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

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42 **Abstract**

43 The enemy release hypothesis states that the absence of effective natural enemies can be a  
44 fundamental aspect leading to the successful establishment of an invasive species. This study  
45 investigates the impact of the native braconid parasitoid *Dinocampus coccinellae* on European  
46 populations of the invasive ladybird *Harmonia axyridis*. The parasitoid attacked adults and  
47 fourth instars of the ladybird more frequently than pupae and third instars. When given a  
48 choice, *D. coccinellae* attacked non-melanic adults and adults of a long term laboratory  
49 population of *H. axyridis* more often than melanic adults and adults of a field population,  
50 respectively. However, in no choice tests the parasitoid attacked individuals of either morph  
51 type and of the field and laboratory populations with the same frequency. Immature  
52 development of *D. coccinellae* took longer at lower temperatures and when less advanced  
53 developmental host stages (larvae and pupae) were successfully parasitized. The parasitoid  
54 emergence rates ranged from 0 to 14.7% on field populations of *H. axyridis* depending on life  
55 stage of the host attacked and up to 16.9% when adults of a long term laboratory population  
56 were attacked. Successfully parasitized ladybirds showed reduced reproductive capacities (6-  
57 12% of unparasitized individuals) and aphid consumption rates (85% of unparasitized  
58 individuals), but these effects together with the low emergence rates of the parasitoid suggest  
59 that *D. coccinellae* may only have a marginal impact on the population growth of *H. axyridis* in  
60 Europe.

61

62 **Keywords:** *Harmonia axyridis*, *Dinocampus coccinellae*, enemy release hypothesis,  
63 establishment, invasive species, morph type, Biological control, coccinellid, braconid

64

65 **Introduction**

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

77         According to the enemy release hypothesis, the absence of effective natural enemies is  
78 assumed to be one of the key factors leading to the successful establishment of an invasive  
79 species (Keane & Crawley 2002; Torchin et al. 2002, 2003; Mitchell & Power 2003). Recorded  
80 natural enemies of *H. axyridis* include the pathogens *Metarhizium anisopliae* (Metchnikoff)  
81 Sorokin (Deuteromycotina: Hyphomycetes), *Beauveria bassiana* (Balsamo) Vuillemin  
82 (Deuteromycotina: Hyphomycetes) and *Hesperomyces virescens* Thaxter (Laboulbeniales:  
83 Ascomycetes), certain bird species (e.g. *Picus canus* Gmelin (Piciformes: Picidae) and *Sitta*  
84 *europaea* L. (Passeriformes: Sittidae)), the parasitoids *Dinocampus coccinellae* (Schrank)  
85 (Hymenoptera: Braconidae), *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae), *Medina*  
86 *luctuosa* (Meigen) (Diptera: Tachinidae), *Medina separata* (Meigen) (Diptera: Tachinidae) and  
87 *Phalacrotophora philaxyridis* Disney (Diptera: Phoridae), the nematodes *Heterorhabditis*  
88 *bacteriophora* Poinar (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Weiser)

89 (Rhabditida: Steinernematidae), and the parasitic mite *Coccipolipus hippodamiae* (McDaniel and  
90 Moril) (Actinedida: Podapolipidae) (Kenis et al., 2008). However, not much is known in the  
91 literature about the potential impact of most of these natural enemies on the coccinellid.

92           During a field survey in Belgium in 2005, three *H. axyridis* adults successfully parasitized  
93 by the braconid parasitoid *D. coccinellae* were found (N. Berkvens, unpublished data),  
94 constituting the first field report of the braconid parasitizing *H. axyridis* in Europe. *Dinocampus*  
95 *coccinellae* is a solitary endoparasitoid that exclusively parasitizes coccinellid species of the  
96 subfamily Coccinellinae (Balduf, 1926; Obrycki, 1989; Hodek and Honěk, 1996; Majerus, 1997).  
97 The species exhibits thelytokous parthenogenesis, with only a handful of male specimens ever  
98 having been recorded (Davis et al., 2006). This cosmopolitan wasp is multivoltine over much of  
99 its geographical range (Majerus, 1997). In Europe, the seven-spotted ladybird *Coccinella*  
100 *septempunctata* L. (Coleoptera: Coccinellidae) is the most common host for *D. coccinellae*  
101 (Obrycki, 1989; Geoghegan et al. 1997, 2000). Whereas several studies have been carried out on  
102 the parasitization of *C. septempunctata* by *D. coccinellae* in different areas (Kadono-Okuda et al.,  
103 1995; Geoghegan et al., 1997, 1998, 2000; Majerus et al., 2000; Okuda and Ceryngier, 2000;  
104 Davis et al., 2006), little is known about the interactions between *D. coccinellae* and its recent  
105 potential host in Europe, the invasive coccinellid *H. axyridis*. Hoogendoorn and Heimpel (2002),  
106 Firlej et al. (2005, 2007) and Koyama and Majerus (2008) have studied interactions between  
107 both species in the USA, Canada and the UK, respectively, and all noted a relatively low  
108 susceptibility of the invasive coccinellid to the parasitoid.

