



AGRARIAN SCIENCES

Larval interactions between two aphidophagous coccinellids in sweet pepper

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Abstract: Antagonistic interactions among predators have the potential to dampen top-down impacts on a shared pest. Strong cannibalism by adults and larvae on eggs along with a stronger intraguild predation by *Eriopis connexa* on *Cycloneda sanguinea* (Coccinellidae) than *vice versa* had been documented. The intensity and direction of these interactions could vary with the larval instar. We calculated aggressiveness, attack, and predation rates between larvae in the laboratory and studied both larval mortality and within-plant distribution in plants. Despite high attack rates, larvae of both species usually escaped from predation, except for the fourth vs. second-instar combinations, where the smaller larvae were more vulnerable to predation than the larger. Successful predation by *E. connexa* fourth-instars on *C. sanguinea* second-instars frequently occurred. All interactions between larvae became relaxed when extraguild prey were present. The larval mortality within the plants was influenced by the availability of extraguild prey only. Larvae of both species promptly dispersed outside the plant when prey became unavailable. With the extraguild prey present, the larvae of both species initially preferred the plant upper stratum in both combinations, but then dispersed. This study suggests that the defensive capability and larval dispersal could prevent cannibalism and intraguild-predation interactions.

Key words: cannibalism, intraguild predation, larval behaviour, *Cycloneda sanguinea*, *Eriopis connexa*.

INTRODUCTION

The spatial and temporal co-occurrence of the aphidophagous ladybird in nature is conducive to intra- and interspecific interactions such as cannibalism and intraguild predation. Cannibalism—intraspecific predation—has potential costs that include a risk of injury in attacking conspecifics and loss of fitness from consuming relatives (Dixon 2000). The widespread nature of cannibalism among these beetles, however, suggests that such activity may also often be considerably beneficial, mainly for development and survival, especially under conditions of low prey availability (Osawa 1992, Snyder et al. 2000). Intraguild predation—defined

as predation on a heterospecific competitor and a common practice among ladybugs—does confer significant benefits such as the elimination of a potential predator or competitor and, concurrently, the consumption of a protein-rich food (Lucas 2012). Both cannibalism and intraguild predation among ladybirds can reduce the beetles' ability to suppress prey, thus making the determination of under what conditions predator diversity will help or hinder pest control difficult (Rosenheim & Harmon 2006, Vance-Chalcraft et al. 2007, Grez et al. 2012).

The developmental stages of coccinellids exhibit different degrees of susceptibility to cannibalism and intraguild predation. For example, immobile eggs, pupal, and molting

stages are especially susceptible. Unhatched eggs are frequently eaten by both conspecific and heterospecific larvae as well as by adults, whereas adults are never successfully attacked (Hodek & Evans 2012). Larval development is strongly dependent on the quality and quantity of food, making this developmental stage extremely active in dispersing and seeking prey; thus, encounters between larvae become highly likely. Moreover, the distribution pattern of the larvae within a plant affects the frequency of encounters among individuals and, therefore, determines the intensity of the interactions. Reciprocally, the degree of interactions among individuals could cause changes in the distribution patterns on the plant (Lucas & Alomar 2002, Walzer et al. 2009, Arnó et al. 2010, Moreno-Ripoll et al. 2012).

Inter-larval cannibalism varies among genera as well as among species (Yasuda et al. 2001, Pervez et al. 2006). The coccinellid larvae have evolved and developed morphological, chemical, and behavioral means of defense for avoiding conspecific or heterospecific aggressors (Hodek & Evans 2012, Lucas 2012). Since aphids are notably only temporary food sources for aphidophagous ladybirds, the predatory larvae of those beetles are frequently driven to cannibalism and intraguild predation in order to obtain resources to complete their development (Noppe et al. 2012). The outcome of inter-larval encounters likely depends on the species involved, their distribution within a plant, the availability of prey, and other conditions such as the relative size and aggressiveness of the larvae and their instar stage (Polis et al. 1989, Lucas et al. 1998, Michaud 2002, 2003, Félix & Soares 2004, Yasuda et al. 2004).

Cycloneda sanguinea L. and *Eriopis connexa* (Germar) are two neotropical aphidophagous coccinellids that are simultaneously present in the horticultural orchards of La Plata (Buenos

Aires, Argentina). In a previous study we found strong cannibalism by adults and larvae on eggs and by adults on larvae in the absence of aphids. The levels of cannibalism by both predatory species decreased when the prey was present. We also found that intraguild predation was bidirectional and asymmetric, with *E. connexa* being the stronger intraguild predator both in the presence and absence of aphids (Rocca et al. 2017). The intensity and direction of these competitive interactions could vary depending upon the larval instars involved. Consequently, the aims of the study reported here were 1) to quantify both the intraspecific (cannibalism) and interspecific (intraguild predation) interactions between *C. sanguinea* and *E. connexa* larvae under laboratory conditions and 2) to assess the effect of these interactions on both larval mortality and the distribution of the larvae within sweet-pepper plants.

