



Potential of predatory Neotropical ladybirds and minute pirate bug on strawberry aphid

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Abstract: Laboratory trials were performed to determine the impact of three Neotropical predatory coccinellids (*Cycloneda sanguinea*, *Eriopis connexa* and *Coleomegilla quadrifasciata*) and a minute pirate bug (*Orius insidiosus*) on *Chaetosiphon fragaefolii*, an important strawberry aphid pest. The predation on *C. fragaefolii* nymphs and adults, as well as the time to the first attack of all predators were compared with predation on *Aphis gossypii*. Predator preferences for prey and aphid defensive behavior were also evaluated. Moreover, the effect of coccinellids on *C. fragaefolii* population growth was assessed in experimental greenhouse conditions. The predation rate varied among predators, being significantly lower for *O. insidiosus* than for the coccinellids. Consumption was higher on *A. gossypii* than on *C. fragaefolii*, regardless of the aphids developmental stage. The time to the first attack of all predators was longer in the presence of *C. fragaefolii*. Walking away and cornicle secretion were the most common antipredator behaviors of aphid against coccinellids and *O. insidiosus*, respectively. Coccinellids preferred *A. gossypii* over *C. fragaefolii*, while *O. insidiosus* showed indifference. *Cycloneda sanguinea* and *E. connexa* exhibited the highest suppression effect on the growth rate of *C. fragaefolii*. Thus, the four predators evaluated could contribute to reduce strawberry aphid populations, especially *C. sanguinea* and *E. connexa*.

Key words: Behavior, Biological control, *Chaetosiphon fragaefolii*, *Fragaria x ananassa*, Predation rate, Coccinellids.

INTRODUCTION

Aphids are important worldwide pests of strawberries, *Fragaria x ananassa* Duchesne (Rosales: Rosaceae) (Rabasse et al. 2001, Thompson et al. 2003, Rondon et al. 2005). In Argentina and other countries (Cross et

al. 2001, Bernardi et al. 2013), the strawberry aphid *Chaetosiphon fragaefolii* Cockerell, as well as *Aphis gossypii* Glover and *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) are commonly found on strawberry (Cédola and Greco 2010, Cingolani and Greco 2018). *Chaetosiphon fragaefolii* is a vector of *Strawberry mild yellow edge virus* (SMYEV), *Strawberry crinkle virus* (SCV) and *Strawberry mottle virus* (SMoV). The

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latter is also transmitted by *A. gossypii* (Krczal 1982, Martin and Tzanetakis 2006, Tzanetakis and Martin 2013, Dughetti et al. 2017). In Argentina, the most frequently used method for aphids control in strawberry fields involves the regular use of broad spectrum insecticides. The development of alternative control strategies, as biological control, is important for more sustainable crop production.

The augmentative and conservation biological control requires knowledge about the relative potential of native or established agents. Parasitoids and predators are common natural enemies of aphids, so they are used for the biological control of agricultural pests in several countries (van Lenteren 2012). Different parasitoid species belonging to the genera *Aphidius*, *Aphelinus*, *Praon* and *Lysiphlebus* attack *A. gossypii* in strawberry crops. While high parasitism occurs on *A. gossypii* in strawberry, a very low parasitism by Hymenoptera parasitoids has been recorded on *C. fragaefolii* around the world (Oatman et al. 1983, Rondon and Cantliffe 2004, Cingolani and Greco 2018). Therefore, other mortality factors such as predation deserve more attention for the latter aphid species. Among aphid predators, coccinellids have the highest biocontrol potential (Hodek and Evans 2012), so they are widely used in pest management (van Lenteren 2012). Other natural enemies present in the crops, like predatory bugs of the genus *Orius* (Hemiptera: Anthocoridae), may also contribute to reduce aphid populations (Cross et al. 2001). These omnivorous insects, which are used worldwide for the control of thrips, can also attack aphids, whiteflies and mites, among other arthropods (Coll and Ridgeway 1995, Mendes et al. 2002, Rondon et al. 2004, Bonte et al. 2015).

