

Oviposition Site Selection Structures Niche Partitioning Among Coccinellid Species in a Tropical Ecosystem

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Abstract

The competitive exclusion hypothesis suggests that coexisting related species using similar resources in nature should partition their realized niches. This hypothesis has direct implications for conservation strategies using biological control, taking into consideration the shifts caused by the introduction of natural enemies in a local community. Such introductions typically lead to disruptions in species interactions and interfere with community structure. In this study, we asked whether community structure of aphidophagous lady beetles is determined by the distribution of specific plants and aphids. To answer this question, we describe the distribution patterns of lady beetles (adults, larvae, and egg clusters) relative to plants and aphids in eight crop ecosystems in a central region of Brazil. We used canonical correspondence analysis to evaluate lady beetle distribution relative to selected habitat variables. *Cycloneda sanguinea* L., *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* Pallas, and *Eriopis connexa* Germar (Coleoptera: Coccinellidae) differed in their use of plants and aphids. The association of egg clusters with specific plants/aphids was stronger than that of larvae or adults. In conclusion, lady beetle species occupied different niches, indicating different patterns of habitat use that may facilitate their coexistence in crop ecosystems. Furthermore, immature individuals had more specific environmental associations than adults, likely because female choice of oviposition sites influences their distribution and thus lady beetle community structure.

Introduction

Species' distribution across the landscape depends on conditions that define their fundamental ecological niche, which can be understood as a multidimensional space of resources and conditions that allows persistence of individuals of a species in explicit sites (Hutchinson 1957). Competition, predation, and parasitism are the major interactions between species that can result in restricted occupation of their fundamental niches, a condition known as the species' realized niche (Ricklefs 2003). For species that coexist, competition is probably the main interaction that reduces their fundamental niche (Hutchinson 1959, Townsend *et al* 2006).

The effects of competition on the distribution of species are usually stronger when these are closely related and use similar resources (Darwin 1859). Given the competitive exclusion principle, similar species that coexist should differ to some extent along their realized niches; if not, one species typically excludes the other in sites where they co-occur (Hardin 1960). Interspecific interactions are important determinants of community structuring and, as such, act as selective pressures that shape adaptive individual behaviors (Tilman 1982, Morris 2003).

The spatial distribution of insect populations is often influenced by female selection of sites to lay their eggs (Ellis 2008). Maternal choice of oviposition site at least partly

determines insect distribution, and thus influences community structure (Resitarits 1996, Seagraves 2009). Behavioral studies that focus on how female insects select specific habitats for their offspring may improve our understanding of insect community structure. A heightened understanding of community structure is important to maximize ecosystem services such as biological control of pest species.

Aphids are agricultural pests of economic importance because they are phytophagous and vectors of viruses that infect many crops (van Emden & Harrington 2007). Various predators are specialized in the consumption of aphids (i.e., aphidophagy), providing natural biological control in many cases (van Emden & Harrington 2007). Aphid colonies grow fast and their population peaks are typically followed by a drastic decrease within a narrow temporal window (Powell *et al* 2006, van Emden & Harrington 2007). Due to this aggregated and ephemeral distribution in aphid abundance, many aphidophagous insects forage on aphid colonies simultaneously.

Dense aggregations of different aphidophagous species result in their greater susceptibility to local population collapse due to predation, infections, and parasitism (Riddick *et al* 2009, Hodek *et al* 2012). When considering death causes, intraguild predation and cannibalism are the main causes of mortality among aphidophagous species in their immature stages. Risks can be high due to the simultaneous presence of multiple individuals of different aphidophagous species, in different developmental stages, all of which depend on a highly ephemeral local prey, i.e., aphids (Hagen 1962, Fox 1975, Polis *et al* 1989, Osawa 1993, Lucas 2005, Seagraves 2009).

