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Intra- and interspecific interactions between aphidophagous ladybirds: the role of prey in predator coexistence

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Abstract

Cannibalism (CANN) and intraguild predation (IGP) may provide energy and nutrients to individuals and eliminate potential competitors. These negative competitive interactions could also affect the coexistence of predatory species. The co-occurrence of aphidophagous ladybird species in crops creates opportunities for CANN and IGP, especially when aphids become scarce. The Lotka–Volterra model predicts the coexistence of two species if intraspecific competition is stronger than interspecific interference interactions. *Cycloneda sanguinea* L. and *Eriopis connexa* (Germar) (both Coleoptera: Coccinellidae) coexist in sweet pepper crops in La Plata (Argentina) consuming mainly *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). The present study used laboratory experiments to estimate levels of CANN and IGP by adults and larvae on eggs, and by adults on larvae, in both the presence and absence of prey (i.e., *M. persicae*), to explain the effect of prey on coexistence of these two predators. Levels of CANN by *C. sanguinea* and *E. connexa* were high in the absence of aphids, and decreased when prey was present. Intraguild predation was bidirectional and asymmetric. Adults and larvae of *E. connexa* were more voracious IG predators of *C. sanguinea* than vice versa, the former being the stronger IG predator and interference competitor. *Eriopis connexa* always won when larvae of the same instar were compared, whereas the larger larva always won when larvae were of different instars, regardless of species. In the presence of prey, CANN by both species decreased, but IGP by *E. connexa* on *C. sanguinea* remained high, suggesting that *E. connexa* could displace *C. sanguinea* via interspecific interference competition. Other factors potentially affecting the coexistence of *C. sanguinea* and *E. connexa* in sweet pepper crops are discussed.

Introduction

Cannibalism (CANN) and intraguild predation (IGP) have important theoretical and practical implications for the dynamics of interacting populations, community structure, and the efficacy of natural enemies in biological control. Cannibalism is defined as predation on other individuals of the same species (Fox, 1975) and is widespread in arthropod populations. On the other hand, IGP occurs when two consumers of different species share a resource and engage in interference competitions by one preying on the other, or both consuming each other (Polis et al., 1989). The spatial and temporal co-occurrence of aphidophagous ladybird species creates opportunities for CANN and IGP, especially when aphids become scarce (Schellhorn & Andow, 1999; Burgio et al., 2002; Michaud, 2002). Cannibalism propensity varies among aphidophagous species and can be influenced by food availability and quality, developmental stage, and encounter rates among individuals (Michaud, 2003; Bayoumy & Michaud, 2015). Intraguild predation interactions among ladybirds are usually bidirectional and asymmetric (Michaud & Grant, 2003). Intraguild prey choice can be based on species, size, mobility, palatability, behavior, or protective devices, and is also affected by encounter rates (Lucas et al., 1998; Lucas, 2012).

Cannibalism and IGP may benefit individual fitness by providing energy and nutrition, and/or by eliminating potential competitors (Mills, 2006; Hodek & Evans, 2012).

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However, both these behaviors may affect the coexistence of predatory species. The Lotka–Volterra model predicts the coexistence of two competing species if intraspecific competition is stronger than interspecific competition (Mills, 2006). In the case of CANN as a form of intraspecific competition and IGP as a form of interspecific competition, the theory predicts that if both species are weak competitors, coexistence becomes more likely. Models predict that a necessary additional condition for coexistence of IG predators is that the IG prey is a superior competitor for the shared resource (Polis & Holt, 1992; Holt & Polis, 1997; Janssen et al., 2006). The intensity of both intraspecific (CANN) and interspecific (IGP) competition could relax with the increase of the extraguild prey, thus contributing to the persistence of both species in the system (Lucas & Rosenheim, 2011; Mirande et al., 2015). However, under this scenario other outcomes are possible, such as the exclusion of the IG prey due to the combined effects of exploitative and apparent competition (Holt & Polis, 1997; Janssen et al., 2006).

The ladybirds *Cycloneda sanguinea* L. and *Eriopis connexa* (Germar) (both Coleoptera: Coccinellidae) are neotropical aphidophagous generalists common in horticultural landscapes around La Plata, Buenos Aires, Argentina. Both species have been recorded together consuming aphids in sweet pepper throughout the growing season. We studied CANN and IGP in both species, in both the presence and absence of extraguild prey to estimate the relative intensity of intra- and interspecific competition and the role of extraguild prey in fostering their coexistence. A series of laboratory experiments was conducted to determine (1) the levels of CANN and IGP by adults and larvae on eggs, and by adults on larvae, in both the presence and absence of prey; (2) the intensity, symmetry, and direction of IGP between various larval instars of *C. sanguinea* and *E. connexa*; and (3) the relative strength of CANN vs. IGP for each species.