The ladybirds *Cycloneda sanguinea* L., *Eriopis connexa* Germar and *Coleomegilla quadrifasciata* Schöenherr (Coleoptera: Coccinellidae), as well as the minute pirate bug *Orius insidiosus* (Say) are Neotropical predators, naturally occurring in horticultural crops around La Plata, Buenos Aires,

Argentina (Carrizo et al. 1999, Olivo et al. 2015, Rocca et al. 2017), such as sweet pepper, eggplant, tomato and leaf vegetables. In general, the predation rate is influenced by the type of prey offered (Mendes et al. 2002, Rondon et al. 2004, Desneux and O'Neil 2008). These coccinellids consume different aphid species, including *A. gossypii*, and *C. quadrifasciata* is even more polyphagous consuming other types of prey or also pollen (Noda et al. 2002, Isikber and Copland 2002, Isikber 2005, Hodek and Evans 2012). The predation of *O. insidiosus* on *A. gossypii* and other aphid species has been also reported (Lattin 2000, Mendes et al. 2002, Rondon and Cantliffe 2004); however, up to now there are no reports of predation of any of the above mentioned predators on *C. fragaefolii*.

The predator ability to search for different preys can determine a differential rate of consumption (Isenhour and Yeargan 1981, Evans 2003). Different prey species, prey sizes or prey developmental stages, as well as the presence of specific defensive mechanisms in aphids (Butler and O'Neil 2006, Nelson 2007, Barry and Ohno 2016) could affect predation capacity. The defensive responses of aphids to predators include dropping from the plant, kicking, or walking away if an appendage is grasped, and attacking predators with substances secreted from the cornicles (Hagen et al. 1999). The pea aphid *Acyrtosiphon pisum* (Harris) responds to the presence of predators by walking away or dropping off the plant (Losey and Denno 1998). *Aulacorthum solani* (Kaltenbach) drops from the sweet pepper plant in the presence of natural enemies (Rocca and Messelink 2017) and the soybean aphid *Aphis glycines* (Matsumura) produces exudates when attacked by *O. insidiosus* (Butler and O'Neil 2006).

Our initial hypothesis is that the rate of consumption of aphidophagous predators would be higher than that of generalist predators, and that these latter would not show preference among aphid species. The aims of this study were: 1) to estimate

the predation rate of coccinellids and *O. insidiosus* on *C. fragaefolii*; 2) to compare the predation rate of the different predators on *C. fragaefolii* and *A. gossypii*; 3) to measure the time to the first attack of each predator against each prey species; 4) to identify the aphid antipredator behaviors; 5) to evaluate the preference of the different predators for each aphid species; 6) to evaluate the population growth of *C. fragaefolii* in presence of coccinellids in strawberry.

The results of this study will be very useful to implement IPM strategies for pests in strawberry crop (Greco et al. 2011) in order to obtain premium pesticide-free strawberries.

MATERIALS AND METHODS

The insect rearing and all laboratory experiments were carried out under controlled environmental conditions ($25 \pm 2^\circ\text{C}$, $70 \pm 5\%$ HR and 16:8 L:D).

INSECT REARING

Aphids (*C. fragaefolii* and *A. gossypii*) and coccinellids (*C. sanguinea*, *E. connexa* and *C. quadrifasciata*) were collected from organic greenhouse crops in La Plata, Argentina ($34^\circ56'04''\text{S}$ $58^\circ10'14''\text{W}$) and kept in quarantine. The progeny of each insect species was used to start the laboratory colonies.

Aphids were reared in plastic cylinders (6 cm diameter x 5 cm high) on strawberry leaf discs adaxially embedded in water agar (1%). The plastic cylinders were placed upside-down, thus ensuring that the abaxial side of the leaf discs (where the aphids feed) faced downwards as they would on intact plants. Ventilation was possible through a hole in the lid covered with insect gauze (80 μm mesh) and because the boxes were placed on a wire gridwall shelf.

The coccinellids, adults and larvae, were reared separately in ventilated plastic cages (15 cm high x 15 cm long x 25 cm wide) containing *Triticum*

aestivum seedlings (standard substrate fertile soil and perlite 1:1 v/v) infested with *Rhopalosiphum padi* L. (Hemiptera: Aphididae) and covered with voile. Ten individuals were placed in each plastic cage, and the proportion of adult female to male was 1:1. Seedlings were previously germinated in plastic pots (6 cm high and 4 cm diameter) with standard substrate (fertile soil and perlite 1:1 v/v) and infested with aphids at germination. Plants were maintained in ventilated plastic boxes (13 cm high x 30 cm long x 23 cm wide) until more than 80% of each seedling was infested with aphids. Water was provided on a sponge inserted into an Eppendorf tube. For adults, the bottom of each container was lined with paper towel as an oviposition substrate. The maintenance of rearing conditions was done twice weekly and the paper towel was transferred to a new plastic box until the larvae hatched.

