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Native preimaginal parasitism of *Harmonia axyridis*: new record of association with *Phalacotrophora fasciata* in Italy

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Abstract

Since 1988 *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae) has been established in more of 50 countries in the world, where concern has been aroused by its invasiveness and the possible negative effects on indigenous aphidophagous species. This study was focused on the research of a new association with indigenous parasitoids of ladybirds and this exotic host. Field samples of *H. axyridis* larvae and pupae were collected during 2013 and 2014 seasons and maintained under observation until emergence of adult ladybirds or parasitoids. Some larvae of *Phalacrotophora fasciata* (Fallen) (Diptera Phoridae) emerged from the collected pupae of *H. axyridis*, the first record of this new association for Italy. Samples of *Dinocampus coccinellae* (Schrank) (Hymenoptera Braconidae) emerged from adult ladybird collected as larvae.

Key words: *Harmonia axyridis*, indigenous parasitoid, pupal parasitism, *Phalacrotophora fasciata*, *Dinocampus coccinellae*.

Introduction

The multicoloured Asian or harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera Coccinellidae), originating from Asia (Kuznetsov, 1997), is a well-known predator of numerous aphid species in its native range (Hodek, 1996), but it also feed on other insect species (Kock, 2003). For this reason it has been utilized in augmentative biological control in Asia (Seo and Youn, 2000), Europe (Trouve *et al.*, 1997) and North America (LaRock and Ellington, 1996).

In Europe early introductions of *H. axyridis* occurred in the east (Katsoyannos *et al.*, 1997); in western Europe *H. axyrids* was first used in 1982 in France (Iperti and Bertand, 2001), after this first introduction *H. axyridis* was largely used in many countries in Europe as an agent for biological control (Brown *et al.*, 2011; Sighinolfi *et al*., 2013). In Italy, it was used in greenhouses in the north from 1995 to 1999 (Burgio *et al.*, 2008), but in 2000 commercialization was interrupted due to the concern aroused by its invasiveness (Roy *et al.*, 2012) and its impacts on the indigenous coccinellids and other predators (Burgio *et al.*, 2002; Santi *et al.*, 2003; Nedvěd *et al*., 2013).

Several parasitoids attack *H. axyridis* in its native Asian range. In Korea two parasitoids of adult ladybirds were found: *Medina luctuosa* (Meigen) (Diptera Tachinidae) (Kenis *et al.*, 2008) and *Dinocampus coccinellae* (Schrank) (Hymenoptera Braconidae) (Park *et al.*, 1996). Maeta (1969) and Osawa (1992) reported a phorid of genus *Phalacrotophora* which parasitized *H. axyridis* pupae; Disney (1997) described this phorid as a new species: *Phalacrotophora philaxyridis* Disney.

In previous studies I found that the indigenous *D. coccinellae*, already known as parasitoid of adult ladybird (Riddick *et al.*, 2009), has adapted in Italy to this new host (Francati, 2013) as found in other countries like England (Browdrey and Mabbott, 2005), Belgium (Berkvens *et al.*, 2010) and Canada (Firlej *et al.*, 2005).

Due to the high reproductive capacity of *H. axyridis*, from two (Osawa, 2000; Koch and Hutchison, 2003) to five (Katsoyannos *et al.*, 1997; Lanzoni *et al.*, 2004) generations per year, the larval and pupal parasitoids could have a important role in restricting the population of this species as with other introduced species (Dindo *et al*., 2013). In several countries various larval and pupal parasitoids of coccinellidae were found, including *H. axyridis* where present (Riddick *et al.*, 2009). The list includes different species of Hymenoptera (Kenis *et al.*, 2008) and Diptera (Koch and Galvan, 2008) which show a large range of success in parasitization rate (Kenis *et al.*, 2008).

To try to expand the list of its parasitoid I chose to collect larvae and pupae of *H. axyridis* with the aim of finding some indigenous parasitoids of the different stages of the coccinellids (Riddick *et al.*, 2009).

Materials and methods

For this study I decided to focus the research on the last instars of preimaginal development of *H. axyridis*: four instar larva, pre-pupa and pupa. This choice was linked to the biology of these parasitoids which usually develop in the instar subsequent to that of the egg laying (Riddick *et al.*, 2009). I collected samples using the sight insect catch system; the captures were made in late Spring of 2013 and 2014 in the Bologna area (Italy) (44°48'39"N 11°37'84"E), and the samples were collected on different trees (oaks, peaches, plums). The collected specimens were transferred to the laboratory and stored in rearing chambers at 25 ± 1 °C, 65 ± 5 RH and L16:D8. The specimens were placed individually in Petri dishes (9 cm diameter) and they were maintained under observation until the emergence of adult ladybirds or of parasitoids. The larvae were fed, until pupation, with *Myzus persicae* Sulzer. The adult ladybirds obtained were maintained under observation in the same conditions as the larvae, for a month, with the aim of

obtaining data on larval parasitization of *D. coccinellae* (Kadono-Okuda *et al.*, 1995). For all species of parasitoids found I calculated the percentage of parasitism, as the number of parasitoids found (per species) on the total number of samples.