Materials and methods

Stock colonies

Laboratory colonies of *C. sanguinea* (Cs) and *E. connexa* (Ec) were established with adults collected from sweet pepper crops in La Plata, Buenos Aires province, Argentina (35°00′S, 58°00′W). Taxonomic determination of the species was confirmed by Dr. Nora Cabrera, Coleoptera specialist from the Museum of Natural Science of La Plata. Cultures were held in a climate-controlled chamber at 25 ± 2 °C, 70 ± 10% r.h., and L16:D8 photoperiod; all experiments were conducted under these conditions. Adult and larval stages were held separately in plastic containers (15 × 15 × 25 cm) covered with voile fabric to allow ventilation. Both life stages were fed yeast, pollen, and *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) reared on wheat seedlings, all refreshed thrice weekly. Water was provided on a sponge inserted into an Eppendorf tube. For adults, the bottom of each container was lined with a paper towel as an oviposition substrate. Egg clusters (usually 20–30 eggs each) were collected daily, stored at 10 ± 1 °C to maintain their viability (Miller, 1995), and used in the experiments when they were ≤10 days old (Cottrell, 2004, 2005). Adult females in reproductive age of 3–4 weeks old and larvae of specific instars were selected from the stock colony and starved for 24 h prior to use in experiments.

Laboratory assays

CANN and IGP by adults and larvae on eggs in the absence of prey. Adult females of each species were isolated in Petri dishes (10 cm diameter) with either an egg cluster of their own species (n = 24 for Cs; n = 18 for Ec) or one of the other species (n = 15 for Cs; n = 21 for Ec). Similarly, one fourth-instar larva was confined in a Petri dish (10 cm diameter) with either an egg cluster of its own species (n = 10 for Cs and Ec) or one of the other species (n = 10 for Cs and Ec). After 24 h, the number of eggs consumed was recorded and number of replicates in which CANN or IGP occurred was tallied. We then calculated the proportion of conspecific and heterospecific eggs consumed by both adults and larvae (as the number of eggs consumed/total number of eggs).

CANN and IGP by adults on larvae of different instars in the absence of prey. Two experiments were conducted to evaluate the effect of prey instar on CANN and IGP. Synchronous cohorts of neonate larvae of each species were reared until they reached the required instars (second and fourth). Adult females of each species were then isolated in Petri dishes (10 cm diameter) with either one conspecific larva (Cs + L2, n = 20; Cs + L4, n = 19; Ec + L2, n = 11; Ec + L4, n = 10) or one heterospecific larva (Cs + L2, Cs + L4, Ec + L2, Ec + L4, all n = 10) for 24 h, whereupon larval mortality was tallied.

Cannibalism and IGP in the presence of prey. *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) was chosen as extraguild prey because it is the most abundant and frequent aphid in sweet pepper crops of the study area. The adult *M. persicae* apterae were placed in plastic cylinders (5 × 6 cm diameter), on a sweet pepper leaf disk that was embedded, abaxial side uppermost, in water agar (1%). Ventilation was possible through a hole in the lid covered with insect gauze (mesh size 80 μm) (Figure 1). The plastic cylinders were upside down on a

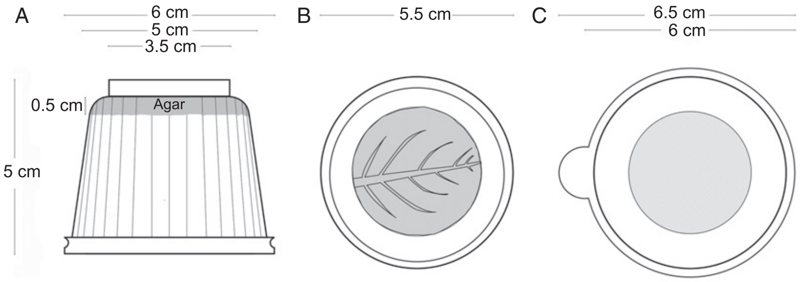

Figure 1 Experimental unit used to study cannibalism and intraguild predation in the presence of prey (*Myzus persicae* aphids). (A) Lateral and (B) top view of the plastic cup: sweet pepper leaf disk embedded in water agar. (C) Plastic lid with a hole covered with insect gauze.

grid, ensuring that the abaxial side of the leaf disks (where the aphids feed) faced downward, simulating the natural position at the plant. The adults were allowed to reproduce until there were ca. 250 aphids on the leaf, more than the number that a predator could consume in 24 h. The following combinations of coccinellids were then added to the containers: One Cs adult + eggs (n = 16), one Ec adult + eggs (n = 13), one Cs L4 + eggs (n = 14), one Ec L4 + eggs (n = 20), one Cs adult + L2 (n = 13), one Ec adult + L2 (n = 13), one Cs adult + L4 (n = 19), and one Ec adult + L4 (n = 15). Because of very low IGP by Cs on Ec in the absence of extraguild prey, this combination was not tested in subsequent experiments. The four IGP combinations tested in the presence of aphids were: one Ec adult + Cs eggs (n = 15), one Ec L4 + Cs eggs (n = 14), one Ec adult + Cs L2 (n = 22), and one Ec adult + Cs L4 (n = 21). The frequency of CANN and IGP was recorded in all replicates after 24 h.

