



Lady Beetle Oviposition Site Choices: Maternal Effects on Offspring Performance

Authors: Sicsú, Paula Ramos, Macedo, Regina Helena, and Sujii, Edison Ryoiti

Source: Florida Entomologist, 103(2) : 228-235

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.103.0212>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Lady beetle oviposition site choices: maternal effects on offspring performance

Paula Ramos Sicsú¹, Regina Helena Macedo², and Edison Ryoiti Sujii^{1,*}

Abstract

Maternal effects can result in adaptive phenotypic responses of offspring that increase their fitness. Several recent studies report a positive relationship between maternal choices and offspring fitness. However, few studies have assessed the associations between aphidophagous lady beetle oviposition site selection, the adequacy of food resources for their offspring, and offspring performance. To clarify these issues, we explored whether aphidophagous lady beetle oviposition site selection can influence offspring performance, thereby reflecting adaptive maternal behavior. To assess the effect of food resources on offspring performance, we fed the larvae of 3 lady beetle species: *Cycloneda sanguinea* (L.), *Harmonia axyridis* (Pallas), and *Hippodamia convergens* (Guérin-Méneville) (all Coleoptera: Coccinellidae), with different types of aphids (*Uroleucon* and *Brevicoryne*) (both Hemiptera: Aphididae) then measured development time, survival, adult body mass, brightness, and chromatic coloration of the adult beetles. We found that *Ha. axyridis* larvae fed *Brevicoryne* exhibited a higher survival rate than those fed *Uroleucon*. Also more adults emerged from *C. sanguinea* larvae fed *Uroleucon* aphids, and these adults were heavier, shinier, with more chroma, and developed faster than those fed *Brevicoryne*. Overall, lady beetles performed better when fed aphids commonly found near their respective oviposition sites. We conclude that aphidophagous lady beetle oviposition site selection reflects adaptive maternal choices associated with an adequate food resource positively affecting offspring fitness. Elucidating the links between lady beetle oviposition site choice and optimal resource for their offspring enhances our understanding of habitat use and co-existence of aphidophagous lady beetle communities in Brazilian agroecosystems.

Key Words: *Cycloneda sanguinea*; *Harmonia axyridis*; *Hippodamia convergens*; *Uroleucon*; *Brevicoryne*; adaptive maternal behavior

Resumo

Os efeitos maternos podem resultar em respostas fenotípicas adaptativas da prole que aumentam sua aptidão. Vários estudos recentes relatam uma relação positiva entre escolhas maternas e aptidão dos filhos. No entanto, poucos estudos avaliaram as associações entre a seleção do local de oviposição de besouros afidófagos, a adequação dos recursos alimentares para a prole e o desempenho da prole. Para esclarecer essas questões, avaliamos se a seleção do local de oviposição de besouros afidófagos pode influenciar o desempenho da prole, refletindo assim o comportamento materno adaptativo. Para avaliar o efeito dos recursos alimentares no desempenho da prole, alimentamos as larvas de três espécies de besouros: *Cycloneda sanguinea* (L.), *Harmonia axyridis* (Pallas), e *Hippodamia convergens* (Guérin-Méneville) (todos Coleoptera: Coccinellidae), com diferentes tipos de pulgões (*Uroleucon* e *Brevicoryne*) (ambos Hemiptera: Aphididae), medimos o tempo de desenvolvimento, a sobrevivência, a massa corporal adulta, o brilho e a coloração cromática dos besouros adultos. Foi observado que larvas de *Ha. axyridis* alimentadas com *Brevicoryne* exibiram uma taxa de sobrevivência mais alta do que aquelas alimentadas com *Uroleucon*. Também emergiram mais adultos de larvas de *C. sanguinea* quando alimentadas com pulgões *Uroleucon* e esses adultos eram mais pesados, mais brilhantes, com mais cromagem e se desenvolveram mais rapidamente do que aqueles alimentados com *Brevicoryne*. No geral, as joaninhas tiveram melhor desempenho quando alimentados com pulgões comumente encontrados perto de suas respectivas posturas. Concluímos que a seleção do local de oviposição de besouros afidófagos reflete escolhas maternas adaptativas associadas a um recurso alimentar adequado que afeta positivamente a aptidão da prole. A elucidação dos vínculos entre a escolha do local de oviposição do besouro e o recurso ideal para seus filhotes aprimora nossa compreensão do uso do habitat e a coexistência de comunidades de besouros afidófagos nos agroecossistemas brasileiros.

Palavras Chaves: *Cycloneda sanguinea*; *Harmonia axyridis*; *Hippodamia convergens*; *Uroleucon*; *Brevicoryne*; comportamento adaptativo materno

Maternal effects that modify offspring phenotype can influence population dynamics and may strongly influence a species' evolutionary trajectory (Marshall et al. 2010). Maternal decisions also involve a series of choices including mate choice, selection of oviposition site, and maternal care, often resulting in considerable effects upon offspring phenotype and fitness (Mousseau & Dingle 1991; Bernardo 1996; Mousseau & Fox 1998). Female insects behave adaptively to increase fertility by laying a considerable number of eggs in repeated oviposition events in different sites that are thought to be chosen due

to specific characteristics (Daloze et al. 1995; Obrycki & Kring 1998; Seagraves 2009; Hodek et al. 2012). Because insect eggs and larvae have high probabilities of being preyed upon, females should select oviposition sites that increase the chances of survival and rapid development of offspring, thereby increasing their own maternal fitness (Mousseau & Dingle 1991; Mousseau & Fox 1998).

Lady beetles (Coleoptera: Coccinellidae) do not exhibit active parental care after oviposition (Grimaldi & Engel 2005). This behavior reinforces the importance of understanding the influence of pre-hatch

¹Universidade de Brasília, Laboratório de Comportamento Animal, Departamento de Zoologia, Campus Universitário Darcy Ribeiro, Brasília, Distrito Federal, 70910-900, Brazil; E-mail: paulasicsu@gmail.com (P. R. S.), rhfmacedo@unb.br (R. H. F. M.)

²Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica - PqEB - Av. W5 Norte (final), Brasília, Distrito Federal, 70770-917, Brazil; E-mail: edison.sujii@embrapa.br (E. R. S.)

