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RESEARCH PAPER

The invasive coccinellid *Harmonia axyridis* (Coleoptera: Coccinellidae) is a less suitable host for parasitism than resident species

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Abstract

V. Romero, T. Zaviezo, and A.A. Grez. 2020. The invasive coccinellid *Harmonia axyridis* (Coleoptera: Coccinellidae) is a less suitable host for parasitism than resident species. Int. J. Agric. Nat. Resour. 312-323. *Harmonia axyridis* (Pallas) has invaded most continents in the world, including South America. In Chile, it became invasive after 2010, turning into a dominant species in coccinellid guilds in alfalfa crops, where it coexists with the introduced species *Hippodamia variegata* (Goeze) and the native species *Eriopis chilensis* Hofmann, the populations of which declined after the invasion of *H. axyridis*. One of the mechanisms attributed to the success of invasive species is the enemy release hypothesis (ERH), which predicts that natural enemies will have a lesser impact on the invasive species in the newly invaded areas than on resident species. *Dinocampus coccinellae* (Hymenoptera: Braconidae) is a cosmopolitan parasitoid of coccinellids, which vary in their suitability to parasitism according to species and location. The present study evaluated whether *H. axyridis* is a less suitable host than *H. variegata* and *E. chilensis* for *D. coccinellae* by assessing each step in the parasitism process, from oviposition to adult emergence in field and laboratory conditions. In the field, successful parasitism in *H. axyridis* (7%) was significantly lower than in *H. variegata* (33%) and *E. chilensis* (36%). *H. axyridis* was also the only species in which failed parasitoid larval development was greater than successful parasitism (adult emergence). In the laboratory, *D. coccinellae* never successfully oviposited in *H. axyridis*, while it did in the other two species. These results suggest that in Chile, *H. axyridis* is a less suitable host than the resident species for *D. coccinellae*. This mechanism, among others, may explain the dominance of this species in this early stage of invasion.

Keywords: *Dinocampus coccinellae, Eriopis chilensis, Hippodamia variegata,* ladybird beetles, natural enemies.

Introduction

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) is considered an invasive species of great success worldwide. It is native to Asia but is now established on all continents except Antarctica (Camacho-Cervantes, Ortega-Iturriaga, & Del-Val, 2017; Roy *et al.*, 2016). *H. axyridis* was introduced to South America for biological control in the late 1980s, but accidental introductions of invasive populations have also played a role in the spread

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of this species on this continent (Lombaert *et al.*, 2014, 2011). The first wild populations in Chile were reported in 2003 in the central part of the country (Grez, Zaviezo, González, & Rothmann, 2010), but this species has begun to dominate coccinellid communities only since 2011–2012, especially in alfalfa fields (*Medicago sativa* L.) (Fabaceae) (Grez, Zaviezo, Roy, Brown, & Bizama, 2016). In these fields, *H. axyridis* coexists with other coccinellids, such as the native *Eriopis chilensis* Hofmann and the exotic *Hippodamia variegata* (Goeze), the latter of which was introduced to the country in the 1970s and has become one of the most abundant species in these fields during recent years, although it has not been considered an invasive species yet (Grez *et al.*, 2016). After the establishment of *H. axyridis*, populations of other coccinellids, particularly native species such as *E. chilensis*, *Adalia angulifera* Mulsant, *Adalia deficiens* Mulsant, and *Cycloneda sanguinea* (L.), among others, decreased in alfalfa fields (Grez *et al.*, 2016).

