop successfully, however less effectively in relation to other native species of Coccinellidae (Koyama and Majerus 2008). In North America, the first report of *H. axyridis* being parasitized by *D. coccinellae* was by Hoogendoorn and Heimpel (2002).

In Europe, *Coccinella septempunctata* (L. 1758) (Coleoptera: Coccinellidae) is the most common host for *D. coccinellae* (Davis et al. 2006), while *H. axyridis* is considered a poor host and with low rate of parasitoid emergence in Canada (Firlej et al. 2005).

In Brazil, *Homolotylus flaminus* (Dalman, 1820) (Hymenoptera: Encyrtidae), *Phalacrotophora neda* (Malloch, 1912) (Diptera: Phoridae), and *D. coccinellae* have been reported as parasitoids of *Cycloneda sanguinea* (Linnaeus, 1763) (Coleoptera: Coccinellidae) larvae, pupae and adults, respectively (Gravena 1978, Santos and Pinto 1981). According to Silva et al. (2012), *D. coccinellae* can even parasitize *Coleomegilla maculata* (De Geer, 1775), *Eriopsis connexa* (Germar, 1824), and *Olla v-nigrum* (Mulsant, 1866), which are native species that can influence in the development time of this parasitoid.

Therefore, the objective of this article was to investigate the interactions between *D. coccinellae* parasitoids and the invasive species *H. axyridis* and to determine the impact this parasitoid has on the establishment capacity of *H. axyridis*. It was also investigate the influence of host on the development of *D. coccinellae* using other Coccinellidae species as hosts: *C. sanguinea*, *Cycloneda pulchella* (Klug, 1829), *E. connexa*, and *O. v-nigrum*.

## Materials and Methods

### Insect rearing

#### *Cinara atlantica*

Aphids were first collected in the field, on *Pinus taeda* L. and *Pinus elliottii* Engelm branches infested with *C. atlantica* in Curitiba, Paraná State, and taken to the “Prof. Renato C. Marini” Insect Rearing Laboratory of Universidade Federal do Paraná, where they were kept at  $21 \pm 1^\circ\text{C}$  and  $70 \pm 10\%$  RH, with a 24-h photoperiod.

The aphids were removed from the field-collected pine branches and transferred to new pine branches acquired from a

commercial nursery. Additional aphids were added weekly to maintain the stock population and later used to feed coccinellids, using the same transfer technique.

*Coccinellidae*. The laboratory population originated from 20 to 30 adults of *H. axyridis*, *C. sanguinea* (L.), *C. pulchella* (Klug), *E. connexa* (Germar), *O. v-nigrum* (Mulsant), and *Hippodamia convergens* (Guérin-Meneville, 1842) collected in the field, on pine trees, in Curitiba, PR, and taken to the insect rearing laboratory.

The insects were reared in 500-ml plastic dishes in rearing chambers (BOD) at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and 12-h photoperiod. The food, *C. atlantica*, was supplied daily to maintain the population stock.

The adults were later sexed and matched, and the eggs obtained were transferred to Petri dishes. When the larvae hatched, they were individually placed in Petri dishes lined with filter paper and with a moistened cotton swab with a drop of honey and fed *ad libitum* daily.

After prepupa formation until adult emergence, the insects were kept under the same conditions, but without food. The containers were cleaned and changed every 48 h and the observations made daily. Individuals of the third generation of each species were used in the experiments.

For the experiments with *H. axyridis*, two populations were used: a third laboratory population and a field population. The field population was obtained from individuals collected in a plantation area of *P. taeda* L. and *P. elliottii* Engelm, in the Centro Politécnico Campus of Universidade Federal do Paraná. The specimens were collected randomly and used in the same day in the experiments.

#### *Dinocampus coccinellae*

The selected Coccinellidae species were collected to obtain parasitized insects and to use these to start the rearing of *D. coccinellae*. Adults from the laboratory population were used to establish the parasitoid rearing. As *D. coccinellae* is a telitokous parthenogenetic solitary parasitoid, only females were used.

Five groups of five females of *D. coccinellae* were individualized and placed in 500-ml plastic containers, lined with filter paper and fed with 1:1 water and honey. Ten 10-day-old Coccinellidae adults were placed in each container, fed *ad libitum* with *C. atlantica*, and kept in contact with the parasitoid for 24 h. Afterward, the Coccinellidae adults were removed and individualized as couples.

The coccinellids were monitored daily for ~1 month after parasitism, to obtain *D. coccinellae* pupae, which were subsequently removed and placed in separate Petri dishes.

After parasitoid emergence, adults were individualized and reared at  $25 \pm 1^\circ\text{C}$  and  $70 \pm 10\%$  RH, with a 12-h photoperiod. For the experiments, we used *D. coccinellae* adults from the third generation and aged 2–3 days.

Parasitoid rearing was carried out with all six Coccinellidae species as hosts, so that there was no interference in the results.

*Experimental Design*. Tests with and without choice were conducted to evaluate the influence of host characteristics on acceptance and *H. axyridis* parasitism rate. In each test, the number of oviposition attacks by *D. coccinellae* was used as the host acceptance measure (Richerson and DeLoach 1972), as well as the rate of parasitoid emergence, which was ~30 days.

The hosts which the parasitoid entered its ovipositor were considered “attacked.” It is noteworthy that an attack did not necessarily lead to the emergence of a parasitoid. Parasitism was considered

successful only in those individuals in which a *D. coccinellae* adult emerged.

The attacked insects were individualized, fed on *C. atlantica* and monitored daily for a month until the emergence of pupae and subsequent adult emergence. We also determined the total larval and pupal development times and longevity for emerged parasitoids.

All experiments were performed in 300-ml plastic containers, in brood chambers (BOD) at  $25 \pm 1^\circ\text{C}$  and  $70 \pm 10\%$  RH, with a 12-h photoperiod. Twenty replicates were performed for each test.

**No-Choice Tests.** Each female adult of *D. coccinellae* was exposed for 20 min to parasitize a single specimen of *H. axyridis* of different stages (third and fourth instars, pupa, and adult), populations (field and laboratory), and sexes.

**Choice Tests.** Each female adult of *D. coccinellae* was exposed for 20 min to parasitize two individuals of *H. axyridis* of different stages (third and fourth instars, pupa, adult male, and female), populations (laboratory female with field female and laboratory male with field male), sexes, and adult species (*H. axyridis*, *C. sanguinea*, *C. pulchella*, *E. connexa*, *H. convergens*, and *O. v-nigrum*).