Orius insidiosus individuals were purchased from a commercial supplier (Brometán SRL, Argentina). These were reared according to the methodology proposed by Bueno (2000) and Bueno et al. (2006), in glass vials of 3 liters capacity, with a top hole covered with voile to allow ventilation. Crumpled paper strips were placed inside to provide shelters, together with green bean pods for oviposition and eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) *ad libitum* as food. Twice a week the bean pods were removed from the oviposition glass vials and transferred to new vials for nymph hatching. These were fed *ad libitum* with frozen *E. kuehniella* eggs twice a week until maturity, when bean pods were incorporated for oviposition.

LABORATORY EXPERIMENTS

The experimental unit consisted of plastic cylinders (6 cm diameter x 5 cm high) sealed with parafilm, and containing a strawberry leaf embedded in water agar (1%). A constant number of aphids and a recently mated female predator (a 4–5 weeks old coccinellid or a 5–7 days old *O. insidiosus*) starved

for 24 hours were placed in each experimental unit. To evaluate predation rates, the treatments with coccinellids were: a) control (without predator); b) 150 4th instar *C. fragaefolii* nymphs; c) 150 4th instar *A. gossypii* nymphs; d) 100 *C. fragaefolii* adults; e) 100 *A. gossypii* adults. In the case of *O. insidiosus* the same treatments were conducted but the number of prey offered was 12. Ten replications per treatment were performed. The initial number of aphids used in all treatments was based on similar experiments performed by other authors with *A. gossypii* as prey (Noda et al. 2002, Rondon et al. 2004). The variables recorded were: a) the number of prey alive after 24 hours, b) the time to the first attack (seconds), and c) the number of replicates in which antipredator behaviors (walking away and cornicle secretion) were observed. Predators and aphids behaviors were recorded by direct observation during the first 10 minutes of the experiment. The number of aphids consumed in 24h was estimated as: (the initial number of aphids) - (the number of aphids alive after 24h + the number of aphids dead by other causes). The mortality from other causes was due only to the fact that the aphids that left the leaf were trapped in the agar of the experimental unit. The predation rate was calculated using Abbott correction (Abbott 1925) as: (number of aphids alive in the control at the end of the assay - number of aphids alive in the treatment at the end of the assay) / (number of aphids alive in the control at the end of the assay) and analyzed with factorial ANOVA, with predator species, aphid species and developmental stage of aphids as factors. Data were arcsine transformed to meet the required assumptions of parametric statistical methods. Differences amongst treatments were tested using Tukey HSD method ($P < 0.05$). The time to the first attack was analyzed by factorial ANOVA, with the previously mentioned factors. The square-root transformation was used to meet parametric assumptions. The aphid behavior (walking away and cornicle secretion) was evaluated separately. The number of replicates in which antipredator behavior occurred was analyzed

for both aphid species by a two-way (antipredator behavior and predator species) χ^2 contingency test. The null hypothesis was that this behavior is independent of the predator species.

To evaluate predators preference, the experimental unit was similar to that in the previous experiment (*cf.* below predation rate and predators and aphid behavior for more details). The treatments with coccinellids were: a) control (without predator); b) 75 *C. fragaefolii* and 75 *A. gossypii* 4th instar nymphs; c) 75 *C. fragaefolii* and 75 *A. gossypii* adults. The same treatments were conducted with *O. insidiosus* but the total number of prey offered was 12 (6 *C. fragaefolii* and 6 *A. gossypii* nymphs or adults). Ten replications per treatment were performed. Prey preference of predators was estimated using the Manly's index without replacement of prey (Manly 1974), according to the following formula: $\alpha_i = \ln_{p_i} / \sum_{i=1}^m p_j$; where α_i is a Manly's preference index for prey i ; p_i and p_j are the proportions of prey i or j alive at the end of the experiment (i.e. the number of prey i or j alive at the end of the experiment/the initial number of prey i or j); m is the number of types of prey. This index takes values between 0 and 1. In this study, because two different preys were offered, α values > 0.5 indicate preference and α values < 0.5 rejection. The prey preference of each predator was analyzed estimating whether α_i and α_j values were significantly different from 0.5 using the two-tailed t test for differences between an estimated mean and a hypothesized value.