It has been established that interspecific exploitative competition may be one of the causes of the higher success of *H. axyridis* over other resident coccinellid species (Zaviezo, Soares, & Grez, 2019), but another possible mechanism is a reduced impact of natural enemies on this invasive species in the newly invaded areas compared to that on resident species (i.e., the enemy release hypothesis, ERH) (Roy *et al.*, 2016). *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) is a cosmopolitan parasitoid of many coccinellid species, mainly of the tribe Coccinellini (Ceryngier *et al.*, 2017; Ceryngier, Roy, & Poland, 2012). This parasitoid has a parthenogenetic mode of reproduction, and males are very rarely found (Balduf, 1926; Ceryngier & Hodek, 1996). The parasitism process begins with the oviposition of one or several eggs in advanced developmental stages (fourth instar larvae and adults) of the coccinellid. Although several larvae can hatch inside the host, only one survives and completes its development, emerging from the adult coccinellid and then weaving a cocoon between the legs of the coccinellid (Balduf, 1926; Ceryngier *et al.*, 2012). During the development of the parasitoid larva, food is provided by teratocytes, and the larva does not feed directly on the internal coccinellid tissues (Ceryngier *et al.*, 2012). Paralysis and changes in the behavior of the coccinellids occur before the parasitoid larva emerges due to replication of the *Dinocampus coccinellae* paralysis virus (DcPV) in the brain of the host (Dheilly *et al.*, 2015). Finally, the parasitoid adult emerges from the pupa, completing a successful parasitism event, after which the coccinellid usually dies (Balduf, 1926; Ceryngier et al., 2012).

Parasitism by *D. coccinellae* can vary depending on host species, location and time since invasion, among other factors, due to genetic differences between species or populations and the adaptation of the parasitoid to the new host (Ceryngier *et al.*, 2017, 2012; Knapp *et al.*, 2019; Paula *et al.*, 2020). The complexity of different interactions in the field, in turn mediated by the abundance of coccinellid species and parasitoids, could modulate the parasitism rate by this natural enemy, so laboratory experiments are an important complement to field assessments to better understand this process. Successful parasitism is generally used to assess the suitability of different coccinellid species for the parasitoid (Ceryngier *et al.*, 2017, 2012; Dindo *et al.*, 2016; Knapp *et al.*, 2019). Studies in several regions of the world have shown that even though *H. axyridis* is similarly parasitized (i.e., oviposited) to other species, there is high parasitoid larval mortality and thus a lower frequency of successful parasitism in this invasive species (Berkvens *et al.*, 2010; Castro-Guedes & Almeida, 2016; Ceryngier *et al.*, 2017, 2012; Comont *et al.*, 2014; Hoogendoorn & Heimpel, 2002).

The lower suitability of *H. axyridis* for the parasitoid has been explained by several mechanisms involving behavior, the immune system and physiology (Firlej, Girard, Brehélin, Coderre, & Boivin, 2012; Firlej, Lucas, Coderre, & Boivin, 2007, 2010). However, in some invaded regions, *H. axyridis* has the same or even greater rates of successful parasitism by *D. coccinellae* than resident species (Dindo *et al*., 2016; Knapp *et al*., 2019; Paula *et al*., 2020). Some authors have drawn attention to the dynamism of biological invasions, expecting an adaptation by natural enemies to invasive species over time (Knapp *et al*., 2019; Roy, Lawson Handley, Schönrogge, Poland, & Purse, 2011). Thus, the extent to which parasitism occurs in *H. axyridis* relative to resident coccinellid species is not a general phenomenon and should be studied in each invaded region.

In South America, where *H. axyridis* invaded more recently than in North America (Lombaert *et al*., 2014), only one study under laboratory conditions in Brazil assessed the relative parasitism of *H. axyridis* compared to that of other coccinellid species, finding that the invader is a poor host for *D. coccinellae* (Castro-Guedes & Almeida, 2016). However, in a more recent field study in a different region of Brazil, *H. axyridis* suffered equal or more parasitism by *D. coccinellae* than other resident species (Paula *et al*., 2020). However, these results do not necessarily apply to other regions of the continent, such as Chile, since Brazilian and Chilean *H. axyridis* populations are genetically different (Lombaert *et al*., 2014), as the parasitoid populations may also be. Consequently, the aim of this study was to evaluate in the field and in the laboratory whether *H. axyridis* is a less suitable host for *D. coccinellae* in central Chile than the resident *E. chilensis* and *H. variegata*.