For the first bioassay, all stages were combined with each other, and for the last, all species were combined with each other.

**Effect of Parasitization on the Reproductive Capacity of *H. axyridis*.** In this test, we analyzed the reproductive capacity of parasitized *H. axyridis* adults, examining the variables pre-oviposition, oviposition, post-oviposition, fecundity, fertility, eggs/day, egg mass, and eggs/egg mass.

We used insects from the laboratory population that had been exposed to females of *D. coccinellae* for 20 min. All attacked individuals were kept in Petri dishes, on *C. atlantica* diet, at  $25 \pm 1^\circ\text{C}$  and  $70 \pm 10\%$  RH, with a 12-h photoperiod.

Due to the low rate of parasitism exhibited by the host, only four successfully parasitized *H. axyridis* females were observed throughout the cycle, with daily monitoring. Each female was paired with a non-parasitized male. A control group of 15 non-parasitized coccinellids was monitored under the same conditions.

**Influence of Host on the Development of *D. coccinellae*.** In this test, we analyzed the influence of host on the development time (egg-pupa, pupa, and egg-adult) and longevity of *D. coccinellae*. Five Coccinellidae species were used as hosts: *H. axyridis*, *C. sanguinea*, *C. pulchella*, *E. connexa*, and *O. v-nigrum*.

We used adults of the laboratory population that had been exposed to *D. coccinellae* females by 20 min. The successfully parasitized individuals were transferred to Petri dishes, and fed on a *C. atlantica* diet, at  $25 \pm 1^\circ\text{C}$  and  $70 \pm 10\%$  RH, with 12-h photoperiod.

Each parasitized adult coccinellid was paired with an individual of the opposite sex, not parasitized, and observed until parasitoid emergence. Monitoring was carried out daily for ~30 days.

For this bioassay, 10 repetitions were performed for each species, except *H. axyridis*, for which only four repetitions were possible.

**Statistical Analysis.** We evaluated the differences between the number of parasitoid attacks in no-choice tests with regard to development stages, effect of parasitism on *H. axyridis* variables and influence of the host on the development of *D. coccinellae* was adjusted a generalized linear model (GLM) using an error distribution of the inverse Gaussian family. The Tukey contrasts ( $P \leq 0.05$ ) were used for comparisons *a posteriori*.

The analysis of no-choice tests was carried out for the four larval instars, pupa, and adult. The analysis of reproductive variables (pre-oviposition, oviposition, post-oviposition, fecundity, fertility, eggs/day, egg mass, eggs/egg mass) was performed for females, and longevity analysis was analyzed for adults.

For GLM, we used the MASS library (Venables and Ripley 2002) and effects (Fox 2003), and for a *posteriori* analysis, we used the multcomp library (Hothorn et al. 2008).

To determine whether there was difference between the eggs/egg mass parameter to parasitized or not *H. axyridis*, a GLM was adjusted using an error distribution of the inverse Gaussian family.

In choice tests, to analyze the preference of *D. coccinellae* between the stages of development, between populations, between the sexes, and between species, Chi-square test was performed.

All statistical analyses were performed with R 2.1.2 (R Development Core Team 2011).

## Results

When found a potential host, *D. coccinellae* first examined it with antennae. Then folded its abdomen with the ovipositor directed to the host. In one swift movement, the female inserted the ovipositor in the host abdomen and than two situations can occur: the parasitoid accepted the host and oviposited or rejected by removing the ovipositor, without laying eggs.

Even with the parasitoid pupa formed externally, the host remains alive. However, in some cases, the parasitoid uses the host legs during the process of pupa formation, trapping and preventing coccinellid to feed, causing its death, possibly by starvation.

In all experiments, the parasitoid interacted with at least one type of host offered.

### No-Choice Tests

In no-choice tests, the pupae were attacked less often than all the other stages, and adults were the most attacked, followed by the fourth and third instars, respectively (Table 1). There was no significant difference in the number of attacks between males and females, for any of the stages analyzed (Table 1).

The parasitoid developed to adulthood only when the adult host was attacked, with an emergence rate of 10% when females were attacked, and 5% when males were attacked (Table 1).

**Table 1.** Mean number of attacks ( $\pm$  SD) and emergence percentage of the parasitoid *D. coccinellae* (Schrank, 1802) when the host were different stages and sexes of *H. axyridis* (Pallas, 1773), in no choice tests.

Stage	Number of attacks	Parasitoid emergence (%)
Third instar female	2.75 $\pm$ 0.55a	0
Third instar male	2.50 $\pm$ 0.51a	0
Fourth instar female	3.75 $\pm$ 0.64bd	0
Fourth instar male	3.05 $\pm$ 1.01ab	0
Pupa female	1.20 $\pm$ 0.77c	0
Pupa male	0.80 $\pm$ 0.77c	0
Adult female	4.50 $\pm$ 0.69d	0
Adult male	3.95 $\pm$ 0.22d	5

Lower case letters within the lines indicate differences between the number of attacks by the parasitoid.

## Choice Tests

### Different Development Stages

*Harmonia axyridis* third instar larvae combined with pupae were attacked more often than when combined with fourth instar larvae and adults (Fig. 1, Table 2).

The pupa was the least attacked stage, where the lowest number of attacks was when combined with adult males (Fig. 1, Table 2). The fourth instar larvae and adults were the most attacked stages, where the fourth instar was more frequently attacked when combined with third instar larvae and pupae (Fig. 1, Table 2).

Parasitism was successful only when *H. axyridis* adults were the hosts, with the highest rate of *D. coccinellae* emergence (15%) occurring in adult females when combined with pupae. The parasitism rate when the male adult was combined with third and fourth instars was 5%, while adult females combined with third and fourth instars showed a 10% rate.

### Different Populations

There was no statistical difference in the number of attacks between the two populations tested (Fig. 2, Table 2). The parasitoid emergence rate was 3 and 7% for laboratory males and females, respectively, and 5 and 8% for field males and females, respectively.

### Different Sexes

Females were statistically more attacked than males ( $P$ -value 0.002038), where the parasitoid emergence rate was 5% with males as host, and 10% when females were the host (Fig. 3, Table 2).