MATERIALS AND METHODS

Insect rearing

Laboratory colonies of *C. sanguinea* (Cs) and *E. connexa* (Ec) were established from adults collected from sweet-pepper crops located in La Plata, Buenos-Aires province, Argentina (35° 00' S, 58° 00' W). The taxonomic determinations of the species were made by a Coleoptera specialist from the Museum of Natural Science of La Plata. Cultures were maintained in a climate-controlled room at 25 ± 2 °C, 70 ± 10% relative humidity, and a 16- to 8-h light-dark photoperiod. Adults and larvae of coccinellids were reared separately in plastic containers (15 x 15 x 25 cm) closed with voile on top to enable ventilation. Both life stages were fed yeast, pollen, and the bird-cherry aphid *Rhopalosiphum padi* (L.) reared on wheat seedlings, all replenished twice weekly. Water was provided on a sponge lodged in an Eppendorf tube. For adults, the

bottom of each container was lined with paper toweling to serve as an oviposition substrate. The resulting egg clusters were collected daily and placed in plastic containers as above until hatching. Particular larval instars were obtained by rearing neonatal larvae until they reached the desired instar. The larvae were starved for 12 h before the larval behavior experiments.

Larval behavior experiments

To perform the trials for observation cannibalism (CANN) and intraguild predation (IGP) between larvae of *C. sanguinea* and *E. connexa*, we used second- (L2), third- (L3), and fourth-instar (L4) larvae in all possible pairings—*i.e.*, to assess both interactions as well as to test species combinations—with larvae both at the same instar and at different instars, though in the latter instance we used only the combinations L4 and L2. The two larvae selected were placed in a 10-cm-diameter plastic Petri dish without food. We performed between 10 and 16 replicates of each combination and made behavioral observations at ambient temperature in the climate-controlled room. During the first 90 min of the interactions, we recorded by direct observation: a) simple contact between the larvae; b) aggressiveness, defined as one larva attacking the other; and c) whether the attacked larva was killed or escaped. From these data we calculated: the rate of aggressiveness as the number of aggressive interactions of each individual in a given replicate divided by the total number of interactions, the rate of attack as the number of replicates where an attack occurred divided by the total number of replicates, and the predation rate as the number of replicates where a larva was killed divided by the total number of replicates (Yasuda et al. 2001, 2004). We then selected those treatments in which many aggressive interactions occurred followed

by death and repeated those treatments with the inclusion of an extraguild prey to evaluate if the intensity of the interactions relaxed in the presence of an additional extraguild prey. The treatments chosen were: L3–L3 and L4–L2 (CANN of both species), L3–L3 and L4–L4 (IGP) and L4 Ec on L2 Cs. In the reverse combination, L4 Cs on L2 Ec, only relatively few aggressive interactions had occurred in the original trials, and thus this pairing was not included (*cf.* Figure 1, Panel c). The experimental unit was a 10-cm-diameter plastic Petri dish in which *Myzus persicae* (Sulzer) (Hemiptera: Aphidae) adults were placed *ad libitum* on the disk of a sweet-pepper leaf that had been laid abaxial side up on a thin layer of 1% (w/v) water agar. We performed 10 or 11 replicates of each combination.

We compared the rates of aggressiveness, attack, and predation among the larval instars in each interaction—CANN Cs, CANN Ec, IGP Cs on Ec, and IGP Ec on Cs—and then between species—CANN (Cs vs. Ec) and IGP (Cs on Ec vs. Ec on Cs)—taking into account all the combinations of larval instars mentioned above. The rate of aggressiveness was analyzed by a one-way analysis of variance (ANOVA) and the mean differences separated by means of a Tukey's multiple-comparisons test. Data were arcsine-square-root transformed before analysis. When the requirements for the use of parametric tests were not met, a non-parametric test was used. The rates of attack and predation were analyzed by the Chi-square contingency tests or Fisher's exact test. Each response variable was then compared between treatments with and without extraguild prey by the one-way ANOVA (for the rate of aggressiveness) or by Chi-square contingency tests or Fisher's exact test (for the rates of attack and predation).