Materials and methods

Field tests

Adults were collected in alfalfa fields near Santiago (33° 40' 45.6" S 70° 34' 59.2" W). Sweep nets were used during spring and autumn, when alfalfa has abundant aphid populations and the aphidophagous coccinellids *H. axyridis, E. chilensis* and *H. variegata* occur frequently in central Chile. In the 2016–2017 season, this was done every 15 days in two alfalfa fields, for a total of 10 surveys. All the coccinellids captured in each survey (10–40 per survey) were kept in the laboratory in acrylic breeding boxes, separated by species, with fava bean (*Vicia faba*, Fabaceae) plants infested with *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), thus keeping food available *ad libitum*. Individuals were maintained under controlled conditions (21 °C, 70% relative humidity and a 16:8 hour (light:dark) photoperiod) until the emergence of parasitoid pupae or for a maximum of 30 ± 5 days. This time allowed all the viable parasitoid larvae to finish their development, given that the estimated development time from egg to last instar larva is three to four weeks (Balduf, 1926; Ceryngier *et al*., 2012). The coccinellids from which parasitoid larvae emerged and pupae developed during this period were counted, extracted from the boxes and kept individually in Petri dishes. The parasitoid pupae were followed until adult emergence, and then the percentages of parasitoid pupa formation and successful parasitism for each coccinellid species were calculated. The difference between successful parasitism and pupal formation represents pupal mortality. Even though in Chile, the wasp *Perilitus stuardoi* Porter has been reported to parasitize *A. deficiens*, *Adalia bipunctata* (L.), *Eriopis connexa* (Germar), *Cryptolaemus montrouzieri* Mulsant and *Coleomegilla quadrifasciata* (Schöenherr) (Porter, 1936; Smith, 1953), its taxonomic status and possible synonymy with *D. coccinellae* is not clear (Hodek & Evans, 2012). Therefore, a confirmation of the identity of the parasitoids was made following the taxonomic key for the family Braconidae (Hymenoptera) (Zack, 1999). The coccinellids from which a parasitoid pupa did not develop after 30 days were dissected to determine whether larval development failed (i.e., the larva that died within the coccinellid) or there was an absence of parasitism (i.e., individuals that lacked larvae). The percentages of the occurrence of these events (pupal formation, successful parasitism and failed larval development) were calculated over the total number of individuals captured of each species. The coccinellids that died during rearing in the laboratory were not considered in these percentages. We summed the percentages of parasitoid pupal formation and failed larval development to estimate the total percentage of parasitism. Captures were made in two additional alfalfa fields every 30 days during the 2018–2019 season to obtain additional measurements of parasitism rates in the field. Individuals of the three coccinellid species were collected and dissected to check for the presence of *D. coccinellae* larvae.

Laboratory tests

Insect rearing

Coccinellids were collected in alfalfa fields, brought to the laboratory and put in boxes with fava bean plants infested with *A. pisum* under the same environmental conditions mentioned above. Eggs of each species were collected periodically and kept in Petri dishes, separated by species, until larval emergence. Once the larvae emerged, they were transferred to new breeding boxes (separated by species), which contained fava bean plants with *A. pisum ad libitum* and water-soaked cotton, until they reached adulthood. We used several Petri dishes and breeding boxes per species, with aphids provided *ad libitum* and a reduced number of larvae in each dish to reduce the possibility of cannibalism. Adults were used in the experiment because this stage is generally the most highly attacked by *D. coccinellae* and has a higher successful parasitism rate than other stages (Firlej *et al.*, 2010; Obrycki, Tauber, & Tauber, 1985). Thus, we obtained individuals that we could ensure were not parasitized. Parasitoids were obtained from coccinellids captured in the field. Once female adult parasitoids emerged from the beetles, they were fed honey and water supplied on cotton and kept individually in Petri dishes for 2–3 days, the time needed to reach sexual maturity, before being exposed to hosts (Balduf, 1926).

