Different parasitization parameters of pupae of native (Coccinella septempunctata) and invasive (Harmonia axyridis) coccinellid species

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Abstract

We studied the parasitism of the pupae of two predatory coccinellid species of similar body size, i.e. native Coccinella septempunctata and invasive Harmonia axyridis (Pallas). The pupae were collected through the period of coccinellid eclosion and parasitoid emergence from a patch of nettles (n = 660) and from a pea crop (n = 1293) in Central Europe where both coccinellid species developed together. Three parasitoid species were recorded: Phalacrotophora fasciata (Fallen), Phalacrotophora bero-linensis Schmitz and Oomyzus scaposus (Thomson). The first species was the dominant parasitoid of C. septempunctata and the only one recorded in H. axyridis pupae. Parasitism by Phalacrotophora spp. was higher on nettle patches, with 42% of C. septempunctata and 5% of H. axyridis parasitized, and lower in pea crops, where the percentage of parasitized pupae was 11% and 0.5%, respectively. At both sites, the proportion of H. axyridis in the total coccinellid population increased during the 13-16-day period of adult eclosion, but the proportion of parasitized individuals of particular coccinellid species giving rise to parasitoids remained similar during the entire period. In C. septempunctata, the mean number of Phalacrotophora puparia that originated from a coccinellid pupa was 7.2 ± 0.30 (range 1-21) on nettle patches, which was significantly more than on pea crops (3.9 ± 0.21 puparia, range 1-9). The mean mass of Phalacrotophora puparia decreased with increasing number per host pupa. The results confirmed a greater (10-20 times) rate of parasitism of native C. septempunctata than of invasive H. axyridis and also revealed large differences in the rate of parasitism between the sites.

Key words: parasitoid, Phalacrotophora, Diptera, Phoridae, Oomyzus, Hymenoptera, Eulophidae, abundance, Urtica, Pisum.

Introduction

Coccinellids are plagued by several parasitic insect species (Ceryngier et al., 2012), including several fly species of the genus Phalacrotophora (Diptera Phoridae). Phalacrotophora fasciata (Fallen) is the most common in Europe (Disney et al., 1994; Ceryngier and Hodek, 1996; Ceryngier et al., 2012). This species is known to parasitize mainly predatory but also mycophagous species of coccinellids (Disney et al., 1994; Ceryngier and Hodek, 1996). General features of the biology of Phalacrotophora are well known (Ceryngier et al., 2012). Females wait close by the coccinellid praeypupa and lay eggs at the beginning of pupation (Hurst et al., 1998). Larvae develop very quickly; and after a few days, the fully grown larvae leave the coccinellid pupa, fall to the ground, pupate and, if not in diapause, emerge as adults 2-4 weeks later (Ceryngier et al., 2012).

Phalacrotophora spp. are the important parasitoids of more than 20 Palearctic coccinellid species (Disney et al., 1994; Ceryngier and Hodek, 1996; Durska et al., 2003; Ceryngier et al., 2012; Francati, 2015), including Coccinella septempunctata L., a dominant aphi-dophagous coccinellid species of herbaceous crop stands (Honek, 1985; Hodek and Michaud, 2008). In the study area, the rate of parasitism differs between years and host plant stands (A. Honek, Z. Martinкова, J. Skuhrovec unpublished data). C. septempunctata may be a preferred host of Phalacrotophora because of its relatively large body size, which makes possible the development of more parasitoid individuals than in smaller hosts (Filatova, 1974; Ceryngier and Hodek, 1996). Among the other coccinellid species, Phalacrotophora also parasitize Harmonia axyridis (Pallas), a non-native species that has recently expanded in Europe (Steenberg and Harding, 2010; Ware et al., 2010; Romanov, 2017; Ceryngier et al., 2018). The history of invasion and the biology of this species in the invaded area is the subject of major work recently summarized by Roy et al. (2016).