### Different Species of Coccinellidae

*Harmonia axyridis* was less frequently attacked when combined with all other species. The most attacked coccinellids were *C.*

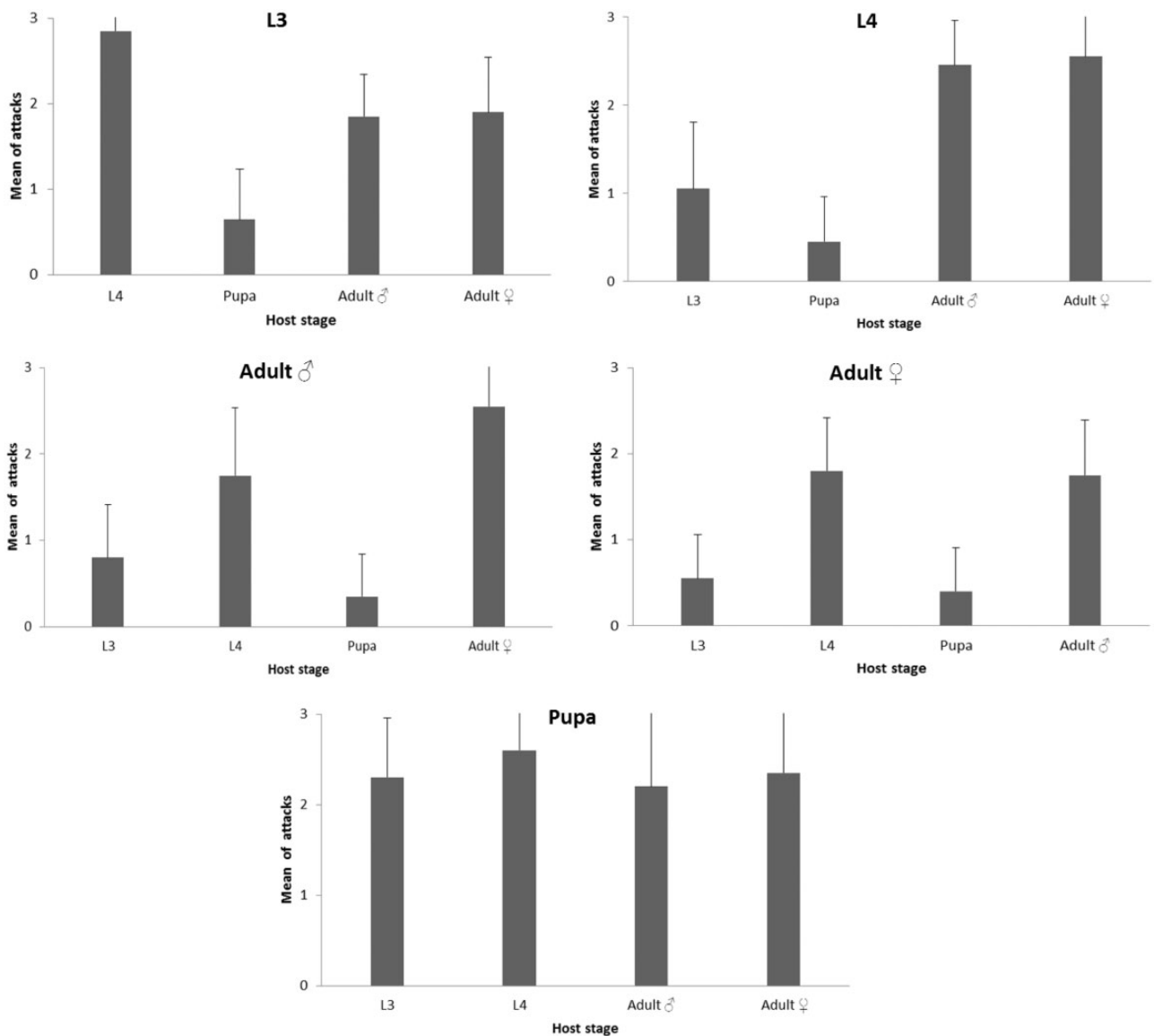


Fig. 1. Mean number of attacks ( $\pm$ SE) by *D. coccinellae* (Schrank, 1802) adult on *H. axyridis* (Pallas, 1773) during 20 min period in choice tests, according to the combined host. L3 = 3rd instar; L4 = 4th instar ( $n=20$ , for each combination).

*sanguinea* and *C. pulchella*, followed by *O. v-nigrum*, *E. connexa*, and *H. convergens*, respectively (Fig. 4, Table 2).

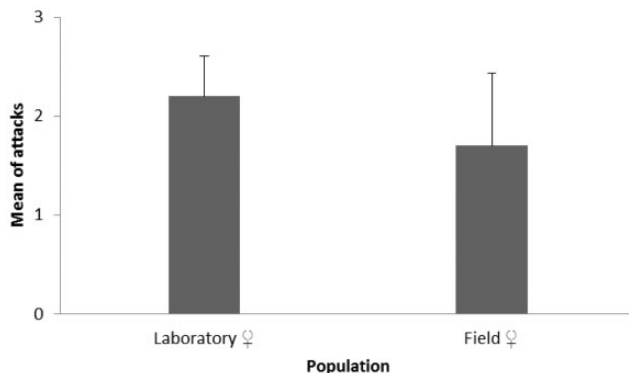
#### Effect of Parasitization on the Reproductive Capacity of *H. axyridis*

Comparing the reproductive variables of *H. axyridis* parasitized by *D. coccinellae* and the non-parasitized control group, there was a statistical difference only for fecundity ( $P$ -value 0.003822), fertility ( $P$ -value 0.012676), and number of eggs/day ( $P$ -value 0.002472), where higher values were found in the control group (Table 3).

**Table 2.** Chi-square values for choice-tests with the parasitoid *D. coccinellae* (Schrank, 1802), in function of development stage, population, gender and species, where df = degrees of freedom.