*Corresponding author; Email: edison.sujii@embrapa.br

choices, such as selection of oviposition sites, on offspring fitness and population distribution. Usually, lady beetles develop through 4 larval instars before pupating, generally increasing in size and weight after each molt. Many lady beetle species in the Coccinellinae subfamily are polyphagous predators that feed preferentially on aphids during larval and adult stages (Völkl et al. 2007; Giorgi et al. 2009). Lady beetle eggs and larvae often suffer intraguild predation (i.e., killing and eating individuals of another species that use similar, often limiting, resources) as well as cannibalism, especially when contiguous populations occur and are abundant (Seago et al. 2011). This is because eggs are not mobile and larvae have no flight ability.

Environmental conditions and resource quality are essential determinants of female lady beetles' reproductive success (Hodek et al. 2012). The nutritional quality of aphids, which are lady beetles' main prey item, also is critical for larval survival because high quality resources allow faster development, decreasing chances of predation during the most vulnerable developmental stages (Lundgren 2009; Hodek et al. 2012). Moreover, appropriate diets during the developmental period allow adult lady beetles to express valuable survival and reproductive traits, including aposematic coloration (Prudic et al. 2006) and higher mass (Lundgren 2009; Hodek et al. 2012), respectively. Most lady beetles are aposematic and their bright colors conspicuously advertise their unpalatability to other predators, particularly birds (Dolenská et al. 2009; Arenas et al. 2015). Interactions between aphid populations and host plants also are important factors that structure the relative abundance of aphidophagous lady beetles in their assemblages (Sicsú et al. 2015).

In Brazilian heterogeneous agroecosystems, native lady beetles *Cycloneda sanguinea* (L.), *Hippodamia convergens* (Guérin-Méneville), *Eriopis connexa* (Germar), and exotic *Harmonia axyridis* (Pallas) (all Coleoptera: Coccinellidae) display different habitat use patterns that appear to facilitate their coexistence through niche partitioning (Sicsú et al. 2015). The distribution patterns of these populations are closely associated with female oviposition sites, reflecting a strong link between immature stages (eggs and larvae) with specific plants (Sicsú et al. 2015). However, few studies have explored the associations between aphidophagous lady beetle oviposition site selection and adequate food resources for offspring as it relates to subsequent fitness. Offspring performance is a key element to understand how maternal choices for specific oviposition sites are determinant factors for the survival and reproduction (fitness) of aphidophagous lady beetle offspring. The link between female behavioral choices and offspring performance also has consequences for community structure because this may be associated with the co-occurrence of species through niche partitioning and reduction of intraguild predation (Sicsú et al. 2015). Thus, examining lady beetle oviposition site choice effects will enhance our understanding about differential habitat use and coexistence of different species in Brazilian agroecosystems.

Based on lady beetle interactions formerly observed under field conditions (Sicsú et al. 2015), we formulated the following questions: (1) do lady beetle preferences for specific oviposition sites in the field affect offspring performance, suggesting adaptive maternal behaviors?, and (2) what is the influence of adaptive maternal behavior on the co-existence of native and exotic species of aphidophagous lady beetles in Brazilian agroecosystems? We hypothesized that females should choose to lay their eggs closest to an optimal food resource that optimizes offspring performance. Therefore, in laboratory experiments, we expected that (1) lady beetle larvae would fare better when fed an aphid species most frequently associated with their clutches in the field, and (2) lady beetle fitness would be adversely affected when reared on aphid species rarely found near their egg patches in the field (Sicsú et al. 2015).

Materials and Methods

EFFECT OF DIET ON THE DEVELOPMENT AND QUALITY OF BEE-TLE OFFSPRING

The effect of food resources on immature development and adult traits was tested with the 3 most abundant Coccinellinae lady beetle species found in the Federal District agroecosystems in Brazil: *C. sanguinea*, *Ha. axyridis*, and *Hi. convergens* (Sicsú et al. 2015). Larvae were fed exclusively on aphids of the same genus during their entire development, which could be either *Uroleucon* aphids or mealy cabbage (*Brassica*) aphids (*Brevicoryne brassicae*) (L.) (both Hemiptera: Aphididae). These aphids typically are found associated with the lady beetle species used in this experiment, but with different distributional patterns in the field. Sicsú et al. (2015) showed that the highest number of egg clutches of *C. sanguinea* were associated with Asteraceae plants, i.e., Spanish needle flower (*Bidens pilosa* L.), Mexican sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray), and sowthistle (*Sonchus oleraceus* L.) when infested with *Uroleucon* aphids. Few egg clutches of this beetle species were associated with *Brassica oleracea* L. (Brassicaceae), kale and cabbage infested with *Brevicoryne* aphids. *Harmonia axyridis* egg clutches, on the contrary, were associated commonly with *Brassica* plants when infested with *Brevicoryne* aphids. *Hippodamia convergens* clutches were distributed evenly on Asteraceae plants infested with *Uroleucon* aphids or on *Brassica* plants infested with *Brevicoryne* aphids.

Studies were conducted from Aug to Nov 2012. Field collected females (22 *C. sanguinea*, 21 *Ha. axyridis*, and 23 *Hi. convergens*) were kept individually in 250 mL jars. They were mated with different males from a breeding colony established at Embrapa Genetic Resources and Biotechnology, Brasília, Brazil. All lady beetles were provisioned with a diet based on cotton hydrated with honey solution (70%) and a variety of aphids from different species collected in organic vegetable farms around the Embrapa Research Station. Four newly hatched larvae from each female beetle species were selected randomly to be used in the experiment; 2 of which were randomly chosen to be reared exclusively with *Uroleucon* aphids and 2 to be reared exclusively with *Brevicoryne* aphids. Some individuals died due to external factors, but across the experiment we used 83 *C. sanguinea*, 80 *Ha. axyridis*, and 86 *Hi. convergens* larvae.

Each larva was individually identified and kept separately in a 50 mL plastic container with an acrylic cover (CEPEL, São Paulo, São Paulo, Brazil) containing a moistened cotton swab and a strip of paper towel. The paper towel strips were changed every 2 d and provided a walking surface for larvae and aphids, thereby increasing aphid survival in the containers (Paula Sicsú, personal observation). Larvae were provisioned with an ad libitum diet consisting of 0.1 g of aphids every other d (aphid rearing methods adapted from Abdel-Salam & Abdel-Baky 2001; Berkvens et al. 2008). *Brevicoryne* aphids were field collected from *B. oleracea* plants and the *Uroleucon* aphids on Asteraceae plants (*S. oleraceus*, *B. pilosa*, and *T. diversifolia*). Larvae were monitored daily to collect data for the following variables: development stage (larval instar, pupae, or adult), development time of immature stages, and mortality. Mortality was used to assess survival and total development time (period between hatching and d of death). Larval instar and pupae periods were estimated in number of d. Exuviae and dead lady beetles were removed daily. Pupae were individually kept in containers with moist cotton until adult emergence because they do not feed during this period.