Another common parasitoid of coccinellids is Oomyzus scaposus (Thomson) (Hymenoptera Eulophidae Tetrastichinae), parasitizing the species of tribes Coccinellini, Chilocorini and Scymnini. The female lays eggs inside 3rd and 4th instar larvae, and mature parasitoids emerge from pupae (Ceryngier et al., 2012). This species parasitizes both C. septempunctata and H. axyridis in its native and recently invaded areas (Ceryngier et al., 2018).

Using published data, Ceryngier et al. (2018) calculated that the rates of parasitism of invasive H. axyridis by Phalacrotophora and Oomyzus are significantly less than in native coccinellid species. The invasive species was also resistant to Dinocampus coccinellae (Schrank) (Hymenoptera Braconidae), a parasitoid of mostly the adult stage (Comont et al., 2014), although Dindo et al. (2016) found H. axyridis as a relatively suitable host of D. coccinellae. As the calculation performed by Ceryngier et al. (2018) used data from different native coccinellid species and from many localities in a large geographic area, the results may be further refined using data available for one locality and similar coccinellid species. C. septempunctata is a convenient complement species for a parasitization rate comparison with H. axyridis. Both species are of similar size and are...
now dominant in Central European coccinellid fauna. Both species prefer different habitats, trees in the case of *H. axyridis* (Honek et al., 2015) and crops in the case of *C. septempunctata* (Honek and Rejmanek, 1982; Honek, 1985), which presents a difficulty. This differentiation of habitat preference is typical of adults and is highly expressed in larvae. The only sites where both species converge during pre-adult development are low-growing vegetation stands very abundantly populated by aphids (Lundgren et al., 2004; Kandel et al., 2015; Hesler, 2014). In stands of wild herbs, excessive aphid incidence may develop, e.g., on nettles (*Urtica dioica* L.) populated by *Microlophium carnosum* Buckton (*Homoptera* Aphididae) or on scentless mayweed - *Tripleurospermum inodorum* (L.) Schultz-Bip.- populated by *Macrospionella tapuskae* (Hottes et Frison) (*Homoptera* Aphididae). In crops, high population densities may develop, e.g., on Fabaceae, such as peas and beans, populated by *Acyrisphion pisum* Harris (*Homoptera* Aphididae). However, excessive aphid populations on crops are rarely tolerated by farmers, except in organic farming. An alignment of several favourable circumstances is thus necessary to observe the parallel development of *C. septempunctata* and *H. axyridis* pre-adult populations: finding stands of convenient host plants with abundant aphid populations and the presence of abundant parasitoid populations, timely detection of the site when the pupation of coccinellid populations start, and the personal conditions of the researcher to allow continued observation until the end of the presence of coccinellid immatures. This alignment of natural and personal circumstances manifested in June 2018, a very warm year typical of high aphid and natural enemy abundance. This paper describes quantitative and temporal aspects of coccinellid and parasitoid frequency at two sites colocated of high aphid and natural enemy abundance. This alignment of several favourable circumstances is thus tolerated by farmers, except in organic farming. An *Acyrthosiphon pisum* (Hemiptera *Aphididae*) or on scentless mayweed - *Tripleurospermum inodorum* (L.) Lundgren et al. (1985), which presents a difficult alignment of several favourable circumstances is thus necessary to observe the parallel development of two sites colocated of high aphid and natural enemy abundance.

### Materials and methods

#### Sampling sites

Coccinellid pupae were collected in June 2018 at two sites at Prague Ruzyne. Site 1 (centred at 50.089N 14.260E, area 250 m²) was comprised of *U. dioica* (nettles, populated by *M. carnosum*) interspersed with *Artemisia vulgaris* L., *Bailotula nigra* L., *Cirsium arvense* (L.) Scop., *Dactylis glomerata* L. and *Leonurus cardica* L. The small ruderal patch of site 1 was bordered by a large crop of winter wheat on one side and a grassy area of the Prague airport on the other side. Site 2 (centred at 50.082N 14.306E, area 20,000 m²) was an organi-farm crop of *Pisum sativum* L. (pea, populated by *A. pisum*) containing weeds of *C. arvense* and *Avena fatua* L. The field of site 2 was bordered by a mixture of small fields sown with barley, oats, buckwheat and sugar beet, a mixed tree stand along a small stream, and an urban area with small houses and gardens. At site 1, sampling started from June 4, at the beginning of coccinellid pupation, and continued until June 15, when pupation ceased. At site 2, the date of the start of pupation was unknown. Sampling started on June 14 and terminated on June 25 when coccinellid pupation ceased. Intraguild predation by coccinellid larvae and Heteroptera was recorded when observed during sampling sessions.