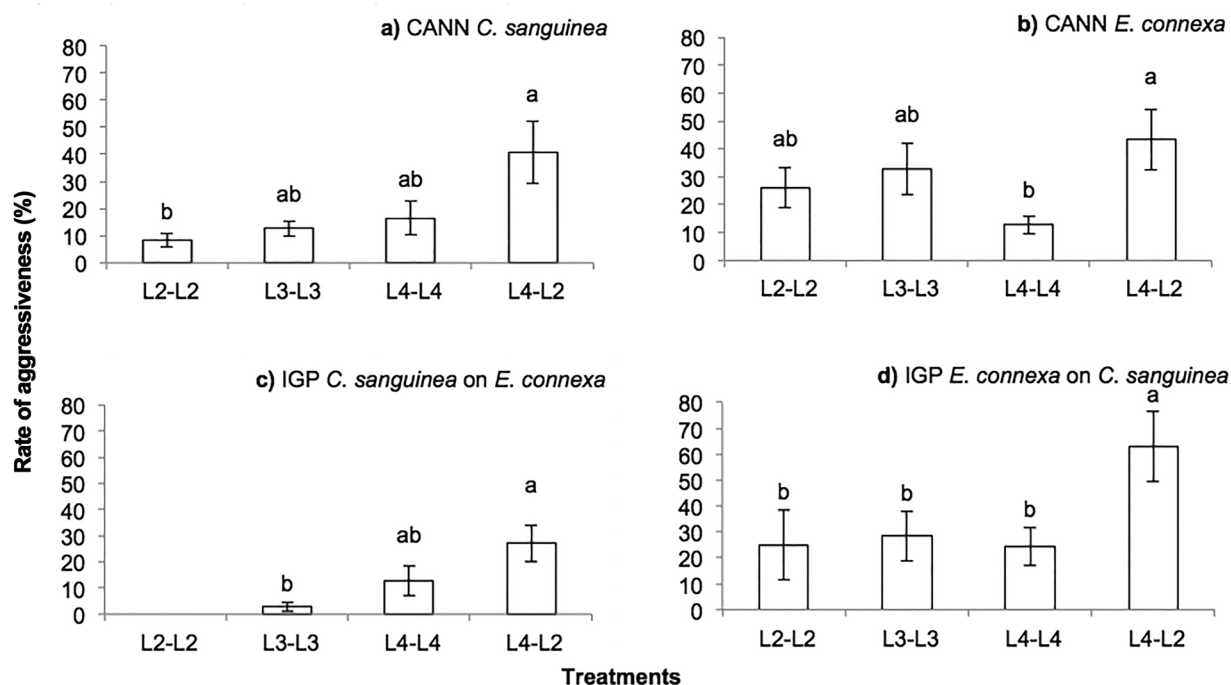


Figure 1. Mean rate of aggressiveness (\pm SE) between different combinations of conspecific or heterospecific larvae in the absence of extraguild prey. Panel a, *Cycloneda sanguinea* cannibalism; Panel b, *Eriopis connexa* cannibalism; Panel c, *C. sanguinea* intraguild predation on *E. connexa*; Panel d, *E. connexa* intraguild predation on *C. sanguinea*. CANN, cannibalism; IGP, intraguild predation; L2, second-instar larvae; L3, third-instar larvae; L4, fourth-instar larvae. Different letters within each panel indicate significant differences ($P < 0.05$).

Effect of larval interference interactions on larval mortality and distribution within the sweet-pepper plant

We studied the effect of the conspecific and heterospecific interactions of *C. sanguinea* and *E. connexa* on larval mortality and distribution within sweet-pepper plants grown in an experimental greenhouse. The experimental unit consisted of one potted plant having 9 to 12 leaves placed in a tray located inside a voile cage (35 x 35 x 35 cm). The treatments, with and without additional prey, were: (a) six L1 *C. sanguinea*, (b) six L1 *E. connexa*, and (c) three L1 of each species. In the treatments with prey, the initial density was ≈ 400 aphids per plant. To obtain this initial density, 50 to 60 adults of *M. persicae* were randomly located on the plant and the total of aphids was counted a week later. Some aphid colonies were removed to

achieve ≈ 400 aphids per plant, maintaining its natural spatial distributions. No further aphids were added throughout the experiment. Ten replicates were conducted per treatment. Taking into account that larval instar body size and voracity vary between instars, the number of surviving larvae and the position on the plant were recorded after two and four days, when the larvae reach instars L2 and L3, respectively. The position of each larva was defined as: in the upper stratum (from the middle of the plant upward), in the lower stratum (from the middle of the plant downward) or outside the plant (on the pot, tray, or voile of the cage). The larval mortality was compared by a two-way repeated-measures ANOVA, with the treatments and prey availability being the predictor variables. The proportion of larval mortality of *E. connexa* and *C. sanguinea* in the heterospecific treatment

was compared, for each treatment separately, with a two-tailed Z-test of proportions.

In order to ascertain if the distribution of *C. sanguinea* and *E. connexa* larvae within the plant over time was affected by conspecific interactions, we analyzed the number of larvae of each species separately, using a generalized linear model with Poisson distribution and a loglink function. The position of the larvae—*i. e.*, the upper or lower strata or outside the plant—and the time of the observation—after two or four days—were the explanatory variables. To examine the larval distribution in the heterospecific treatment, we employed a generalized linear model with binomial distribution and logitlink function. The presence or absence of both species together—there involving at least one larva of each species—was the binary-response variable, with the position and time being the explanatory variables.

All analyses were performed with the statistical package R, v.3.5.0 (R Development Core Team, Vienna, Austria).

RESULTS

Larval behavior experiments

The rate of *C. sanguinea* conspecific aggressiveness—*i. e.*, cannibalism (CANN, Figure 1, Panel a)—was higher in the L4–L2 than in the L2–L2 combinations ($F = 3.84$; $df = 3, 39$; $P = 0.016$). A similar result being obtained from the conspecific *E. connexa* combinations (Figure 1, Panel b), and the rate of aggressiveness was higher in the L4–L2 than in the L4–L4 combinations ($F = 3.47$; $df = 3, 46$; $P = 0.02$).