Intensity and symmetry of IGP. Heterospecific pairs of predators were confined in Petri dishes (10 cm diameter) in the following combinations: L2 + L2 (n = 21), L3 + L3 (n = 15), L4 + L4 (n = 25), L4 Cs + L2 Ec (n = 14), and L4 Ec + L2 Cs (n = 9). Numbers of predation events after 24 h were used to estimate the intensity and symmetry of IGP; IGP intensity was estimated as the percentage of replicates in which IGP occurred, and the index of symmetry was computed as the number of replicates in which a given predator was prey divided by the total number of replicates in which IGP occurred. This value was compared to an expected value of 50% (symmetric interactions) with the χ^2 goodness of fit test.

CANN vs. IGP. To determine the relative strength of CANN vs. IGP, we compared the CANN of Cs vs. IGP by Ec on Cs, and the CANN of Ec vs. IGP by Cs on Ec, all in the absence of prey. Only the first combination was conducted also in the presence of prey, because IGP by Cs on Ec in the absence of extraguild prey was very low, so it is not likely to occur in the presence of prey. Dependent variables were the proportion of eggs preyed upon by adults and larvae, and the frequency of adult predation on larvae.

Data analysis

Frequencies of CANN and IGP in all experiments were analyzed by χ^2 contingency tests. Egg consumption was analyzed as a binary response variable using multivariate logistic models with type of interaction (CANN or IGP), species (*E. connexa* or *C. sanguinea*), developmental stage (larva or adult), and prey availability (presence or absence) as the explanatory variables. In each test full models were reduced by discarding all variables lacking explanatory power until the most parsimonious model remained (Agresti, 2015).

We estimated odds ratios (OR) to quantify the likelihood of one event relative to the other (Agresti, 2015). The OR is defined as the probability an event occurs divided by the probability it does not. For example, the OR for the variable 'species' is the ratio of the odds of consumption (or no consumption) of *E. connexa* divided by the odds of consumption (or no consumption) of *C. sanguinea*.

The initial model for analyzing CANN of eggs by larvae and adults in the absence or presence of prey, and IGP of heterospecific eggs by larvae and adults in the absence of prey, was as follows:

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 I_S + \beta_2 I_{DS} + \beta_3 I_S ID_S,$$

where π is the probability of consuming eggs, β the regression parameter, $I_S = 1$ for *E. connexa*, $I_S = 0$ for *C. sanguinea*, $I_{DS} = 1$ for larva, $I_{DS} = 0$ for adult, $I_S I_{DS} = 1$ for *E. connexa* + larva, and $I_S I_{DS} = 0$ for all other combinations of species and developmental stages.

The initial model for analyzing CANN of eggs by larvae and adults in the presence and absence of prey was:

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 I_S + \beta_2 I_P + \beta_3 I_{DS} + \beta_4 I_S I_P + \beta_5 I_S I_{DS} + \beta_6 I_P I_{DS} + \beta_7 I_S I_P I_{DS},$$

where π is the probability of consuming eggs, β the regression parameter, $I_S = 1$ for *E. connexa*, $I_S = 0$ for *C. sanguinea*, $I_P = 1$ for prey absent, $I_P = 0$ for prey present,

$I_{DS} = 1$ for larva, $I_{DS} = 0$ for adult, $I_S I_P = 1$ for *E. connexa* + prey absent, $I_S I_P = 0$ for all other combinations of species and prey availability, $I_P I_{DS} = 1$ for prey absent + larva, $I_P I_{DS} = 0$ for all other combinations of prey availability and developmental stage, $I_S I_{DS} = 1$ for *E. connexa* + larva, $I_S I_{DS} = 0$ for all other combinations of species and developmental stage, $I_S I_P I_{DS} = 1$ for *E. connexa* + prey absent + larva, and $I_S I_P I_{DS} = 0$ for all other combinations of species, prey availability, and developmental stage.