109           The aim of this study is to investigate a number of interactions between the native  
110 parasitoid *D. coccinellae* and the invasive coccinellid *H. axyridis* as to determine which impact  
111 the parasitoid could potentially have on the establishment capacity of the coccinellid in western

112 Europe. We have investigated the host acceptance and the parasitization success of *D.*  
113 *coccinellae* on *H. axyridis* in the laboratory. Furthermore, we have examined the impact of  
114 parasitization on the reproductive capacity and food uptake of the coccinellid. Previous studies  
115 have shown that compared to native coccinellids *H. axyridis* is less susceptible to the impact of  
116 several natural enemies, including the tachinid fly *S. triangulifera* (Katsoyannos & Aliniaze, 1998),  
117 the nematode *S. carpocapsae* (Shapiro-Ilan & Cottrell, 2005) and the fungus *B. bassiana*  
118 (Roy et al., 2008). By broadening the range of examined natural enemies of *H. axyridis* with the  
119 braconid *D. coccinellae*, we set out to further investigate the significance of the enemy release  
120 hypothesis for the colonization success of this invasive coccinellid in Europe.

121

## 122 **Materials and Methods**

### 123 ***Insect Populations***

#### 124 ***Harmonia axyridis***

125 Three populations of *H. axyridis* were used: a long term melanic laboratory population, a non-  
126 melanic or 'red' field population and a melanic or 'black' field population. The laboratory  
127 population was started with larvae acquired from Biobest NV (Westerlo, Belgium) in September  
128 1998. The field populations originated from individuals of an established wild population  
129 collected between August 2006 and May 2008 in Ghent, Belgium. Individuals were identified  
130 using the morphological characteristics described in Chapin and Brou (1991) and Adriaens et al.  
131 (2003). All populations were established and reared as described by Berkvens et al. (2008a,b).  
132 All populations were reared on frozen *Ephestia kuehniella* Zeller eggs (Lepidoptera: Pyralidae).  
133 The experiments were conducted using individuals of the 61<sup>st</sup> to 65<sup>th</sup> generation (choice and no  
134 choice tests, development of *D. coccinellae* at different temperatures, aphid predation

135 experiments) and of the 82<sup>nd</sup> generation (host fitness experiments) of the laboratory population  
136 and of the 3<sup>rd</sup> to 6<sup>th</sup> generation of the field populations.

137 **Dinocampus coccinellae**

138 A culture of *D. coccinellae* was started in April 2006 with two adult wasps, one emerging from a  
139 *C. septempunctata* adult and another from a *H. axyridis* adult, both collected during field  
140 sampling in Ghent, Belgium. The adult coccinellids were identified using morphological  
141 characteristics described in Ipert (1964). *Harmonia axyridis* adults from the laboratory  
142 population were used to establish a culture of the braconid. At the start of each generation  
143 single females of *D. coccinellae* were confronted with five adult ladybirds (4-21 days old) in a  
144 plastic Petri dish (14 cm diameter, 1.5 cm high) during a period of 30 min. The ladybirds were  
145 then transferred to rearing containers (30 x 17 x 9 cm), holding about 30 individuals. The bottom  
146 of the containers was covered with paper toweling and the lid had a large rectangular opening  
147 screened with fine mesh for ventilation. The rearing containers were kept in an incubator at 23 ±  
148 1 °C, 65 ± 5% relative humidity (RH) and a 16:8 h (L:D) photoperiod. The ladybirds were provided  
149 daily with *E. kuehniella* eggs ad libitum as food. A moist paper plug fitted into a plastic dish  
150 served as a water source. During the 4 weeks after parasitization, the containers and ladybirds  
151 were checked daily for *D. coccinellae* cocoons. Cocoons were collected and placed individually in  
152 a Petri dish (9 cm diameter, 1.3 cm high). After adult emergence, a paper plug fitted into a  
153 plastic dish was added to the Petri dish, which was then moistened daily with a 15% honey-  
154 water solution. *Dinocampus coccinellae* wasps of the 7<sup>th</sup> to 13<sup>th</sup> generation (choice and no choice  
155 tests, development of *D. coccinellae* at different temperatures, aphid predation experiments)  
156 and of the 34<sup>th</sup> and 35<sup>th</sup> generation (host fitness experiments) of laboratory rearing were used in  
157 the study; all *D. coccinellae* adults used in the experiments were 2-4 days old. In each generation  
158 approximately 120 *H. axyridis* adults of the melanic laboratory population were allowed to be