Pairing	$\chi^2$	df	$P$ -value
L3 × L4	16.6154	1	4.578e <sup>-05</sup>
L3 × Pupa	18.4576	1	1.737e <sup>-05</sup>
L3 × Adult ♂	1.9206	1	0.003919
L3 × Adult ♀	2.25	1	0.0001147
L4 × Pupa	30.3115	1	3.679e <sup>-08</sup>
L4 × Adult ♂	2.3333	1	0.1266
L4 × Adult ♀	2.5862	1	0.1078
Pupa × Adult ♂	26.8431	1	2.207e <sup>-07</sup>
Pupa × Adult ♀	27.6545	1	1.450e <sup>-07</sup>
L ♀ × C ♀	1.4678	1	0.2273
L ♂ × C ♂	1.2821	1	0.2575
Male × female	9.5149	1	0.002038
Ha × Cs	50.5806	1	0.144e <sup>-12</sup>
Ha × Cp	61.4932	1	4.443e <sup>-15</sup>
Ha × Ec	47.6102	1	5.2e <sup>-12</sup>
Ha × Hc	37.3556	1	9.844e <sup>-10</sup>
Ha × Ov	44.5873	1	2.433e <sup>-11</sup>
Cs × Cp	0.4098	1	0.52221
Cs × Ec	16.2881	1	5.44e <sup>-05</sup>
Cs × Hc	7.0435	1	0.007955
Cs × Ov	1.3913	1	0.2382
Cp × Ec	5.6667	1	0.01729
Cp × Hc	4.9	1	0.02686
Cp × Ov	2.0833	1	0.1489
Ec × Hc	0.1233	1	0.7255
Ec × Ov	0.0137	1	0.9068
Hc × Ov	15.5172	1	8.176e <sup>-5</sup>

L3 = 3rd instar; L4 = 4th instar; L = laboratory population; C = field population; Ha = *Harmonia axyridis*; Cs = *Cycloneda sanguinea*; Cp = *Cycloneda pulchella*; Ec = *Eriopis connexa*; Hc = *Hippodamia convergens*; Ov = *Olla v-nigrum*.



**Fig. 2.** Mean number of attacks ( $\pm$ SE) by *D. coccinellae* (Schrank, 1802) adult on *H. axyridis* (Pallas, 1773) during 20 min period in choice tests, according to the population ( $n=20$ , for each combination).

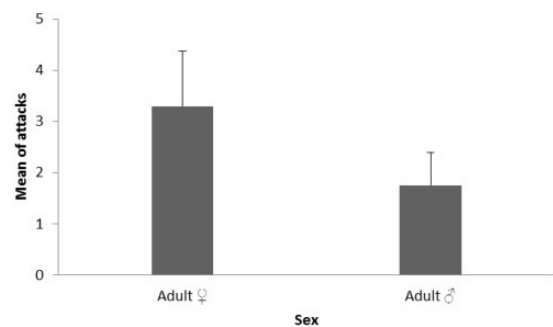
#### Influence of Host on the Development of *D. coccinellae*

The parasitoid development time from egg to pupa was significantly less with *H. axyridis* as host ( $P$ -value 0.0023812). For pupa and egg–adult phases, the time was significantly shorter with *H. axyridis* ( $P$ -value 0.004675 and 0.003924) and *C. sanguinea* ( $P$ -value 0.002988 and 0.002783) as hosts. *D. coccinellae* longevity was significantly longer when the host was *H. axyridis*, *C. sanguinea* ( $P$ -value 0.0027652) or *O. v-nigrum* ( $P$ -value 0.003956), where a statistical difference was seen for the last two species (Table 4).

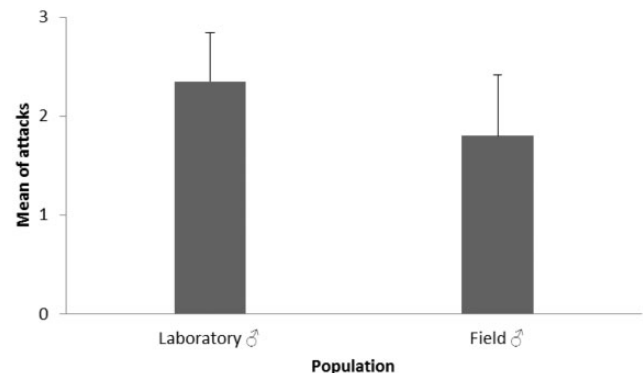
*Dinocampus coccinellae* did not complete its development with *H. axyridis* as host. For *C. sanguinea*, *C. pulchella*, *H. convergens*, and *E. connexa*, the parasitoid emergence rate was 67, 65, 51, and 43%, respectively, and all parasitized coccinellids died after parasitoid emergence (Table 4).