Results

I collected overall 223 specimens (among larvae and pupae): 98 in 2013 and 125 in 2014. In 2013 some maggots emerged from the pupae, identified as *Phalacrotophora fasciata* (Fallen) (Diptera Phoridae); the percentage of parasitism was 5.10%. Although the pupae were the same size, the number of larvae that emerged from every pupa was different (respectively: 3-2-5-6-9), confirming the trend of gregariousness of this parasitoid (Ceryngier *et al.*, 2013). The other samples collected completed their development and some larvae of *D. coccinellae* emerged from the adult ladybirds; the percentage of parasitism was 4.08%. In 2014, the percentage of parasitism by *P. fasciata* decreased to 0.80%. Indeed, I found only a single parasitized *H. axyridis* pupa, from which 5 larvae emerged. Despite the fact that the percentage of emerged ladybirds was lower, the rate of parasitism by *D. coccinellae* was higher; 19.20% of the individuals were parasitized. From 2013 to 2014 the rate of pupae parasitized by *P. fasciata* was reduced to one-fifth. On the other hand, *H. axyridis* larvae parasitized by *D. coccinellae* presented a six-fold increase (figure 1).

Discussion and conclusions

Various European *Phalacrotophora* species attack coccinellids (Disney and Beuk, 1997); three of them, *P. fasciata*, *Phalacrotophora berolinensis* Schmitz and *Phalacrotophora delageae* Disney, are parasitoids of ladybird pupae (Disney and Beuk, 1997). Several authors have suggested that some Phoridae species could adapt to *H. axyridis* in new areas (Kenis *et al.*, 2008; Roy *et al.*, 2011); *P. fasciata* is in fact only known as a parasitoid of *H. axyridis* in Europe (Ware *et al.*, 2010; Steenberg and Harding, 2010); this is the first record in Italy.

D. coccinellae is already known as a parasitoid of *H. axyridis* in the native areas (Park *et al.*, 1996) and in those of introduction (Firlej *et al.*, 2005; Berkvens *et al.*, 2010), including Italy (Francati, 2013). Although *D. coccinellae* is known as a parasitoid of adult ladybirds it was reported that it can sporadically lay eggs inside larvae and complete its development when the host has reached the adult stage (Filatova, 1974; Kadono-Okuda *et al.*, 1995); this can occur especially when the natural hosts are scarce. During the sampling *H. axyridis* was the most abundant species in the collecting locations, and the larvae and the pupae were more abundant than adults. The rate of parasitism for the immature stages, in 2013, was similar to what I found for the adults in previous years (Francati, 2013); the significant increase obtained in 2014 was totally unexpected.

Hokkanen and Pimentel (1984) suggested that a new association between exotic insect species and native antagonists, especially parasitoids, may over time provide more successful parasitism compared to former associations. As reported by Hoogendoorn and Heimpel (2002) the abundance of *H. axyridis* could have a positive effect on the population dynamics involving the interaction between the coccinellid complex and *D. coccinellae*, as this parasitoid could prefer the new host to the native ones. Similar effects could also occur on other parasitoids, such as *P. fasciata*.

These data on preimaginal parasitism seem to confirm these hypotheses, and refute others which considered *H. axyridis* a marginal host for *D. coccinellae* (Burling *et al.*, 2010; Berkvens *et al.*, 2010). In particular *D. coccinellae* shows a great capacity of adaptation to this host, attacking both larvae and adults. The ability of *D. coccinellae* to parasitize larvae of *H. axyridis* could be

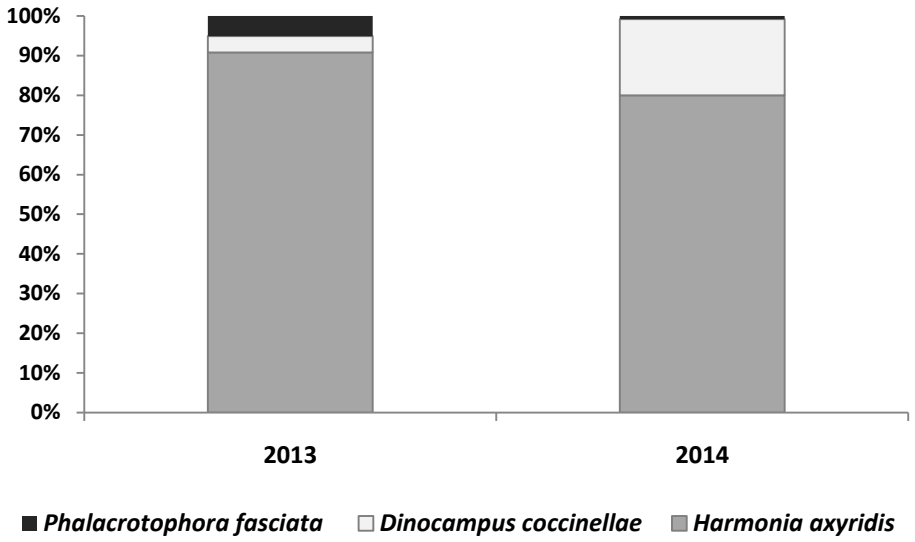

Figure 1. Parasitoids emerged from pupae and adults of *H. axyridis* collected in 2013 (n. = 98) and 2014 (n. = 125). Percentages of pupae parasitized by *P. fasciata*; percentages of *D. coccinellae* emerged from adult ladybirds; percentages of *H. axyridis* adults not parasitized.

the reason for the decrease in parasitism rate of *P. fasciata*, which is otherwise difficult to explain. Kadono-Okuda *et al.* (1995) reported that, if the host stage parasitized by *D. coccinellae* is larvae or pupae, the larval development is arrested at the first instar until the host moult to adult. *P. fasciata* could lays egg when the other parasitoid larva is quiescent, but during its development some defensive responses could be activated. In fact, the interactions between these two parasitoids, and between these and others, are not totally known; we do not know if the different species may or may not discriminate whether a host has already been parasitized, and if the eggs laid (or the larvae) have some form of protection against other species. Other studies will be needed to better understand the interactions between *P. fasciata* and this new host and also between the two parasitoids, *P. fasciata* and *D. coccinellae*.

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