Dinocampus coccinellae as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe

Nick Berkvens [a], Joachim Moens [a], Dirk Berkvens [b], Mohammad Amin Samih [c], Luc Tirry [a], Patrick De Clercq [a,*]

[a] Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[b] Department of Animal Health, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium
[c] Department of Plant Protection, College of Agriculture, Vali e Asr University, P.O. Box 771393641, Rafsanjan, Iran

ARTICLE INFO

ABSTRACT

The enemy release hypothesis states that the absence of effective natural enemies can be a fundamental aspect leading to the successful establishment of an invasive species. This study investigates the impact of the native braconid parasitoid *Dinocampus coccinellae* on European populations of the invasive ladybird *Harmonia axyridis*. The parasitoid attacked adults and fourth instars of the ladybird more frequently than pupae and third instars. When given a choice, *D. coccinellae* attacked non-melanic adults and adults of a long term laboratory population of *H. axyridis* more often than melanic adults and adults of a field population, respectively. However, in no choice tests the parasitoid attacked individuals of either morph type and of the field and laboratory populations with the same frequency. Immature development of *D. coccinellae* took longer at lower temperatures and when less advanced developmental host stages (larvae and pupae) were successfully parasitized. The parasitoid emergence rates ranged from 0% to 14.7% on field populations of *H. axyridis* depending on life stage of the host attacked and up to 16.9% when adults of a long term laboratory population were attacked. Successfully parasitized ladybirds showed reduced reproductive capacities (6–12% of unparasitized individuals) and aphid consumption rates (85% of unparasitized individuals), but these effects together with the low emergence rates of the parasitoid suggest that *D. coccinellae* may only have a marginal impact on the population growth of *H. axyridis* in Europe.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

The multicolored Asian ladybird or harlequin ladybird, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), is a predatory coccinellid native to central and eastern Asia (Coderre et al., 1995; Nalepa et al., 1996; Koch, 2003). The species was introduced as a biological control agent of aphid and coccid pests, first in North America and later on in Europe. The predator succeeded to establish in North America by the end of the 1980's and since establishment several undesired side-effects were reported, including impacts on non-target arthropods, adverse effects on human health and contamination in fruit production (see references in Koch, 2003; Koch and Galvan, 2008). *Harmonia axyridis* is currently now established in many European countries and expanding across the European continent on a large scale (Coutanceau, 2006; Brown et al., 2008) and it is predicted that it will have similar adverse impacts as reported in North America (Adriaens et al., 2008).

According to the enemy release hypothesis, the absence of effective natural enemies is assumed to be one of the key factors leading to the successful establishment of an invasive species (Keane and Crawley, 2002; Torchin et al., 2002, 2003; Mitchell and Power, 2003). Recorded natural enemies of *H. axyridis* include the pathogens *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycotina: Hyphomycetes), *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) and *Hesperomyces virescens* Thaxter (Laboulbeniales: Ascomycetes), certain bird species (e.g., *Picus canus* Gmelin (Piciformes: Picidae) and *Sitta europaea* L. (Passeriformes: Sittidae)), the parasitoids *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae), *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae), *Medina luctuosa* (Meigen) (Diptera: Tachinidae), *Medina separata* (Meigen) (Diptera: Tachinidae) and *Phalacrotophora philaxyridis* Disney (Diptera: Phoridae), the nematodes *Heterorhabditis bacteriophora* Poinar (Rhabitida: Heterorhabditidae) and *Steinernema carpocapsae* (Weiser) (Rhabitida: Steinernematidae), and the parasitic mite *Coccipolipus hippodamiae* (McDaniel and Moril) (Actinedida: Podapolipidae) (Kenis et al., 2008). However, not much is known in the literature about the potential impact of most of these natural enemies on the coccinellid.

* Corresponding author. Fax: +32 (0)9 264 62 39.
E-mail address: Patrick.Declercq@UGent.be (P. De Clercq).

During a field survey in Belgium in 2005, three *H. axyridis* adults successfully parasitized by the braconid parasitoid *D. coccinellae* were found (Berkvens, unpublished data), constituting the first field report of the braconid parasitizing *H. axyridis* in Europe. *Dinocampus coccinellae* is a solitary endoparasitoid that exclusively parasitizes coccinellid species of the subfamily Coccinellinae (Balduf, 1926; Obrycki, 1989; Hodek and Honek, 1996; Majerus, 1997). The species exhibits thelytokous parthenogenesis, with only a handful of male specimens ever having been recorded (Davis et al., 2006). This cosmopolitan wasp is multivoltine over much of its geographical range (Majerus, 1997). In Europe, the seven-spotted ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is the most common host for *D. coccinellae* (Obrycki, 1989; Geoghegan et al., 1997, 2000). Whereas several studies have been carried out on the parasitization of *C. septempunctata* by *D. coccinellae* in different areas (Kadono-Okuda et al., 1995; Geoghegan et al., 1997, 1998, 2000; Majerus et al., 2000; Okuda and Ceryngier, 2000; Davis et al., 2006), little is known about the interactions between *D. coccinellae* and its recent potential host in Europe, the invasive coccinellid *H. axyridis*. Hoogendoorn and Heimpel (2002), Firlej et al. (2005, 2007) and Koyama and Majerus (2008) have studied interactions between both species in the USA, Canada and the UK, respectively, and all noted a relatively low susceptibility of the invasive coccinellid to the parasitoid.

The aim of this study is to investigate a number of interactions between the native parasitoid *D. coccinellae* and the invasive coccinellid *H. axyridis* as to determine which impact the parasitoid could potentially have on the establishment capacity of the coccinellid in western Europe. We have investigated the host acceptance and the parasitization success of *D. coccinellae* on *H. axyridis* in the laboratory. Furthermore, we have examined the impact of parasitization on the reproductive capacity and food uptake of the coccinellid. Previous studies have shown that compared to native coccinellids *H. axyridis* is less susceptible to the impact of several natural enemies, including the tachinid fly *S. triangulifera* (Katsoyannos and Aliniazee, 1998), the nematode *S. carpocapsae* (Shapiro-Ilan and Cottrell, 2005) and the fungus *B. bassiana* (Roy et al., 2008). By broadening the range of examined natural enemies of *H. axyridis* with the braconid *D. coccinellae*, we set out to further investigate the significance of the enemy release hypothesis for the colonization success of this invasive coccinellid in Europe.