Asymmetry in intraguild predation and cannibalism between aphidophagous individuals leads to a high mortality of eggs and newly hatched larvae. Females need to lay their eggs close to adequate food resources (aphids) to ensure successful offspring development (Fox 1975, Polis *et al* 1989, Evans 2003, Lucas 2005, Seagraves 2009). Moreover, females use several cues from the environment to assess suitable oviposition sites. For example, cues indicating coccinellid presence, such as larval residues and adult pheromones, may indicate high predation risk and deter lady beetle oviposition (Hemptinne & Dixon 1991, Michaud & Jyoti 2007, Seagraves 2009). Therefore, oviposition site selection exemplifies a tradeoff situation, since laying eggs too close to aphids may result in their predation, but if laid too far away may decrease the chances of their larvae finding food. It is suggested that the heightened mortality risk of eggs laid close to aphid colonies leads larvae on the verge of pupation to disperse from aphid colonies, which may reduce their vulnerability to intraguild predation, cannibalism, and other mortality factors associated with dense aggregations (Lucas *et al* 2000).

Coccinellids are important aphidophagous insects and some species may sometimes help to maintain aphid populations under control. Lady beetles are predators that can naturally control populations of aphids and other insects that are considered agricultural pests of crops in different regions of the world (Obrycki & Kring 1998, Valério *et al* 2007, Caballero-López *et al* 2012, Vantaux *et al* 2012). Various species from the Coccinellinae subfamily use aphids as an essential resource during adult and larval stages, although they also feed on pollen, nectar, and honeydew as alternative food sources (Pemberton & Vandenberg 1993, Evans *et al* 1999, Michaud 2000, van Emden & Harrington 2007, Giorgi *et al* 2009). Exceptionally, some ladybeetles feed exclusively on fungi or plants such in the Halyzini group and Epilachninae subfamily, respectively (Giorgi *et al* 2009).

Despite the considerable literature on the distribution of lady beetles in crop ecosystems (e.g., Caballero-López *et al* 2012), few studies address the distribution of all developmental stages (Kindlmann & Houdková 2006, Seagraves 2009, Smith & Gardiner 2013, Liere *et al* 2014). Management practices that improve biological control of aphids rely on information about the distribution of aphidophagous individuals in different developmental stages (Ferran & Dixon 1993, Kindlmann & Dixon 1993, Evans 2003). The environment where immatures develop may strongly influence adult fitness (Craig *et al* 1989, Resitarits 1996). Consequently, the study of oviposition site selection by lady beetles can provide information about population dynamics and community structure, which in turn may prove important for their use as biological control agents.

The question we address here is whether aphidophagous lady beetle species that coexist in the environment have different realized niches. We studied a tropical community of lady beetles to evaluate whether tritrophic interactions (plant-herbivore-predator) influence community structure and distribution patterns of individuals according to their developmental stage. We test the hypothesis that aphidophagous lady beetle species differ in their distribution patterns and that each developmental stage requires different foods and varies relative to mortality, which probably reflects their susceptibility to intraguild predation and cannibalism.

Material and Methods

We conducted fieldwork from June to October 2012 in eight crop ecosystems in central Brazil (Table 1), within the tropical savanna biome known as “Cerrado.” The local climate, according to the Köppen-Geiger classification, is Cwa semi-humid with seasonal variation, a dry mild winter and a rainy hot summer (Klink & Machado 2005). All survey localities are cultivated areas managed without conventional pesticides.

Table 1 Field site sampling locations of lady beetle communities.

	Crop ecosystem	Longitude (west °)	Latitude (south °)	Type of site
1	Sítio Vida Verde	48°15'9.00"	15°49'28.92"	Organic farm
2	Chácara Frutos da Terra	48°4'5.24"	15°49'48.72"	Organic farm
3	Sítio Geranium	48°4'26.40"	15°50'42.72"	Organic farm
4	Chácara Guarujá	48°9'51.92"	15°40'25.22"	Organic farm
5	Chácara Santa Cecília	47°38'48.12"	15°45'12.96"	Organic farm
6	Embrapa Hortaliças	48°8'27.96"	15°56'26.52"	Research farm
7	Embrapa Cenargen	47°54'0.00"	15°43'45.48"	Research farm
8	Fazenda Água Limpa	47°56'0.96"	15°56'57.84"	Research farm