#### Detecting parasitism

At both sites, pupae of *C. septempunctata* and *H. axyridis* were sampled at two- to three-day intervals. During each sampling session at site 1, the entire area was carefully searched, and all pupae were collected. At site 2, the pupae were collected along a 50 to 200 m long transect randomly placed across the field. Pupae of both coccinellid species were sampled, giving scrupulous care to sample unselectively and not bias the natural species ratio. The pupae were placed individually in glass tubes (5 cm long, 1 cm diameter) plugged by a piece of cotton and placed in the laboratory at 23 ± 1 °C and natural photoperiod. Because the mean temperature over the sampling period in the field (20.6 ± 0.36 °C) was similar to the laboratory temperature, we assumed that the date of eclosion of coccinellids and parasitoids in the laboratory was the same as in the field. Therefore, regardless of the date of sampling, coccinellid adults and parasitoids were grouped according to the date when they eclosed in the laboratory. The number and cumulative fresh mass of *Phalacrotophora* puparia emerging from particular coccinellid pupae was determined within 24 h after pupation with 10⁻⁶ g precision, and the mean mass of the puparium was calculated. After eclosion of the last coccinellid adult and emergence of the last *Phalacrotophora* larvae, the remaining coccinellid pupae were preserved for 15 days, during which *Oomyzus* emergence was recorded, and the coccinellid was then considered dead. The dead coccinellid pupae were dissected to establish unclosed parasitoids. Adults of *Phalacrotophora* were identified to species using keys by Disney and Beuk (1997), Durska et al. (2003) and Lengyel (2009).

#### Data evaluation

The number of coccinellid pupae from which eclosed coccinellid adults (NA) emerged and the number of coccinellid pupae from which *P. fasciata* parasitoids emerged (NP) were recorded daily. The percentage of parasitism (PP) by *Phalacrotophora* on particular dates was calculated as PP = NP / (NP + NA) × 100. The temporal trend in the percentage of *Phalacrotophora* parasitism was approximated using linear regression PP = a + bD, where PP was the percentage of parasitism by *Phalacrotophora* (angular transformation) and D was the day of emergence. Using data from site 1, we further calculated linear regression M = a + bN, where M was
the average fresh mass of parasitoid puparium and N was the number of puparia emerged per coccinellid pupa. The differences in puparium size (M) at sites 1 and 2 were tested using the Mann-Whitney test. All calculations were performed using SigmaStat 3.5 (Systat Software, 2006).

Results

Three species of parasitoids were identified in the parasitization of the collected coccinellid pupae: the flies, *P. fasciata* and *Phalacrotophora berolinensis* Schmitz, and the wasp, *O. scaposus* (table 1). The former species was the only parasitoid recorded in *H. axyridis* and very clearly dominant among the parasitoids of *C. septempunctata*. *P. fasciata* and *O. scaposus* emerged only from *C. septempunctata* pupae.