159 attacked by individual wasps from which generally 15 to 30 adult parasitoids successfully  
160 emerged (i.e., an emergence rate of 10 to 25%).

## 161 **Experiments**

### 162 **Choice and no choice tests**

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

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

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



182 in the no choice tests the suitability of *H. axyridis* as a host for the development of *D. coccinellae*  
183 was assessed by determining the emergence rate of the parasitoid. Attacked ladybirds were  
184 placed in an individual Petri dish, fed *E. kuehniella* eggs and monitored daily during the following  
185 month for the appearance of *D. coccinellae* cocoons and the subsequent emergence of adult  
186 wasps. Further, total larval and pupal developmental time of each successfully emerged *D.*  
187 *coccinellae* wasp was determined, as was the sex of each successfully parasitized ladybird  
188 (McCornack et al., 2007). ‘Attacked’ ladybirds are defined in this study as ladybirds that were  
189 stung by *D. coccinellae* wasps (i.e. an oviposition attack was observed). However, being stung  
190 does not imply that adult wasps successfully emerge from the coccinellid. ‘Successfully  
191 parasitized’ ladybirds are defined in this study as attacked ladybirds from which adult wasps  
192 emerged.

### 193 ***Influence of temperature on the development of D. coccinellae***

194 Adults of *H. axyridis* from the laboratory population were confronted individually with a single  
195 wasp in a Petri dish (9 cm diameter, 1.3 cm high) during 9 minutes at 23°C. Attacked hosts were  
196 then transferred to individual Petri dishes and exposed to one of 4 temperatures (16, 20, 23 and  
197 27 °C), 65 ± 5% RH and a 16:8 h (L:D) photoperiod. Ladybirds were provided daily with food and  
198 water as described above. A cohort of 45 attacked adults was used per temperature regimen.  
199 For 30 days after parasitization, the adults were checked daily for emerged *D. coccinellae* pupae.  
200 Cocoons were collected, transferred to individual Petri dishes and maintained at the same  
201 temperature as during their immature development until adult emergence.

### 202 ***Effects of parasitization on host fitness***

203 In preliminary experiments, using the non-melanic field population, some successfully  
204 parasitized females were observed to produce eggs. Because emergence rates of the parasitoids  
205 from *H. axyridis* adults of field populations were near to zero in this study (see further in section

206 3.3 of the results), the reproductive capacity of successfully parasitized *H. axyridis* adults was  
207 investigated in more detail using adults of the laboratory population. A group of 68 emerged  
208 adult ladybirds of the laboratory population were each allowed to be stung twice by a *D.*  
209 *coccinellae* female and then paired in Petri dishes (9 cm diameter, 1.3 cm high). All attacked  
210 individuals were maintained on *E. kuehniella* eggs and held at 23°C, 65 ± 5% RH and a 16:8 h  
211 (L:D) photoperiod. Survival and oviposition of the attacked *H. axyridis* were monitored on a daily  
212 basis. The experiment was terminated 28 days after parasitization (i.e. 5 days more than the  
213 average egg-pupa period of *D. coccinellae* when attacking fourth instar *H. axyridis* at 23°C in this  
214 study). A control group of 40 non-attacked adults was monitored under the same conditions.

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

### 223 **Data analysis**

224 The statistical program Stata/SE 9.2 was used for all statistical analyses in the study (StataCorp,  
225 2005). None of the experimental data were normally distributed. Countable data were analyzed  
226 using a generalized linear model (Poisson distribution if applicable and negative binomial  
227 distribution in case of overdispersion: overdispersion was determined using the deviance and  
228 the Pearson goodness-of-fit statistics (i.e. deviance and Pearson chi-square divided by the  
229 number of degrees of freedom) (Hilbe, 2008)). Each analysis started with a saturated model and

230 interactions and non-significant main factors were dropped at a significance level of 0.05. The  
231 most parsimonious model is reported, using likelihood ratios to assure model fit. Experiments  
232 studying the emergence rate (number of emerged adult wasps) were analyzed by means of a 2-  
233 sided Fisher-exact-test because in some treatments zero *D. coccinellae* adults emerged.