No-choice test

Four adult coccinellids of the same species (a mix of males and females) between 15 and 35 days old were exposed to one *D. coccinellae* adult female for 1 hour in transparent 755 cm^3 plastic containers, as in similar studies (Dindo *et al.*, 2016; Geoghegan, Majerus, & Majerus, 1998). We chose 1 hour of exposure because female *D. coccinellae* have the potential to parasitize a large number of coccinellids in this time period (Balduf, 1926). In each of the experiments, we also verified that the parasitoids made at least one oviposition attempt on any of the coccinellids, thus verifying that they were sexually mature and active. Three or four days after exposure to the parasitoid, two coccinellids were dissected to search for parasitoid eggs (to find evidence of the first event of the parasitoid life cycle: oviposition). The other two coccinellids were kept in transparent containers for 30 ± 5 days with the same feeding and environmental conditions mentioned above to assess the subsequent events of the parasitoid life cycle: failed larval development, pupal formation and successful parasitism (i.e., adult emergence). The coccinellids that developed a parasitoid pupa were counted, extracted from the cage and individually maintained in Petri dishes to monitor for adult wasp emergence (i.e., successful parasitism). Those individuals that did not present parasitoid pupal formation after 30 ± 5 days were dissected in search of larvae, and if larvae were present, they were recorded as having failed larval development. The experiments were repeated 13 times for *H. variegata* and *E. chilensis* and 10 for *H. axyridis*, with a total of 52 individuals of *H. variegata* and *E. chilensis* and 40 individuals of *H. axyridis* exposed to the parasitoid.

Oviposition was calculated as the number of individuals with parasitoid eggs from those dissected 3–4 days after exposure (half of the exposed individuals). The percentages of pupal formation, successful parasitism and failed larval development were calculated from the other half of the individuals that were kept for 30 ± 5 days. To calculate parasitism in this latter group of coccinellids, we summed the percentages of pupal formation and failed larval development.

Finally, the total percentage of parasitism was calculated using both groups of individuals (i.e., those dissected 3–4 days after exposure and those reared for 30 ± 5 days) as the sum of the number of coccinellids oviposited with pupal formation or failed larval development divided by the total number of coccinellids exposed per species.

Statistical analyses

Differences in the percentage of failed larval development among the coccinellid species, successful parasitism and total parasitism in the field were compared through chi-square tests, while the variation among coccinellid species in the percentage of oviposition, failed larval development, pupal formation, successful parasitism and total parasitism in no-choice tests in the laboratory were analyzed using Fisher´s exact test. When significant species effects were found, chi-square or Fisher´s exact tests between pairs of species were carried out using a Bonferroni adjustment for the p-values. Additionally, for the field data, statistical resampling was carried out to evaluate possible effects due to differences in the sample size of coccinellid species captured. For this, a bootstrap analysis was applied to the percentages of total parasitism in each species. A fixed sample size of 100 coccinellids per species was used, generating 200 new samples for each. The average percentage of parasitism for each species in these 200 samples was calculated and compared again using chi-square tests. All the statistical analyses were performed with R (R Core Team, 2015).

Results

Field tests

As expected, *D. coccinellae* was the only parasitoid species detected in the coccinellids in both seasons. During the 2016–2017 season, a total of 737 adult coccinellids of the studied species were captured in the field and then monitored in the laboratory in search of parasitoids. *Hippodamia variegata* was the most frequently collected species, followed by *E. chilensis* and *H. axyridis* (Figure 1). Of the total coccinellids that were captured, *H. axyridis* (7%) had four to five times less successful parasitism than the other species, significantly less than *E. chilensis* and *H. variegata* (36 and 33%, respectively, chi-square = 32.77, P<<0.001, Figure 1). Additionally, *H. axyridis* along with *E. chilensis* had significantly less the failed larval development, with approximately half as much as *H. variegata* (12, 14 and 24%, respectively, chi-square = 14.67, P<<0.001, Figure 1). The percentage of failed pupal development was similar in all three species (Fisher´s exact tests for the three species, P=0,695, Figure 1) and did not exceed 2% (difference between successful parasitism and pupal formation), meaning that almost all pupae developed to adults. The total parasitism of *H. axyridis* (successful parasitism + failed pupal development + failed larval development) was 20%, less than half that of *E. chilensis* and *H. variegata* (52 and 59%, respectively, Figure 1). The average parasitism by species, calculated from the 200 samples generated by bootstrap resampling, had the same pattern as that obtained with the original samples, with 60%, 52% and 19% total parasitism for *H. variegata*, *E. chilensis* and *H. axyridis*, respectively, with *H. axyridis* having a significantly lower percentage of parasitism (chi-square = 28.50, P<<0.001).