No aggressive interactions of L2 Cs on the L2 Ec occurred and significantly less L3 Cs attacked L3 Ec ($F = 5.89$; $df = 2, 30$; $P = 0.007$), whereas the aggressiveness of L4 Cs on L2 Ec and L4 Ec were somewhat greater, but statistically similar (Figure 1, Panel c). The rates of aggressiveness of *E.*

connexa on *C. sanguinea* (Figure 1, Panel d) were significantly different among the combinations ($F = 2.79$; $df = 3, 37$; $P = 0.05$), and a greater number of aggressive interactions of the L4s on the L2s were observed. The aggressiveness rate between conspecific larvae of both species was similar (Cs: $19.17 \pm 3.71\%$ vs. Ec: $26.98 \pm 3.92\%$, $F = 1.68$, $df = 1, 91$, $P = 0.19$), but differed between the heterospecific larvae (IGP Cs on Ec: $15.26 \pm 3.76\%$ vs. IGP Ec on Cs: $34.48 \pm 5.77\%$, $F = 6.8$; $df = 1, 74$; $P = 0.01$) with *E. connexa* being more aggressive on *C. sanguinea* than *vice versa*.

The attack rates between conspecific larvae of both species (Figure 2, Panel a) were independent of the larval instar involved (Cs: $\chi^2 = 3.55$; $df = 3$; $P = 0.31$; Ec: $\chi^2 = 6.71$; $df = 3$, $P = 0.08$). In contrast, the attack rates involving IGP varied among the larval instar combinations. A higher frequency of attacks occurred in the combination L4 Cs on L2 Ec than in the other pairings ($\chi^2 = 13.99$; $df = 3$; $P = 0.003$). In the IGP of *E. connexa* on *C. sanguinea*, the lowest frequency of attacks was observed in the L2 Ec larvae on L2 Cs ($\chi^2 = 8.33$; $df = 3$; $P = 0.039$). The average values for the attack rates among all the conspecific or heterospecific larval combinations for both species (Figure 2, Panel b) ranged between 35 and 80%, but the average of the conspecific rates was similar between the two species ($\chi^2 = 0.26$; $df = 1$; $P = 0.60$). In contrast, the average attack rate of *E. connexa* on *C. sanguinea* within the IGP category was higher than that recorded for aggression in the opposite direction ($\chi^2 = 36.67$; $df = 1$, $P < 0.001$).

The conspecific predation rates for both species (Figure 3, Panel a)—during the 90 min permitted for their interaction—were independent of the larval instar involved (Cs: $\chi^2 = 3.92$; $df = 3$; $P = 0.27$; Ec: $\chi^2 = 7.42$; $df = 3$; $P = 0.06$). With respect to heterospecific predation, the rate of *C. sanguinea* on *E. connexa* was similar among the different larval instar