The concept of crop ecosystem, as we apply here, comprises the organisms and environment at these field sites, as well as the surrounding spontaneous native vegetation. Crop ecosystems 1 to 5 are commercial organic farms, and 6 to 8 are research areas used for scientific purposes (Table 1). The major crops grown at these sites are lettuce (*Lactuca sativa*); carrots (*Daucus carota*); tomatoes (*Lycopersium esculentum*); brassica vegetables such as kale, broccoli, and cabbage (*Brassica oleracea*); and corn (*Zea mays*). The most common spontaneous plants (personal observation) that occur surrounding cultivated crops in the studied sites include sowthistle (*Sonchus oleraceus*), Spanish needle (*Bidens pilosa*), Mexican sunflower (*Tithonia diversifolia*), and billygoat-weed (*Ageratum conyzoides*).

Before initiating data collection, we visited all field sites and actively searched the vegetation for aphids and lady beetles in all developmental stages on different plant species during 1 month. We subsequently selected a subsample of nine plant species/genera in which we had found at least 20 aphids and 20 coccinellids, regardless of prey and predator species, and which were abundant in the sampled crop ecosystems. The following plants were selected: *Coriandrum sativum* (Apiaceae), *Brassica oleracea* (Brassicaceae), *Z. mays* (Poaceae), *Citrus* spp. (Rutaceae), *Lactuca sativa* (Asteraceae), all of which were cultivated; and the following spontaneous Asteraceae plants: *Ageratum conyzoides*, *Bidens pilosa*, *Sonchus oleraceus*, and *Tithonia diversifolia*.

Sampling in the crop ecosystems was conducted during non-rainy days between 0800 and 1300 hours and lasted, on average, 3 h. Sampling consisted of walking along unidirectional transects randomly determined in each crop ecosystem and examining the plants from the selected species for the presence of aphids and lady beetles. We found few lady beetles in the pupal stage; thus, we excluded this life stage from analyses.

The variables we used to determine the populational distribution and structuring pattern of the Coccinellidae community on the selected plant species were as follows: abundance of individuals of specific aphid genera (when present), and species and abundance of lady beetles in different life stages (adult, larval, and egg). Further, we used presence/

absence of lady beetle eggs and not their number because the latter is influenced by female condition and quality of the oviposition environment, such as presence of other lady beetle individuals (Minkenberg *et al* 1992, Kindlmann & Houdková 2006, Seagraves 2009). Thus, except for egg clusters, we considered total number of individuals (aphids or lady beetles) per developmental stage per species and per plant host species.

We collected aphid and lady beetle specimens to identify to genus and species levels, respectively. We identified aphids with the assistance of a specialist and with identification keys (Denmark 1990, Rees *et al* 1994, Liu & Sparks 2001). To identify lady beetle species from the egg stage, we collected egg clusters and their plant substrates in the field, kept them in an environmental chamber in the laboratory until eggs hatched, and reared the larvae until they could be identified (Rees *et al* 1994, Rhoades 1996). Larvae were reared in 50 mL plastic cups and fed with aphids from different species, *Anagastha kuheniella* (Zeller) (Lepidoptera: Pyralidae) eggs, honey diluted in water (30%), and a Spanish needle flower containing pollen. We replaced rearing cups and food every couple of days, on average. The environmental chamber was maintained at 24±1°C, 70±7% RH, and 12 h photoperiod.

Statistical analysis

We conducted a canonical correspondence analysis (CCA) to determine lady beetle community structure and distribution of individuals in different developmental stages in relation to the surveyed plants and aphids. The dependent variable was abundance of lady beetles of each species in adult, larval, and egg stages. The explanatory variables were aphid abundance (from different genera) and plant species used by lady beetles. We considered that each plant inspected represented a data sample, and results allowed us to evaluate the association of the lady beetle community and the biotic variables (i.e., plants and aphids).