Adult beetles were sexed 12 h after emergence with a stereoscopic microscope (Zeiss Stemi 2000 C) (Zeiss, Jena, Germany). To assess the effect of food resources on offspring quality we determined adult weight with an electronic microbalance (BIOPRECISA® JA3003N; accuracy of 0.001 g) (Bioprecisa, Curitiba, Paraná, Brazil). Adults were main-

tained in containers with moistened cotton for 72 ± 12 h, the required period for their elytra to attain the typical adult coloration (Blount et al. 2012). Adults were then sacrificed by being subjected to a temperature of -20 °C for approximately 20 min. Following this, elytra were carefully removed with entomological tweezers and kept in freezers at -40 °C until elytra reflectance measurements were taken. Elytra reflectance in relation to a standard white WS-1-SS (a diffuse white plastic that provides a Lambertian reference surface for reflectance experiments) was measured using a USB 4000 spectrophotometer coupled to a PX-2 pulsed xenon light source in relation to a standard white (WS-1SS) (all from Ocean Optics, Dunedin, Florida, USA), with the support of an optical fiber positioned at an angle of 45° relative to the flat surface where the elytra were positioned. The elytra were exposed individually on black suede paper with a standardized distance of 20 mm between the fiber and measuring surface. Three measurements were recorded per elytron (i.e., 6 spectral measures per adult). Thirty individual readings were completed for each measurement, generated with an integration time of 100,000 msec. Measurements were performed with the Spectra Suite program (Ocean Optics, Dunedin, Florida, USA).

STATISTICAL ANALYSIS

The effect of diet on larval survival rate of each species was assessed with a survival curve using the software package “survival” (Therneau 2012) with “coxph” function controlling for maternal identity effect. This analysis determines death probability taking into account the effect of diet on individual longevity (in this case, total development time in d), controlling for the effect of maternal identity of larvae (covariate in the analysis). A log-rank test was used to compare survival rates among the diets. A model diagnosis was conducted as suggested by Fox (2002) and the result was consistently $P \geq 0.05$, so that no model assumption was violated.

Elytra spectral data (320–700 nm) were analyzed using the software package “pavo” (Maia et al. 2013). The brightness and chromatic color means derived from carotenoids, i.e., chroma (carotenoids) were generated from the 6 measurements taken for each individual. Aver-

age brightness was obtained from the average reflectance over the entire spectral amplitude. Chroma was measured as $(R_{\lambda 450} - R_{\lambda 700}) / R_{\lambda 700}$, in which R_{λ} is the reflectance percentage at the wavelength in question (Ornborg et al. 2002; Montgomerie 2006; Maia et al. 2013). A multivariate approach was used to evaluate the effect of diet on the set of dependent variables: development time, weight, brightness, and chroma. First, we conducted a Principal Component Analysis of the dependent variables transformed to a standardized normal distribution (Quinn & Keough 2002). For each principal component we conducted a Monte-Carlo randomized t -test (10,000 permutations) between the 2 diets for each dependent variable. All analyses were performed using R (R Core Team 2012), differences were considered significant at $P < 0.05$.

Results

EFFECT OF DIET ON DEVELOPMENT AND QUALITY OF BEETLE OFFSPRING

From the 40 *Ha. axyridis* larvae fed with *Brevicoryne* aphids, 15 (37.5%) emerged as adults, whereas for the 40 larvae fed with *Uroleucon* aphids, none pupated and only 1 larva reached the final fourth instar. *Harmonia axyridis* larvae that fed on *Brevicoryne* had a higher survival rate than those that fed on *Uroleucon* (log rank = 56.4; G.L. = 1; $P < 0.05$; Fig. 1A). The opposite pattern was observed for *C. sanguinea* where 42 larvae fed *Uroleucon* aphids produced 29 (69%) adults, whereas of 41 larvae fed *Brevicoryne* only 4 (9.8%) emerged as adults. This difference in daily survival for larvae fed either aphid species was significant (log rank = 24.68; G.L. = 1; $P < 0.05$; Fig. 1B).

Development patterns associated with either aphid species were not distinct for *Hi. convergens*, where 10 of 41 (24.4%) larvae fed *Uroleucon* emerged as adults as well as 8 of 47 (17%) that had fed on *Brevicoryne*. There was a large overlap between *Hi. convergens* larval survival when fed either aphid species (log rank = 0.03; G.L. = 1; $P = 0.88$). The number of d that predatory beetle larvae spent in each developmental stage did not differ statistically due to aphid prey species (Table 1).