EXPERIMENTAL GREENHOUSE ASSAY

The population growth of *C. fragaefolii* in presence of *C. sanguinea*, *E. connexa* and *C. quadrifasciata* in strawberry was estimated in an experimental greenhouse in December 2017. Strawberry plants (cv. Sweet Ann) were grown individually in 5-liter plastic pots with soil plus humus (50:50) under natural photoperiod (14:10 L:D). Each experimental unit consisted of one plant with five leaves placed

in the center of a voile cage (30 cm high x 30 cm long x 30 cm wide). The average temperature and relative humidity during the experiment was 21.4°C (range 12.4 – 30.3°C) and 48.9% (range 14.2 – 88.6%) respectively.

Plants were infested with 20 *C. fragaefolii* nymphs (mixed-instars). After 7 days, the number of aphids in each plant was counted and approximately 100 individuals were taken as initial density, removing the rest of them. Each experimental unit was distributed within the greenhouse using a randomized block design, and natural enemies were added to achieve each of the following treatments: a) Control (without predator); b) one female of *C. sanguinea*; c) one female of *E. connexa*; d) one female of *C. quadrifasciata*. All females were mated and age ranged between 4-5 weeks. Each treatment was replicated ten times. After 7 days, the number of aphids was counted in order to estimate the growth rate of *C. fragaefolii* in the different treatments.

Assuming that the growth is exponential, the following formula was used to estimate the growth rate (r): $r = \ln(N_t / N_0) / \Delta t$, where N_t : number of aphids at the end of the experiment; N_0 : number of aphids at the beginning of the experiment; Δt : 7 days. The data were analyzed using Kruskal–Wallis test. Subsequently, multiple comparisons were made of the average rank for each pair of groups, calculating the z-average value for each comparison, and the corresponding probability (corrected for the number of comparisons) for a two-tailed significance test.

RESULTS

LABORATORY EXPERIMENTS

Predation rate

The predation rate varied depending on predator species and also the aphid species. Significant interaction was found between predators and aphids species (Table I). *O. insidiosus* predation rate was significantly smaller than that of the three

coccinellids. The predation rate by all species of predators on *C. fragaefolii* was lower than on *A. gossypii*. All predators consumed the aphid nymphs and adults at similar rates (Table II).

Time to the first attack

Every predator took the same time to attack nymphs and adults but the time to the first attack varied depending on prey and predator species. There was no significant interaction between any of the three variables (predator, prey and developmental stage of the prey) evaluated (Table III). The time to the first attack by all predators was consistently higher when *C. fragaefolii* was the prey. Regarding this parameter, no significant differences were observed among predators on *C. fragaefolii* nymphs. However, *O. insidiosus* was faster than *C. sanguinea* and *C. quadrifasciata* in attacking *C. fragaefolii* adults. In presence of *A. gossypii*, *O. insidiosus* and *C. sanguinea* were faster than *C. quadrifasciata* in attacking nymphs and *O. insidiosus* was faster than *C. quadrifasciata* in attacking adults. The rest of the evaluated predator/prey interactions showed statistically overlapping results (Table IV).

TABLE I
Analysis of variance of the effects of predator species, prey species and development stage on the predation rate of *Cycloneda sanguinea*, *Eriopsis connexa*, *Coleomegilla quadrifasciata* and *Orius insidiosus* on *Chaetosiphon fragaefolii* and *Aphis gossypii* preys.

Source of variation	df	F	P
Predator	3	13.7	< 0.001
Prey	1	69.9	< 0.001
Development stage of prey	1	0.2	0.694
Predator * Prey	3	4.9	< 0.001
Predator * Development stage of prey	3	0.3	0.802
Prey * Development stage of prey	1	0.0	0.955
Predator * Prey * Development stage of prey	3	1.0	0.395
Error	144		

TABLE II
Predation rate and number of aphids consumed (in parentheses) in 24h by different predator species. The data correspond to the means \pm the standard error.

Predators	<i>Chaetosiphon fragaefolii</i>		<i>Aphis gossypii</i>	
	nymphs	adults	nymphs	adults
<i>Cycloneda sanguinea</i>	0.38 \pm 0.05 a (57.2 \pm 7.1)	0.37 \pm 0.08 a (37 \pm 7.8)	0.84 \pm 0.02 c (126.6 \pm 3.1)	0.86 \pm 0.04 c (85.8 \pm 3.9)
<i>Eriopis connexa</i>	0.46 \pm 0.08 a (69.8 \pm 12.5)	0.46 \pm 0.10 a (46.4 \pm 9.9)	0.67 \pm 0.05 c (101 \pm 8.4)	0.81 \pm 0.04 c (80.6 \pm 4.1)
<i>Coleomegilla quadrifasciata</i>	0.45 \pm 0.07 a (67.7 \pm 10.9)	0.46 \pm 0.09 a (46 \pm 9.2)	0.61 \pm 0.04 c (92.6 \pm 6.9)	0.6 \pm 0.05 c (60 \pm 4.9)
<i>Orius insidiosus</i>	0.21 \pm 0.04 b (2.3 \pm 0.57)	0.28 \pm 0.06 b (3 \pm 0.53)	0.50 \pm 0.08 d (5.6 \pm 0.74)	0.39 \pm 0.08 d (4 \pm 0.83)