Considering only the parasitized individuals (*E. chilensis* = 144, *H. variegata* = 208, *H. axyridis* = 22), parasitoids had significantly higher failed larval development in *H. axyridis* and *H. variegata* than in *E. chilensis* (59%, 41% and 27%, respectively) (chi-square = 12.39, P=0.002).

In the 2018–2019 captures, 361 adult coccinellids of the three species studied were captured (150 *Hippodamia variegata*, 143 *E. chilensis* and 68 *H. axyridis*), and similar to the 2016–2017 season, *H. axyridis* had a lower percentage (2%) of parasitism (i.e. larval presence) than *E. chilensis* and *H.*

variegata (9% and 17%, respectively) (chi-square = 9.80, P=0.007).

Laboratory

Oviposition by *D. coccinellae*

Of the 20 *H. axyridis* exposed to the parasitoid and dissected 3–4 days after exposure to evaluate oviposition, no individual had *D. coccinellae* eggs (0%), while of the 26 *E. chilensis* and 26 *H. variegata* dissected, 42% and 38%, respectively, had parasitoid eggs. These percentages were similar and significantly larger than that observed in *H. axyridis* (Fisher's exact test, P<<0.001).

Development of *D. coccinellae*

No *H. axyridis* presented parasitoid pupal formation among the individuals exposed to the parasitoid and kept in the laboratory for 30 ± 5 days, and dissection revealed that there were no failed larval development events (Figure 2). In contrast, for *E. chilensis* and *H. variegata*, parasitoid development occurred, and more than 30% of individuals were successfully parasitized (35 and 31%, respectively, Figure 2). Even though failed larval development in *E. chilensis* was twice as frequent as in *H. variegata* (42 and 19%, respectively), these percentages were not significantly different (Figure 2). Considering both successful parasitism and failed larval development, more than 50% of *E. chilensis* and *H. variegata* individuals were parasitized (77 and 50%, respectively, Figure 2).

Total parasitism

Considering all the individuals exposed to the parasitoid (*E. chilensis* = 52, *H. variegata* = 52, *H. axyridis* = 40), the total percentages of parasitism (i.e., the individuals with parasitoid oviposition, successful parasitism or failed larval development) were 43% for *H. variegata* and 58% for *E. chilensis*, with no significant difference (Fisher's exact test, P=0.16). No parasitism was observed in *H. axyridis*; therefore, the parasitism rate was significantly lower for this species than for the other two species (Fisher's exact test, P<<0.001).

Discussion

Our results support previous research in other regions that describe *H. axyridis* in invaded areas as a less suitable host than resident coccinellids for *D. coccinellae* (Berkvens *et al.*, 2010; Castro-Guedes & Almeida, 2016; Comont *et al.*, 2014; Hoogendoorn & Heimpel, 2002). We found that

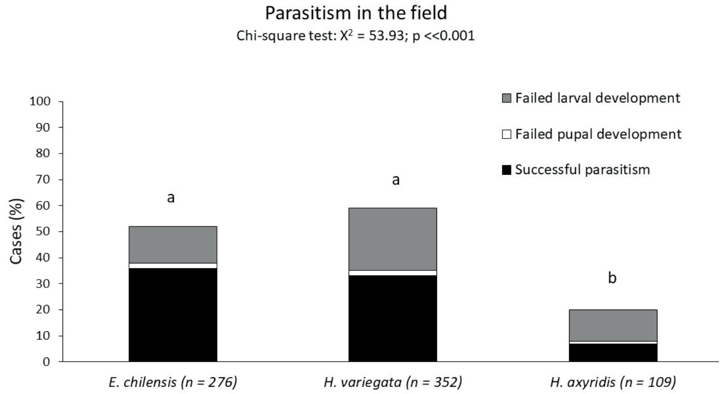

Figure 1. Percentages of total parasitism, successful parasitism, failed larval development and failed pupal development of *D. coccinellae* in *E. chilensis*, *H. variegata* and *H. axyridis* captured in alfalfa fields during 2016–2017. Different letters between the species indicate significant differences (chi-square tests between pairs of species, α=0.02).