All analyses regarding lady beetle community structure were performed using the “vegan” package (Oksanen *et al* 2012) on R (R Core Team 2012). The most parsimonious

general model was generated using the function “cca.” For variable selection, we applied a stepwise technique with bi-directional variable elimination from the comparisons between the full and simple model using “add1” and “drop1” functions. The full model consisted of all plant species and aphid genera associated with lady beetles, and the simple model consisted of only lady beetle data, without the biotic variables. The variables were sequentially added and excluded in the simple model using the Akaike information criterion (AIC), and the variables maintained in the most parsimonious models were the ones that, when excluded, significantly changed the models’ power of explanation. As a premise to conduct this analysis, in order to detect collinear constraints, we calculated the Variance Inflation Factors (VIF) of complete and also final models to confirm that the variables selected had VIF values below 10 and, therefore, were not strongly dependent on other variables (Oksanen *et al* 2012). The significance of all terms in the final model was assessed using permutation tests with the function “anova.cca,” setting the analyses by marginal effects of the terms when each term was excluded from the model encompassing all other terms (Oksanen *et al* 2012). The graph of the final model was generated with symmetric scaling.

We conducted monotonic correlation analyses using a Spearman coefficient to describe the covariance between aphid abundance and plant species associated to lady beetles (Legendre & Legendre 1998). The correlations were generated with the command “rcorr,” “type=spearman” from the package Hmisc (Harrell & Dupont 2012). We report only the significant correlations ($p \leq 0.05$) with Spearman $r_s \geq |0.5|$ between the variables selected in the final model and non-selected variables.

Results

We sampled a total of 8389 plants in the set of selected species distributed among the following genera: *Bidens*=30% ($n=2548$), *Brassica*=19% ($n=1580$), *Sonchus*=14% ($n=1211$), *Tithonia*=12% ($n=1004$), *Zea*=11% ($n=960$), *Ageratum*=6% ($n=519$), *Coriandrum*=3% ($n=244$), *Lactuca*=3% ($n=235$), and *Citrus*=1% ($n=88$). However, most sampled plants did not harbor either aphids or lady beetles; in fact, only approximately 6% ($n=530$ plants) hosted lady beetles. These, when present, usually occurred in small groups (mean \pm SE, 3 ± 10). Thus, the evaluation of the lady beetle community and population dynamics is restricted to the small subgroup of plants where we found lady beetles.

The lady beetle species sampled included *Cycloneda sanguinea* (L.), *Hippodamia convergens* Guérin-Ménéville, *Harmonia axyridis* (Pallas), and *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). We also found a single adult and three larvae of *Azya luteipes* Mulsant as well as four

Olla v-nigrum (Mulsant) adults on *Citrus* plants. Given the small sample size and the lack of egg clusters of these two species, they were discarded from the dataset. Although *Scymnus* and *Chilocorus* lady beetles (i.e., scale predators) were also recorded during the surveys, we did not include them in the dataset since our objective was to study community structure of the aphidophagous coccinellid guild.

Lady beetle adults and immatures from all species were found on Mexican sunflowers and Spanish needle plants, and only lady beetle adults occurred on *Zea*, *Ageratum*, and *Lactuca* species (Table 2). Considering all lady beetle individuals, regardless of developmental stage, most (69%) were found on plants infested with aphids. About half of the lady beetle adults were in plants infested with aphids (55%), whereas 88% of larvae and 81% of egg clusters were found in plants that were also harboring aphids.

Sampled aphids belonged to the following nine genera: *Aphis*, *Brevicoryne*, *Hyadaphis*, *Hyperomyzus*, *Lipaphis*, *Myzus*, Macrosiphini gen. sp., *Toxoptera*, and *Uroleucon*. We found *Uroleucon* and *Aphis* on more than one plant genus, whereas the remaining genera of aphids were each found on only one plant genus. Thus, the plants associated with aphids included the following: *Coriandrum* with *Hyadaphis*; *Ageratum* with *Uroleucon*; *Bidens* with *Uroleucon* and *Aphis*; *Lactuca* with *Uroleucon*; *Sonchus* with *Uroleucon* and *Hyperomyzus*; *Tithonia* with *Uroleucon* and Macrosiphini gen. sp.; *Brassica* with *Brevicoryne*, *Myzus*, and *Lipaphis*; *Citrus* with *Toxoptera*; and *Aphis*; and no aphids sampled on *Zea* plants.