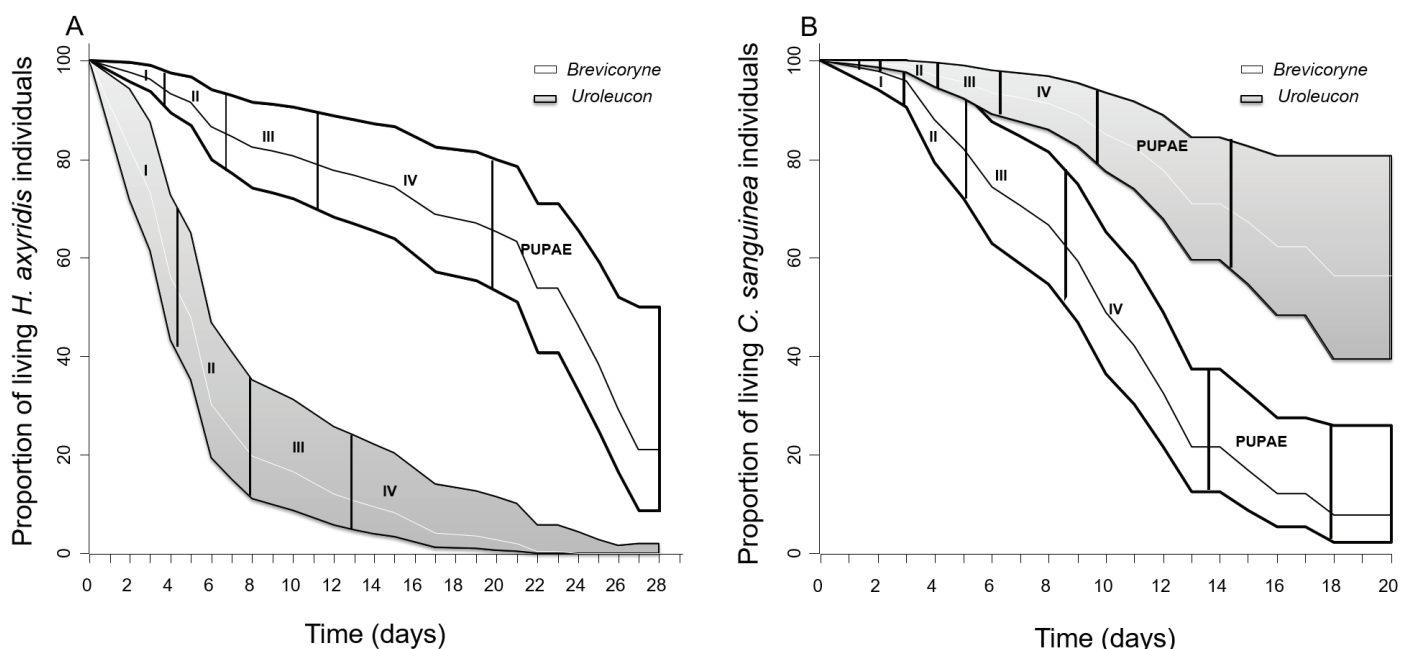


Fig. 1. Survival curves as a function of time for *Harmonia axyridis* (A) and *Cycloneda sanguinea* (B) immatures fed with aphids *Brevicoryne* (white) or *Uroleucon* (grey). Straight lines demarcate the average periods for instars (first to fourth in Roman numerals) and pupae.

Table 1. Duration in d (mean \pm SD) of each larval developmental stage in *Cycloneda sanguinea*, *Harmonia axyridis*, and *Hippodamia convergens* when fed with aphids of the genus *Uroleucon* (U) or *Brevicoryne* (B). Roman numerals indicate larval instars.

Lady beetle	Diets	I-II	II-III	III-IV	IV-Pupae	Pupae-Adult
<i>Cycloneda sanguinea</i>	B	2.9 \pm 0.7	2.2 \pm 0.8	3.5 \pm 1.0	5.0 \pm 1.2	4.3 \pm 0.5
	U	2.1 \pm 0.4	2.0 \pm 0.4	2.2 \pm 0.8	3.4 \pm 1.4	4.7 \pm 0.6
<i>Hippodamia convergens</i>	B	2.8 \pm 0.8	2.2 \pm 0.6	2.8 \pm 0.6	4.9 \pm 1.0	5.0 \pm 0.0
	U	3.1 \pm 0.9	2.6 \pm 0.8	2.9 \pm 0.4	4.6 \pm 1.5	5.3 \pm 0.5
<i>Harmonia axyridis</i>	B	3.7 \pm 1.4	3.1 \pm 1.5	4.5 \pm 1.2	8.5 \pm 2.6	5.5 \pm 0.5
	U	4.3 \pm 1.8	4.0 \pm 1.0	5.0	–	–

No *Ha. axyridis* individuals reached the adult stage when fed *Uroleucon* during larval development. Also we did not test for an effect of aphid diet on male and female attributes because the number of *C. sanguinea* and *Hi. convergens* fed either aphid species was small (Fig. 2). For all beetle species, males tended to be lighter in mass than females. Moreover, larvae that took more time to develop tended to develop into lightweight adults compared with those that developed during shorter periods (Fig. 2). *Cycloneda sanguinea* larvae tended to be heavier and developed faster when fed *Uroleucon*. However, for *Hi. convergens*, there appeared to be no difference in weight and development time when fed either aphid species.

The spectra of adult elytra reflectance of both lady beetle species when fed *Brevicoryne* or *Uroleucon* during the larval stage is illustrated in Figure 3. The brightness and chroma (carotenoids derived) of *C. sanguinea* adults fed *Brevicoryne* tended to be lower than that of adults fed *Uroleucon* (Fig. 4A). *Hippodamia convergens* adults did not differ for these attributes between either aphid prey (Fig. 4B).

Principal Component Analyses of adult *C. sanguinea* adults fed *Uroleucon* or *Brevicoryne* as larvae was significantly different only for component 1 (PC1) ($P < 0.001$). Thus, *C. sanguinea* individuals reared with *Uroleucon* were heavier on average, shinier, with more chroma, and developed in less time than those fed *Brevicoryne* (Fig. 5B). The correlation between these variables and PC1 are detailed in Table 2 and the variance explained by this component was 50%.

None of the other comparisons among larvae fed either aphid prey species was significant for *C. sanguinea* ($P > 0.05$) for all other comparisons.

There also was no difference in weight, brightness, chroma, or developmental time for either adult predator when fed *Uroleucon* or *Brevicoryne* as larvae ($P > 0.05$) (Fig. 5A). The correlation between all these variables and PC1 to PC4 and the variance explained for each component are detailed in Table 3.