2. Materials and methods

2.1. Insect populations

2.1.1. Harmonia axyridis

Three populations of *H. axyridis* were used: a long term melanic laboratory population, a non-melanic or 'red' field population and a melanic or 'black' field population. The laboratory population was started with larvae acquired from Biobest NV (Westerlo, Belgium) in September 1998. The field populations originated from individuals of an established wild population collected between August 2006 and May 2008 in Ghent, Belgium. Individuals were identified using the morphological characteristics described in Chapin and Brou (1991) and Adriaens et al. (2003). All populations were established and reared as described by Berkvens et al. (2008a,b). All populations were reared on frozen *Ephestia kuehniella* Zeller eggs (Lepidoptera: Pyralidae). The experiments were conducted using individuals of the 61st to 65th generation (choice and no choice tests, development of *D. coccinellae* at different temperatures, aphid predation experiments) and of the 82nd generation (host fitness experiments) of the laboratory population and of the 3rd to 6th generation of the field populations.

2.1.2. Dinocampus coccinellae

A culture of *D. coccinellae* was started in April 2006 with two adult wasps, one emerging from a *C. septempunctata* adult and another from a *H. axyridis* adult, both collected during field sampling in Ghent, Belgium. The adult coccinellids were identified using morphological characteristics described in Iperti (1964). *Harmonia axyridis* adults from the laboratory population were used to establish a culture of the braconid. At the start of each generation single females of *D. coccinellae* were confronted with five adult ladybirds (4–21 days old) in a plastic Petri dish (14 cm diameter, 1.5 cm high) during a period of 30 min. The ladybirds were then transferred to rearing containers (30 × 17 × 9 cm), holding about 30 individuals. The bottom of the containers was covered with paper toweling and the lid had a large rectangular opening screened with fine mesh for ventilation. The rearing containers were kept in an incubator at 23 ± 1 °C, 65 ± 5% relative humidity (RH) and a 16:8 h (L:D) photoperiod. The ladybirds were provided daily with *E. kuehniella* eggs ad libitum as food. A moist paper plug fitted into a plastic dish served as a water source. During the 4 weeks after parasitization, the containers and ladybirds were checked daily for *D. coccinellae* cocoons. Cocoons were collected and placed individually in a Petri dish (9 cm diameter, 1.3 cm high). After adult emergence, a paper plug fitted into a plastic dish was added to the Petri dish, which was then moistened daily with a 15% honey–water solution. *Dinocampus coccinellae* wasps of the 7th to 13th generation (choice and no choice tests, development of *D. coccinellae* at different temperatures, aphid predation experiments) and of the 34th and 35th generation (host fitness experiments) of laboratory rearing were used in the study; all *D. coccinellae* adults used in the experiments were 2–4 days old. In each generation approximately 120 *H. axyridis* adults of the melanic laboratory population were allowed to be attacked by individual wasps from which generally 15–30 adult parasitoids successfully emerged (i.e., an emergence rate of 10–25%).

2.2. Experiments

2.2.1. Choice and no choice tests

Choice and no choice tests were performed to study the influence of host characteristics on acceptance and rate of parasitization of *H. axyridis* by *D. coccinellae*. All experiments were conducted in Petri dishes (9 cm diameter, 1.3 cm high) held in controlled environmental conditions (23 ± 1 °C, 65 ± 5% RH and 16:8 h (L:D) photoperiod).

In the no choice tests, single *D. coccinellae* females were confronted for 5 min with individual *H. axyridis* of different morphs, life stages or populations. Fourth instars and adults (both sexes) from the field populations were used to study the effect of host morph type (melanic versus non-melanic). The influence of host developmental stage was studied using third and fourth instars, pupae and adults (both sexes) of the non-melanic field population. Parasitization of the field versus the laboratory population was compared using melanic adults (both sexes) of the coccinellid. In a subsequent series of choice tests, single *D. coccinellae* adults were confronted for 5 min with two individuals of *H. axyridis* of different morphs, stages, or populations as described above. In addition, the influence of host sex (adult male versus adult female) was also studied in a choice test using individuals of the non-melanic field population of *H. axyridis*. Adult sex was determined as described in McCornack et al. (2007).

For all choice and no choice tests 30 replications were used. In each test the number of oviposition attacks by *D. coccinellae* was assessed as a measure of host acceptance (Richerson and Deloach, 1972). According to Okuda and Ceryngier (2000) effective egg deposition by *D. coccinellae* can be recognized by a powerful thrust of the ovipositor into the host. Additionally, in the no choice tests the

suitability of H. axyridis as a host for the development of D. coccinellae was assessed by determining the emergence rate of the parasitoid. Attacked ladybirds were placed in an individual Petri dish, fed E. kuehniella eggs and monitored daily during the following month for the appearance of D. coccinellae cocoons and the subsequent emergence of adult wasps. Further, total larval and pupal developmental time of each successfully emerged D. coccinellae wasp was determined, as was the sex of each successfully parasitized ladybird (McCornack et al., 2007). 'Attacked' ladybirds are defined in this study as ladybirds that were stung by D. coccinellae wasps (i.e., an oviposition attack was observed). However, being stung does not imply that adult wasps successfully emerge from the coccinellid. 'Successfully parasitized' ladybirds are defined in this study as attacked ladybirds from which adult wasps emerged.

2.3. Influence of temperature on the development of D. coccinellae

Adults of H. axyridis from the laboratory population were confronted individually with a single wasp in a Petri dish (9 cm diameter, 1.3 cm high) during 9 min at 23 °C. Attacked hosts were then transferred to individual Petri dishes and exposed to one of 4 temperatures (16, 20, 23 and 27 °C), 65 ± 5% RH and a 16:8 h (L:D) photoperiod. Ladybirds were provided daily with food and water as described above. A cohort of 45 attacked adults was used per temperature regimen. For 30 days after parasitization, the adults were checked daily for emerged D. coccinellae pupae. Cocoons were collected, transferred to individual Petri dishes and maintained at the same temperature as during their immature development until adult emergence.