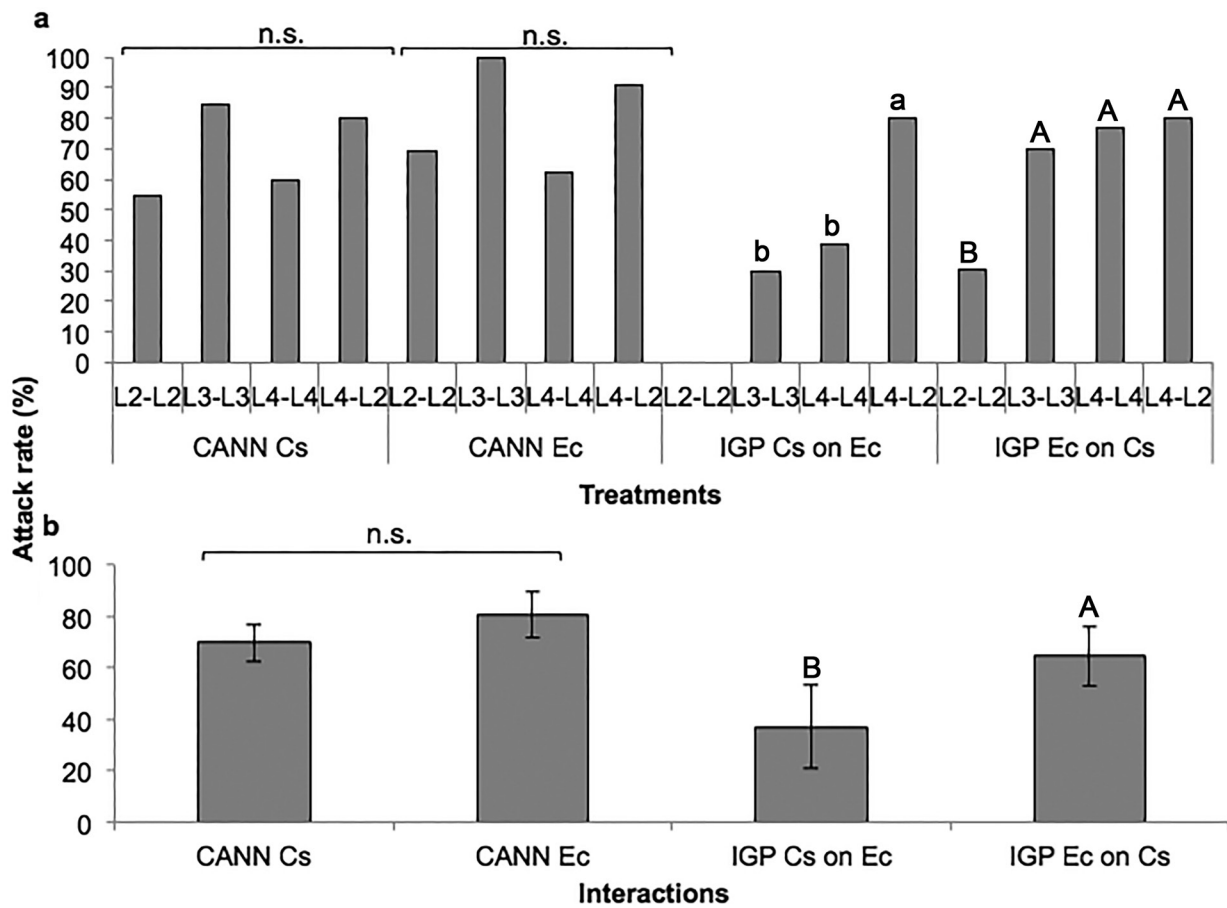


Figure 2. Occurrences of attack between different combinations of larval instars (Panel a) and mean rates of attack (Panel b) by larvae of *Cycloneda sanguinea* (Cs) and *Eriopis connexa* (Ec) on conspecific or heterospecific larva during 90 min of interaction, in the absence of extraguild prey. CANN, cannibalism; IGP, intraguild predation; L2, second-instar larvae; L3, third-instar larvae; L4, fourth-instar larvae. Different letters indicate significant differences (lower case, among larval combinations of IGP Cs on Ec; upper case, among larval combinations of IGP Ec on Cs). The brackets denote \pm standard error. Different letters indicate significant differences ($P < 0.05$).

combinations ($\chi^2 = 6.92$; $df = 2$; $P = 0.07$), but that of *E. connexa* on *C. sanguinea* was dependent on the larval instars involved, with the value being higher for the pairing L4 Ec on L2 Cs than in the other combinations ($\chi^2 = 10.73$; $df = 3$; $P = 0.013$). The predation rates among all the larval combinations (Figure 3, Panel b) were similar between conspecific pairings ($\chi^2 = 0.67$; $df = 1$; $P = 0.41$), but differed between heterospecific combinations (IGP: $\chi^2 = 14.37$; $df = 1$; $P < 0.001$), with higher values being obtained with the pairing *E. connexa* on *C. sanguinea* than with the reverse predation.

In the presence of extraguild prey, no aggressive interactions were recorded in the combinations L3–L3 or L4–L2 with respect to CANN in both species or in the pairings L3–L3 or L4–L4 in terms of IGP between the two species. Aggressive larval interactions were recorded in only three replicates of the treatment L4 Ec on L2 Cs (at a rate of aggressiveness of $27.27 \pm 14\%$) and were similar to the interference obtained in the absence of extraguild prey ($F = 2.77$; $df = 1, 18$; $P = 0.11$). All the aggressive interactions within this pairing ended in the death of the L2 Cs by predation of the L4 Ec and were likewise similar