Discussion

Aphids usually are an essential resource for larval development of aphidophagous lady beetles. Morphological and chemical differences among aphid species may determine how this food resource is more or less suitable for each beetle species (Michaud 2005). Enhanced development of *Ha. axyridis* larvae was achieved when fed *Brevicoryne* aphids reared on *Brassica*, whereas this same scenario generated high mortality in *C. sanguinea* larvae. Ingestion of *Brevicoryne* by some ladybug species may disrupt their development via accumulation of glucosinolates obtained by the aphids from their host plant (Francis et al. 2001). Glucosinolates, particularly sinigrin, are the main secondary metabolites accumulated by cruciferous plants as a chemical defense against pests (Wink 1988). *Harmonia axyridis* may

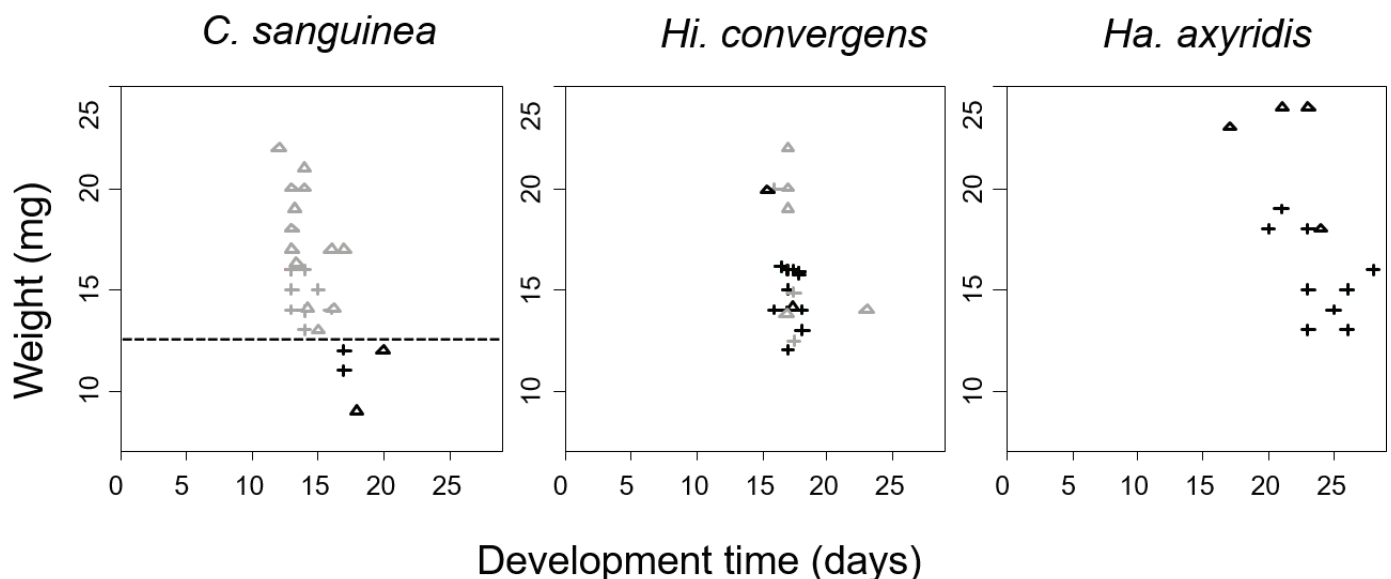


Fig. 2. Individual adult weight (mg) for *Cycloneda sanguinea*, *Hippodamia convergens*, and *Harmonia axyridis* that emerged after the *Uroleucon* (grey) and *Brevicoryne* (black) larvae diets as a function of development time (d). Females are indicated with triangles and males with crosses. The dashed line indicates the weight difference of *Cycloneda sanguinea* in each diet.

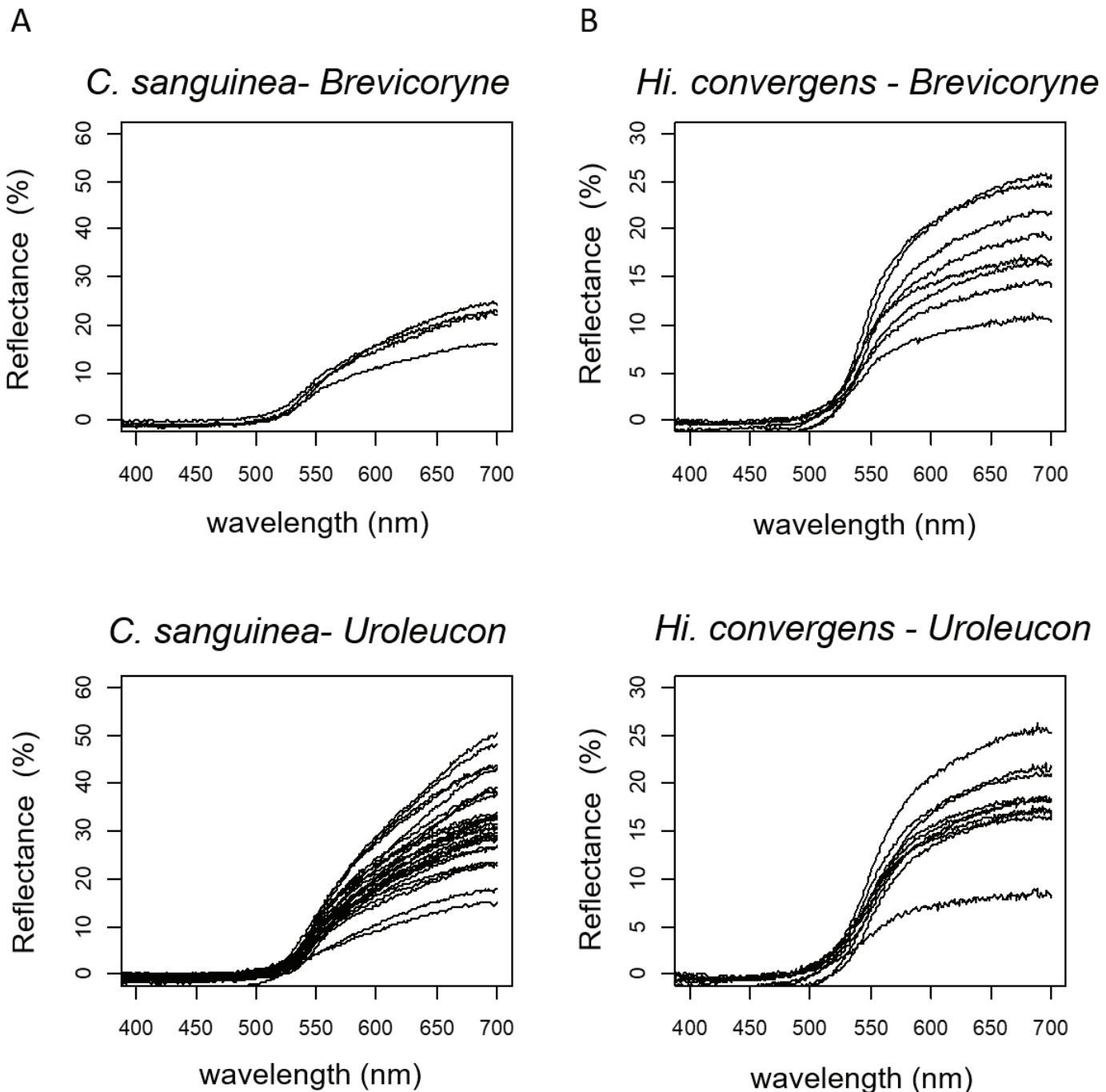


Fig. 3. Reflectance spectrum for the elytra of *Cycloneda sanguinea* ($n = 33$) (A) and *Hippodamia convergens* ($n = 18$) (B) adults when fed with *Uroleucon* or *Brevicoryne* aphids during the immature stages. Each curve represents the average of 6 spectral measures (3 in each elytra) performed for each individual.