Despite differences in the host plants and aphid prey, the trends in distribution and abundance of eclosed coccinellid adults and *P. fasciata* parasitism were similar at both sites. The parasitism of *Phalacrotophora* and mortality of pupae due to parasitism of *O. scaposus* and other causes were higher in *C. septempunctata* than in *H. axyridis* (table 2) and higher at site 1 (nettle patch) than at site 2 (pea crop) (table 2). The temporal trends in the eclosion of adult coccinellid species were similar at both sites. *C. septempunctata* eclosed earlier than *H. axyridis* at site 1 (figure 1A) as well as at site 2 (figure 2A). In contrast, the frequency of *Phalacrotophora* emergence had no temporal trend either at site 1 (figure 1B) (*C. septempunctata*: a = 2.156, b = −0.0765, R² = 0.2432, F₁,₁₀ = 2.289, P = 0.123; *H. axyridis*: a = 0.470, b = −0.0136, R² = 0.0329, F₁,₁₀ = 0.306, P = 0.593) or at site 2 (figure 2B) (*C. septempunctata*: a = 0.770, b = −0.00798, R² = 0.0117, F₁,₁₃ = 0.142, P = 0.712; *H. axyridis*: a = 0.144, b = 0.00405, R² = 0.0152, F₁,₁₃ = 0.185, P = 0.675). The numbers of *P. fasciata* puparia established per parasitized pupa of *C. septempunctata* (figure 3) were significantly greater at site 1 (7.2 ± 0.30, median 6) than at site 2 (3.9 ± 0.21, median 4) (P_{Mann-Whitney} < 0.001, U = 9533.500). The average mass of *P. fasciata* puparia decreased with an increased number of puparia per *C. septempunctata* pupa (a = 1.550, b = −0.0528, R² = 0.484, F₁,₁₄₂ = 132.5, P < 0.001) (figure 4).

Discussion

Asynchrony in coccinellid species eclosion

The data showed both unexpected and predicted trends. Earlier eclosion of *C. septempunctata* than *H. axyridis* adults was established at both the sites. Identical differences were observed in the native area of the invasive species (Hironori and Katsumi, 1997) as well as in area recently invaded by *H. axyridis* (Jansen and Hautier, 2008). The first possible explanation of this asynchrony is a difference in the development time of both species caused by different thermal requirements for their development. According to published information, the requirements for development in both species are similar. A lower development threshold (LDT) between 10.2-13.7 °C and a sum of effective temperatures (SET) between 191-297 degree days were established for *C. septempunctata* (Hodek, 1958; Obrycki and Tauber, 1981; Butler, 1982; Katsarou et al., 2005) and an LDT of 10.4-11.2 °C and an SET of 232-279 degree days were established for *H. axyridis* (Schanderl et al., 1985; LaMana and Miller, 1998; Stathas et al., 2010). Using these published data, we calculated the expected length of pre-imaginal development at 23 °C (a temperature close to natural and laboratory conditions at the time of experiment) to be 20.2 ± 1.09 days in *C. septempunctata* and 20.8 ± 0.99 days in *H. axyridis*. Thus, it is unlikely that the difference in eclosion time was caused by differences in pre-imaginal development time. Another cause may be differences in the terms of

<table>
<thead>
<tr>
<th>Host</th>
<th>Hostplant</th>
<th>N (%) parasitized by:</th>
<th>N (%)</th>
<th>P. f</th>
<th>P. b</th>
<th>P. sp.</th>
<th>O. s</th>
<th>O. s + P. f</th>
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</thead>
<tbody>
<tr>
<td><em>C. septempunctata</em></td>
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<td></td>
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<tr>
<td>Nettles</td>
<td>375</td>
<td>285 (78.9)</td>
<td>91 (10.5)</td>
<td>426</td>
<td>404 (94.8)</td>
<td>2 (0.5)</td>
<td>20 (4.7)</td>
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<tr>
<td>Peas</td>
<td>867</td>
<td>684 (78.9)</td>
<td>92 (10.6)</td>
<td>426</td>
<td>404 (94.8)</td>
<td>2 (0.5)</td>
<td>20 (4.7)</td>
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<td><em>H. axyridis</em></td>
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<tr>
<td>Nettles</td>
<td>310</td>
<td>2 (0.6)</td>
<td>5 (1.6)</td>
<td>426</td>
<td>1 (0.2)</td>
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<td>Peas</td>
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Table 1. Species composition of parasitoids that emerged from *C. septempunctata* and *H. axyridis* pupae collected at two sites. *P. f* - *Phalacrotophora fasciata*, *P. b* - *P. berolinensis*, *P. sp.* - undetermined Phalacrotophora (adults did not emerge from puparia), *O. s* - *Oomyzus scaposus*, *O. s + P. f* - multiparasitism by *O. scaposus* and *P. fasciata*.