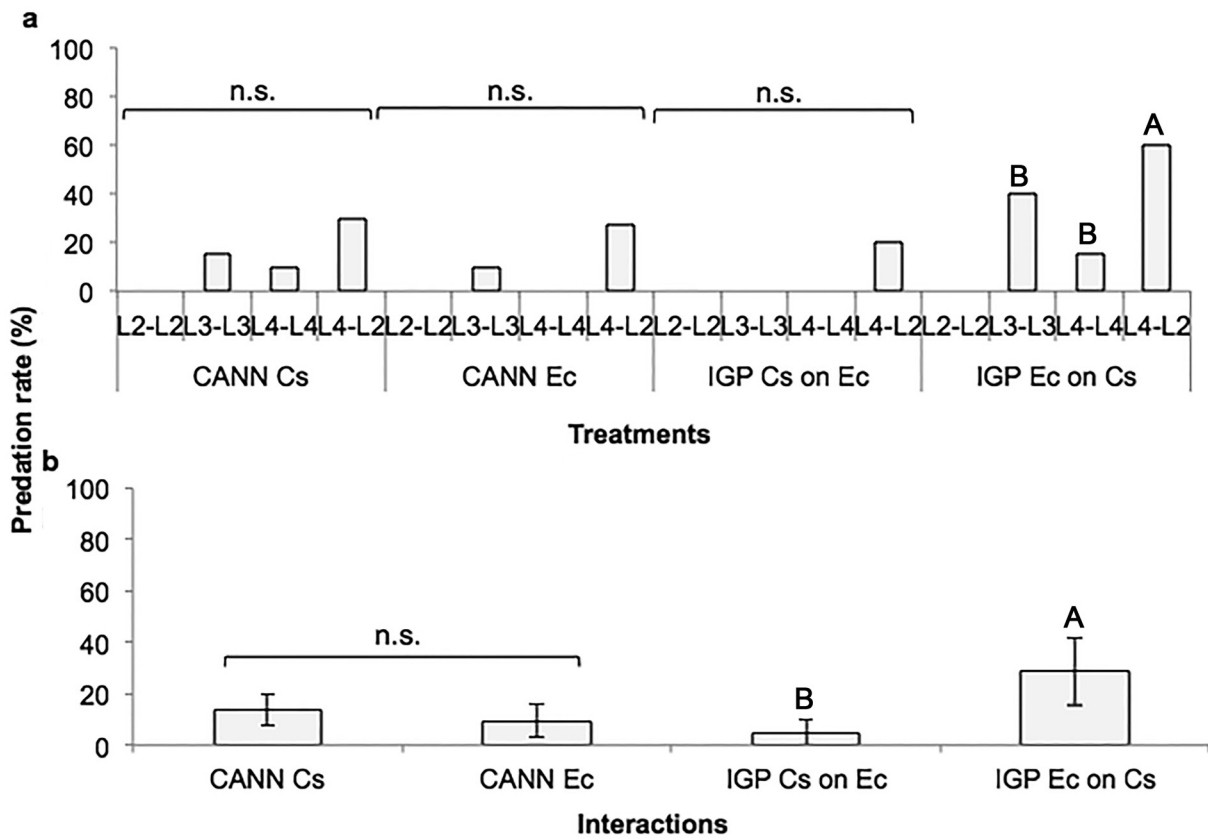


Figure 3. Occurrences of predation between different combinations of larval instars (Panel a) and mean rates of predation (Panel b) by conspecific or heterospecific larvae of *Cycloneda sanguinea* (Cs) and *Eriopis connexa* (Ec) during 90 min of interaction, in the absence of extraguild prey. CANN, cannibalism; IGP, intraguild predation; L2, second-instar larvae; L3, third-instar larvae; L4, fourth-instar larvae. Different letters indicate significant differences. The brackets denote \pm standard error. Different letters indicate significant differences ($P < 0.05$).

either with or without extraguild prey (Fisher's exact test: $P = 0.14$).

In conclusion (cf. Figure 4), these results have indicated that, in the larval stage, *E. connexa* was more often the intraguild predator with *C. sanguinea* as the intraguild prey than was *C. sanguinea* the predator in the opposite direction. Hence, *E. connexa* would be more successful than *C. sanguinea* in escaping from heterospecific aggressors. Moreover, in the presence of extraguild prey, intraguild predation by *E. connexa* on *C. sanguinea* nevertheless remained high, whereas acts of cannibalism by both species no longer occurred.

Effect of larval interference interactions on both larval mortality and within-plant distribution

At the plant level, larval mortality was similar among the different treatments and over time, but was far greater in the absence than in the presence of extraguild prey (Figure 5; Table I).

The proportion of larval mortality of *E. connexa* and *C. sanguinea* in the heterospecific treatment was similar on the second (0.52 and 0.48, respectively; $Z = 0.31$, $P = 0.76$; not shown) and the fourth days in the absence (at 0.6 and 0.4 respectively; $Z = 1.55$, $P = 0.12$; not shown) as well as in the presence of extraguild prey (second day: 0.46 and 0.54, respectively, $Z = 0.62$, $P = 0.53$;

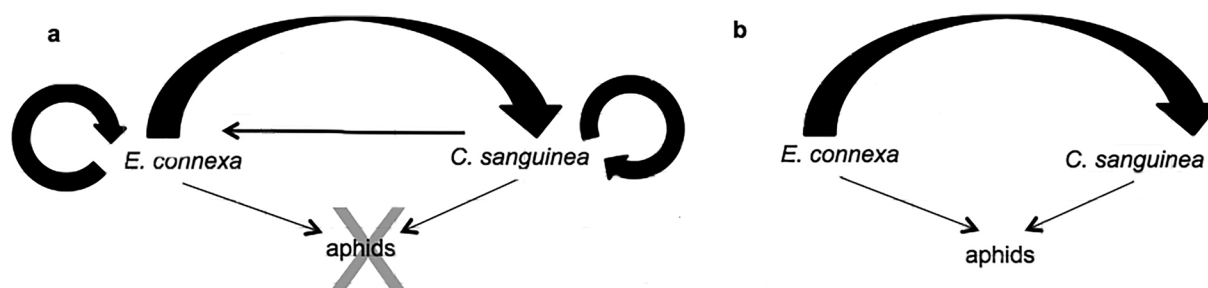


Figure 4. Semi-quantitative schematic illustration of the relative intensities of cannibalism and intraguild predation in conspecific (circular arrows) and heterospecific (adjoining arrows) interactions, respectively, between larval instars of *Cycloneda sanguinea* and *Eriopis connexa*, in (a) the absence or (b) the presence of prey (*Myzus persicae* aphids). The thicker the arrows, the higher the relative intensity of the interaction.

and fourth day: 0.45 and 0.55, respectively, $Z = 0.77$, $P = 0.44$; not shown).

On the plants, in the absence of extraguild prey, the larvae promptly dispersed and were always found outside the plant, both on the second and the fourth day after release. With extraguild prey present, *C. sanguinea* alone was recorded mainly in the upper stratum on the second day and in the upper stratum and outside the plant on the fourth day, whereas *E. connexa* alone became located mainly in the upper stratum and outside the plant throughout the experiment. In the heterospecific encounter, both species of predators were together in the upper stratum as well as outside the plant on both the second and the fourth days (Table II).

DISCUSSION

In the present investigation of coccinellid-larval interactions, *E. connexa* proved to be the stronger intraguild predator when the larvae were older than those of *C. sanguinea*. Larval cannibalism was also observed in both species with the larger larvae tending to be more aggressive toward the smaller ones. In most instances of cannibalism and intraguild predation, the difference in size determined the outcome of the interactions, in

which older larval instars were more voracious and aggressive than younger ones. However, such interactions, in general, can vary when different species are involved (Yasuda et al. 2001, N6ia et al. 2008, F6lix & Soares 2004). If one species acts as a predator more often than the other, the potentially bidirectional IGP becomes skewed in favor of the larger or stronger species (Lucas 2005).