2.4. Effects of parasitization on host fitness

In preliminary experiments, using the non-melanic field population, some successfully parasitized females were observed to produce eggs. Because emergence rates of the parasitoids from H. axyridis adults of field populations were near to zero in this study (see further in section 3.3 of the results), the reproductive capacity of successfully parasitized H. axyridis adults was investigated in more detail using adults of the laboratory population. A group of 68 emerged adult ladybirds of the laboratory population were each allowed to be stung twice by a D. coccinellae female and then paired in Petri dishes (9 cm diameter, 1.3 cm high). All attacked individuals were maintained on E. kuehniella eggs and held at 23 °C, 65 ± 5% RH and a 16:8 h (L:D) photoperiod. Survival and oviposition of the attacked H. axyridis were monitored on a daily basis. The experiment was terminated 28 days after parasitization (i.e., 5 days more than the average egg–pupa period of D. coccinellae when attacking fourth instar H. axyridis at 23 °C in this study). A control group of 40 non-attacked adults was monitored under the same conditions.

In addition, prey consumption by successfully parasitized H. axyridis was examined. Individuals of a cohort of 42 fourth instars from the non-melanic field population of H. axyridis were each stung twice by D. coccinellae. Each attacked host larva was then placed in an individual 9 cm Petri dish and kept for 30 days. Water was provided as described above and the feeding stages of H. axyridis were daily supplied with 40 fourth instar and adult Acyrthosiphon pisum (Harris) aphids. The number of aphids consumed by each coccinellid during a 24 h period was determined 2, 14, 17 and 23 days after parasitoid attack. Aphid predation by a control group of 40 non-attacked H. axyridis was also measured.

2.5. Data analysis

The statistical program Stata/SE 9.2 was used for all statistical analyses in the study (StataCorp, 2005). None of the experimental data were normally distributed. Countable data were analyzed using a generalized linear model (Poisson distribution if applicable and negative binomial distribution in case of overdispersion: overdispersion was determined using the deviance and the Pearson goodness-of-fit statistics (i.e., deviance and Pearson chi-square divided by the number of degrees of freedom) (Hilbe, 2008)). Each analysis started with a saturated model and interactions and non-significant main factors were dropped at a significance level of 0.05. The most parsimonious model is reported, using likelihood ratios to assure model fit. Experiments studying the emergence rate (number of emerged adult wasps) were analyzed by means of a 2-sided Fisher-exact-test because in some treatments zero D. coccinellae adults emerged.

3. Results

3.1. No choice tests

Mobile stages were most frequently attacked in the no choice tests assessing attack rates on different host stages (Fig. 1(a)). Based on the Poisson regression, pupae of H. axyridis ($n = 30$) were less frequently attacked than third instars ($n = 30$) ($p = 0.005$), fourth instars ($n = 30$) ($p < 0.001$) and adults ($n = 30$) ($p < 0.001$). Third instars were less frequently attacked than fourth instars ($p < 0.001$) and adults ($p = 0.0035$), and attack rates on adults were lower than those on fourth instars ($p = 0.005$). The parasitoid successfully developed to adulthood only when larval instars were attacked, with an emergence rate of $10.0 ± 5.6\%$ from third instars and $6.7 ± 4.6\%$ from fourth instars.

The Poisson regression and 2-sided Fisher-exact-test determined that morph type did not affect the number of attacks by the wasp nor its emergence rate, respectively (Fig. 1(b)) ($n = 30$ per stadium and per morph type). Again, in this experiment successful emergence of D. coccinellae adults was only observed when larvae (fourth instars) were attacked ($6.3 ± 4.4\%$ and $7.1 ± 5.0\%$ for non-melanic and melanic fourth instars, respectively). Female non-melanic beetles ($n = 13$) were attacked more than male non-melanic beetles ($n = 17$) ($p = 0.007$) in this experiment.

The number of attacks by the wasp was similar on adults of the laboratory and melanic field population (Fig. 1(c)). Parasitoid emergence rate was slightly higher on the laboratory population than on the melanic field population ($10.0 ± 5.6\%$ versus $3.3 ± 3.3\%$, respectively), but according to the 2-sided Fisher-exact-test this difference was not statistically significant.

3.2. Choice tests

Based on the Poisson regression third instars of H. axyridis paired with a pupa ($n = 30$) were attacked more frequently than when paired with a fourth instar ($n = 30$) or adult ($n = 30$) ($p < 0.001$) (Fig. 2). Third instars combined with an adult were attacked more than when paired with a fourth instar ($p = 0.017$). Pupae placed together with a third instar ($n = 30$) were attacked more frequently than when paired with a fourth instar ($n = 30$) or adult H. axyridis ($n = 30$) ($p = 0.013$). Fourth instars and adults were attacked with the same frequency, independent of the type of developmental stage they were paired with.