Within and between the columns, different letters denote significant differences among treatments ($P \leq 0.05$).

TABLE III
Analysis of variance of the effects of predator species, prey species and development stage on the time of the first attack of *Cycloneda sanguinea*, *Eriopis connexa*, *Coleomegilla quadrifasciata* and *Orius insidiosus*.

Source of variation	df	F	P
Predator	3	18.50	< 0.001
Prey	1	35.45	< 0.001
Development stage of prey	1	2.63	0.106
Predator * Prey	3	1.88	0.134
Predator * Development stage of prey	3	2.44	0.097
Prey * Development stage of prey	1	0.75	0.385
Predator * Prey * Development stage of prey	3	0.92	0.428
Error	144		

Aphid antipredator behavior

In relation to antipredator behavior, the frequency of walking away (i.e. number of replicates in which this behavior was recorded) was similar for both aphid species ($\chi^2 = 2.20$, $df = 1$, $P = 0.13$) (Fig. 1a) and dependent on the predator species ($\chi^2 = 28.49$; $df = 3$, $P < 0.0001$). Neither aphid species showed this behavior in the presence of *O. insidiosus*. In contrast, the highest frequency was recorded in the presence of *C. sanguinea* (Fig. 1b). The frequency of cornicle secretion (i.e. number of replicates in

which this behavior was recorded) was different between the two aphid species ($\chi^2 = 5.83$, $df = 1$, $P = 0.016$) being higher for *A. gossypii* (Fig. 1a). Moreover, it was more frequent in presence of *O. insidiosus* than in the presence of coccinellids ($\chi^2 = 29.23$, $df = 3$, $P < 0.0001$) (Fig. 1c).

Preference of predators for aphids

In the preference assays, all coccinellids preferred to feed on both nymphs and adults of *A. gossypii* (*C. sanguinea*: $t_{nymphs} = 9.81$, $df = 9$, $P < 0.0001$ and $t_{adults} = 4.90$, $df = 9$, $P < 0.0001$; *E. connexa*: $t_{nymphs} = 7.50$, $df = 9$, $P < 0.0001$ and $t_{adults} = 6.70$, $df = 9$, $P < 0.0001$; and *C. quadrifasciata*: $t_{nymphs} = 4.96$, $df = 9$, $P < 0.0001$ and $t_{adults} = 5.88$, $df = 9$, $P < 0.0001$). Instead, *O. insidiosus* showed indifference for both aphid species ($t_{nymphs} = 0.97$, $df = 9$, $P = 0.35$ and $t_{adults} = 0.25$, $df = 9$, $P = 0.80$) (Fig. 2 a, b).

EXPERIMENTAL GREENHOUSE ASSAY

The growth rate of *C. fragaefolii* was significantly lower in presence of *C. sanguinea* and *E. connexa* than in presence of *C. quadrifasciata* or in the absence of predators (control treatment) ($H_{(3, N=38)} = 27.93$, $P < 0.0001$) (Fig. 3).

TABLE IV
Time (seconds) to the first attack by different predator species in presence of aphids species. The data correspond to the means ± the standard error.

Predators	<i>Chaetosiphon fragaefolii</i>		<i>Aphis gossypii</i>	
	nymphs	adults	nymphs	adults
<i>Cycloneda sanguinea</i>	460.10 (± 75.39) aA	391.40 (± 79.61) aA	77.10 (± 21.33) bA	165 (± 75.19) bAB
<i>Eriopis connexa</i>	410.10 (± 68.38) aA	304.50 ± (70.81) aAB	203.40 (± 62.06) bAB	185 (± 56.32) bAB
<i>Coleomegilla quadrifasciata</i>	439.90 (± 68.75) aA	549.60 (± 49.43) aA	431.20 (± 86.63) bB	339.70 (± 68.54) bA
<i>Orius insidiosus</i>	275.80 (± 47.69) aA	111.20 (± 55.08) aB	78.70 (± 22.38) bA	41.80 (± 26.97) bB

Different lowercase letters between columns and different uppercase letters within columns denote significant differences among treatments ($P \leq 0.05$).