present counter adaptations that enable them to feed on chemically defended *Brevicoryne* without apparent negative effects. *Harmonia axyridis* fed *Uroleucon* exhibited high mortality in newly hatched larvae in addition to an inability to develop to adulthood. These results thus indicate that *Uroleucon* aphids are not an adequate food for this invasive coccinellid, confirming previous findings (Snyder et al. 2000). Morales and Burandt (1985) reported that frozen *Uroleucon* aphids appeared to be a suitable resource for the development of *C. sanguinea* as well as *Hi. convergens* as shown in a 24-h no-choice laboratory test (Hesler 2013). Our study is the first to demonstrate that *Uroleucon* aphids are suitable for complete holometabolous development of *C. sanguinea*.

We found *C. sanguinea* larvae fed *Uroleucon* aphids developed faster than those fed on *Brevicoryne*, whereas development time of *Hi. convergens* larvae was similar for individuals reared with either aphid species. Rapid growth of immatures may be adaptive if development time is inversely related to survival rate, conferring advantages to individuals that become adults more quickly (Slansky & Rodriguez 1986; Clancy & Price 1987; Polis et al. 1989). Although the presence of potential predators and competitors has an important effect on female behavior, adequate resource also is extremely important in oviposition site selection, especially when considering that adequate food will allow immatures to develop quickly and escape from enemies (Seagraves 2009; Hodek et al. 2012).

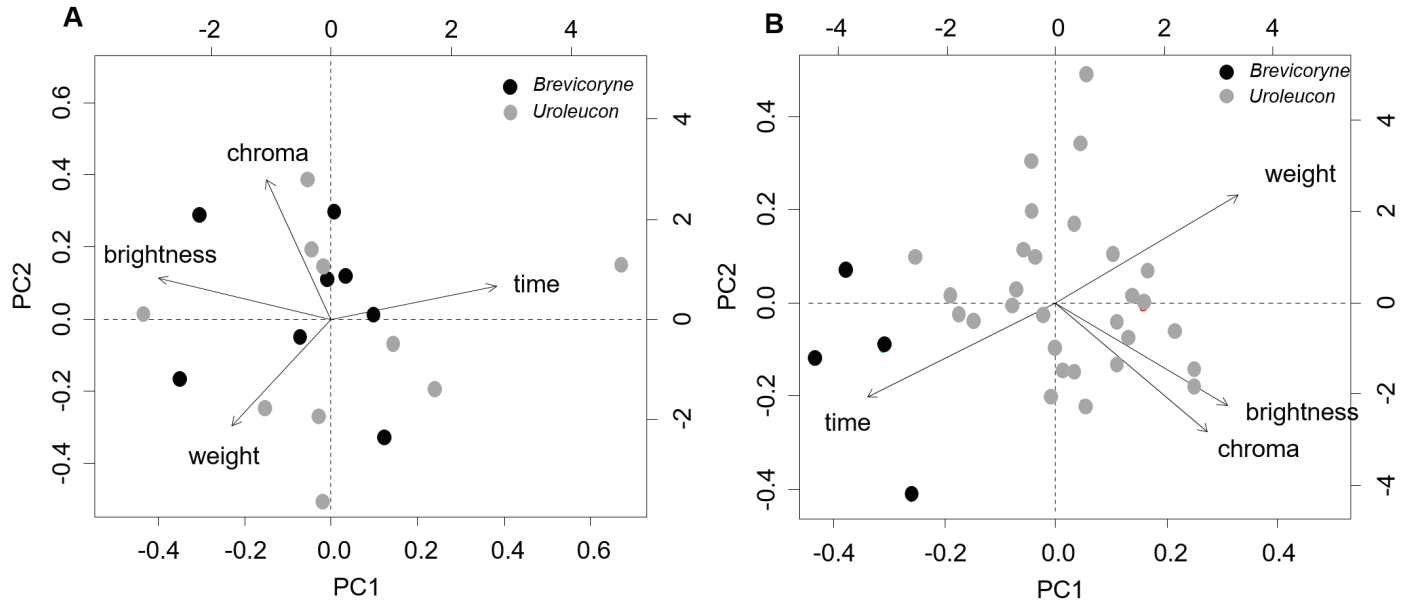


Fig. 5. PCA graph performed for the following variables: development time (time), brightness, weight, and chroma derived from carotenoids, with the position of *Hippodamia convergens* (A) and *Cycloneda sanguinea* (B) adults which have emerged from the *Uroleucon* (grey) and *Brevicoryne* (black) diet during the immature stages, when sorted in function of the 2 main axes of data variation.

Lady beetle weight usually is correlated negatively with development time; the quality of larval food may have a significant impact on the size achieved by the adult (Michaud 2005; Evans 2000). Adult body size often reflects differential success of individuals as immatures in obtaining food because they may accumulate more nutrients needed for reproduction when they become adults (Slansky & Rodriguez 1986; Evans 2000). In addition to development time, other phenotypic features of adults have significant effects on fitness (Slansky & Rodriguez 1986). In our study we found that *C. sanguinea* adults fed *Uroleucon*

were heavier, brighter, and exhibited greater chromatic coloration derived from carotenoids than those fed *Brevicoryne*. However, the features of *Hi. convergens* adults fed *Uroleucon* in the larval stage did not differ from those of adults fed *Brevicoryne* aphids. Thus, both aphid species were adequate for the development of this lady beetle species. These data complement field observations in which *C. sanguinea* egg clutches were associated with plants infested with *Uroleucon*, whereas *Hi. convergens* egg clutches were associated with plants infested with *Uroleucon* and *Brevicoryne* (Sicsú et al. 2015). Thus, we can conclude