When given a choice, based on the Poisson regression, the parasitoid preferred to attack adults of the non-melanic field population over those of the melanic population ($n = 30$) ($p = 0.01$ for males and $p = 0.008$ for females). No difference was found in its preference for fourth instars of either the melanic or non-melanic field population ($n = 30$) (Fig. 3). Furthermore, melanic fourth instars were attacked more frequently than melanic adults
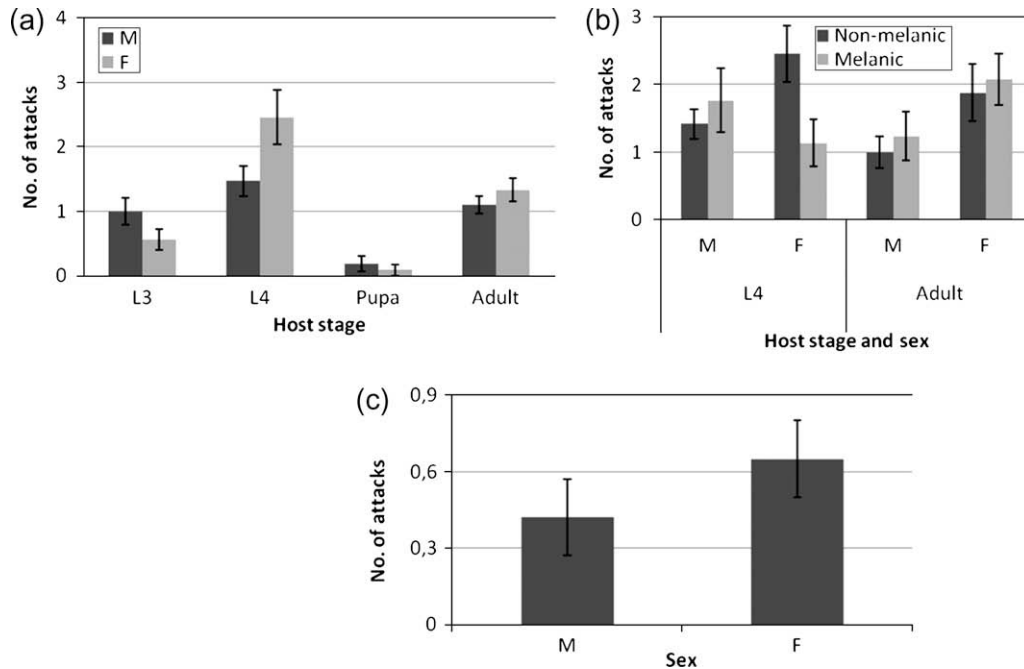
Fig. 1. Mean number of attacks (±SE) by adult *D. coccinellae* wasps on *H. axyridis* during a 5 min period in no choice tests as a function of: (a) developmental stage (b) morph type and (c) population of the host. M, male; F, female; L3, third instar; L4, fourth instar.
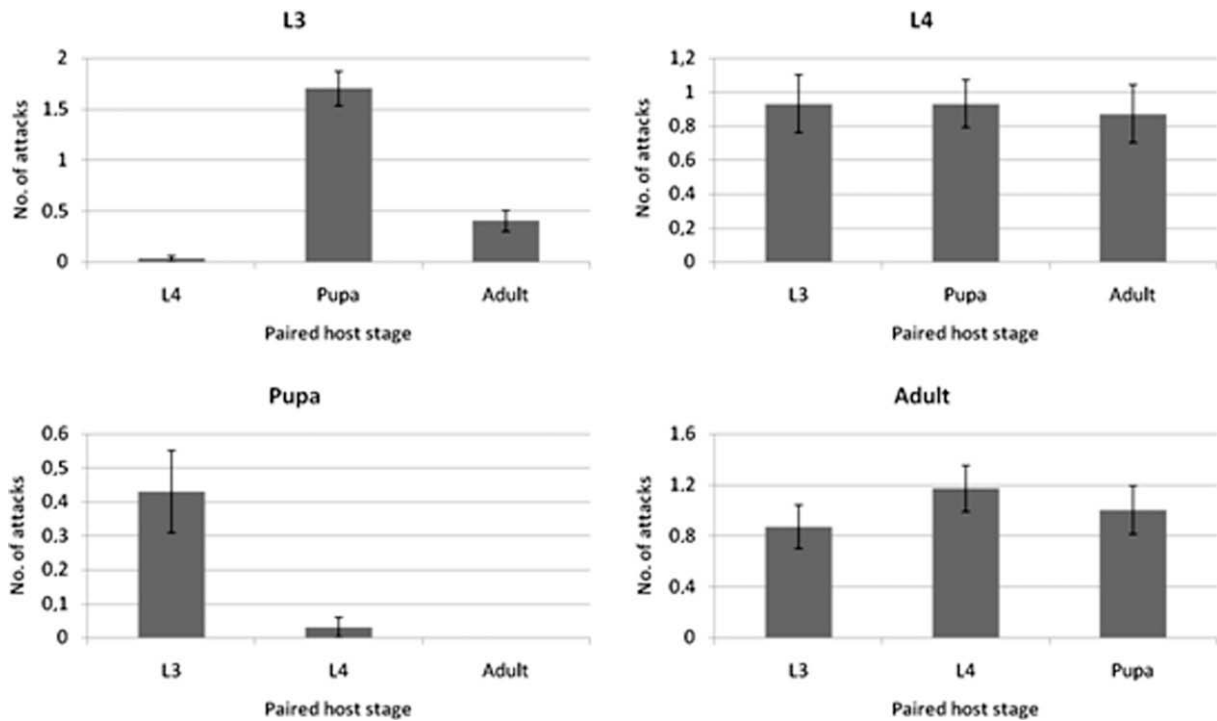

Fig. 2. Mean number of attacks (±SE) by adult *D. coccinellae* wasps on *H. axyridis* during a 5 min period in choice tests as a function of the paired host stage. L3, third instar; L4, fourth instar.

($p = 0.015$) and melanic male adults ($n = 18$) were attacked more frequently than melanic female adults ($n = 12$) ($p = 0.002$).

The Poisson regression determined that *D. coccinellae* preferred to attack melanic adults of the laboratory population ($1.00 ± 0.17$ attacks) over those of the field population ($0.33 ± 0.11$ attacks) ($n = 30$) ($p = 0.003$). The parasitoid showed no preference for males or females of the non-melanic field population ($n = 30$), with an average of $0.42 ± 0.15$ and $0.65 ± 0.15$ attacks, respectively.

3.3. Development of D. coccinellae

The developmental time of the wasp decreased with increasing temperature according to the negative binomial regression (Table 1). At 16 ($n = 9$) and 20 °C ($n = 6$) the development of *D. coccinellae* from oviposition to pupa took 27 and 25 days, respectively, and this period decreased to 20 and 15 days at 23 ($n = 10$) and 27 °C ($n = 1$), respectively ($p < 0.001$ for 16 °C compared to 23 and
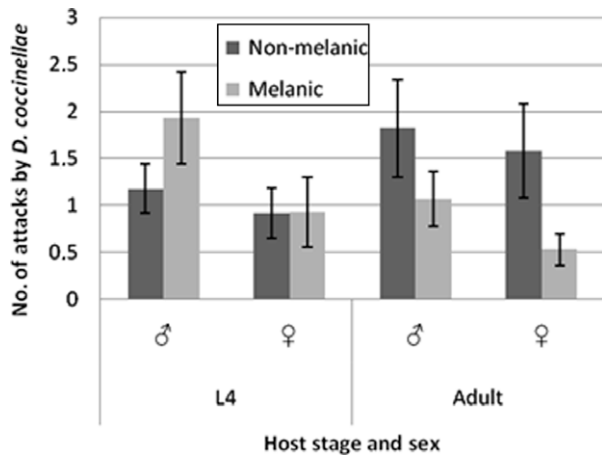
Fig. 3. Mean number of attacks (±SE) by adult *D. coccinellae* wasps on *H. axyridis* during a 5 min period in choice tests as a function of morph type of the host. L4, fourth instar.