The proportion of IGP eggs consumed by adults and larvae of *E. connexa* in the presence and absence of prey was analyzed as follows:

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 I_P,$$

where π is the probability of consuming eggs, β the regression parameter, $I_P = 1$ for prey absent, and $I_P = 0$ for prey present.

Cannibalism vs. IGP was then compared, in the presence or absence of prey, using the following model:

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 I_I + \beta_2 I_{DS} + \beta_3 I_I I_{DS},$$

where π is the probability of consuming eggs, β the regression parameter, $I_I = 1$ for IGP, $I_I = 0$ for CANN, $I_{DS} = 1$ for larva, $I_{DS} = 0$ for adult, $I_I I_{DS} = 1$ for the interaction between IGP and larva, and $I_I I_{DS} = 0$ for all other combinations of interactions and developmental stage.

All analyses using Generalized Linear Models (GLM) with binomial distribution were performed with the statistical package R, v.3.2.1 (R Development Core Team, Vienna, Austria).

Results

Cannibalism on eggs in the presence and absence of prey

In the absence of prey, adults of both species consumed conspecific eggs at similarly high frequencies ($\chi^2 = 1.66$, d.f. = 1, P = 0.2) (Figure 2A), and larvae of both species consumed eggs in all replicates (Figure 2B). Similar proportions of eggs were cannibalized by adults of *C. sanguinea* (mean ± SE = 0.75 ± 0.08) and *E. connexa* (0.83 ± 0.07), and by larvae (*C. sanguinea*: 0.96 ± 0.04; *E. connexa*: 1.0 ± 0). The only indicator retained by the model was 'developmental stage' (Table 1A). For eggs, the OR of being cannibalized by a larva was 17× greater than the OR of being cannibalized by an adult.

In the presence of prey, conspecific eggs were consumed at similar frequencies by *C. sanguinea* and *E. connexa* adults ($\chi^2 = 0.1$, d.f. = 1, P = 0.75; Figure 2A) and larvae ($\chi^2 = 2.35$, d.f. = 1, P = 0.12; Figure 2B). Similar proportions of eggs were cannibalized by adults of *C. sanguinea* (mean ± SE = 0.04 ± 0.02) and *E. connexa* (0.06 ± 0.04) and the same was true for larvae (*C. sanguinea*: 0.70 ± 0.07; *E. connexa*: 0.63 ± 0.1). Again 'developmental stage' was the only indicator retained by the model (Table 1B). For eggs, the OR of being cannibalized by a larva was 41× greater than the OR of being cannibalized by an adult.

Cannibalism of eggs by adults of *C. sanguinea* diminished in the presence of aphids prey ($\chi^2 = 11.3$, d.f. = 1, $P<0.01$), whereas larvae cannibalized in 100% of replicates whether aphids were present or not. Similarly, the presence of aphids diminished egg CANN by *E. connexa* adults ($\chi^2 = 17.7$, d.f. = 1, $P<0.01$), but not CANN by larvae ($\chi^2 = 1.47$, d.f. = 1, P = 0.22; Figure 2). The model retained both 'prey availability' and 'developmental stage' as explanatory variables (Table 1C). When 'prey availability' was fixed in the model, the OR of an egg being cannibalized by a larva was 35× higher than that of being cannibalized by an adult. When 'developmental stage' was fixed, the OR of an egg being cannibalized was 77× larger when prey was absent than when it was present.

IGP on eggs in the presence and absence of prey

The frequency of IGP by *E. connexa* on *C. sanguinea* eggs was higher in the absence of prey for both adults and larvae

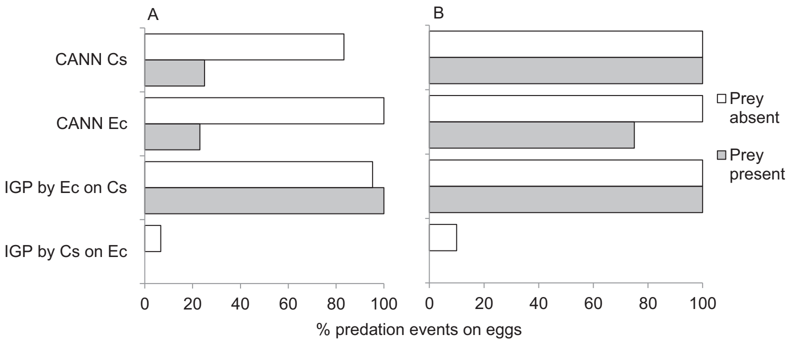

Figure 2 Frequency of conspecific (cannibalism, CANN) and heterospecific (intraguild predation, IGP) egg consumption by (A) adults and (B) larvae of *Cycloneda sanguinea* (Cs) and *Eriopis connexa* (Ec), in the presence and absence of prey (*Myzus persicae* aphids).