Lady beetle community structure

Cycloneda sanguinea had a stronger association with plants of the family Asteraceae, *Harmonia axyridis* with *Citrus*, *Eriopis connexa* with *Brassica*, and *Hippodamia convergens* with *Brassica* and Asteraceae (Fig 1). Immature lady beetles from the different species clearly occupied distinct habitats and had a closer association with plants and aphids than did the adults of their species (Fig 1). In contrast, lady beetle adults were associated with more plant genera than were larvae and eggs of their species (Fig 1, Table 2).

Variation in abundance of lady beetle species, considering all developmental stages together, was determined by two aphid genera, *Toxoptera* ($F_{3,525}=32.45$, $p < 0.005$; VIF=1.06) and *Myzus* ($F_{3,525}=10.00$, $p < 0.05$; VIF=1.05), and two plant genera, *Tithonia* ($F_{3,525}=18.62$, $p < 0.005$; VIF=1.27) and *Brassica* ($F_{3,525}=12.56$, $p < 0.05$; VIF=1.30) (Fig 1 and Table 3). The following tested variables were correlated: *Citrus* plants with aphids from the *Toxoptera* genus ($r_s=0.94$, $p < 0.001$) and aphids from the *Myzus* genus with aphids of the *Brevicoryne* genus ($r_s=0.72$; $p < 0.001$). Immature stages of all lady beetle species were more associated with specific studied variables (aphids and plants) than

Table 2 Abundance of lady beetles *Cycloneda sanguinea*, *Hippodamia convergens*, *Harmonia axyridis*, and *Eriopis connexa* as adults (A), larvae (L), and egg clusters (E), according to their associations with plant and aphid genera.

	<i>C. sanguinea</i>			<i>H. convergens</i>			<i>H. axyridis</i>			<i>E. connexa</i>		
	A	L	E	A	L	E	A	L	E	A	L	E
Plants												
<i>Zea</i>	22	0	0	3	0	0	25	0	0	0	0	0
<i>Ageratum</i>	7	0	0	7	0	0	1	0	0	2	0	0
<i>Lactuca</i>	1	0	0	2	0	0	0	0	0	0	0	0
<i>Brassica</i>	10	0	2	136	37	48	27	2	9	25	22	6
<i>Coriandrum</i>	2	0	0	103	1	8	6	0	0	4	0	0
<i>Citrus</i>	6	1	13	0	0	0	19	9	25	0	0	0
<i>Tithonia</i>	90	206	74	109	167	2	66	1	0	3	1	0
<i>Bidens</i>	84	43	4	64	20	4	7	1	0	1	1	0
<i>Sonchus</i>	25	3	21	61	10	22	6	1	4	0	2	2
Aphids												
<i>Brevicoryne</i>	6	0	2	11	8	13	3	0	5	4	6	4
<i>Myzus</i>	3	0	1	11	2	11	1	0	4	3	4	4
<i>Lipaphis</i>	2	0	1	9	9	7	3	0	0	1	3	3
<i>Uroleucon</i>	152	249	98	191	178	27	22	2	4	2	4	2
Macrosiphini gen. sp.	1	5	1	0	0	0	1	0	0	0	0	0
<i>Aphis</i>	1	0	0	8	5	0	1	0	0	0	0	0
<i>Hyperomyzus</i>	8	0	11	15	0	14	1	0	3	0	0	0
<i>Hyadaphis</i>	2	0	0	78	1	6	2	0	0	2	0	0
<i>Toxoptera</i>	6	1	13	0	0	0	17	9	25	0	0	0

were the adults. The first two axes explained 65% of the variation in the final model (CCA1=0.34, CCA2=0.31). Therefore, both axes of Fig 1 are almost equally important in explaining lady beetle distribution in relation to plants and aphids. According to our final model, which included the most important variables to describe lady beetle distribution, *Harmonia axyridis* were more associated with *Toxoptera* aphids and, consequently, *Citrus* plants. This association is

stronger for immature individuals than adults. Individuals of *Cycloneda sanguinea*, predominantly larvae and eggs, were strongly associated with *Tithonia* plants. Egg clusters of *Cycloneda sanguinea* were also commonly associated with *Citrus* plants infested with *Toxoptera* aphids. Most *E. connexa* individuals were found on *Brassica* plants, usually infested by aphids of the *Myzus* and *Brevicoryne* genera, and the association with these variables was stronger for larvae

Fig 1 Canonical correspondence analysis (CCA1 and CCA2) of the community of lady beetles associated with plants and aphids in crop ecosystems of central Brazil. The selected habitat variables (plants and aphids) are represented by vectors in gray. Lady beetle species are abbreviated as follows: Cyc = *Cycloneda sanguinea*; Eri = *Eriopis connexa*; Hip = *Hippodamia convergens*; and Har = *Harmonia axyridis*. Lady beetle developmental stages are represented by the letters following the abbreviations: adults = _A; larvae = _L; and eggs = _E.