## 234 **Results**

### 235 ***No choice tests***

236 Mobile stages were most frequently attacked in the no choice tests assessing attack rates on  
237 different host stages (Fig. 1(a)). Based on the Poisson regression, pupae of *H. axyridis* (n = 30)  
238 were less frequently attacked than third instars (n = 30) (p = 0.005), fourth instars (n = 30) (p <  
239 0.001) and adults (n = 30) (p < 0.001). Third instars were less frequently attacked than fourth  
240 instars (p < 0.001) and adults (p = 0.0035), and attack rates on adults were lower than those on  
241 fourth instars (p = 0.005). The parasitoid successfully developed to adulthood only when larval  
242 instars were attacked, with an emergence rate of  $10.0 \pm 5.6$  % from third instars and  $6.7 \pm 4.6$ %  
243 from fourth instars.

244 The Poisson regression and 2-sided Fisher-exact-test determined that morph type did  
245 not affect the number of attacks by the wasp nor its emergence rate, respectively (Fig. 1(b)) (n =  
246 30 per stadium and per morph type). Again, in this experiment successful emergence of *D.*  
247 *coccinellae* adults was only observed when larvae (fourth instars) were attacked ( $6.3 \pm 4.4$ % and  
248  $7.1 \pm 5.0$ % for non-melanic and melanic fourth instars, respectively). Female non-melanic  
249 beetles (n = 13) were attacked more than male non-melanic beetles (n = 17) (p = 0.007) in this  
250 experiment.

251 The number of attacks by the wasp was similar on adults of the laboratory and melanic  
252 field population (Fig. 1(c)). Parasitoid emergence rate was slightly higher on the laboratory

253 population than on the melanic field population ( $10.0 \pm 5.6$  % versus  $3.3 \pm 3.3\%$ , respectively),  
254 but according to the 2-sided Fisher-exact-test this difference was not statistically significant.

255 **Choice tests**

256 Based on the Poisson regression third instars of *H. axyridis* paired with a pupa ( $n = 30$ ) were  
257 attacked more frequently than when paired with a fourth instar ( $n = 30$ ) or adult ( $n = 30$ ) ( $p <$   
258  $0.001$ ) (Fig. 2). Third instars combined with an adult were attacked more than when paired with  
259 a fourth instar ( $p = 0.017$ ). Pupae placed together with a third instar ( $n = 30$ ) were attacked  
260 more frequently than when paired with a fourth instar ( $n = 30$ ) or adult *H. axyridis* ( $n = 30$ ) ( $p =$   
261  $0.013$ ). Fourth instars and adults were attacked with the same frequency, independent of the  
262 type of developmental stage they were paired with.

263         When given a choice, based on the Poisson regression, the parasitoid preferred to  
264 attack adults of the non-melanic field population over those of the melanic population ( $n = 30$ )  
265 ( $p = 0.01$  for males and  $p = 0.008$  for females). No difference was found in its preference for  
266 fourth instars of either the melanic or non-melanic field population ( $n = 30$ ) (Fig. 3).  
267 Furthermore, melanic fourth instars were attacked more frequently than melanic adults ( $p =$   
268  $0.015$ ) and melanic male adults ( $n = 18$ ) were attacked more frequently than melanic female  
269 adults ( $n = 12$ ) ( $p = 0.002$ ).

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

275 **Development of *D. coccinellae***

276 The developmental time of the wasp decreased with increasing temperature according to the  
277 negative binomial regression (Table 1). At 16 (n = 9) and 20°C (n = 6) the development of *D.*  
278 *coccinellae* from oviposition to pupa took 27 and 25 days, respectively, and this period  
279 decreased to 20 and 15 days at 23 (n = 10) and 27 °C (n = 1), respectively ( $p < 0.001$  for 16°C  
280 compared to 23 and 27 °C;  $p = 0.015$  and  $p = 0.001$  for 20°C compared to 23 and 27 °C,  
281 respectively). The 2-sided Fisher-exact-test determined that the adult emergence rates of *D.*  
282 *coccinellae* were higher at 16 and 23 °C than at 27°C, averaging  $20.0 \pm 6.0$ ,  $22.2 \pm 6.3$  and  $2.2 \pm$   
283  $2.2$  %, respectively ( $p = 0.015$  for 16 versus 27°C and  $p = 0.007$  for 23 versus 27°C). Emergence  
284 rate at 20°C averaged  $13.3 \pm 5.1\%$  and was not statistically different from that at the other  
285 temperatures.