#### Discussion

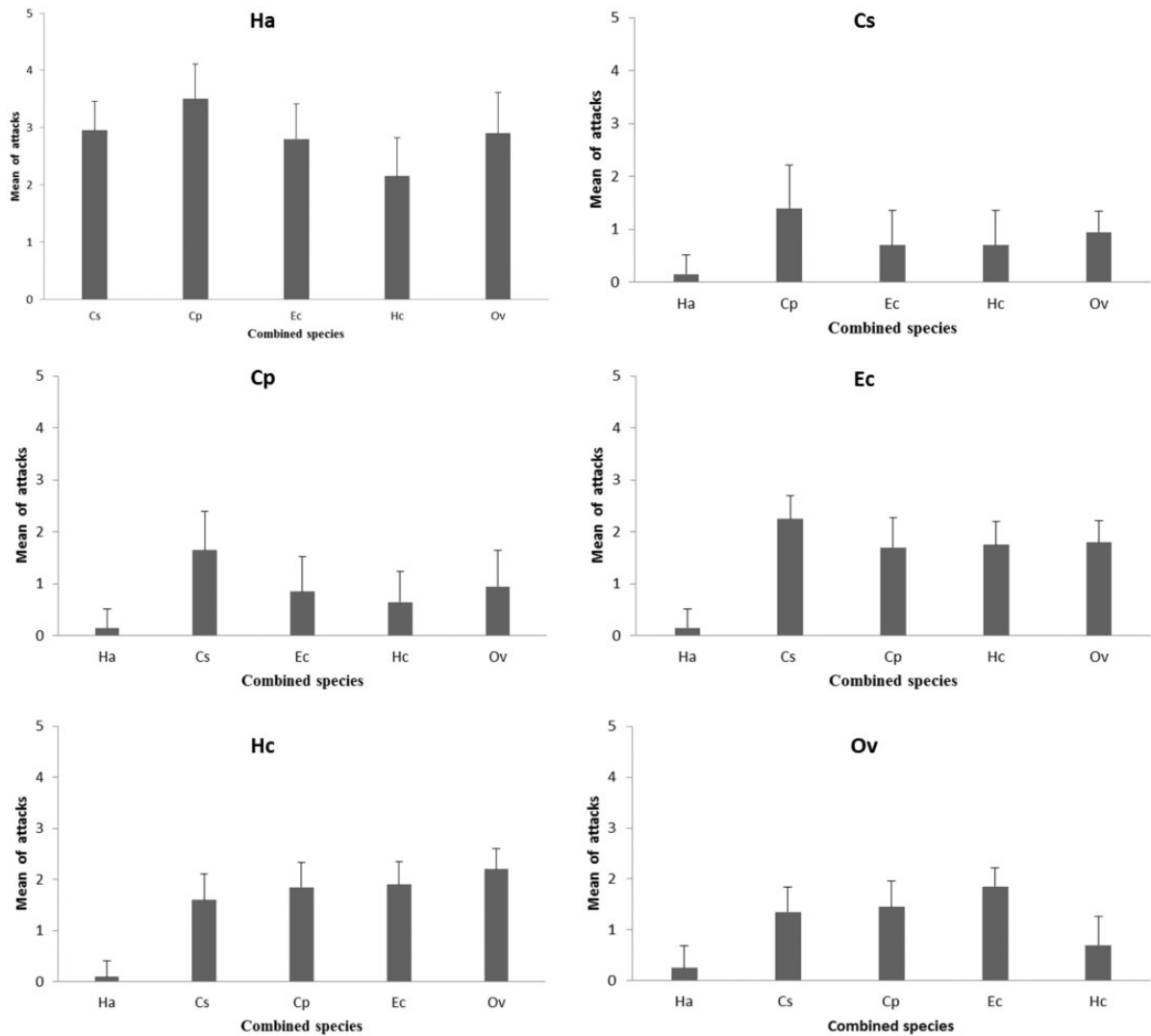
Some studies have shown that parasitoids can differentiate hosts of low and high quality on the basis of species, size, and developmental stage (Godfray 1994, Davis et al. 2006). For *D. coccinellae* there are some host characteristics that can influence the parasitism success, such as species and developmental stage (Okuda and Ceryngier 2000), adult age (Majerus et al. 2000), and sex (Majerus et al. 2000, Davis et al. 2006). In this way, the development stage affects the suitability of *H. axyridis* as a host for *D. coccinellae*. In both choice and no-choice tests, the pupa and third instar stages were attacked less often than the fourth instar and the adult.



**Fig. 3.** Mean number of attacks ( $\pm$ SE) by *D. coccinellae* (Schrank, 1802) adult on *H. axyridis* (Pallas, 1773) during 20 min period in choice tests, according to the sex ( $n=20$ , for each combination).







**Fig. 4.** Mean number of attacks ( $\pm$ SE) by *D. coccinellae* (Schrank, 1802) adult on *H. axyridis* (Pallas, 1773) during 20 min period in choice tests, according to the species (the number of attacks reported related to the coccinellid species in the title of the figure in combination with the coccinellid species reported on the x-axis) ( $n=20$ , for each combination). HA = *H. axyridis*; CS = *Cycloneda sanguinea*; CP = *Cycloneda pulchella* EC = *Eriopis connexa*; HC = *Hippodamia convergens*; OV = *Olla v-nigrum*.

**Table 3.** Reproductive variables (days) (mean  $\pm$  SD) of *H. axyridis* (Pallas, 1773) parasitized and control group no-parasitized.

Variables	<i>H. axyridis</i>	
	Parasitized	Control
Fecundity	490.00 $\pm$ 5.67a	747.53 $\pm$ 71.75b
Fertility	397.75 $\pm$ 0.93a	671.07 $\pm$ 87.26b
Eggs/day	6.75 $\pm$ 0.52a	9.33 $\pm$ 0.40b
Egg mass	29.00 $\pm$ 2.75a	32.87 $\pm$ 8.22a
Egg/egg mass	18.47 $\pm$ 2.39a	24.08 $\pm$ 6.30a
Longevity	89.00 $\pm$ 4.69a	95.47 $\pm$ 9.72a
Pre-oviposition	7.25 $\pm$ 0.5a	6.93 $\pm$ 1.22a
Oviposition	73.75 $\pm$ 4.50a	80.47 $\pm$ 10.36a
Post-oviposition	7.75 $\pm$ 0.5a	8.07 $\pm$ 0.88a

In choice tests, there was no statistical difference in relation to parasitoid preference when fourth instar larva and adult of either sex were combined. However, the parasitoid only emerged after parasitizing adults. In no-choice tests, the number of attacks was significantly higher for adults, in either sex.

Host detection by *D. coccinellae* is largely based on visual detection, such as by host movement and also by olfactory signs (Berkvens et al. 2010). Several studies have shown that this parasitoid displays preference for moving individuals (Berkvens et al. 2010, Silva et al. 2012). Therefore, the low number of attacks in the third instar, fourth instar and pupa stages may be related to the lower mobility of these stages.

In studies of *H. axyridis* in Europe, Berkvens et al. (2010) obtained similar results for choice and no-choice tests. *D. coccinellae* was found to attack adults almost exclusively, avoiding laying eggs in immatures in presence of adults (Geoghegan et al. 1998, Majerus et al. 2000, Firlje et al. 2005). Davis et al. (2006) observed that *D. coccinellae* had a preference for parasitizing adult Coccinellidae. In theory, this preference is adaptive, because parasitism is more successful and development time is shorter in adult hosts (Obrycki et al. 1985, Firlje et al. 2007).

Several authors have noted that *D. coccinellae* generally attacks larger species, and shows higher survival rates when the host is bigger. Hypothetically, this occurs because larger individuals contain

**Table 4.** Development period and longevity (mean  $\pm$  SD) of *D. coccinellae* (Schrank, 1802) when the hosts were different Coccinellidae species.