Table 1
Developmental times (mean ± SE, days) of *D. coccinellae* parasitizing *H. axyridis* adults of a laboratory population at different temperatures.

Stage	Temperature (°C)			
	16 ($n = 9$)	20 ($n = 6$)	23 ($n = 10$)	27 ($n = 1$)
Egg–pupa	26.90 ± 0.42	25.50 ± 0.22	19.80 ± 0.36	15.00
Pupa–adult	13.44 ± 0.17	13.50 ± 0.22	10.60 ± 0.27	9.00
Total	40.34 ± 0.50	39.00 ± 0.37	30.40 ± 0.31	24.00

27 °C; $p = 0.015$ and $p = 0.001$ for 20 °C compared to 23 and 27 °C, respectively). The 2-sided Fisher-exact-test determined that the adult emergence rates of *D. coccinellae* were higher at 16 and 23 °C than at 27 °C, averaging 20.0 ± 6.0, 22.2 ± 6.3 and 2.2 ± 2.2%, respectively ($p = 0.015$ for 16 versus 27 °C and $p = 0.007$ for 23 versus 27 °C). Emergence rate at 20 °C averaged 13.3 ± 5.1% and was not statistically different from that at the other temperatures.

When the results of all experiments performed at 23 °C were pooled ($n = 87$), the negative binomial regression indicated that the attacked host stage also had an influence on developmental time of *D. coccinellae*. The egg–adult developmental time of the wasp decreased as the host stage was more advanced ($p = 0.038$ for adult ($n = 57$) versus third instar ($n = 8$) and $p = 0.081$ for adult versus fourth instar ($n = 22$)) (Table 2).

Further, *D. coccinellae* had very low emergence rates when attacking individuals of the field populations. Only 10.0 ± 5.6% of non-melanic third instars, 14.7 ± 3.5% and 7.1 ± 5.0% of non-melanic and melanic fourth instars, respectively, and 0% and 1.7 ± 1.7% of non-melanic and melanic adults, respectively, were successfully parasitized. Parasitoid emergence rate amounted up to 16.9 ± 2.9% when adults of a long term laboratory population were attacked.

Table 2
Developmental times (mean ± SE, days) of *D. coccinellae* parasitizing different stages of *H. axyridis*.

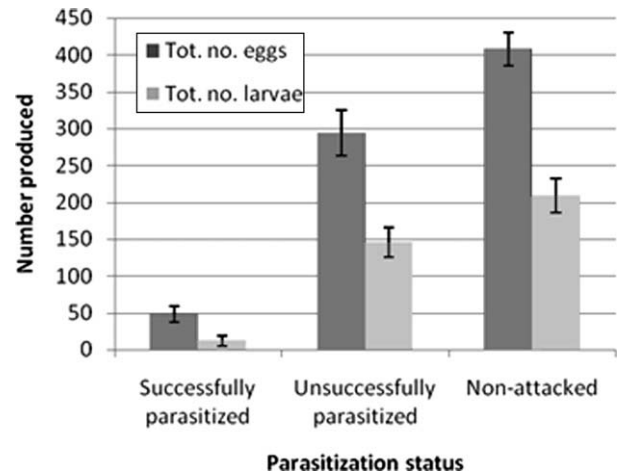| Parasitoid stage	Host stage		
	Third instar ($n = 8$)	Fourth instar ($n = 22$)	Adult ($n = 57$)
Egg–pupa	25.50 ± 0.65	23.18 ± 0.36	21.68 ± 0.55
Pupa–adult	10.38 ± 0.26	10.77 ± 0.17	9.16 ± 0.32
Egg–adult	35.88 ± 0.85	33.96 ± 0.38	30.84 ± 0.59

Fig. 4. Mean (±SE) number of eggs and larvae produced by successfully parasitized couples (successfully parasitized), attacked but not successfully parasitized couples (unsuccessfully parasitized) and non-attacked couples (non-attacked) of the laboratory population.

3.4. Effects of parasitization on host fitness

In this experiment adults that turned out to be successfully parasitized by *D. coccinellae* were able to produce fertile eggs (Fig. 4); out of the 8 couples of successfully parasitized ladybirds, the females of only 2 couples failed to oviposit. Couples with a successfully parasitized male partner laid a similar number of eggs as couples with a successfully parasitized female partner (there were no couples with both partners successfully parasitized in this experiment). Based on the negative binomial regression, the number of eggs and larvae produced by successfully parasitized couples was, however, lower than that produced by the non-attacked couples of the control group ($n = 20$) ($p \leqslant 0.001$ and $p = 0.001$, respectively) and by couples attacked by *D. coccinellae* but without successful parasitization (i.e., defined as the unsuccessfully parasitized group in this experiment) ($n = 26$) ($p = 0.006$ and $p = 0.001$). In addition, egg production by the unsuccessfully parasitized couples was lower than that of the non-attacked couples of the control group ($p = 0.026$). Egg hatch of the successfully parasitized couples (16.35 ± 8.36%) was lower than that of the unsuccessfully parasitized couples (43.17 ± 4.44%) and the non-attacked couples of the control group (49.77 ± 4.63%) ($p = 0.006$ and $p \leqslant 0.001$, respectively).

Based on the negative binomial regression, successfully parasitized fourth instars of *H. axyridis* ($n = 9$) consumed more pea aphids than did non-attacked fourth instars of the control group ($n = 42$) and the unsuccessfully parasitized group ($n = 33$) ($p = 0.067$ and $p = 0.011$, respectively) (Fig. 5). After becoming adults, successfully parasitized adults ($n = 9$) consumed similar amounts of aphids as non-attacked ($n = 38$) and unsuccessfully parasitized ($n = 31$) ladybirds in the first days after their emergence. However, about 23 days after having been attacked by *D. coccinellae* (i.e., 0–2 days prior to parasitoid emergence), the successfully parasitized *H. axyridis* adults consumed less aphids than the control adults ($p = 0.006$).