<table>
<thead>
<tr>
<th>Host - Hostplant</th>
<th>C. septempunctata</th>
<th>H. axyridis</th>
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<tbody>
<tr>
<td>Site 1 - Nettles</td>
<td>N Total</td>
<td>N Eclosed</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>178 (47.5)</td>
</tr>
<tr>
<td>Site 2 - Peas</td>
<td>867</td>
<td>684 (78.9)</td>
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</table>
settling and/or the start of reproduction in the both species. Aphid densities necessary for settling *C. septempunctata* at crop stands (10 aphids per m² of field area) and starting ovariole maturation (1 aphid per 200-400 cm² of host plant leaf area) are low (Honek, 1980). Unfortunately, we do not know the corresponding information for *H. axyridis*; however, it is very likely that the aphid abundance necessary for attracting *H. axyridis* populations to herbaceous stands is greater. This is because the preferred habitat of *H. axyridis* are trees (Vandereycken *et al.*, 2012); the population may be lured to low growing stands only if aphid densities are
high (Nault and Kennedy, 2003; Lundgren et al., 2004). Additionally, Hironori and Katsumi (1997) observed that the oviposition period of *C. septempunctata* started earlier and was shorter than that of *H. axyridis*. The difference in requirements for settling at a habitat and starting oviposition may contribute to asynchrony in the timing of development of both species.

**Host-specific differences in pupal mortality**

In accordance with hypothesis (i), the parasitism rates were higher in *C. septempunctata* than *H. axyridis*. The difference (10-20 times higher) in the percentage of parasitized pupae between both species in our study was greater than the difference calculated from published data of many coccinellid species collected in a large geographic area (Ceryngier et al., 2018). The standardized conditions of our observation (using data from similarly sized coccinellid species collected simultaneously from the same site) likely contributed to estimating the real difference in parasitism of both coccinellid species. Interestingly, the proportion of unparasitized pupae that remained uneclosed was greater in *C. septempunctata* than in *H. axyridis*. One of the possible factors of pupal mortality may be predation (Devee et al., 2018). Predation by *Deraeocoris ruber* (L.) (Heteroptera Miridae) was observed several times during sampling sessions at site 1. Damage by sucking predators, uneasy to detect at sampling, may increase the number of dead pupae. Intraguild predation of coccinellid larvae on pupae was also observed but did not substantially contribute to the number of uneclosed pupae because pupae with visible external damage were excluded from the experiment. During sampling sessions at site 1, *H. axyridis* larvae were observed eating *C. septempunctata* pupae in 17 cases, while an *H. axyridis* larva eating a conspecific pupa was observed only once. A *C. septempunctata* larva eating a conspecific pupa was observed in one case, while larvae eating *H. axyridis* pupae were never observed. Further possible factors of pupal mortality (Ceryngier et al., 2012) were not established.

**Host-specific differences in parasitism**

Most important among the recorded species of parasitoids was *P. fasciata*, with only few hosts found to be parasitized by *P. berolinensis* or *O. scaposus*. However, *Phalacrotophora* puparia obtained from some coccinellids (especially from site 2) did not give rise to adult flies, and their species identity was not determined. It cannot be excluded that those unhatched puparia contained dead or diapausing individuals of species other than *P. fasciata*. Durska et al. (2003) found that *Phalacrotophora heuki* Disney, a parasitoid of *Anatis ocellata* (L.), did not generally emerge from puparia under laboratory conditions.