Host	Development period				
	Egg-pupa	Pupa	Egg-adult	Longevity	Total
<i>H. axyridis</i>	8.50 $\pm$ 0.58a	5.00 $\pm$ 0.00a	13.70 $\pm$ 1.15a	7.00 $\pm$ 0.00ab	20.70 $\pm$ 1.15
<i>C. sanguinea</i>	10.10 $\pm$ 1.29b	5.00 $\pm$ 0.67a	15.10 $\pm$ 1.60ab	7.30 $\pm$ 0.48b	22.40 $\pm$ 1.90
<i>C. pulchella</i>	9.80 $\pm$ 0.92b	6.10 $\pm$ 0.87c	15.80 $\pm$ 1.47b	5.60 $\pm$ 0.52c	21.40 $\pm$ 1.78
<i>E. connexa</i>	12.90 $\pm$ 0.99b	6.50 $\pm$ 0.53c	19.40 $\pm$ 1.07c	6.00 $\pm$ 0.67c	25.40 $\pm$ 1.50
<i>O. v-nigrum</i>	16.90 $\pm$ 0.74c	7.90 $\pm$ 0.74d	24.80 $\pm$ 1.40d	6.10 $\pm$ 0.57ac	30.90 $\pm$ 1.37

Lower case letters within the line indicate differences between the hosts.

greater resources, and thus offer the parasitoid offspring better chances to successfully complete the life cycle (Davis et al. 2006, Berkvens et al. 2010). Majerus et al. (2000) also noted the preference for oviposition in younger (pre-winter) than older (overwintered) adults, increasing the chances of offspring fitness, since newly emerged hosts are more likely to survive and allow full parasitism, compared with older adults.

Adult insects are better protected against natural enemies due to their chitinous exoskeleton and greater mobility compared to immatures. However, the successful oviposition of *D. coccinellae*, especially in native species, in well-protected individuals such as Coccinellidae is facilitated by host movement, when the vulnerable areas of the body, such as the softer parts of the abdomen, are more easily accessible (Richerson and DeLoach 1972, Okuda and Ceryngier 2000).

For other Coccinellidae hosts (Obrycki et al. 1985, Geoghegan et al. 1998), the adults were more susceptible to *D. coccinellae* development. However, according to some authors, *H. axyridis* larvae are more susceptible (Firlej et al. 2007, 2010), suggesting that adults parasitized by *D. coccinellae* found in the field may have been attacked as larvae (Hoogendoorn and Heimpel 2002, Firlej et al. 2005, 2010).

The emergence rate observed for the parasitoid in this study, in both tests, was low and only occurred when adults were attacked, demonstrating that *H. axyridis* is not a good host for *D. coccinellae*. In choice tests, there was no difference in the number of attacks by the parasitoid in adult individuals of the laboratory and field populations, and the emergence rate of the parasitoid was similar. However, Berkvens et al. (2008a, b) reported differences between laboratory and field populations of *H. axyridis* in response to food and photoperiod conditions, highlighting that conclusions based solely on laboratory experiments very often cannot be simply extrapolated to the field.

In no-choice tests, there was no difference in the number of parasitoid attacks between *H. axyridis* males and females, for any of the stages. However, the emergence rate of the parasitoid was higher when females were parasitized. In choice tests, there was a preference for females, and with a higher rate of parasitism when they were the hosts. Other authors also observed the preference of *D. coccinellae* for females of other Coccinellidae species, in choice tests (Obrycki 1989, Davis et al. 2006).

This preference for females can be explained by the fact that they are bigger than males, providing higher rates of encounter. Also, since the female feeds more than the male, Davis et al. (2006) suggested that the preference for oviposition in females is adaptive, providing the parasitoid larva more resources to develop. Furthermore, when the *D. coccinellae* host is a female, it is possible for the parasitoid to feed on two sources: the ovaries and fat of the host (Geoghegan et al. 1997).

This study demonstrated that, based on two measures of suitability (development time and successful parasitism), all species tested except *H. axyridis* are suitable as a host for *D. coccinellae*.

In choice tests, the parasitoid always preferred to attack other coccinellid species hosts than *H. axyridis*. Emergence rates were high for all species, but again, this was not the case for *H. axyridis*, in which the parasitoid showed 5% adult emergence.

The species that were more susceptible to the parasitoids were *C. sanguinea* and *C. pulchella*, which are native aphidophagous species that can be displaced by *H. axyridis* through competition for food resources (Martins et al. 2009). Therefore, there is a possibility that over time *D. coccinellae* adapts more to *H. axyridis* (Koyama and Majerus 2008, Berkvens et al. 2010).

It is noteworthy that since *D. coccinellae* is a generalist and is known to attack both native and exotic species, it can be assumed that the parent host does not influence the development of the parasitoid (Hoogendoorn and Heimpel 2002). Furthermore, parasitoid rearing was tested with several species of Coccinellidae, but very few emerged with *H. axyridis* as host. Since a low rate of parasitism was observed for *H. axyridis* in other experiments, it is believed that in this case there was no actual interference.

*Dinocampus coccinellae* has been shown to attack *H. axyridis* laboratory populations in North America (Hoogendoorn and Heimpel 2002). However, Firlej et al. (2007) and Hoogendoorn and Heimpel (2002) also demonstrated that the development success of *D. coccinellae* was fairly low with *H. axyridis* as host.

According to Firlej et al. (2010), *H. axyridis* has a greater number of defensive behaviors (such as escape and movements with metathoracic legs to release the parasitoid ovipositor) in relation to *C. maculata*. This fact may also indicate that the parasitoid is more suited to overcome the chemical defenses of native/established species than those of exotic species, in the case *H. axyridis*.

The defensive behaviors of the host influence the success of parasitoid parasitism, because they directly affect its fitness (Firlej et al. 2010). In extreme cases, defensive behavior can harm or even cause the death of the parasitoid (Firlej et al. 2010), affecting the acceptance of the host and decreasing the rate of parasitism (Firlej et al. 2010).

*Harmonia axyridis* adults show more defensive behaviors than do fourth instar larvae and usually can break oviposition sequence (Firlej et al. 2010). This fact supports previous results that show that *H. axyridis* acts as the most aggressive species within a coccinellid guild (Michaud 2002, Sato et al. 2005) and that immature stages have fewer defenses against parasitoids than do adults (Firlej et al. 2010).

Even with oviposition, what probably happens is that the parasitoid eggs are destroyed by the host immune system. In the case of *H. axyridis*, Firlej et al. (2010) observed that no egg of *D.*

*coccinellae* was found in the abdomen of the host after five days of oviposition, suggesting that the eggs were destroyed by its immune system.

These results demonstrate that, unlike other species of Coccinellidae, such as *C. maculata*, *H. axyridis* was able to reproduce even when parasitized (Firlej et al. 2010). Moreover, given the low rate of parasitism of *H. axyridis* adults by *D. coccinellae* and with an average fertility of this parasitized coccinellid of 490 eggs, it is assumed that the suppressive effect of the parasitoid on reproductive variables of *H. axyridis* is small.