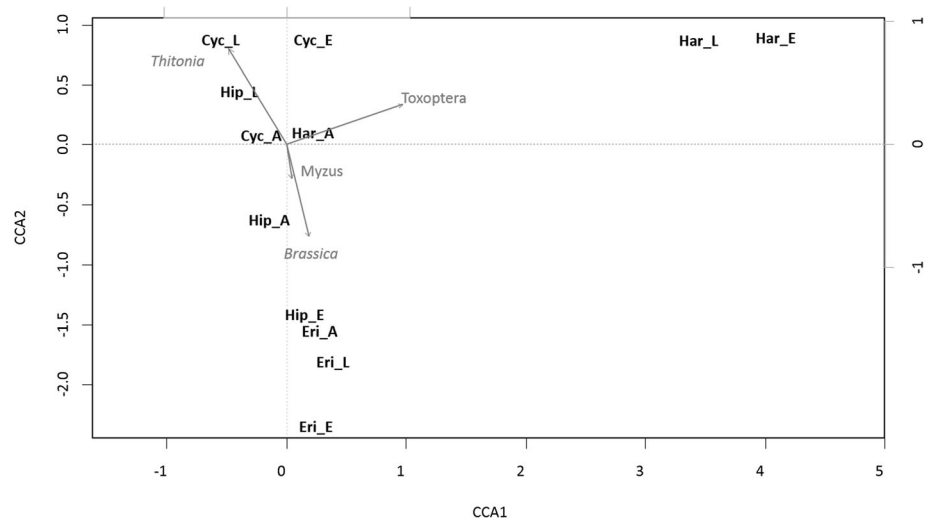


Table 3 Canonical correspondence analysis model selection via stepwise bidirectional variable inclusion/exclusion using Akaike Information Criterion (AIC). The final model is highlighted in bold, ANOVA significant results are represented by asterisks and non-significant results as NS.

Variables	AIC model:			
	Without variable	With variable	ANOVA	Decision
<i>Toxoptera</i>	1577	1549	*	Kept
<i>Toxoptera</i> + <i>Tithonia</i>	1549	1525	*	Kept
<i>Toxoptera</i> + <i>Tithonia</i> + <i>Brassica</i>	1525	1514	*	Kept
<i>Toxoptera</i> + <i>Tithonia</i> + <i>Brassica</i> + <i>Coriandrum</i>	1514	1505	NS	Excluded
<i>Toxoptera</i> + <i>Tithonia</i> + <i>Brassica</i> + <i>Myzus</i>	1514	1506	*	Kept
<i>Toxoptera</i> + <i>Tithonia</i> + <i>Brassica</i> + <i>Myzus</i> + <i>Citrus</i>	1506	1503	NS	Excluded
<i>Toxoptera</i> + <i>Tithonia</i> + <i>Brassica</i> + <i>Myzus</i> + <i>Bidens</i>	1506	1500	NS	Excluded

and eggs, respectively. *Hippodamia convergens* had a weaker association with specific plants and aphids. Individuals were commonly found on *Tithonia* and *Brassica* plants, yet immatures had a stronger association with these plants than did the adults (Fig 1).

Discussion

Lady beetles had an aggregated distribution whenever they were sampled. Aphids frequently occurred on the same plants where we found lady beetles, which highlights the importance of food resources in determining the distribution of these lady beetle populations, reinforcing the pattern also found in other studies (Valério *et al* 2007, Caballero-López *et al* 2012, Hodek *et al* 2012, Vantaux *et al* 2012).