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

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

297 ***Effects of parasitization on host fitness***

298 In this experiment adults that turned out to be successfully parasitized by *D. coccinellae*  
299 were able to produce fertile eggs (Fig. 4); out of the 8 couples of successfully parasitized  
300 ladybirds, the females of only 2 couples failed to oviposit. Couples with a successfully parasitized  
301 male partner laid a similar number of eggs as couples with a successfully parasitized female  
302 partner (there were no couples with both partners successfully parasitized in this experiment).  
303 Based on the negative binomial regression, the number of eggs and larvae produced by  
304 successfully parasitized couples was, however, lower than that produced by the non-attacked  
305 couples of the control group ( $n = 20$ ) ( $p \leq 0.001$  and  $p = 0.001$ , respectively) and by couples  
306 attacked by *D. coccinellae* but without successful parasitization (i.e. defined as the  
307 unsuccessfully parasitized group in this experiment) ( $n = 26$ ) ( $p = 0.006$  and  $p = 0.001$ ). In  
308 addition, egg production by the unsuccessfully parasitized couples was lower than that of the  
309 non-attacked couples of the control group ( $p = 0.026$ ). Egg hatch of the successfully parasitized  
310 couples ( $16.35 \pm 8.36\%$ ) was lower than that of the unsuccessfully parasitized couples ( $43.17 \pm$   
311  $4.44\%$ ) and the non-attacked couples of the control group ( $49.77 \pm 4.63\%$ ) ( $p = 0.006$  and  $p \leq$   
312  $0.001$ , respectively).

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

321 **Discussion**

322 Several host characteristics have been reported to influence the success of parasitization by *D.*  
323 *coccinellae* on *C. septempunctata*, including host stage and species (Geoghegan et al., 1988;  
324 Kadono-Okuda et al., 1995; Okuda and Ceryngier, 2000), adult age (Majerus et al., 2000) and sex  
325 (Majerus et al., 2000; Davis et al., 2006).

326 Developmental stage also affected the suitability of *H. axyridis* as a host for *D.*  
327 *coccinellae*. Both in choice and no choice tests, pupae and third instars were attacked less  
328 frequently than fourth instars and adults. Host detection by *D. coccinellae* is largely based on  
329 visual detection, i.e. sensing host movement (Balduf, 1926; Richerson and Deloach, 1972), and  
330 detection of olfactory cues (Orr et al., 1992; Al Abassi et al., 2001). The lower attack rates of the  
331 parasitoid on pupae and to a lesser extent on third instars of the coccinellid could be related to  
332 the lower mobility of these life stages. However, based on differences in mobility alone, the  
333 more active adults are expected to be attacked more frequently than fourth instars, as was  
334 found by Obrycki et al. (1985) and Geoghegan et al. (1998) for *Coleomegilla maculata* Lengi  
335 (Coleoptera: Coccinellidae) and *C. septempunctata*, respectively. In our experiments, however,  
336 we observed that in contrast to fourth instars, adults of *H. axyridis* were often too agile for the  
337 parasitoid female to successfully insert its ovipositor. Body size may be a further factor  
338 explaining the parasitoid's preference for fourth instars and adults over third instars. Richerson  
339 and Deloach (1972) found that *D. coccinellae* adults prefer larger coccinellid species,  
340 hypothetically because larger individuals contain greater resources and thus offer the  
341 parasitoid's offspring better chances to successfully complete larval development (Davis et al.,  
342 2006). Like Firlej et al. (2005), we found that parasitoid emergence rate decreased, as the  
343 attacked life stage of *H. axyridis* was more advanced. Obrycki et al. (1985) and Geoghegan et al.  
344 (1998), however, found that the emergence rates from adults of *C. maculata* and *C.*

345 *septempunctata*, respectively, were higher than those from pupae and larvae. Development of  
346 *D. coccinellae* took longer when larvae of *H. axyridis* were parasitized than when adults were  
347 attacked. Kadono-Okuda et al. (1995) reported similar observations in ~~for~~ *C. septempunctata*  
348 and attributed prolonged development to a pause in the development of the parasitoid's first  
349 instar, which is continued when the adult host emerges from the pupa. This is not fully  
350 corroborated by our findings, as the increase in development time of *D. coccinellae* when  
351 parasitizing fourth instar versus adult hosts, is shorter than the host's pupal stage.