Results of this study demonstrated that the intensity of predation in the larval stage was low, despite the high number of aggressive interactions and high rates of attack observed. This minimal frequency would indicate that the larvae of *C. sanguinea* and *E. connexa* had in general succeeded in escaping the majority of the attacks; an observation consistent with that of Lucas et al. (1997), who found that an escape response was the principal defensive tactic employed by *Coleomegilla maculata lengi* Timberlake larval instars. Indeed, these authors reported that a biting and wriggling behavior was more frequent in third and fourth instars. We observed that the larvae of both coccinellid species had similar escape tactics upon attack: they wriggled the body, made sudden movements of the abdomen while warding off the aggressor with the legs, and in that way

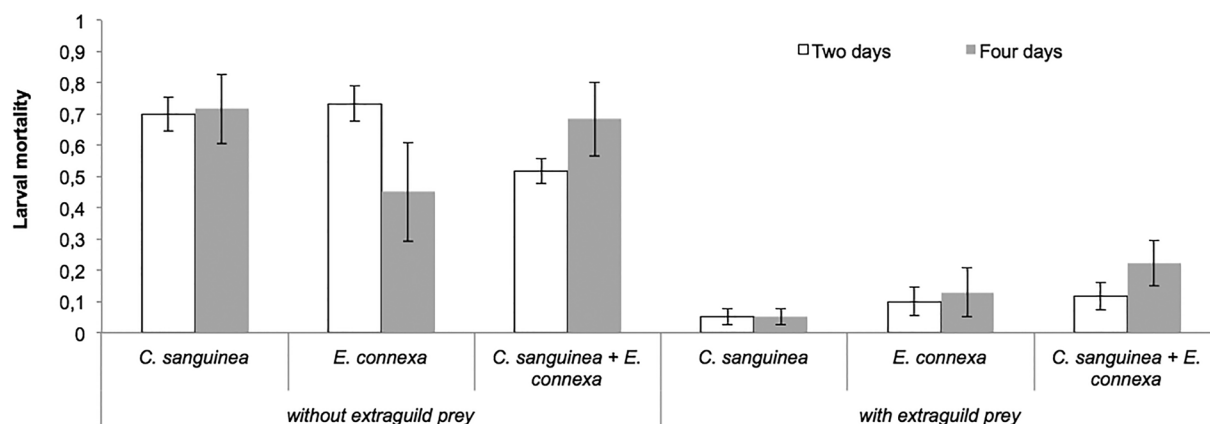


Figure 5. Mean larval mortality of *Cycloneda sanguinea* (six larvae), *Eriopis connexa* (six larvae), and both species together (three *C. sanguinea* + three *E. connexa* larvae) at two (white bars) and four (gray bars) days after the start of the experiment, either with or without additional prey (*Myzus persicae* aphids). In the figure, larval mortality, expressed as a fraction of the total larvae present, is plotted on the vertical axis for each of the experimental groups indicated on the horizontal axis, with a single species denoting cannibalism and both species intraguild predation. The initial larval stage was L1. The brackets indicate the standard errors.

managed to escape. Similarly, Yasuda et al. (2001) observed that *Harmonia axyridis* (Pallas) was more able to escape from the attacks of *Coccinella septempunctata* L. than *vice versa*, with the former being the more aggressive and stronger intraguild predator. Asymmetry in interspecific competition in insects has been found to occur more frequently than symmetric competition (Lawton & Hassell 1981, Ridsdill-Smith 1993).

Interactions (*i. e.*, aggressiveness, attack, and predation) between both conspecific and heterospecific larvae were notably different when extraguild prey were available. In the absence of extraguild prey, in all the experimental combinations, the larvae of both species exhibited mutually aggressive interactions attacking other conspecific or heterospecific larvae. Various studies had reported that cannibalism and intraguild predation by ladybirds became more frequent when aphid populations were scarce (Osawa 1989, Agarwala & Dixon 1992, Schellhorn & Andow 1999, Burgio et al. 2002, Cottrell 2005, Takizawa & Snyder 2011, Rondoni et al. 2012). Accordingly, the collapse of an aphid population

is seen to result in an increasing competition for food that drives coccinellid larvae to conspecific and heterospecific predation in order to complete their development. The increase in larval hunger enhances aggression between conspecifics (Agarwala 1991, Michaud 2003). Conversely, Agarwala & Dixon (1992) reported that starvation increased the vulnerability of *Adalia bipunctata* (L.) larvae to predation by other coccinellid larvae. This effect may have occurred in our study: the *C. sanguinea* larvae may have been stressed by not having prey for food, thus becoming weakened and as such more vulnerable to predation by the larvae of *E. connexa*. Several authors had recorded different consumption rates for those two species: Isikber (2005) found that *C. sanguinea* consumed around 344 *A. gossypii* per day, whereas Grez et al. (2007) reported that *E. connexa* consumed only 12 *A. craccivora* per day. Likewise, we observed that *C. sanguinea* consumed 40% more *M. persicae* than did *E. connexa* in 24 h (not shown). An increase in the density of extraguild prey would accordingly lead to a reduction in cannibalism and intraguild predation via an