4. Discussion

Several host characteristics have been reported to influence the success of parasitization by *D. coccinellae* on *C. septempunctata*, including host stage and species (Geoghegan et al., 1998; Kadono-Okuda et al., 1995; Okuda and Ceryngier, 2000), adult age (Majerus et al., 2000) and sex (Majerus et al., 2000; Davis et al., 2006).

Developmental stage also affected the suitability of *H. axyridis* as a host for *D. coccinellae*. Both in choice and no choice tests,
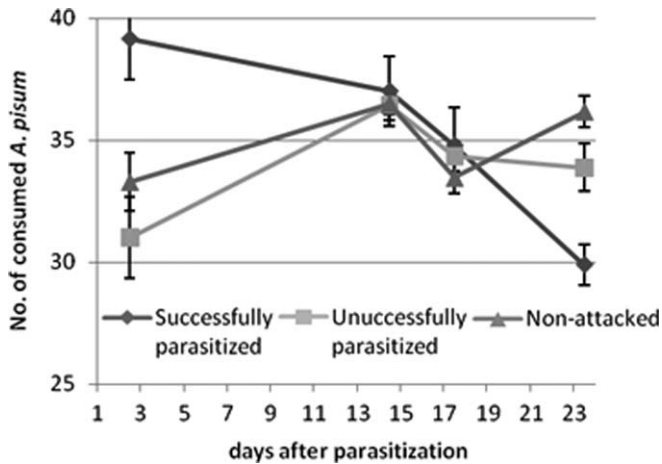
Fig. 5. Number (mean ± SE) of *A. pisum* consumed during 24 h by fourth instars (2 days after attack) and adults (14, 17 and 23 days after attack) of successfully parasitized (successfully parasitized), attacked but not successfully parasitized (unsuccessfully parasitized) and non-attacked (non-attacked) *H. axyridis* by *D. coccinellae*.

pupae and third instars were attacked less frequently than fourth instars and adults. Host detection by *D. coccinellae* is largely based on visual detection, i.e., sensing host movement (Balduf, 1926; Richerson and Deloach, 1972), and detection of olfactory cues (Orr et al., 1992; Al Abassi et al., 2001). The lower attack rates of the parasitoid on pupae and to a lesser extent on third instars of the coccinellid could be related to the lower mobility of these life stages. However, based on differences in mobility alone, the more active adults are expected to be attacked more frequently than fourth instars, as was found by Obrycki et al. (1985) and Geoghegan et al. (1998) for *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae) and *C. septempunctata*, respectively. In our experiments, however, we observed that in contrast to fourth instars, adults of *H. axyridis* were often too agile for the parasitoid female to successfully insert its ovipositor. Body size may be a further factor explaining the parasitoid's preference for fourth instars and adults over third instars. Richerson and Deloach (1972) found that *D. coccinellae* adults prefer larger coccinellid species, hypothetically because larger individuals contain greater resources and thus offer the parasitoid's offspring better chances to successfully complete larval development (Davis et al., 2006). Like Firlej et al. (2005), we found that parasitoid emergence rate decreased, as the attacked life stage of *H. axyridis* was more advanced. Obrycki et al. (1985) and Geoghegan et al. (1998), however, found that the emergence rates from adults of *C. maculata* and *C. septempunctata*, respectively, were higher than those from pupae and larvae. Development of *D. coccinellae* took longer when larvae of *H. axyridis* were parasitized than when adults were attacked. Kadono-Okuda et al. (1995) reported similar observations in *C. septempunctata* and attributed prolonged development to a pause in the development of the parasitoid's first instar, which is continued when the adult host emerges from the pupa. This is not fully corroborated by our findings, as the increase in development time of *D. coccinellae* when parasitizing fourth instar versus adult hosts, is shorter than the host's pupal stage.

In the no choice tests, *D. coccinellae* indifferently attacked the melanic and non-melanic morphs of *H. axyridis*, whereas, when given a choice, the parasitoid attacked the non-melanic morphs more often than the melanic ones. Thompson et al. (2002) and Hagen et al. (2003) stated that melanism may strengthen the cuticle and consequently make the penetration by fungi, bacteria and parasitoids more difficult. However, morph type had no effect on the emergence rates of *D. coccinellae*.

When not been given a choice, *D. coccinellae* similarly attacked melanic individuals of the laboratory and field populations. However, in a choice situation the adults of the laboratory population were attacked more frequently than those of the field population. This may be due to genetic and/or non-genetic adaptation of the host to its laboratory environment, in which defense mechanisms against natural enemies are redundant. On the other hand, the origin of the examined population had no influence on the emergence rates of the parasitoid. Berkvens et al. (2008a,b) revealed differences between laboratory and field populations of *H. axyridis* in response to food and photoperiodic conditions and cautioned that conclusions based on experiments with laboratory-reared individuals cannot simply be extrapolated to the field.

Majerus et al. (2000) and Davis et al. (2006) found female adults of *C. septempunctata* to be attacked more often than male adults. They suggested that females would provide more resources for the development of *D. coccinellae* larvae due to being larger and having a greater food intake than males. However, our experiments suggest that for *H. axyridis* the sex of larvae and adults did not affect the host preference of *D. coccinellae*.

Emergence rates of the parasitoid from the field populations of *H. axyridis* varied between 0% and 15% depending on developmental stage, whereas much higher emergence rates have been noted for other species: 58% for *Cycloneda munda* (Say) (Coleoptera: Coccinellidae) (Obrycki, 1989), 30–58% for *C. maculata* (Obrycki, 1989; Orr et al., 1992; Firlej et al., 2005), 12.5–47% for *C. septempunctata* (Obrycki, 1989; Orr et al., 1992; Triltsch, 1996) and ca. 30% for *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) (Obrycki, 1989). The low parasitoid emergence rates of *D. coccinellae* in European *H. axyridis* are analogous to the near zero rates found after arrival of this invasive coccinellid in the USA (Hoogendoorn and Heimpel, 2002) and Canada (Firlej et al., 2005). However, emergence rates may increase in the future due to selective adaptation of the parasitoid to its new host (Firlej et al., 2005; Koyama and Majerus, 2008). Firlej et al. (2007) hypothesized that the low parasitization rates on *H. axyridis* could be caused by an inhibition of the development of the teratocytes, resulting in insufficient food support for larval growth and development of the parasitoid. Although we found very low rates of successful parasitization, the parasitoid readily attacked the ladybird in our experiments, supporting the hypothesis of Hoogendoorn and Heimpel (2002) that *H. axyridis* may represent an egg-sink for *D. coccinellae* eggs. The attack of the abundant but unsuitable host *H. axyridis*, could result in a dead-end for *D. coccinellae* eggs, which, given the expansive colonization of *H. axyridis* in Europe (Adriaens et al., 2008; Brown et al., 2008), may then lead to population declines of the parasitoid in these areas. This could in turn lead to a lower parasitism pressure on other indigenous coccinellid species.