As lady beetle individuals develop from eggs to adults, their specific food requirements vary, as well as their susceptibility to different sources of mortality. Thus, we expected them to exhibit different distribution patterns through their life cycles. In fact, our results support this prediction and the data show that the lady beetle community structure has a clear ordination pattern with unmistakable separation of the species in the multidimensional space evaluated. This suggests that coexisting lady beetle species occupy distinct niches, each associated with specific plant and aphid groups. Consequently, specific tritrophic interactions were important in structuring the studied community.

When similar species coexist and differ in their realized niches, it is reasonable to assume that niche differentiation occurs due to competitive exclusion. Competition tends to be more intense when the different species are phylogenetically close and when they share food resources, which was the case for the lady beetles in this study (Darwin 1859, Harvey & Pagel 1991, Wiens & Graham 2005, Donoghue 2008). The four lady beetle species found in the plants examined belong to the Coccinellinae subfamily, a lineage that is specialized in aphid predation (Giorgi *et al* 2009). The lady

beetle niche differentiation we found is analogous to that found in other studies, indicating that coexisting species exhibit spatial differences in habitat use (reviewed by Snyder 2009). Lady beetles exhibit intraguild predation (Cottrell & Yeargan 1998, Kindlmann & Houdková 2006, Kajita *et al* 2006), and the differences we found in habitat use among species and among individuals in different developmental stages probably reflect predator avoidance behavior.

Mexican sunflower (*Thitonia diversifolia*) and Spanish needle (*Bidens pilosa*) were the only ones that harbored adults and immatures of all lady beetle species studied. Thus, these two spontaneous Asteraceae species may be important for the maintenance of local populations of lady beetles in the crop ecosystems of central Brazil. The strategy of maintaining these spontaneous plants within crop fields may enhance the efficiency of the biological control provided by aphidophagous lady beetles in aphid-infested crops (Horn 1981, Tscharrntke *et al* 2007, Letourneau *et al* 2011, Altieri 2012). Moreover, habitats with increased plant diversity and structure complexity may reduce negative interactions among predators and enhance pest suppression (Janssen *et al* 2007, Finke & Snyder 2008, Pell *et al* 2008). Habitat heterogeneity apparently enhances the potential for coexistence of the exotic *Harmonia axyridis* and native aphidophagous predators in invaded areas (Osawa 2011). This may be the case in our study, where lady beetle species coexistence was possibly enhanced by the presence of *T. diversifolia* and *B. pilosa* plants.

Another objective of this study was to evaluate the distribution of lady beetle individuals of the different species according to their developmental stage. We tested the hypothesis that immature individuals (larvae and eggs) would have stronger specific associations with particular plants or plant/aphid combinations when compared with adults. The results obtained in the final model support this expectation. According to the model generated, adult distribution patterns for all lady beetle species showed weaker and less specific associations with the selected plant-aphid interactions when compared with patterns exhibited by larvae and

eggs. Moreover, only lady beetle adults occurred on all plant species evaluated, including *Zea*, *Ageratum*, and *Lactuca* plants, whereas immature lady beetles were not found on any plant of these genera.

Factors such as diet, adult morphological traits, and inter-specific interactions may explain the weak association between lady beetle adults with specific plants and aphids (Giorgi *et al* 2009, Riddick *et al* 2009). Although aphidophagous lady beetle adults and larvae feed essentially on aphids, adult lady beetles use nectar and pollen as alternative resources since these food items can give them enough energy (nectar) and protein (pollen) during periods of prey scarcity. Thus, adult lady beetles may exploit resources other than aphids and are frequently found on plants devoid of aphids (Schmidt 1992, Evans 2009, Giorgi *et al* 2009, Seago *et al* 2011). However, we did not record whether sampled plants were flowering because some species open and close their flowers at different moments during the day and for some species flower opening only happens for a brief period (van van Doorn & van Meeteren 2003). Moreover, it would be very difficult to measure whether flowers on each inspected plant had pollen and nectar available at the sampling moment. Lastly, the occasional presence of adults on flowering plants may not be predictable, since several plant species can be fortuitously used by adults, and these data are not necessarily useful for determining niche pattern.