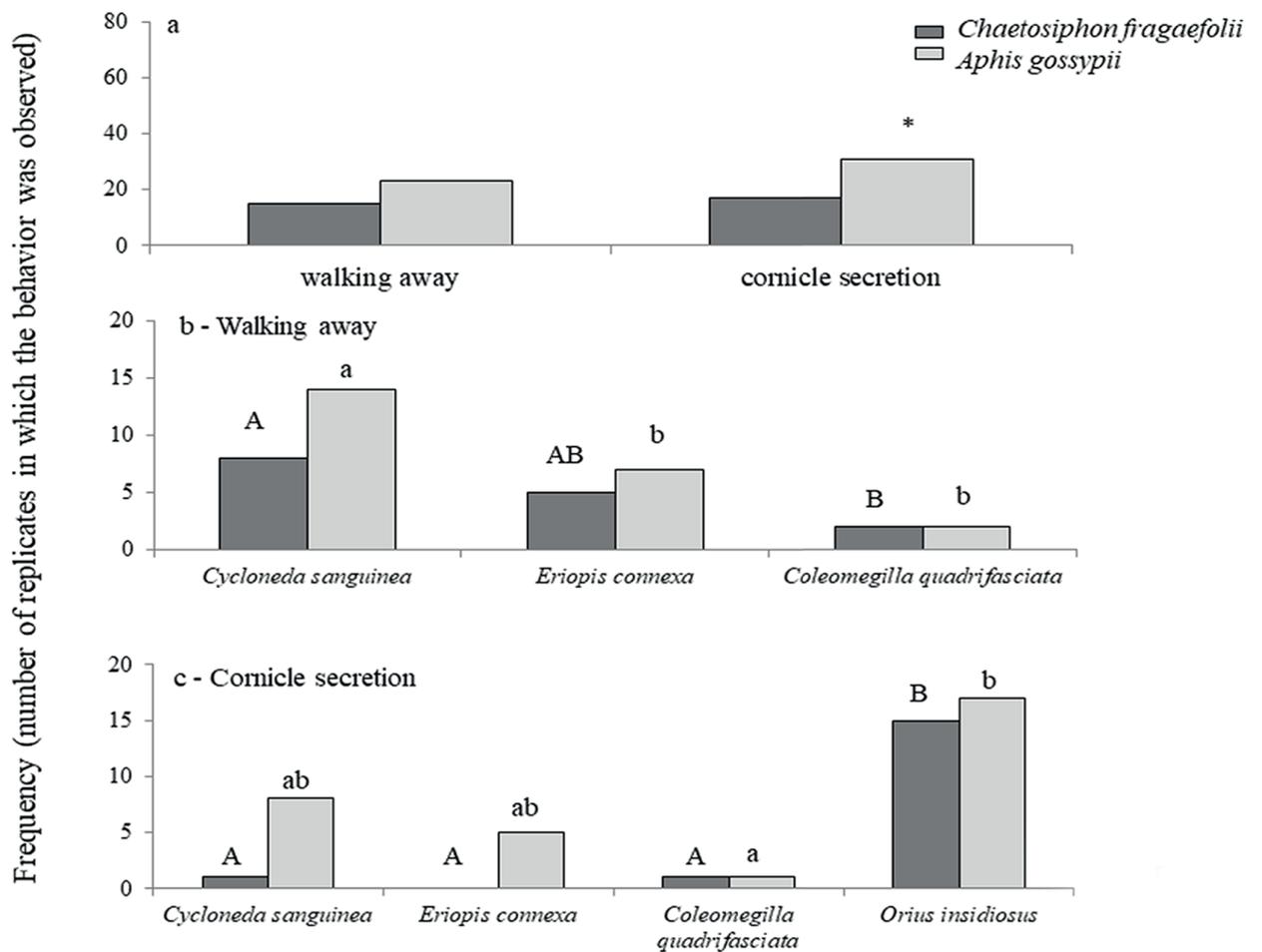


Figure 1 - Results of antipredator behavior assay showing the frequency (number of replicates) of **a**) total walking away and cornicle secretion behaviors, **b**) walking away and **c**) cornicle secretion behaviors of aphid species in the presence of different predator species. Asterisk indicates significant differences between aphid species for each behavior; uppercase letters and lowercase letters show significant differences for *C. fragaefolii* and *A. gossypii*, respectively, in the presence of different predator species ($P < 0.05$).