Table 1 Results of the GLM binomial distribution model. (A) Cannibalism (CANN) of *Cycloneda sanguinea* and *Eriopis connexa* eggs in the absence of prey (*Myzus persicae* aphids); (B) CANN of *C. sanguinea* and *E. connexa* eggs in the presence of prey; (C) comparison of egg CANN between species in the presence and absence of prey; (D) intraguild predation (IGP) of *C. sanguinea* eggs by *E. connexa* and vice versa, in the absence of prey; (E) CANN of *C. sanguinea* eggs and IGP of *C. sanguinea* eggs by *E. connexa*, in the absence of prey; (F) CANN of *E. connexa* eggs and IGP of *E. connexa* eggs by *C. sanguinea*, in the absence of prey; (G) CANN of *C. sanguinea* eggs and IGP of *C. sanguinea* eggs by *E. connexa*, in the presence of prey

	Variables	β	SE	P
A	Intercept	1.4095	0.3083	2.5e-05
	Larva	2.81	1.469	0.061
B	Intercept	−3.0957	0.6194	5.2e-06
	Larva	3.7153	0.6658	5.9e-07
C	Intercept	−2.9577	0.6401	9.6e-06
	No prey	4.3435	0.6881	4.6e-09
	Larva	3.5546	0.6865	9.0e-07
D	Intercept	−3.3086	0.8345	0.00022
	E. connexa	4.9478	0.9181	1.6e-06
E	Intercept	1.2467	0.2791	3.4e-05
	Larva	1.3457	0.7099	0.063
F	Intercept	2.3198	0.5496	0.0001
	IGP	−5.6284	1.1099	5.6e-06
G	Intercept	−3.2815	0.7381	4.3e-05
	IGP	4.7445	0.8165	3.3e-07
	Larva	4.1408	0.8136	4.5e-06
	IGP*larva	−4.1402	0.9664	7.4e-05

β, regression parameter.

(χ^2 = 24.72 and 12.93, respectively, both d.f. = 1, P<0.01) (Figure 2). The proportion of *C. sanguinea* eggs consumed by *E. connexa* IGP was also much higher (mean ± SE = 0.80 ± 0.06 and 0.89 ± 0.06 eggs for adults and larvae, respectively) than vice versa (0.01 ± 0.01 and 0.11 ± 0.11 eggs for adults and larvae, respectively). The only indicator retained in the model was 'species' (Table 1D). An egg of *C. sanguinea* was 141× more likely to be preyed upon by *E. connexa* than vice versa.

In the presence of prey, the frequency of IGP on *C. sanguinea* eggs by adults and larvae of *E. connexa* was 100% (Figure 2). Intraguild predation by *E. connexa* adults did not decrease even when extraguild prey was available (χ^2 = 0.03, d.f. = 1, P = 0.86) and larvae consumed eggs in 100% of replicates in both cases (Figure 2). Likewise, the proportion of *C. sanguinea* eggs eaten by adults and larvae of *E. connexa* (mean ± SE = 0.83 ± 0.06 and 0.80 ± 0.07, respectively) remained high in the presence of prey. Because the model detected colinearity between the explanatory variables 'prey availability' and 'development stage', the first variable was selected, whereupon the model rejected 'prey availability', so these proportions were compared by a two-proportion z-test. Consumption of *C. sanguinea* eggs by *E. connexa* occurred independent of prey availability (z = −1.27, P = 0.21).

CANN on larvae in the presence and absence of prey

The frequency of larval cannibalism by adults in the absence of prey was independent of larval stage (Cs on L2 vs. L4: χ^2 = 3.08, d.f. = 1, P = 0.08; Ec on L2 vs. L4: χ^2 = 0.39, d.f. = 1, P = 0.53) and species (Cs vs. Ec on L2: χ^2 = 0.08, d.f. = 1, P = 0.77; Cs vs. Ec on L4: χ^2 = 0.64, d.f. = 1, P = 0.43; Figure 3). Adults of both species preyed on conspecific larvae in the presence of aphids, but at low frequencies. When extraguild prey was present, adult cannibalism by both species occurred independent of larval stage (Cs on L2 vs. L4: χ^2 = 0.22, d.f. = 1, P = 0.64; Ec on L2 vs. L4: χ^2 = 0.44, d.f. = 1, P = 0.51) and species (Cs vs. Ec on L2: χ^2 = 0.99, d.f. = 1, P = 0.32; Cs vs. Ec on L4: χ^2 = 0.05, d.f. = 1, P = 0.83) (Figure 3). Cannibalism on larvae became less frequent in both species when adults had aphids to eat (Cs on L2: χ^2 = 9.95, P<0.01; Cs on L4: χ^2 = 3.96, P = 0.04; Ec on L2: χ^2 = 4.37, P = 0.03; Ec on L4: χ^2 = 4.05, P = 0.04, all d.f. = 1).