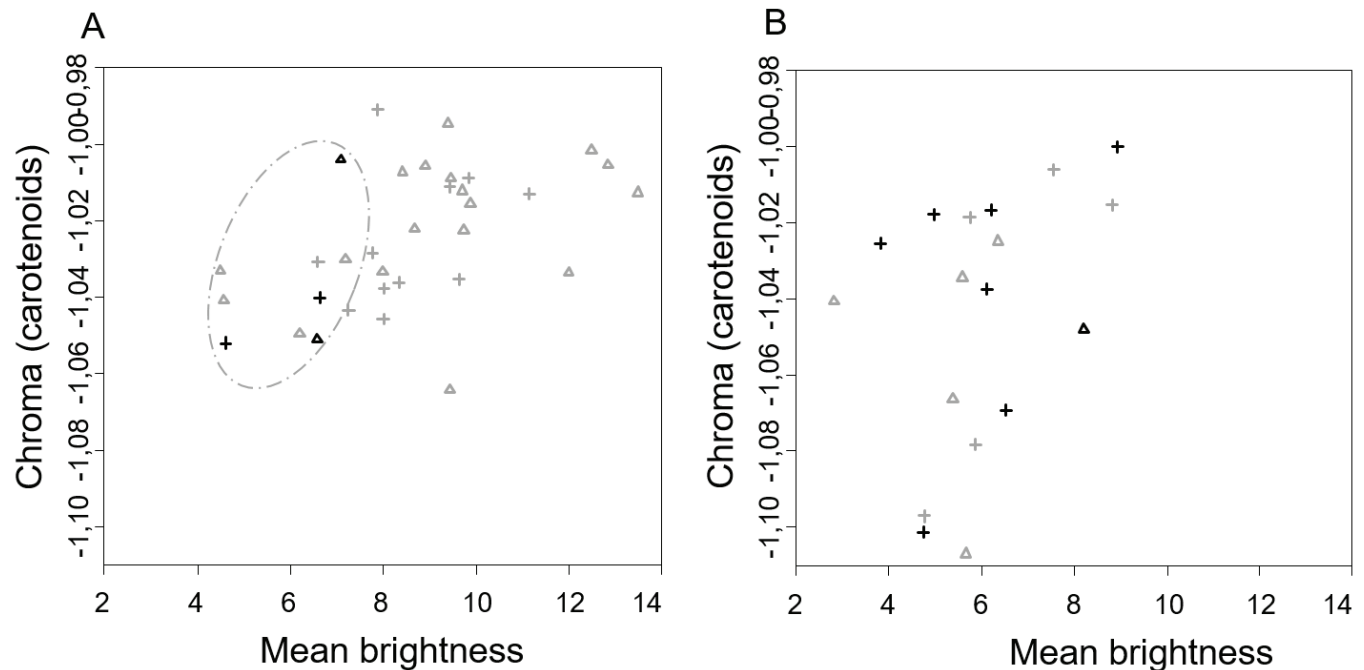


Fig. 4. Chroma (carotenoids) of *Cycloneda sanguinea* (A) and *Hippodamia convergens* (B) adults that developed with the *Uroleucon* (grey) and *Brevicoryne* (black) diets during the immature stages as a function of the mean brightness. Females are indicated with triangles and males with crosses. The dashed ellipse highlights the difference in mean brightness of *Cycloneda sanguinea* adults for each diet.

Table 2. Correlation of variables measured for the *Cycloneda sanguinea* adults with the 4 principal components (PC) generated by Principal Component Analysis for larvae fed *Uroleucon* or *Brevicoryne* aphids and the proportion of variance explained for each component.

<i>Cycloneda sanguinea</i>	PC1	PC2	PC3	PC4
Weight	0.52	0.49	0.69	0.00
Brightness	0.49	-0.47	0.72	-0.13
Chroma for carotenoids	0.44	-0.59	-0.65	0.18
Development time	-0.54	-0.43	0.21	0.69
Proportion of variance	0.50	0.28	0.12	0.10

Table 3. Correlation of variables measured for the *Hippodamia convergens* adults with the 4 principal components (PC) generated by Principal Component Analysis for larvae fed *Uroleucon* or *Brevicoryne* aphids and the proportion of variance explained for each component.

<i>Hippodamia convergens</i>	PC1	PC2	PC3	PC4
Weight	-0.37	-0.58	0.72	0.00
Brightness	-0.65	0.22	-0.12	0.72
Chroma for carotenoids	-0.24	0.76	0.47	-0.38
Development time	0.63	0.18	0.50	0.58
Proportion of variance	0.45	0.30	0.16	0.09

that diet in the immature stages can influence adult beetle weight with potential effects on fecundity success. We suggest that oviposition site choice is an adaptive maternal behavior in aphidophagous lady beetles.

Lady beetles acquire their aposematic coloration through their aphid prey, because the latter synthesize carotenoids (Moran & Jarvik 2010; Blount et al. 2012). We found adult *C. sanguinea* exhibited a tendency to possess brighter elytra and more chroma when fed *Uroleucon* aphids. It is possible that the difference in brightness and chromatic variation of newly emerged *C. sanguinea* individuals reared on *Uroleucon* increased their conspicuousness for predatory birds. As for *Hi. convergens*, we found that spectral features of adult elytra that fed on *Uroleucon* or *Brevicoryne* aphids were similar. Thus, our results indicate that maternal choice for adequate food sites is an adaptive strategy not only for larval development, but also influences the adaptive value of adults, directly affecting female lady beetle fitness.

Acknowledgments

With our most profound gratitude, we thank Lucas Machado de Souza for inestimable assistance throughout the entire process of this study. We thank Cláudio Lúcio Costa for his most valuable help in identifying the aphids, and Marcos Robalinho and Rafael Maia for their worthy contributions to data analysis. We thank Raphael Igor Dias, Glauco Machado, and Raul Laumann for their thorough revision of a previous version of this manuscript. We acknowledge Bruno Rocha, Yuri Prestes, Amanda Vieira, and Caroline Almeida for daily support in the laboratory and in the field. We thank all the agroecosystem farm owners who kindly allowed our constant visits, and the Embrapa and Laboratório de Comportamento Animal (University of Brasília) personnel for supporting our activities. We would also like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico for providing financial aid during the period of this study.