successful parasitism was significantly lower in *H. axyridis* than in *H. variegata* and *E. chilensis* among individuals collected in the field during both seasons. Parasitoid larvae suffered greater mortality inside the bodies of both introduced species (failed larval mortality: 59% in *H. axyridis*, 41% in *H. variegata*) than in the native species (27% in *E. chilensis*) among those parasitized in the 2016–2017 season. Although parasitism levels were much lower in 2018–2019 than in 2016–2017, both seasons showed a significantly lower percentage of parasitism by *D. coccinellae* in *H. axyridis* than in *E. chilensis* and *H. variegata*. Furthermore, in the laboratory, no oviposition or parasitism of adults was detected in *H. axyridis*, while total parasitism was 43% in *H. variegata* and 58% in *E. chilensis*. Different sampling sizes could have led to a different probability of detecting parasitism by *D. coccinellae* in the three coccinellid species. Nevertheless, when resampling our field and laboratory dataset with bootstrapping, the results were the same, with significantly lower parasitism on *H. axyridis* than on the other two species.

It should be noted that in Japan (the native range of *H. axyridis*), *D. coccinellae* attacks *H. axyridis* at the same rate as in some invaded areas (Koyama & Majerus, 2008). Therefore, several other mechanisms, such as a defensive behavior of *H. axyridis* during oviposition (Firlej *et al.*, 2010), the encapsulation of parasitoid eggs by the immune system (Firlej *et al.*, 2012) or the low suitability of *H. axyridis* during the development of parasitoid larvae (Firlej *et al.*, 2007), could determine the lower successful parasitism of *H. axyridis* by *D. coccinellae* relative to that of other coccinellid species. After carrying out laboratory experiments and field sampling, our results suggest that these three mechanisms play a role in the escape of *H. axyridis* from this natural enemy.

Observations made during coccinellid exposure to *D. coccinellae* showed that *H. axyridis* displayed several aggressive behaviors, including biting, kicking, hiding the abdomen, spinning around or escaping (see Suppl. Material); these behaviors were less frequent in *H. variegata* and *E. chilensis*. Furthermore, these behaviors, which result in increased handling time of the host by the parasitoid and less successful parasitism, have also been observed in *H. axyridis* in other invaded regions of the world (Firlej *et al.*, 2010). In preliminary tests, we exposed the coccinellids to the parasitoids for a longer time (24 hours); nevertheless, *H. axyridis* was not successfully oviposited, while in the other two species, several parasitoid eggs were found when they were dissected. However, parasitism was recorded

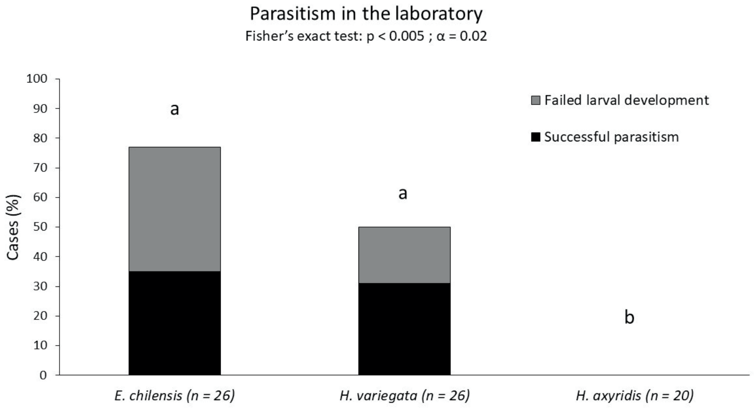

Figure 2. Percentages of total parasitism, successful parasitism (Fisher´s exact tests for the three species, P<0.004) and failed larval development (Fisher´s exact tests for the three species, P<0.002) of *D. coccinellae* in *E. chilensis*, *H. variegata* and *H. axyridis* exposed to the parasitoid under laboratory conditions. Different letters between the species indicate significant differences (Fisher´s exact tests between pairs of species, α=0.02).