The parasitoid development time varied with the host species, such that the total period was lower when *H. axyridis* was the host, however, close to *C. sanguinea*. Parasitoid longevity was also higher with these host species.

Obrycki (1989) observed that the development of the egg until the end of the larval phase of *D. coccinellae* was also significantly influenced by the Coccinellidae host species. The author recorded a faster development with native species as host (*C. maculata*: 20.8; *H. convergens*: 21.8; and *Cycloneda munda* (Say, 1835): 22.1 d) in relation to the introduced species (*C. septempunctata*: 23.6 and *P. quatuordecimpunctata*: 26.5 d). The pupal period was similar as in all species, with a mean of 10.04 d.

The total development period of *D. coccinellae* was longer when *Coleomegilla maculata* was the host (32.4 d), where longevity was similar for the three species compared: 6.9 d for *C. maculata*; 7.1 d for *E. connexa*, and 6.8 d for *O. v-nigrum* (Silva et al. 2012).

Berkvens et al. (2010) observed that *D. coccinellae* development, when *H. axyridis* was the host, was 19.8 d for egg–pupa and 10.6 d for pupa–adult, totaling 30.4 d, at 23°C, while at 27°C, it was 15.0, 9.0, and 24.0 d, respectively, where these values were higher than those obtained in this study. Saito and Bjørnson (2013) reported for *D. coccinellae* a mean total development time of 26.3 d, with *H. convergens* as host.

At long last, the results of this study confirm the assumption that, as in other continents where it has been introduced, *H. axyridis* exhibits low susceptibility to *D. coccinellae*, where it is not a suitable host for it.

Introduced Coccinellidae species may display different interactions with native species. One of these interactions may be mediated through the activities of the parasitoid *D. coccinellae*. For example, the addition of species can increase the availability of hosts in an ecosystem and thereby modify the existing host–parasitoid relationship between native coccinellids and *D. coccinellae* (Obrycki 1989).

In Japan populations of *H. axyridis* and *D. coccinellae* co-exist, where the parasitoid can develop successfully having this coccinellid as host, but with a lower parasitism rate when compared with other native species (Koyama and Majerus 2008). This suggests that the *D. coccinellae* populations in Brazil have not yet had enough time to adjust and overcome the defense mechanisms of *H. axyridis*.

More studies are needed to determine the capacity of *H. axyridis* to defend itself against its natural enemies. It is known that this species has chemical defenses, but the defensive role of these components is still unclear. Despite of the biology of *D. coccinellae* in the laboratory is known, further studies of the impact on population dynamics and ecology of Coccinellidae communities are needed, especially when it comes to invasive alien species, which can alter the community of native coccinellids.

Due to the low rate of *H. axyridis* parasitism by *D. coccinellae*, it is believed that the parasitoid has little impact on the populations of this coccinellid in Brazil. However, it is noteworthy that the increase in *H. axyridis* coverage areas can affect the populations of *D. coccinellae*, since in some places of occurrence, *H. axyridis* has become the

predominant Coccinellidae species. The result can be a decrease in this parasitoid species or its better adaptation to the new host.

## Acknowledgments

We thank Dr. Mauricio Osvaldo Moura and Dr. Mário Luis Pessoa Guedes for help in the statistical analyses and Dr. John Winder and Francisco Pessoa Guedes who helped with the English translation of the manuscript. We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research fellowships CFCG (150155/2015-8), LMA (306772/2006-0).

## References Cited

- Adriaens, T., G. M. Y. Gomez, and D. Maes. 2008. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. *BioControl* 53: 69–68.
- Balduf, W. V. 1926. The bionomics of *Dinocampus coccinellae* Schrank. *Ann. Entomol. Soc. Am.* 19: 465–498.
- Berkvens, N., J. Bonte, D. Berkvens, K. Deforce, L. Tirry, and P. De Clercq. 2008a. Pollen as an alternative food for *Harmonia axyridis*. *BioControl* 53: 201–210.
- Berkvens, N., J. Bonte, D. Berkvens, L. Tirry, and P. De Clercq. 2008b. Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *BioControl* 53: 211–221.
- Berkvens, N., J. Moens, D. Berkvens, M. A. Samih, L. Tirry, and P. De Clercq. 2010. *Dinocampus coccinellae* as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe. *BioControl* 53: 92–99.
- Brown, P. M. J., H. E. Roy, P. Rothery, D. B. Roy, R. L. Ware, and M. E. N. Majerus. 2008. *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *BioControl* 53: 55–68.
- Brown, P. M. J., C. E. Thomas, E. Lombaert, D. L. Jeffries, A. Estoup, and L.-J. L. Handley. 2011. The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl* 56: 623–641.
- Castro, C. F., L. M. Almeida, and S. R. C. Penteado. 2011. The impact of temperature on biological aspects and life table of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *Fla. Entomol.* 94: 923–932.
- Comont, R. F., B. V. Purse, W. Phillips, W. E. Kunin, M. Hanson, O. T. Lewis, R. Harrington, C. R. Shortall, G. Rondoni, and H. E. Roy. 2013. Escape from parasitism by the invasive alien ladybird, *Harmonia axyridis*. *Insect. Conserv. Diver.* 7: 334–342.
- Davis, D. S., S. L. Stewart, A. Manica, and M. E. N. Majerus. 2006. Adaptive preferential selection of female coccinellid hosts by the parasitoid wasp *Dinocampus coccinellae* (Hymenoptera: Braconidae). *Eur. J. Entomol.* 103: 41–45.
- Firlej, A., G. Boivin, É Lucas, and D. Coderre. 2005. First report of parasitism of *Harmonia axyridis* parasitism by *Dinocampus coccinellae* Schrank in Canada. *Biol. Invasions.* 7: 553–556.
- Firlej, A., É Lucas, and D. Coderre. 2010. Impact of host behavioral defenses on parasitization efficacy of a larval and adult parasitoid. *BioControl* 55: 339–348.
- Firlej, A., É Lucas, D. Coderre, and G. Boivin. 2007. Teratocytes growth pattern reflects host suitability in a host-parasitoid assemblage. *Phys. Entomol.* 32: 181–187.
- Fox, J. 2003. Effect displays in R for generalised linear models. *J. Stat. Softw.* 8: 27.
- Geoghegan, I. E., T. M. O. Majerus, and M. E. N. Majerus. 1998. Differential parasitisation of adult and pre-imaginal *Coccinella septempunctata* (Coleoptera: Coccinellidae) by *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae). *Eur. J. Entomol.* 95: 571–579.
- Geoghegan, I. E., W. P. Thomas, and M. E. N. Majerus. 1997. Notes on the coccinellid parasitoid *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) in Scotland. *Entomologist* 116: 179–184.
- Gerling, D., B. D. Roitberg, and M. Mackauer. 1990. Instar-specific defense of the pea-aphid, *Acyrtosiphon pisum*: influence on oviposition success of the



- parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). *J. Insect Behav.* 3: 501–514.
- Godfray, H. C. J. 1994. *Parasitoids*. Princeton University Press, Princeton.
- Gravena, S. 1978. Occurrence of parasitism in *Cycloneda sanguinea* (Linnaeus, 1763) in Jaboticabal, SP, Brazil. *An. Soc. Entomol. Bras.* 20: 69–70.
- Guedes, C. F. C., and L. M. Almeida. 2013. The potential of different fruit species as food for *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae). *R. Bras. Frutic.* 35: 1025–1031.
- Hodek, I. 1973. *Biology of Coccinellidae*. Academia, Prague and Dr W. Junk, The Hague.
- Hoogendoorn, M., and G. E. Heimpel. 2002. Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. *Biol. Control* 25: 224–230.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biom. J.* 50: 346–363.
- Katsanis, A., D. Babendreier, W. Nentwig, and M. Kenis. 2013. Intraguild predation between the invasive ladybird *Harmonia axyridis* and non-target European coccinellid species. *BioControl* 58: 73–83.
- Kenis, M., H. E. Roy, R. Zindel, and M. E. N. Majerus. 2008. Current and potential management strategies against *Harmonia axyridis*. *BioControl* 53: 235–252.
- Koyama, K., and M. E. N. Majerus. 2008. Interactions between the parasitoid wasp *Dinocampus coccinellae* and two species of coccinellid from Japan and Britain. *BioControl* 53: 253–264.
- Majerus, M. E. N., I. E. Geoghegan, and T. M. O. Majerus. 2000. Adaptive preferential selection of young coccinellid host by the parasitoid wasp *Dinocampus coccinellae* (Hymenoptera: Braconidae). *Eur. J. Entomol.* 97: 161–164.
- Majerus, M. E. N., and H. E. Roy. 2005. Scientific opportunities presented by the arrival of the harlequin ladybird, *Harmonia axyridis*, in Britain. *Antenna* 29: 196–208.
- Martins, C. B. C., L. M. Almeida, R. C. Zonta-de-Carvalho, C. F. Castro, and R. A. Pereira. 2009. *Harmonia axyridis*: a threat to Brazilian Coccinellidae? *Rev. Bras. Entomol.* 53: 663–671.
- Michaud, J. P. 2002. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. *Environ. Entomol.* 31: 827–835.
- Obrycki, J. J. 1989. Parasitization of native and exotic Coccinellids by *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae). *J. Kan. Entomol. Soc.* 62: 211–218.
- Obrycki, J. J., M. J. Tauber, and C. A. Tauber. 1985. *Perilitus coccinellae* (Hymenoptera: Braconidae): parasitization and development in relation to host-stage attacked. *Ann. Entomol. Soc. Am.* 78: 852–854.
- Okuda, T., and P. Ceryngier. 2000. Host discrimination in *Dinocampus coccinellae* (Hymenoptera: Braconidae), a solitary parasitoid of coccinellid beetles. *Appl. Entomol. Zool.* 35: 535–539.
- R Development Core Team 2011. *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org>. (accessed 10 Oct 2014).
- Richerson, J. V., and C. J. DeLoach. 1972. Some aspects of host selection by *Perilitus coccinellae*. *Ann. Entomol. Soc. Am.* 65: 834–839.
- Riddick, E. W., T. E. Cottrell, and K. A. Kid. 2009. Natural enemies of the Coccinellidae: Parasites, pathogens, and parasitoids. *Biol. Control* 51: 306–312.
- Roy, H. E., T. Adriaens, N. J. B. Isaac, M. Kenis, T. Onkelinx, G. San Martin, P. M. J. Brown, L. Hautier, R. L. Poland, D. B. Roy, et al. 2012. Invasive alien predator causes rapid declines of native European ladybirds. *Divers. Distrib.* 18: 717–725.
- Saito, T., and S. Bjørnson. 2013. The convergent lady beetle, *Hippodamia convergens* Guérin-Méneville and its endoparasitoid *Dinocampus coccinellae* (Schrank): the effect of a microsporidium on parasitoid development and host preference. *J. Invert. Pathol.* 113: 18–25.
- Santos, G. P., and A. C. Q. Pinto. 1981. Biology *Cycloneda sanguinea* and its association with aphids on leaves of mango. *Pesq. Agropec. Bras.* 16: 473–476.
- Sato, S., H. Yasuda, and E. W. Evans. 2005. Dropping behavior of larvae of aphidophagous ladybirds and its effects on incidence of intraguild predation: interactions between the intraguild prey, *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.) and the intraguild predator, *Harmonia axyridis* Pallas. *Ecol. Entomol.* 30: 220–224.
- Silva, R. B., I. Cruz, M. L. C. Figueiredo, A. G. Pereira, and A. M. Penteado-Dias. 2012. Occurrence and biology of *Dinocampus coccinellae* (Schrank, 1802) (Hymenoptera; Braconidae; Euphorinae) parasitising different species of Coccinellidae (Coleoptera) in Neotropical region. *Braz. J. Biol.* 72: 215–219.
- Torchin, M. E., K. D. Lafferty, A. P. Dobson, V. J. Mckenzie, and A. M. Kuris. 2003. Introduced species and their missing parasites. *Nature* 421: 628–630.
- Ukrainsky, A. S., and M. J. Orlova-Bienkowskaja. 2014. Expansion of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) to European Russia and adjacent region. *Biol. Invasions* 16: 1003–1008.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Springer, New York.
- Ware, R., L. Michie, T. Otani, E. Rhule, and R. Hall. 2010. Adaptation of native parasitoids to a novel host: the invasive coccinellid *Harmonia axyridis*. *IOBC Bull.* 58: 175–172.