Observations cited by Hodek and Honek (1996) for other coccinellid species indicate that survival of attacked coccinellids prior to the time of emergence of the *D. coccinellae* adult was lower than that of non-attacked coccinellids. This was not the case in our study; in a preliminary experiment only an insignificant fraction (ca. 5%) of attacked fourth instar *H. axyridis* died either as a larva or an adult before the parasitoid emerged. Dissection of these dead hosts revealed the presence of a dead *D. coccinellae* larva in the haemocoel. Further research is needed to determine if such parasitoid larvae died as a result of immunity reactions within the host; alternatively, parasitization may have weakened the host to the extent that it died prematurely leading in turn to the death of the parasitoid larva. Based on these premature mortality rates and the parasitoid emergence rates corresponding with the fraction of the host population dying as a result of successful parasitization, *D. coccinellae* would in theory be capable of directly killing at best about 20% of the *H. axyridis* populations in Europe. However, such extrapolation based on laboratory experiments is highly uncertain

and most probably leads to an overestimation of the parasitoid's host suppression potential. Further field monitoring of *H. axyridis* parasitism by *D. coccinellae* is imperative to have an accurate interpretation of the parasitoid's impact on European populations of *H. axyridis*.

Fertility and fecundity rates of successfully parasitized *H. axyridis* adults of the laboratory population were about 6% and 12%, respectively, of those of the control group. In contrast, Wright and Laing (1978) reported that *C. maculata* adults parasitized by *D. coccinellae* had no reproductive outputs. Attacked *H. axyridis* in which *D. coccinellae* did not complete development, also appeared to be affected in their reproductive capacity, with reproductive rates amounting up to 70% of those of the control group. Given the near zero parasitization success in adult ladybirds from the field population and the high reproductive output of up to about 4000 eggs in their lifetime (Hodek and Honek, 1996; Koch, 2003; Berkvens et al., 2008b), the suppressive effect of *D. coccinellae* on the productive growth of *H. axyridis* is assumed to be minor.

A reduction in aphid predation rate as a result of parasitoid attack only occurred from about 23 days after parasitization of the fourth instar, i.e., when the host was about 2 weeks in its adult life. Sluss (1968) observed a similar decline in aphid predation by *H. convergens* adults when investigating the effect of parasitization by the braconid. The decline in predation rate occurs at a time when the last instar of *D. coccinellae* is developing within the host. This instar usually paralyzes the host in the short period before emergence by damaging the leg muscles (Hodek and Honek, 1996).

In conclusion, the findings of our laboratory studies suggest that *H. axyridis* is a suboptimal host for *D. coccinellae*, and that the parasitoid may have little impact on the spread of the invasive ladybird in Europe. These findings may provide support for the enemy release hypothesis as a partial explanation for the successful spread of this exotic species in Europe. However, continued monitoring of the parasitization of *H. axyridis* by the braconid in this region may potentially reveal adaptation of the parasitoid to its new host. It is equally important to investigate trends in host preference as the invasive species continues to expand its range in Europe, in order to determine if the "egg-sink" phenomenon will have an impact on the population dynamics of *D. coccinellae* and its native coccinellid hosts.

Acknowledgments

References

Al Abassi, A., Birkett, M.A., Petterson, J., Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 2001. Response of the ladybird parasitoid *Dinocampus coccinellae* to toxic alkaloids from the seven-spot ladybird, *Coccinella septempunctata*. Journal of Chemical Ecology 27, 33–43.

Adriaens, T., Gomez, G.M.Y., Maes, D., 2008. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. BioControl 53, 69–88.

Adriaens, T., Maes, D., Branquart, E., 2003. The multicoloured asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? Belgian Journal of Zoology 133, 195–196.

Balduf, W.V., 1926. The bionomics of *Dinocampus coccinellae* Schrank. Annals of the Entomological Society of America 19, 465–498.

Berkvens, N., Bonte, J., Berkvens, D., Deforce, K., Tirry, L., De Clercq, P., 2008a. Pollen as an alternative food for *Harmonia axyridis*. BioControl 53, 201–210.

Berkvens, N., Bonte, J., Berkvens, D., Tirry, L., De Clercq, P., 2008b. Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). BioControl 53, 211–221.

Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hagg, T., Kenis, M., Klausnitzer, B.E.M., Kovar, I., Loomans, A.J.M., Majerus, M.E.N., Nedved, O.,

Pedersen, Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I.A., Roy, D.B., 2008. *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. BioControl 53, 5–21.

Chapin, J.B., Brou, V.A., 1991. *Harmonia axyridis* (Pallas). The third species of the genus to be found in the United States (Coleoptera: Coccinellidae). Proceedings of the Entomological Society of Washington 93, 630–635.

Coderre, D., Lucas, E., Gagne, I., 1995. The occurrence of *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) in Canada. Canadian Entomologist 127, 609–611.

Coutanceau, J.-P., 2006. *Harmonia axyridis* (Pallas, 1773): une coccinelle asiatique introduite, acclimatée et en extension en France. Bulletin de la Société Entomologique de France 111, 395–401.

Davis, D.S., Stewart, S.L., Manica, A., Majerus, M.E.N., 2006. Adaptive preferential selection of female coccinellid hosts by the parasitoid wasp *Dinocampus coccinellae* (Hymenoptera: Braconidae). European Journal of Entomology 103, 41–45.

Firlej, A., Boivin, G., Lucas, E., Coderre, D., 2005. First report of *Harmonia axyridis* Pallas being attacked by *Dinocampus coccinellae* Schrank in Canada. Biological Invasions 7, 553–556.

Firlej, A., Lucas, E., Coderre, D., Boivin, G., 2007. Teratocytes growth pattern reflects host suitability in a host-parasitoid assemblage. Physiological Entomology 32, 181–187.