in *H. axyridis* in the field, although at a lower proportion than in *E. chilensis* and *H. variegata*. Perhaps the higher habitat complexity in field conditions compared to that in Petri dishes in the laboratory could prevent the host from detecting the parasitoid and deploying defensive behaviors. Another possibility is that in the field, parasitoids attack larvae, which are less mobile and may be less aggressive than adults and therefore are less likely to escape from the parasitoid. In fact, studies have shown that even though adult coccinellids are the stage most attacked by *D. coccinellae*, fourth and third instar larval stages are also parasitized (Firlej, Boivin, Lucas, & Coderre, 2005; Obrycki *et al.*, 1985; Paula *et al.*, 2020). The fourth and third instar larvae of *H. axyridis* would also be more susceptible to parasitoid oviposition than adults, since they have less aggressive defensive behavior (Berkvens *et al.*, 2010; Firlej *et al.*, 2005, 2010; Hoogendoorn & Heimpel, 2002).

Studies in other invaded areas have reported that the *H. axyridis* immune response has a predominant role in its suitability for the development of parasites and diseases. In fact, more genes related to the immune response have been found in this species than in other coccinellid species (Vilcinskas, Mukherjee, & Vogel, 2013). Through one such immune mechanism, *H. axyridis* has the ability to encapsulate *D. coccinellae* eggs, especially when they are deposited in low numbers (Firlej *et al.*, 2012). It is possible that the lack of detection of parasitoid eggs in *H. axyridis* in our laboratory experiments could be due to rapid encapsulation, and since only eggs with more than 4 days of development were clearly visible and included in our results, oviposition would have been underestimated. The use of other techniques, such as fluorescence microscopy, would be necessary to detect the presence of parasitoid eggs more precisely immediately after oviposition (Firlej *et al.*, 2012).

In terms of the lower suitability of *H. axyridis* for the development of the parasitoid larvae, although individuals captured in the field presented parasitism, the larvae failed to develop in more than 50% of these individuals. This suggests that larvae hatching from *D. coccinellae* eggs that were not encapsulated by the immune system of *H. axyridis* died or had delayed development, probably due to their low number and the unsuitable growth pattern of teratocytes in *H. axyridis* (Firlej *et al.*, 2007). These teratocytes play a key nutritive role during the development of parasitoid larvae (Kadono-Okuda, Weyda, & Okuda, 1998).

Therefore, our results suggest the possible occurrence of the three abovementioned mechanisms, resulting in *H. axyridis* having a lower suitability than the other resident species, one introduced and one native, to *D. coccinellae* parasitism in this invaded region of the world. This has also been reported in Europe (Berkvens *et al.*, 2010), the USA (Hoogendoorn & Heimpel, 2002) and Canada (Firlej *et al.*, 2005). However, the effectiveness and frequency of each of these mechanisms could differ from populations of *H. axyridis* in other parts of the world. For example, in Italy, *D. coccinellae* affected the fitness of *H. axyridis* more negatively than that of native *A. bipunctata* (Dindo *et al.*, 2016). Additionally, in the Czech Republic, during 2015–2018, *H. axyridis* was parasitized to a greater extent than the native *C. septempunctata*, and rates of *D. coccinellae* parasitism in *H. axyridis* were significantly higher than earlier assessments (before 2016), suggesting the presence of a *D. coccinellae* phenotype adapted to *H. axyridis* in those regions (Knapp *et al.*, 2019). Recently, a field study in Brazil found that *H. axyridis* was parasitized equally or to a greater extent by *D. coccinellae* and other parasitoids than resident coccinellids, including the natives *E. connexa* and *C. sanguinea*. Therefore, our study is the first report of low suitability in the field in South America and is confirmed by the results of laboratory tests. This, together with the results obtained in laboratory studies in Curitiba in Brazil (Castro-Guedes & Almeida, 2016), but not from field studies in the Distrito Federal in Brazil (Paula *et al.*, 2020), indicate that at least two independent introductions of this invasive alien species to South America from eastern North America (Lombaert *et al.*, 2014) have occurred, corresponding to populations that could better escape this natural enemy in comparison to other resident species (native or introduced). Therefore, even though the adaptation of natural enemies to novel invasive species is thought to occur by the ERH, in Chile, to date, *D. coccinellae* populations have not developed a strong ability to parasitize *H. axyridis*, contrary to what has occurred in some European populations that have had a longer period of interaction (Dindo *et al.*, 2016; Knapp *et al.*, 2019). Therefore, to understand this phenomenon more comprehensively, assessments of the suitability of *H. axyridis* to parasitism in invaded areas should be followed through time to account for any possible parasitoid adaptation.