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

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



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

372           Emergence rates of the parasitoid from the field populations of *H. axyridis* varied  
373 between 0 and 15% depending on developmental stage, whereas much higher emergence rates  
374 have been noted for other species: 58% for *Cycloneda munda* (Say) (Coleoptera: Coccinellidae)  
375 (Obrycki, 1989), 30 - 58% for *C. maculata* (Obrycki, 1989; Orr et al., 1992; Firlej et al., 2005), 12.5  
376 - 47% for *C. septempunctata* (Obrycki, 1989; Orr et al., 1992; Triltsch, 1996) and ca. 30% for  
377 *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) (Obrycki, 1989). The low  
378 parasitoid emergence rates of *D. coccinellae* in European *H. axyridis* are analogous to the near  
379 zero rates found after arrival of this invasive coccinellid in the USA (Hoogendoorn and Heimpel,  
380 2002) and Canada (Firlej et al., 2005). However, emergence rates may increase in the future due  
381 to selective adaptation of the parasitoid to its new host (Firlej et al., 2005; Koyama and Majerus,  
382 2007). Firlej et al. (2007) hypothesized that the low parasitization rates on *H. axyridis* could be  
383 caused by an inhibition of the development of the teratocytes, resulting in insufficient food  
384 support for larval growth and development of the parasitoid. Although we found very low rates  
385 of successful parasitization, the parasitoid readily attacked the ladybird in our experiments,  
386 supporting the hypothesis of Hoogendoorn and Heimpel (2002) that *H. axyridis* may represent  
387 an egg-sink for *D. coccinellae* eggs. The attack of the abundant but unsuitable host *H. axyridis*,  
388 could result in a dead-end for *D. coccinellae* eggs, which, given the expansive colonization of *H.*  
389 *axyridis* in Europe (Adriaens et al., 2008; Brown et al., 2008), may then lead to population

390 declines of the parasitoid in these areas. This could in turn lead to a lower parasitism pressure  
391 on other indigenous coccinellid species.

392 Observations cited by Hodek and Honek (1996) for other coccinellid species indicate  
393 that survival of attacked coccinellids prior to the time of emergence of the *D. coccinellae* adult  
394 was lower than that of non-attacked coccinellids. This was not the case in our study; in a  
395 preliminary experiment only an insignificant fraction (ca 5%) of attacked fourth instar *H. axyridis*  
396 died either as a larva or an adult before the parasitoid emerged. Dissection of these dead hosts  
397 revealed the presence of a dead *D. coccinellae* larva in the haemocoel. Further research is  
398 needed to determine if such parasitoid larvae died as a result of immunity reactions within the  
399 host; alternatively, parasitization may have weakened the host to the extent that it died  
400 prematurely leading in turn to the death of the parasitoid larva. Based on these premature  
401 mortality rates and the parasitoid emergence rates corresponding with the fraction of the host  
402 population dying as a result of successful parasitization, *D. coccinellae* would in theory be  
403 capable of directly killing at best about 20% of the *H. axyridis* populations in Europe. However,  
404 such extrapolation based on laboratory experiments is highly uncertain and most probably leads  
405 to an overestimation of the parasitoid's host suppression potential. Further field monitoring of  
406 *H. axyridis* parasitism by *D. coccinellae* is imperative to have an accurate interpretation of the  
407 parasitoid's impact on European populations of *H. axyridis*.

408 Fertility and fecundity rates of successfully parasitized *H. axyridis* adults of the  
409 laboratory population were about 6% and 12%, respectively, of those of the control group. In  
410 contrast, Wright and Laing (1978) reported that *C. maculata* adults parasitized by *D. coccinellae*,  
411 had no reproductive outputs. Attacked *H. axyridis* in which *D. coccinellae* did not complete  
412 development, also appeared to be affected in their reproductive capacity, with reproductive

413 rates amounting up to 70% of those of the control group. Given the near zero parasitization  
414 success in adult ladybirds from the field population and the high reproductive output of up to  
415 about 4000 eggs in their lifetime (Hodek & Honek, 1996; Koch, 2003; Berkvens et al., 2008b), the  
416 suppressive effect of *D. coccinellae* on the productive growth of *H. axyridis* is assumed to be  
417 minor.