Adults of lady beetle develop more dynamic and wide-ranging foraging strategies than immatures because they can fly and disperse quickly across an ecosystem (Ferran & Dixon 1993, Evans 2003, Hodek *et al* 2012). Thus, as expected, lady beetle adults occupied a wider habitat range as shown by a higher number of plant species used and a less restricted relationship to aphid-infested plants when compared to larvae and eggs.

Aphidophagous lady beetles from various species and in different developmental stages gather on plants infested with aphids due to the aggregated and ephemeral properties of this particular type of prey (Hagen 1962, Lucas 2005, Hodek *et al* 2012). Predators, parasites, and parasitoids attracted to crowded prey/host sites and arrested at such food sites suffer a higher risk of predation and parasitism themselves (Hassell & Southwood 1978, Begon & Mortimer 1996). Lady beetle natural enemies apparently use environmental cues to find aphid-infested plants or lady beetle agglomerations (Riddick *et al* 2009, Ceryngier *et al* 2012). Possible behavioral adaptations of adult lady beetles should include quick departure from aphid-infested plants after feeding to decrease predation and parasitism risks, in addition to well-developed search behavior that increases their chances of finding another aphid infestation to support reproduction (Ricklefs 2003).

Also as expected, larvae and eggs were more strongly associated with specific plants and aphid-plant combinations,

and larvae tended to have an intermediate pattern of distribution when compared to adults and eggs. This pattern may be explained by the strong association of newly hatched larvae with oviposition sites (Hodek *et al* 2012). In contrast, older larvae that are about to pupate disperse from the foraging site where they hatched, weakening the specific association with plants and aphids (Evans & Dixon 1986, Lucas *et al* 2000, Hodek *et al* 2012).

Lady beetle eggs had the strongest association with specific biotic factors which indicates the existence of a tight link between oviposition site and plants with aphids. Many studies have shown that biotic factors influence the behavior of invertebrate females when choosing where to lay their eggs (Craig *et al* 1989, Machado & Oliveira 2002, Wong *et al* 2012). For breeding lady beetle females, resource availability (i.e., aphid presence on the plants) is an important cue to stimulate oviposition (Evans & Dixon 1986, Seagraves 2009). Finally, oviposition decisions also probably reflect the risk of intraguild predation and cannibalism, both of which are highest during the egg stage, followed by the risk to newly hatched larvae (Schellhorn & Andow 1999, Lucas 2005). The probability of mortality from these causes drops when newly hatched larvae feed on adequate food resources because they develop faster, reducing their time of exposure to enemies during one of the most susceptible developmental stages (Slansky & Rodriguez 1986, Lucas 2005, Honěk 2012). It has been suggested that lady beetles usually lay eggs in places with the most adequate aphids and on plants that have sufficient prey for their offspring development (Seagraves 2009, Honěk 2012). Based on our study, we can infer that certain plants and the aphids inhabiting them are both associated with eggs of specific lady beetle species. Thus, such aphids must be suitable for the survival and development of lady beetle offspring.

Lady beetle species, such as *Cycloneda sanguinea*, *Harmonia axyridis*, *Hippodamia convergens*, and *Eriopsis connexa*, that co-occur in crop ecosystems in central Brazil use different plants and aphids suggesting a separation of their realized niches, even when we consider only a subsample of all available plants and aphids. Lady beetles in our study occupied different niches even when we consider their distribution patterns separately as eggs, larvae, and adults. Thus, we conclude that habitat use by the coccinellid community is dictated by interspecific differences and by traits that occur in each developmental stage. The absence of immatures on plants occupied by adults highlights the effects of different specific habitats for each developmental stage, and hence the importance of studies such as this one, evaluating the distribution of individuals in all phases of their life cycle. Moreover, each lady beetle species must have its own specific dietary requirements for development; thus, the more specific distribution of immatures must reflect an adaptive oviposition behavior that determines adequate places for

offspring development. Our study indicates that oviposition site choice affects the population distribution of each lady beetle species and community structure. Therefore, female lady beetle choices about where to lay their eggs are maternal decisions that affect various biological levels, from the individual decision-making process to the spatial distribution of the population and ultimately the community structure.

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