Comparing the suitability of the other two species (the native *E. chilensis* and the exotic *H. variegata*) to *D. coccinellae*, the parasitoid could have adapted to *H. variegata*, a species that has been in Chile for more than four decades (Grez *et al.*, 2016) and has similar rates of parasitism as the native *E. chilensis.* However, even though the two species have similar rates of parasitism, *H. variegata* presented greater rates of failed larval development. This may be partly explained by its smaller body size (up to 5.0 mm) compared to that of *E. chilensis* (up to 5.8 mm), which does not allow proper larval development (Balduf, 1926). This suggests that *H. variegata* may represent a sink for the parasitoid population, because although it is easily oviposited, larvae do not develop successfully.

In summary, our results suggest that the lower successful parasitism observed in *H. axyridis* populations in central Chile may be one of the mechanisms contributing to its establishment and dominance in coccinellid communities (Grez *et al.*, 2016), in addition to others, such as intraguild predation and competition (Roy & Brown, 2015; Zaviezo *et al.*, 2019).

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Resumen

V. Romero, T. Zaviezo, y A.A. Grez. 2020. El coccinélido invasor *Harmonia axyridis* (Coleoptera: Coccinellidae) es un huésped menos adecuado para el parasitismo que las especies residentes. Int. J. Agric. Nat. Resour. 312-323. *Harmonia axyridis* (Pallas) ha invadido la mayoría de los continentes del mundo, incluida América del Sur. En Chile se convirtió en invasora después del 2010, convirtiéndose en una especie dominante en ensambles de coccinélidos en cultivos de alfalfa, donde coexiste con la especie introducida *Hippodamia variegate* (Goeze) y la especie nativa *Eriopis chilensis* Hofmann, cuyas poblaciones disminuyeron después de la invasión. Uno de los mecanismos atribuidos al éxito de las especies invasoras es la hipótesis de "liberación del enemigo", que predice un impacto reducido de los enemigos naturales sobre estas especies en las áreas recientemente invadidas en comparación con las especies residentes. *Dinocampus coccinellae* (Hymenoptera: Braconidae) es un parasitoide cosmopolita de coccinélidos, los cuales varían en su idoneidad para el parasitismo según la especie y la ubicación. El presente estudio evaluó si *H. axyridis* es un huésped menos adecuado para *D. coccinellae* en comparación con *H. variegata* y *E. chilensis*, evaluando cada paso del proceso de parasitismo desde la oviposición hasta la emergencia del adulto en condiciones de campo y laboratorio. En el campo, el parasitismo exitoso en *H. axyridis* (7%)

fue significativamente menor que en *H. variegata* (33%) y *E. chilensis* (36%). *Harmonia axyridis* también fue la única especie en la que el desarrollo larval fallido del parasitoide fue mayor que el parasitismo exitoso (aparición de adultos). En el laboratorio, *D. coccinellae* nunca ovipositó exitosamente en *H. axyridis*, mientras que si lo hizo en las otras dos especies. Estos resultados sugieren que *H. axyridis* en Chile es un hospedero menos adecuado para *D. coccinellae* en comparación con las especies residentes. Este mecanismo, entre otros, puede explicar el dominio de esta especie en esta etapa temprana de la invasión.

Palabras clave: Coccinélidos, *Dinocampus coccinellae,* enemigos naturales*, Eriopis chilensis, Hippodamia variegata*.

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