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

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

434

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440

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567

568 **Fig. 1.** Mean number of attacks ( $\pm$ SE) by adult *D. coccinellae* wasps on *H. axyridis* during a 5 min  
569 period in no choice tests as a function of: (a) developmental stage (b) morph type and (c)  
570 population of the host  
571 M: male, F: female, L3: 3<sup>rd</sup> instar; L4: 4<sup>th</sup> instar.

572

573 **Fig. 2.** Mean number of attacks ( $\pm$ SE) by adult *D. coccinellae* wasps on *H. axyridis* during a 5 min  
574 period in choice tests as a function the paired host stage  
575 L3: 3<sup>rd</sup> instar; L4: 4<sup>th</sup> instar.

576

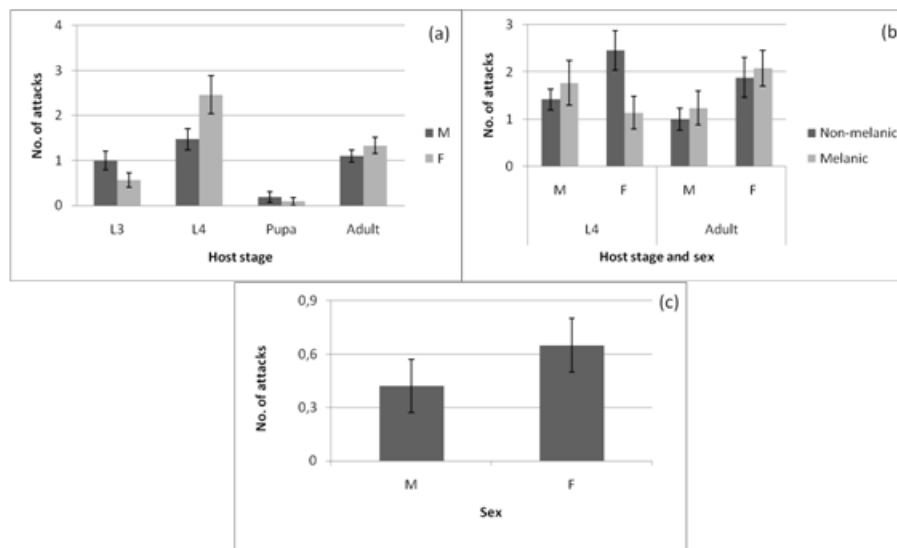
577 **Fig. 3.** Mean number of attacks ( $\pm$ SE) by adult *D. coccinellae* wasps on *H. axyridis* during a 5 min  
578 period in choice tests as a function of morph type of the host  
579 L4: 4<sup>th</sup> instar.

580

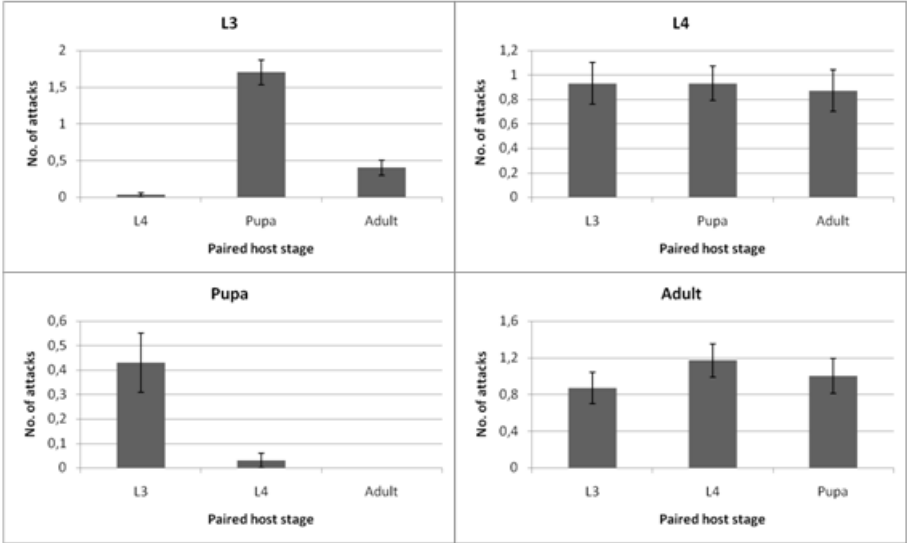
581 **Fig. 4.** Mean ( $\pm$ SE) number of eggs and larvae produced by successfully parasitized couples  
582 (Successfully parasitized), attacked but not successfully parasitized couples (Unsuccessfully  
583 parasitized) and non-attacked couples (Non-attacked) of the laboratory population.