References Cited

Abdel-Salam AH, Abdel-Baky NF. 2001. Life table and biological studies of *Harmonia axyridis* Pallas (Col., Coccinellidae) reared on the grain moth eggs of

- Sitotroga cerealella* Olivier (Lep., Gelechiidae). Journal of Applied Entomology 125: 455–462.
- Arenas LM, Walter D, Stevens M. 2015. Signal honesty and predation risk among a closely related group of aposematic species. Scientific Reports 5: 11021. doi: 10.1038/srep11021
- Berkvens N, Bonte J, Berkvens D, Tirry L, De Clercq P. 2008. Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). BioControl 53: 211–221.
- Bernardo J. 1996. Maternal effects in animal ecology. American Zoologist 36: 83–105.
- Blount JD, Rowland HM, Drijfhout F, Endler JA, Inger R, Sloggett JJ, Hurst GDD, Hodgson DJ, Speed MP. 2012. How the ladybird got its spots: effects of resource limitation on the honesty of aposematic signals. Functional Ecology 26: 334–342.
- Clancy KM, Price W. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology 68: 733–737.
- Daloz D, Braekman JC, Pasteels JM. 1995. Ladybird defence alkaloids: structural, chemotaxonomic and biosynthetic aspects (Col.: Coccinellidae). Chemoecology 5: 173–183.
- Dolenská M, Nedvěd O, Veselý P, Tesařová M, Fuchs R. 2009. What constitutes optical warning signals of ladybirds (Coleoptera: Coccinellidae) towards bird predators: colour, pattern or general look? Biological Journal of the Linnean Society 98: 234–242.
- Evans EW. 2000. Morphology of invasion: body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. European Journal of Entomology 97: 469–474.
- Fox J. 2008. Cox proportional-hazards regression for survival data. Appendix to An R and S-PLUS Companion to Applied Regression. socialsciences.mcmaster.ca/jfox/Books/Companion-1E/appendix-cox-regression.pdf (last accessed 10 Mar 2020).
- Francis F, Lognag G, Wathelet JP, Haubruge E. 2001. Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. Journal of Chemical Ecology 27: 243–256.
- Giorgi JA, Vandenberg NJ, McHugh JV, Forrester JA, Sliński SA, Miller KB, Shapiro LR, Whiting MF. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215–231.
- Grimaldi D, Engel MS. 2005. Evolution of the Insects. Cambridge University Press, Cambridge, United Kingdom.
- Hesler LS. 2013. Risk to native *Uroleucon* aphids (Hemiptera: Aphididae) from non-native lady beetles (Coleoptera: Coccinellidae). Entomologica Americana 119: 14–22.
- Hodek I, van Emden HF, Honěk A. 2012. Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). Blackwell Publishing Ltd., Chichester, United Kingdom.
- Lundgren JG. 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. Biological Control 51: 294–305.
- Maia R, Eliason CM, Bitton PP, Doucet SM, Shawkey MD. 2013. pavo: an R Package for the analysis, visualization and organization of spectral data. Methods in Ecology and Evolution 4: 609–613.
- Marshall DJ, Heppell SS, Munch SB, Warner RR. 2010. The relationship between maternal phenotype and offspring quality: do older mothers really produce the best offspring? Ecology 91: 2862–2873.
- Michaud JP. 2005. On the assessment of prey suitability in aphidophagous Coccinellidae. European Journal of Entomology 102: 385–390.
- Montgomerie R. 2006. Analyzing colors, pp. 90–147 In Hill GE, McGraw KJ [eds.], Bird Coloration: Mechanisms and Measurements. Harvard University Press, Cambridge, Massachusetts, USA.
- Morales J, Burandt CL. 1985. Interactions between *Cycloneda sanguinea* and the brown citrus aphid: adult feeding and larval mortality. Environmental Entomology 14: 520–522.
- Moran NA, Jarvik T. 2010. Lateral transfer of genes from fungi underlies carotenoid production in aphids. Nature 328: 624–627.
- Mousseau TA, Dingle H. 1991. Maternal effects in insect life histories. Annual Review of Entomology 36: 511–534.
- Mousseau TA, Fox CW. 1998. The adaptive significance of maternal effects. Trends in Ecology and Evolution 13: 403–407.
- Obyrcki JJ, Kring TJ. 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology 43: 295–321.
- Ornberg J, Andersson S, Griffith SC, Sheldon BC. 2002. Seasonal changes in an ultraviolet structural colour signal in blue tits, *Parus caeruleus*. Biological Journal of the Linnean Society 76: 237–245.
- Polis GA, Myers CA, Holt RD. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20: 297–330.

- Prudic LK, Skemp AK, Papaj DR. 2006. Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behavioral Ecology* 18: 41–46.
- Quinn GP, Keough MJ. 2002. Introduction to multivariate analyses, pp. 401–472 *In* *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, United Kingdom.
- R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/
- Seago AE, Giorgi JA, Li J, Słipiński A. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Molecular Phylogenetics and Evolution* 60: 137–151.
- Seagraves M. 2009. Lady beetle oviposition behavior in response to the trophic environment. *Biological Control* 51: 313–322.
- Sicsú PR, Macedo RH, Sujii ER. 2015. Oviposition site selection structures niche partitioning among coccinellid species in a tropical ecosystem. *Neotropical Entomology* 44: 430–438.
- Slansky F, Rodriguez JG. 1987. Nutritional ecology of insects, mites, spiders, and related invertebrates: an overview, pp. 1–69 *In* Slansky Jr F, Rodriguez JG [eds.], *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates*. Wiley-Interscience Publications, New York, USA.
- Snyder WE, Joseph SB, Preziosi RF, Moore AJ. 2000. Nutritional benefits of cannibalism for the lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae) when prey quality is poor. *Environmental Entomology* 29: 1173–1179.
- Therneau T. 2012. A package for survival analysis in Survival R package, vers. 2.37-2. cran.r-project.org/web/packages/survival/index.html (last accessed 10 Mar 2020).
- Völkl W, Mackauer M, Pell JK, Brodeur J. 2007. Predators, parasitoids and pathogens, pp. 187–233 *In* van Emden HF, Harrington R [eds.], *Aphids as Crop Pests*. CAB International, Oxfordshire, United Kingdom.
- Wink M. 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics* 75: 225–233.