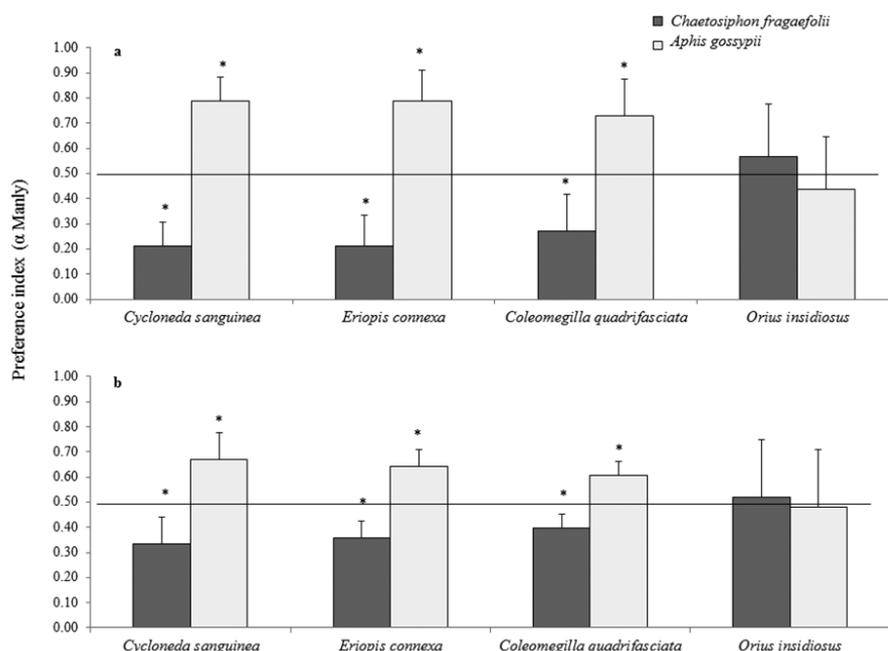


Figure 2 - Manly's index values of preference assay for different predator species on a) nymphs and b) adults of *C. fragaefolii* and *A. gossypii*. Line indicates the reference value of 0.50 that indicates indifference, values > 0.5 preference and values < 0.5 rejection. Asterisk indicates significant differences from 0.5 using the two-tailed t test for a single sample.

DISCUSSION

In this study, we found that all four predators fed on *C. fragaefolii*, but the predation rate was lower on this species than on *A. gossypii*. Other studies had reported predation rates of *A. gossypii* by coccinellids (Noda et al. 2002, Rondon et al. 2005, Isikber 2005). Noda et al. 2002 found that *E. connexa* and *C. quadrifasciata* consumed 31 and 34.5 *A. gossypii* adults per day (44 and 49% of the offered preys, respectively), less than shown in the present study. However, similar predation rates by *C. sanguinea* on *A. gossypii* were observed by Isikber (2005). Experimental conditions could affect the consumption of the same prey, e.g. the host plant and the arrangement of the experimental unit. So, in order to make accurate comparisons, the different predator/prey interactions under study should be evaluated in the same type of experiment. The preference experiments also contribute to determine differential predation on distinct prey

species. The preference of coccinellids for *A. gossypii* found in this study agrees with previous reports involving *Propylea dissecta* Mulsant (Coleoptera: Coccinellidae) and seven aphid species (Pervez and Omkar 2003).

The predation rate of *O. insidiosus* was significantly lower than that of coccinellids, and this predator showed indifference against both developmental stage and aphid species. Minute pirate bugs are able to complete their whole life cycle (including reproduction) feeding on aphids (Mendes et al. 2002), but thrips are their preferred prey (Bonte et al. 2015, Butler and O'Neil 2008, Desneux and O'Neil 2008). Also, this predator preferentially fed on the two spotted spider mite *Tetranychus urticae* Koch rather than on the cotton aphid (Rondon et al. 2004). The predator consumption could be related to prey nutritional quality (Nagai 1991) and/or defensive response (Butler and O'Neil 2006), among other factors.