IGP on larvae in the presence and absence of prey

In the absence of prey, adults of neither species distinguished between second or fourth-instar heterospecific larvae (Cs on Ec L2 vs. L4: χ^2 = 1.07, d.f. = 1, P = 0.3; 100% consumption of Cs L2 and L4 by Ec). *Eriopis connexa* adults preyed more on *C. sanguinea* larvae, both L2 and L4 stages, than vice versa (Ec vs. Cs on heterospecific L2: χ^2 = 12.93, d.f. = 1, P<0.01; Ec vs. Cs on

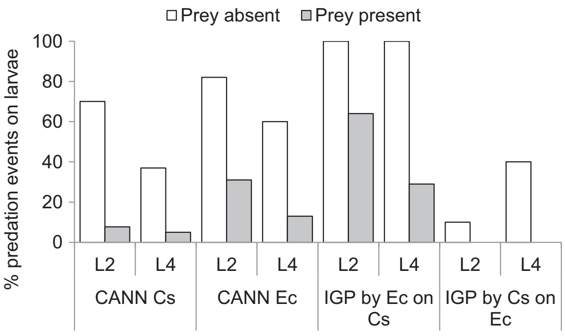

Figure 3 Frequency of adults consuming conspecific (cannibalism, CANN) and heterospecific (intraguild predation, IGP) second-instar (L2) and fourth-instar (L4) larvae of *Cycloneda sanguinea* (Cs) and *Eriopis connexa* (Ec), in the presence and absence of prey (*Myzus persicae* aphids).

heterospecific L4: $\chi^2 = 5.99$, d.f. = 1, P = 0.01; Figure 3). When prey was present, adult *E. connexa* preyed more frequently on *C. sanguinea* L2 than on L4 ($\chi^2 = 3.99$, d.f. = 1, P = 0.04). *Eriopis connexa* adults preyed less often on L4 when aphids were present ($\chi^2 = 11.13$, d.f. = 1, $P<0.001$), but at a similar frequency on Cs L2 ($\chi^2 = 3.1$, d.f. = 1, P = 0.08).

Intensity and symmetry of IGP

Levels of IGP were high in all larval combinations, and highest between larvae of disparate sizes with significant asymmetry between species in all combinations. *Eriopis connexa* always won over *C. sanguinea*, except when the latter was larger (Table 2).

CANN vs. IGP

The model comparing *C. sanguinea* egg consumption due to CANN vs. IGP by *E. connexa*, in the absence of prey, rejected the explanatory variable 'type of interaction', that is, *C. sanguinea* eggs had the same probability of being cannibalized or preyed upon by *E. connexa*. The model retained the indicator variable 'developmental stage' but the high P-value indicated poor explanatory power (Table 1E). The OR of being cannibalized or preyed upon by a larva was 3.8× larger than by an adult.

The model comparing CANN of *E. connexa* eggs vs. IGP by *C. sanguinea*, in the absence of prey, retained only the indicator 'type of interaction' (Table 1F). For an *E. connexa* egg, the OR of being preyed upon by *C. sanguinea* was 99.8% lower than that of being cannibalized.

The model comparing CANN of *C. sanguinea* eggs vs. IGP by *E. connexa* in the presence of prey retained 'type of interaction', 'developmental stage', and 'IGP*larva interaction' (Table 1G). A *C. sanguinea* egg was more likely to be preyed upon by *E. connexa* (OR = 11.5) than to be cannibalized, was as likely (OR = 1) to be preyed upon by a larval or adult *E. connexa*, and more likely (OR = 6.3) to be cannibalized by a larva than by an adult.

When adults preyed on larvae in the absence of prey, *C. sanguinea* suffered less cannibalism than IGP by *E. connexa* ($\chi^2 = 65.06$, d.f. = 3, $P<0.01$), and in both cases L2 were eaten more frequently than L4 ($\chi^2 = 15.32$, d.f. = 3, $P<0.01$). In contrast, *E. connexa* suffered more cannibalism than IGP by *C. sanguinea* ($\chi^2 = 43.64$, d.f. = 3, $P<0.01$). L2 *E. connexa* were most often cannibalized, whereas *C. sanguinea* preyed more often on L4 ($\chi^2 = 9.98$, d.f. = 3, P = 0.04; Figure 3). In the presence of prey, CANN of *C. sanguinea* was lower than IGP by *E. connexa* ($\chi^2 = 23.74$, d.f. = 3, $P<0.01$), and in both cases the L2 was more frequently eaten ($\chi^2 = 13.23$, d.f. = 3, P = 0.01).