Geoghegan, I.E., Chudek, J.A., Mackay, R.L., Lowe, C., Moritz, S., McNicol, R.J., Birch, A.N.E., Hunter, G., Majerus, M.E.N., 2000. Study of anatomical changes in *Coccinella septempunctata* (Coleoptera: Coccinellidae) induced by diet and by infection with the larva of *Dinocampus coccinellae* (Hymenoptera: Braconidae) using magnetic resonance microimaging. 7th International Symposium on Ecology of Aphidophaga. Bromont, Canada. pp. 457–461.

Geoghegan, I.E., Majerus, T.M.O., Majerus, M.E.N., 1997. Notes on the coccinelid parasitoid *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) in Scotland. Entomologist 116, 179–184.

Geoghegan, I.E., Majerus, T.M.O., Majerus, M.E.N., 1998. Differential parasitisation of adult and pre-imaginal *Coccinella septempunctata* (Coleoptera: Coccinellidae) by *Dinocampus coccinellae* (Hymenoptera: Braconidae). European Journal of Entomology 95, 571–579.

Hagen, S.B., Ims, R.A., Yoccoz, N.G., 2003. Density-dependent melanism in sub-arctic populations of winter moth larvae (*Operophtera brumata*). Ecological Entomology 28, 659–665.

Hilbe, J.M., 2008. Negative Binomial Regression. Cambridge University Press, Cambridge.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht.

Hoogendoorn, M., Heimpel, G.E., 2002. Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. 7th European Workshop on Insect Parasitoids. Academic Press Inc. Elsevier Science, Haarlem, Netherlands. pp. 224–230.

Iperti, G., 1964. Les parasites des coccinelles Aphidiphages das les Alpes-Maritimes et les Basses-Alpes. Entomophaga 9, 153–180.

Katsoyannos, P., Aliniazee, M.T., 1998. First record of *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae) as a parasitoid of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in western North America. Canadian Entomology 130, 905–906.

Kadono-Okuda, K., Sakurai, H., Takeda, S., Okuda, T., 1995. Synchronous growth of a parasitoid, *Perilitus coccinellae*, and teratocytes with the development of the host, *Coccinellae septempunctata*. Entomologia Experimentalis et Applicata 75, 145–149.

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecological Evolution 17, 164–170.

Kenis, M., Roy, H.E., Zindel, R., Majerus, M.E.N., 2008. Current and potential management strategies against *Harmonia axyridis*. BioControl 53, 235–252.

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, 16–32.

Koch, R.L., Galvan, T.L., 2008. Bad side of a good beetle: the North American experience with *Harmonia axyridis*. BioControl 53, 23–35.

Koyama, S., Majerus, M.E.N., 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., 1997. Parasitization of Britisch ladybirds by *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae). British Journal of Entomology and Natural History 10, 15–24.

Majerus, M.E.N., Geoghegan, I.E., Majerus, T.M.O., 2000. Adaptive preferential selection of young coccinellid hosts by the parasitoid wasp *Dinocampus coccinellae* (Hymenoptera: Braconidae). European Journal of Entomology 97, 161–164.

McCornack, B.P., Koch, R.L., Ragsdale, D.W., 2007. A simple method for in-field sex determination of the multicolored Asian lady beetle *Harmonia axyridis*. Journal of Insect Science 7, 1–12.

Mitchell, C.E., Power, A.G., 2003. Release of invasive plants from fungal and viral pathogens. Nature 421, 625–627.

Nalepa, C.A., Kidd, K.A., Ahlstrom, K.R., 1996. Biology of *Harmonia axyridis* (Coleoptera: Coccinellidae) in winter aggregations. Annals of the Entomological Society of America 89, 681–685.

Obrycki, J.J., 1989. Parasitization of native and exotic coccinellids by *Dinocampus coccinellae* (Schrank) (Hymenoptera, Braconidae). Journal of the Kansas Entomological Society 62, 211–218.

Obrycki, J.J., Tauber, M.J., Tauber, C.A., 1985. *Perilitus coccinellae* (Hymenoptera: Braconidae): parasitization and development in relation to host-stage attacked. Annals of the Entomological Society of America 78, 852–854.

Okuda, T., Ceryngier, P., 2000. Host discrimination in *Dinocampus coccinellae* (Hymenoptera: Braconidae), a solitary parasitoid of coccinellid beetles. Applied Entomological and Zoology 35, 535–539.

Orr, C.J., Obrycki, J.J., Flanders, R.V., 1992. Host-acceptance behaviour of *Dinocampus coccinellae* (Hymenoptera, Braconidae). Annals of the Entomological Society of America 85, 722–730.

Richerson, J.V., Deloach, C.J., 1972. Some aspects of host selection by *Perilitus coccinellae* Hymenoptera Braconidae. Annals of the Entomological Society of America 65, 834–839.

Roy, H.E., Brown, P.M.J., Rothery, P., Ware, R.L., Majerus, M.E.M., 2008. Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. Biocontrol 53, 265–276.

Shapiro-Ilan, D.I., Cottrell, T.E., 2005. Susceptibility of lady beetles (Coleoptera: Coccinellidae) to entomopathogenic nematodes. Journal of Invertebrate Pathology 89, 150–156.

Sluss, R., 1968. Behavioural and anatomical responses of Convergent lady beetle to parasitism by *Perilitus coccinellae* (Schrank) (Hymenoptera – Braconidae). Journal of Invertebrate Pathology 10, 9.

StataCorp, 2005. Stata Statistical Software: Release 9. StataCorp LP, College Station, TX.

Thompson, J.J.W., Armitage, S.A.O., Siva-Jothy, M.T., 2002. Cuticular colour change after imaginal eclosion is time-constrained: blacker beetles darken faster. Physiological Entomology 27, 136–141.

Triltsch, H., 1996. On the parasitization of the ladybird *Coccinella septempunctata* L. (Col, Coccinellidae). Journal of Applied Entomology 120, 375–378.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630.

Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2002. Parasites and marine invasions. Parasitology 124, 137–151.

Wright, E.J., Laing, J.E., 1978. Effects of temperature on development, adult longevity and fecundity of *Coleomegilla maculata* Lengi and its parasite, *Perilitus coccinellae*. Proceedings of the Entomological Society of Ontario 109, 33–47.