The differences in the parasitism and mortality of *C. septempunctata* and *H. axyridis* pupae may indicate a low ability in host location and a low host acceptance and/or low nutritional suitability of the latter species for parasitoids as demonstrated by Firlej et al. (2007) and Comont et al. (2014) for a braconid, *D. coccinellae*. Effects of *H. axyridis* mechanical, e.g. larval spines (Hau-tier et al., 2017), behavioural (Firlej et al., 2010), chemical (Sloggett et al., 2011) or microbial (Vilkinskas et al., 2013) protection against the natural enemies may also play a part. Anyway in Italy it was found that the acceptance and suitability of *H. axyridis* adults as host for *D. coccinellae* in comparison with the native *A. bipunctata*, which shares the same ecological niche, was higher than in *A. bipunctata* (Dindo et al., 2016). Hence *D. coccinellae* may play a marginal role in controlling the populations of *H. axyridis*, without representing a threat to *A. bipunctata* (Dindo et al., 2016). The effectiveness of defensive mechanisms against *Phalacrotophora* or *Oomyzae* have not yet been investigated. Species-specific selection of pupation sites observed during sampling sessions at both sites might also play a role in the varying rate of parasitism. At both sites, the pupae were often found on plant species other than host plant of the aphid population, more frequently in *H. axyridis* than *C. septempunctata*. Pupation on *C. arvensis* and *A. fatua* leaves was particularly frequent at the pea stand (site 2). This difference only became conspicuous at the end of the pupation period and therefore was not strictly recorded during all sampling sessions. The selection of detached pupation sites may decrease the rate of predation or parasitism. Such a phenomenon was recorded by Osawa (1992) in *H. axyridis* within its native range (Kyoto, Japan), where coccinellid pupae found on host plants were significantly more often cannibalized and parasitized by *Phalacrotophora* sp. than those located outside of the host plants.

**Local differences in parasitism**

In accordance with hypothesis (ii), the rates of parasitism differed at both sites. This was likely influenced by different ratios of available coccinellid pupae to populations of *Phalacrotophora*, and the difference thus may have two concurrent causes: a greater density of *Phalacrotophora* and a smaller abundance of coccinellid pupae at site 1 than at site 2. The density of *Phalacrotophora* (number of individuals per unit of area) at both sites was not established. However, it is likely that it was greater at site 1 (a small patch of nettles hosting aphids and surrounded by a large aphid-free area) than at site 2 (a pea crop approximately 80-times larger than the nettle patch), where the parasitoid population may be diluted over a larger area. In contrast, the supply of coccinellid pupae might be less at site 1 than at site 2. With admission of ca. 50% sampling success of the collector (A. Honek), there were in total ca. 2000 pupae per nettle patch (site 1), i.e., approximately 8 pupae of both species per m². In pea crop (site 2), orientation counting at twenty randomly placed 1 m² plots indicated a population density of ~25 pupae of both species per m². Thus, a 20,000 m² plot might contain half a million pupae available to parasitism. This abundance might contribute to decreasing the parasitoid pressure. The local conditions, abundance of parasitoids and abundance of coccinellid pupae might all influence parasitism rates and parasitism intensity. Proportions of parasitized pupae as well as numbers of *Phalacrotophora* puparia per a parasitized pupa were less at site 2 than at site 1. Higher average numbers of *Phalacrotophora* puparia in *C. septempunctata* pupae collected at site 1 compared to
those from site 2 may suggest that at site 1, with higher densities of the flies and lower densities of coccinellid pupae, the cases of oviposition by more than one fly into the same host were more frequent than at site 2. Multiparasitism is sometimes reported in *Phalacrotophora* (Disney et al., 1994; Ceryngier and Hodek, 1996; Ceryngier et al., 2012), but certainly also simultaneous parasitization of a pupating coccinellid by several females of the same species (e.g. *P. fasciata*) is not uncommon (P. Ceryngier, unpublished observations). Additionally, the frequency of parasitism of *C. septempunctata* in relation to *H. axyridis*, which was 14 times more frequent at site 1 compared to 21 times more frequent at site 2, indicates that *Phalacrotophora* at site 2 most often selected *C. septempunctata* as a host, probably because of its greater availability.

**General trends**

The results confirmed the low susceptibility of *H. axyridis* to *Phalacrotophora* and *Oomyzus* parasitism compared to other coccinellid species, in this case, *C. septempunctata* (Ceryngier et al., 2018). The difference in parasitism rates persisted despite both species being similar in body size, development time, site of occurrence, development rate and probably other characteristics that were not recorded. The study also revealed local variation in parasitism rates, probably due to the abundance of host and parasitoid species. The studies of different rates of parasitism in the field should take into account both species-specific differences in coccinellid sensitivity to parasitism and local variation of parasitoid abundance.

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