584

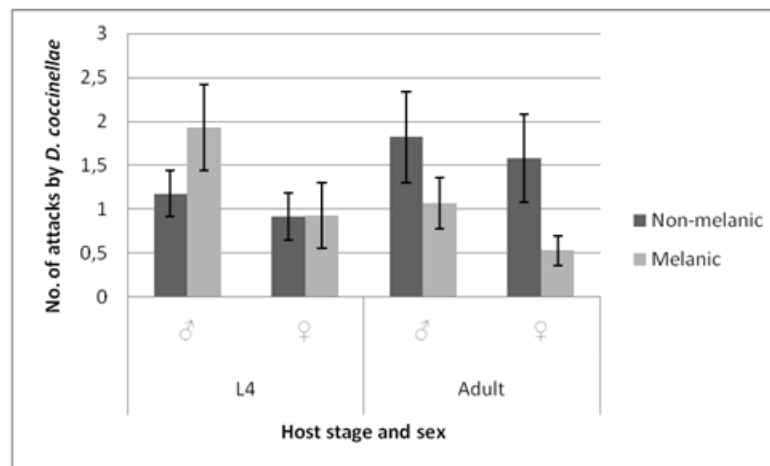
585 **Fig. 5.** Number (mean  $\pm$  SE) of *A. pisum* consumed during 24h by fourth instars (2 days after  
586 attacked) and adults (14, 17 and 23 days after attack) of successfully parasitized (Successfully  
587 parasitized), attacked but not successfully parasitized (Unsuccessfully parasitized) and non-  
588 attacked (Non-attacked) *H. axyridis* by *D. coccinellae*.



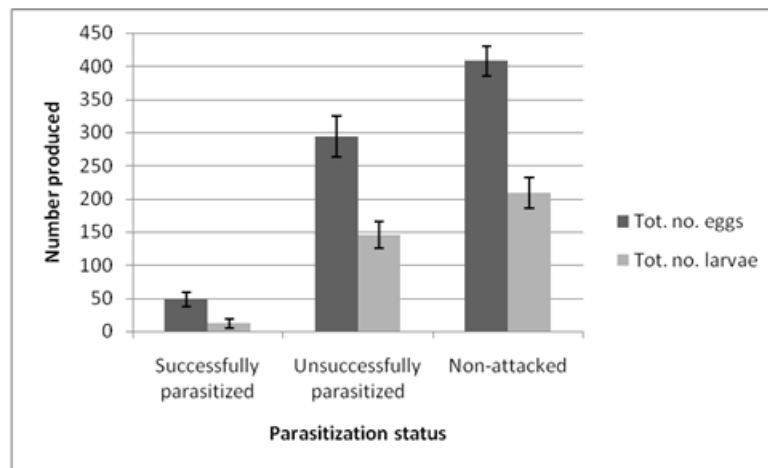
589 Fig. 1



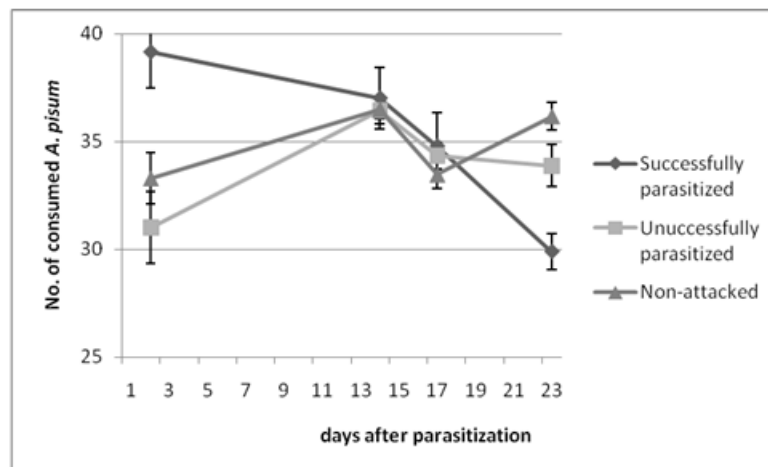
590 Fig. 2



591 Fig. 3



592 Fig. 4



593 Fig. 5