Thus, results indicated that *E. connexa* was more often the intraguild predator and *C. sanguinea* more often the intraguild prey. Intraguild predation by *E. connexa* on *C. sanguinea* remained high in the presence of prey, whereas CANN by both species decreased (Figure 4).

Discussion

As expected, CANN of eggs and larvae by adults and larvae of *C. sanguinea* and *E. connexa* diminished when the extraguild prey was available. Various studies have reported that CANN on eggs and larvae by ladybirds became more frequent when aphid populations crash (Osawa, 1989; Agarwala & Dixon, 1992; Schellhorn & Andow, 1999; Burgio et al., 2002; Cottrell, 2005; Takizawa & Snyder, 2011; Rondoni et al., 2012). The collapse of an aphid population results in increasing competition for food and drives larvae to CANN and IGP in order to complete their development (Agarwala, 1991; Michaud, 2003), with the added advantage of eliminating competitors (Agarwala & Dixon, 1992).

Intraguild predation was asymmetric, with *E. connexa* more successful in preying on *C. sanguinea* than vice versa, suggesting that *C. sanguinea* would suffer from interference competition with *E. connexa*. The latter was always the winner in larval confrontations, except when *C. sanguinea* individuals were larger. Similarly, Lucas et al. (1998) reported that in IGP among the aphid predators *Aphidoletes aphidimyza* Rondani, *Chrysoperla rufilabris*

Table 2 Intensity of intraguild predation (IL) and symmetry indices (SI) in heterospecific interactions among second-instar (L2) and fourth-instar (L4) larvae of *Cycloneda sanguinea* and *Eriopis connexa*, in the absence of extraguild prey (*Myzus persicae* aphids)

Predator	Prey	IL (%)	SI (%)	χ^2 (d.f. = 1)	P	n
E. connexa/L2	*C. sanguinea*/L2	90.5	100	10.29	0.001	21
E. connexa/L3	*C. sanguinea*/L3	66.7	100	4.27	0.04	15
E. connexa/L4	*C. sanguinea*/L4	72	88.9	4.71	0.03	25
C. sanguinea/L4	*E. connexa*/L4		11.1			
E. connexa/L4	*C. sanguinea*/L2	100	100	4.24	0.03	9
C. sanguinea/L4	*E. connexa*/L2	92.9	100	6.36	0.01	13

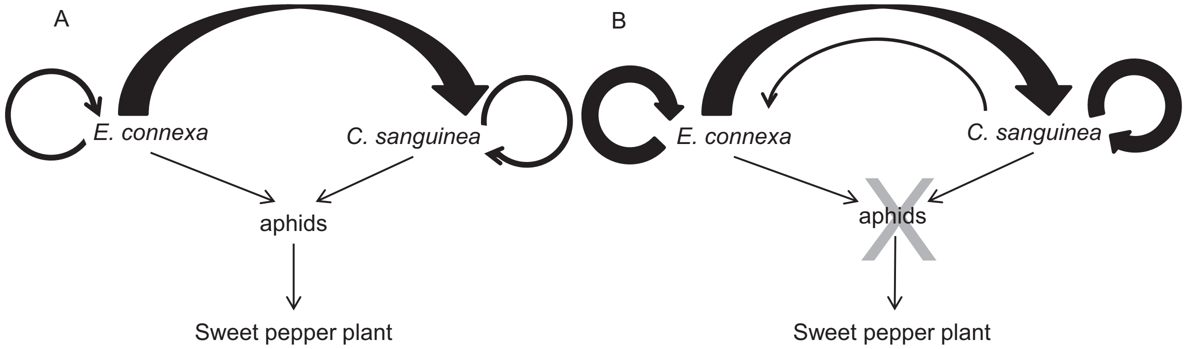

Figure 4 Relative intensities of cannibalism and intraguild predation in conspecific and heterospecific interactions between *Eriopis connexa* and *Cycloneda sanguinea*, in (A) presence and (B) absence of prey (*Myzus persicae* aphids). The thicker the arrows, the higher the relative intensity of the interaction.

(Burmeister), and *Coleomegilla maculata lengi* Timberlake, the largest individuals consistently won confrontations. The relative size of individuals is often mentioned as one of the most important factors determining IGP outcomes (Polis et al., 1989; Félix & Soares, 2004; Gkounti et al., 2015).

The intensity of CANN and IGP varies greatly and depends on the species involved. In general, CANN of coccinellid eggs is more common than IGP, probably because of species-specific chemical defenses that deter other ladybird species more than conspecifics (Hemptinne et al., 2000; Cottrell, 2004, 2005; Kajita et al., 2010). The relative propensity for egg CANN in coccinellids has also been demonstrated to vary in a species-specific manner, in both the presence and absence of food, as a function of life stage and prey density (Bayoumy & Michaud, 2015).