Table I. Results of two-way repeated-measures ANOVA of larval mortality of *Cycloneda sanguinea* and *Eriopis connexa* on sweet-pepper plants for different forms of predation (conspecific and heterospecific) with or without an extraguild prey.

	df	F value	P-value
Intercept	1	243.4121	<0.0001
Treatment	2	0.5278	0.593
Availability of prey	1	94.9218	<0.0001
Treatment × Availability of prey	2	1.8532	0.166
Error	54		
Time	1	0.2728	0.604
Time × Treatment	2	2.8351	0.067
Time × Availability of prey	1	0.1084	0.743
Time × Treatment × Availability of prey	2	1.2450	0.296
Error	54		

increased availability of quality food. In the present experiments, when the extraguild prey was available, all the intraguild interactions became relaxed, except for the combination of *E. connexa*-L4 and of *C. sanguinea*-L2 larvae. Furthermore, Michaud (2003) found that larval cannibalism in three species of ladybirds increased with the rate of encounter between conspecific larvae, independently of the food supply.

Larval mortality at the level of the plant was influenced by the availability of extraguild prey only. Accordingly, by the end of the experiment, the surviving larvae reached L2–L3 instars in the absence and L3–L4 instars in the presence of additional prey.

Moreover, the coexistence of predators can affect their within-plant distribution leading to a wider distribution on the plant, with that of a given species being especially influenced by the search for predator-free patches (Moreno-Ripoll et al. 2012). Depending on the species involved, as well as the prey availability, predators tend to aggregate or disperse in certain parts of the plant, leading to either an increase or a decrease in mutual predation (Lucas & Alomar

2002, Walzer et al. 2009, Arnó et al. 2010, Moreno-Ripoll et al. 2012). In the present study, in the conspecific treatments when extraguild prey was not available, the larvae were always found off the plant (*i. e.*, on the pot, on the floor of the cage, or in the voile). However, when extraguild prey was present, a certain preference for the upper stratum was observed. Such tendency, however, changed throughout the experiment, with *E. connexa* larvae tending to leave the plant promptly, whereas those of *C. sanguinea*, though likewise initially concentrated in the upper stratum, only later became dispersed off the plant—in both instances, probably because of the decrease in the aphid population. In the heterospecific treatment, the larvae of both species were found together in the upper stratum and off the plant. Such a behavior of leaving the plant would be what occurs in the field when the larvae disperse looking for prey. *Eriopis connexa* would thus be seen as the more aggressive predator than *C. sanguinea*, a difference that could also influence larval mortality via intraguild predation, as was observed in the Petri-dish experiments. Since, when the extraguild prey became scarce, both

Table II. Results of the generalized linear model (GLM) for within-plant distribution of *Cycloneda sanguinea* and *Eriopis connexa* larvae after two and four days. For the conspecific treatments, a log-linear Poisson GLM was used, with the position of the larvae in the upper or lower stratum or outside the plant and the time, as the explanatory variables. For the heterospecific treatment, a binomial logistic GLM was performed, with the position of both species together (presence or absence) and time as the explanatory variables. The Akaike information criterion (AIC), likelihood ratio test (L. Ratio χ^2), and adjusted deviance (Deviance/df) are given for the best models fitted (in all instances, $P < 0.0001$). The GLM-Wald statistics and P -values are indicated.

Treatment	Variables	Wald statistic	P-value
Best model AIC = 214.77; L. Ratio χ^2 = 25.48; Deviance/df = 1.61			
<i>C. sanguinea</i>	Interceptor	19.68	<0.0001
	time	1.92	0.17
	position	10.57	0.005
	position*time	13.71	0.001
Best model AIC = 183.11; L. Ratio χ^2 = 33.09; Deviance/df = 1.19			
<i>E. connexa</i>	Interceptor	5.07	0.02
	position	28.47	<0.001
Best model AIC = 68.59; L. Ratio χ^2 = 17.29; Deviance/df = 1.04			
<i>C. sanguinea</i> + <i>E. connexa</i>	Interceptor	5.13	0.023
	position	8.91	0.012

species tended to leave the plant, the encounter rate would necessarily be reduced. For this reason, larval mortality would be more affected by the availability of prey than by conspecific and heterospecific interactions—in contrast to the circumstance in the Petri-dish experiments.

Concordant results had been obtained when other developmental stages of these coccinellid species were considered (Rocca et al. 2017). Larval intraguild predation was also asymmetric between the two species, with *E. connexa* being the more aggressive intraguild predator. In the presence of prey, cannibalism by both species decreased, but the intraguild predation by *E. connexa* on *C. sanguinea* still remained high, suggesting that *E. connexa* could displace *C. sanguinea* via interspecific interference competition. Results of our study suggest that, despite the high attack rates observed, a combination of the defensive abilities and the dispersal of plant-associated larvae could reduce the incidence of cannibalism

and intraguild predation at this stage of larval development, thus explaining the co-occurrence of these two coccinellid species in crops.

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Author contributions

Margarita Rocca carried out the planning of the assays, performed the analysis of data, interpretation and discussion of results, and carried out the writing of the manuscript. Estefanía Rizzo carried out the laboratory assays and collaborated in the data analysis. Nancy M. Greco participated in the planning of the assays, interpretation and discussion of results, as well as in the writing of the manuscript. She also obtained the funds to carry out the investigation.