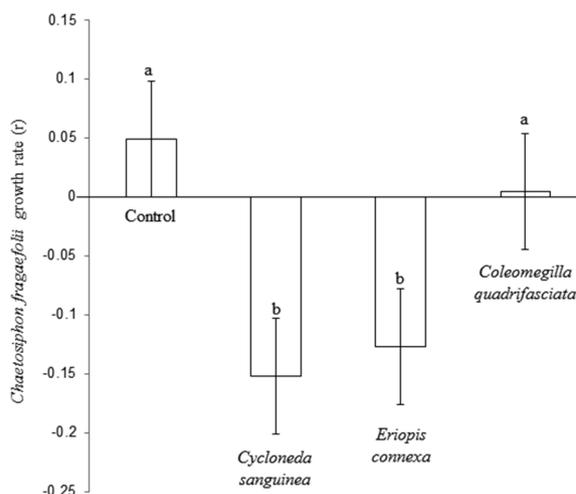


Figure 3 - Results of experimental greenhouse assay showing the reduction in the population growth of *C. fragaefolii* in the presence of *C. sanguinea*, *E. connexa* and *C. quadrifasciata* in strawberry plants. Different letters indicate significant differences ($P < 0.05$).

When given the choice, hemipteran predators prefer to attack highly mobile preys (Venzone et al. 2002). The effect of prey mobility on prey selection seems to be predator-specific (Eubanks and Denno 2000). In our study, *C. fragaefolii* and *A. gossypii* did not show the walking away behavior in the presence of *O. insidiosus*, and Butler and O'Neil (2008) also found that soybean aphids were sedentary when this predator approached them.

The time to the first attack of all predators was consistently higher when the prey was *C. fragaefolii* than when it was *A. gossypii* and, in general, *O. insidiosus* was faster than coccinellids in attacking both aphid species. The most recurrent antipredator behavior of both aphid species in the presence of *O. insidiosus* was cornicle secretion. This could be a response to the feeding mode of the predator, since bugs insert their beak into the host body, usually several times, to absorb the body contents. This behavior was also observed by other authors when *A. gossypii* was exposed to *O. insidiosus* (Butler and O'Neil 2008, Desneux and O'Neil 2008). In contrast, walking away was the most common aphid behavior in the presence of

coccinellids. In all cases, the frequency of walking away increased in the presence of *C. sanguinea*, compared to other predators. Defensive behaviors of *C. fragaefolii* were generally less frequent than those of *A. gossypii*, so factors other than behavior are presumably involved in the differential predation on *C. fragaefolii* and *A. gossypii*. The body of *C. fragaefolii* is covered with conspicuous capitate hairs of unknown function which could be related to defensive mechanisms (Rondon and Cantliffe 2004).

Cycloneda sanguinea and *E. connexa* reduced significantly the rate of population increase of *C. fragaefolii* in experimental greenhouse conditions. Thus, these coccinellids could be regarded as effective biological control agents for this aphid in strawberry. Different genera of Coccinellidae (*Rhyzobius*, *Adalia* and *Coleomegilla*) were found attacking and consuming this aphid in the field (Dicker 1952, van Driesche and Hauschild 1987); however, this is the first report about the effect of ladybirds on its population growth rate.

Cycloneda sanguinea, *E. connexa*, *C. quadrifasciata* and *O. insidiosus* could all play a role in diminishing *C. fragaefolii* populations in strawberry crop, especially the former two species. The ascertained considerably different effect on the population growth of *C. fragaefolii* (Fig. 3) by *Coleomegilla quadrifasciata* could be related to the well known polivory in the genus *Coleomegilla* (Hodek and Evans 2012). However, all the predators' efficiency to reduce *C. fragaefolii* populations in the field would presumably depend on its relative abundance in relation to that of *A. gossypii*. Results of this study may be used as a basis to explain specific predator-aphid interactions in strawberry crop.

Information about *C. fragaefolii* is scarce throughout the world and particularly in Neotropical region. Accordingly, these new findings about potential control agents are useful

to design appropriate management strategies for these aphids.

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AUTHORS CONTRIBUTIONS

Natalia Francesena performed all laboratory tests, she collaborated in the analysis of the data and in the writing of the manuscript. Margarita Rocca performed data analyzes, interpreted the results and collaborated in the writing of the manuscript. Estefanía Rizzo, carried out the insect rearing and collaborated in the laboratory assays. Joel Arneodo participated in the discussion of the results and in the writing of the manuscript. Nancy Greco carried out the planning of the essays and participated in the data analysis and interpretation and discussion of results, as well as in the writing of the manuscript. She also obtained the funds to carry out the investigation.

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