We found high levels of *E. connexa* IGP on eggs and small larvae of *C. sanguinea* even in the presence of aphids. The eating of eggs and larvae of other species of ladybirds is likely to reduce the quality of the diet (Obrycki et al., 1998). An increase in the density of extraguild prey will lead to a reduction in IGP via increased availability of quality food. Lucas et al. (1998) proposed three non-exclusive explanations as to why IGP might occur independent of extraguild prey density: (1) IG predators face no risk in attacking IG prey, (2) predator foraging behavior results in high rates of encounter with intraguild prey, and (3) high densities of prey increase the risk of predator confrontations. Several hypotheses about the benefits of IGP for coccinellids were proposed: the predator attacks IG prey to protect itself before a period of high vulnerability, the predator eliminates the competitor, or the predator selects the prey according to its relative size and prey defensive abilities (Lucas, 2005).

Evidence exists that high availability of extraguild prey can foster population growth of the IG predator, eventually magnifying its impact on the IG prey (Polis et al., 1989; Nóia et al., 2008; Lucas et al., 2009; Chacón & Heimpel, 2010). Laboratory studies can clarify interactions specific to biology and behavior of the species involved. An increase in extraguild prey density potentially also strengthens IGP at spatial and temporal scales larger than those used in laboratory experiments (Polis et al., 1989; Nóia et al., 2008; Lucas et al., 2009; Chacón & Heimpel, 2010). The ability of extraguild prey to relax the intensity of IGP, has been documented in small-scale field experiments (Lucas & Rosenheim, 2011) and may well extend to larger scales under open field conditions. These authors observed that fields with more abundant extraguild prey (aphids and mites) were not associated with higher densities of IG predators, but rather were strongly associated with increased survival of IG prey (lacewing larvae).

Our results suggest that *E. connexa* could displace *C. sanguinea*, at least under the conditions similar to our laboratory study, due to the greater strength of interspecific interference (IGP) relative to intraspecific competition (CANN). Under field conditions, other processes aside from CANN and IGP may influence the population dynamics of these species in ways that favor their coexistence, such as habitat complexity, the competitive ability of the IG prey, and the food web complexity (Janssen et al., 2006). According to habitat complexity many processes could result in spatial and temporal niche partitioning in ways that would not be evident within our experimental setup. For example, coccinellid females tend to select oviposition sites that minimize the probability of CANN and IGP for their progeny (Ruzicka, 1997; Lucas et al., 1998; Michaud & Jyoti, 2007).

A competitive advantage in exploiting the extraguild prey can serve to compensate the disadvantaged species (IG prey) and promote coexistence (Polis & Holt, 1992; Holt & Polis, 1997; Janssen et al., 2006). We observed that

C. sanguinea consumed 40% more *M. persicae* than did *E. connexa* in 24 h (M Rocca, unpubl.). Similarly, Işikber (2005) found that *C. sanguinea* consumed around 344 *A. gossypii* per day, whereas Grez et al. (2007) reported that *E. connexa* consumed 12 *A. craccivora* per day. These data suggest that *E. connexa* is less voracious than *C. sanguinea* and that the latter species might be more competitive in exploitation of extraguild prey.

The presence of other predatory species in the food web can result in other direct and indirect interactions. *Harmonia axyridis* (Pallas) and *Scymnus argentinicus* (Weise) are other coccinellid species occasionally present in the study area but their relative abundance and temporal synchrony with *E. connexa* and *C. sanguinea* have not been yet explored. Mirande et al. (2015) found that *H. axyridis* was the IG predator and *E. connexa* the IG prey in laboratory experiments. Moreover, the food web could include alternative food to predators. Coccinellids are specialists on aphids, but the tendency to include other insect preys in their diet may arise from shortages of aphids (Evans, 2009). Non-prey food – such as pollen, nectar, fungal spores, plant wound exudates, and phloem sap – are important components of most coccinellids' diet (Lundgren, 2009). Therefore, negative interactions could be mitigated in the field if *E. connexa* and *C. sanguinea* feed on other non-aphid prey and no prey food when aphids are absent.

The coccinellids *C. sanguinea* and *E. connexa* exhibit great potential for controlling aphids in horticultural systems (Miller, 1995; Michaud, 2002). The present study improves our understanding of the potential for IG conflict between these species when they contribute together to biological control of aphids. Field experiments are needed to provide information on the other ecological factors that might contribute to their coexistence in sweet pepper crops and their combined impact on aphid populations.

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