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ORIGINAL RESEARCH PAPER

Spatio-temporal variation of strawberry aphid populations and their parasitoids

María F. Cingolani[1] · **Nancy Greco**[1,2]

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Abstract

Aphids are common herbivores in the strawberry crop that can reduce plant vigor and fruit quality and also transmit viruses. Aphid species prefer diverse plant organs, which represent particular habitats of different quality for aphids and for the development of natural enemies' populations. Different habitat units (young leaves, mature leaves, buds, flowers) of strawberry were sampled fortnightly during all seasons. We identified seven aphid species, namely *Chaetosiphon fragaefolii*, *Aphis gossypii*, and *Macrosiphum euphorbiae,* the most abundant. During the autumn, *C. fragaefolli* and *M. euphorbiae* were scarce and *A. gossypii* was denser on mature leaves, while during summer *M. euphorbiae* was absent. During the winter, *C. fragaefolii* predominated on buds and young leaves, *A. gossypii* on flowers, and both species on mature leaves. During the spring, *C. fragaefolii* was even more abundant on buds, *A. gossypii* predominated on mature leaves, and the three species were equally abundant on flowers and young leaves. Parasitoids emerged from *A. gossypii*, *M. euphorbiae* and *Myzus persicae*, but not from *C. fragaefolii*. Three *Aphidius* and two *Aphelinus* species were recovered. All primary parasitoid species emerged from *A. gossypii*, and secondary parasitoids emerged only from this aphid. *Aphis gossypii* parasitism on mature leaves was markedly higher in winter and summer than in autumn and spring. Parasitism of *A. gossypii* was independent of its density, and the number of parasitized aphids was never higher than six. Our results contribute to define the most appropriate sample unit to estimate aphid density of different species and provide information about seasonal natural parasitism.

Keywords Aphelinidae · *Aphis gossypii* · Braconidae · *Chaetosiphon fragaefolii* · Habitat unit · Secondary parasitoids

Introduction

Aphids (Hemiptera: Aphididae) are herbivores commonly present in the strawberry crop, *Fragaria × ananassa* Duchesne (Rosales: Rosaceae). High density populations can reduce plant vigor, making plants susceptible to other pests. Moreover, the honeydew that aphids excrete diminishes photosynthate production and reduces fruit quality because of the development of a black sooty mold on the substrate (Rondon et al. 2005). These insects are often considered secondary pests of the strawberry because their populations usually do not reach high densities (Cédola et al. 2012; Greco et al. 2011). However, they can affect crop yields through the transmission of viruses, or by sucking out plant sap during the feeding on the underside of leaves (Martin and Tzanetakis 2006; Rabasse et al. 2001; Thompson et al. 2003).

In strawberry fields, and also at the leaflet level, aphids show an aggregated distribution and they persist at relatively low densities for most of the crop growing period (Rabasse et al. 2001; Trumble and Oatman 1984). It is well known that aphid species prefer diverse plant organs or structures (Isaacs et al. 2008), which represent different habitat units. The chemical composition of plants, e.g. soluble nitrogen and carbohydrates, and their levels of defensive secondary metabolites vary substantially from one tissue to another (Raupp and Denno 1983; Rosenthal and Berenbaum 1992). Myers and Gratton (2006) found that potassium and nitrogen availability in soil and leaves may play an important role in the dynamic of aphid populations. The organs of plants on which aphids feed may

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✉ Nancy Greco
ngreco@cepave.edu.ar

[1] Centro de Estudios Parasitológicos y de Vectores (CEPAVE), Facultad de Ciencias Naturales y Museo, UNLP, CONICET, Boulevard 120 e/60 y 64, La Plata, Buenos Aires 1900, Argentina

[2] Comisión de Investigaciones Científicas de La Provincia de Buenos Aires, Buenos Aires, Argentina

therefore differ in their quality as substrates for the development of aphid populations (Whitham 1980).

Plant quality could also explain variations in natural enemy populations, as observed by Mace and Mills (2016) who found that increased nitrogen availability to the host plant was correlated with a decrease in the number of mummified aphids produced. On the other hand, different organs or structures of the plant may enable aphids to escape the attack of predators (Grevstad and Klepetka 1992; Kareiva and Sahakian 1990) and parasitoids (Gardner and Dixon 1985; Mace and Mills 2016; Reed et al. 1992), thereby providing refuges for these phytophagous insects (Price et al. 1980, 1988). Thus, plant traits, as well as interactions between biotic and abiotic factors may affect aphid performance (Morris 1992) and within-plant aphid distribution (Gonzáles et al. 2001; Jansson and Smilowitz 1986; Lampert 1989).

The limitation imposed by the action of natural enemies on the population growth of these pests is relevant (Costamagna and Landis 2006), and biological control is presented as an important management tool. Among natural enemies, the family Aphidiidae (Hymenoptera) consists exclusively of solitary endoparasitoids of aphids and they are used as augmentative biological control agents in various countries (van Lenteren 2012).

Several characteristics of host-parasitoid interaction are relevant for successful biological programs such as synchrony, spatial coincidence, aggregation response of control agents, and mortality caused to pest population (Beddington et al. 1978). In addition, the existence of obligated secondary parasitoids may affect the performance of primary parasitoids (Brodeur 2000).

Aphid species recorded in La Plata horticultural belt were *Aphis gossypii*, *Myzus persicae*, *Macrosiphum euphorbiae* and *Chaetosiphon fragaefolii* (Cédola and Greco 2010). In particular, *C. fragaefolii* can affect yields because it transmits viruses such as the "strawberry mild yellow edge virus", the "strawberry crinkle virus" and the "strawberry mottle virus" (Krczal 1982).

The hypotheses of this work are: (1) aphid species in the strawberry crop are located in different plant organs (habitat units) and their abundance varies seasonally; (2) aphids in the strawberry crop have specific interactions with their parasitoids and parasitism varies seasonally.

The general objective of this study is to identify the spatial and temporal variation in populations of aphids and their parasitoids, and the mortality they cause, considering also the effect of secondary parasitoids. The specific objectives are:

1. To determine the spatial and temporal abundance of strawberry aphid species on different habitat units.
2. To determine primary and secondary parasitoids species emerging from each aphid species.
3. To estimate the percentage of field parasitism per habitat unit, in each host-parasitoid interaction.
4. To evaluate the relationship between aphids density and percentage of parasitism per habitat unit, in each host-parasitoid interaction.

Materials and methods

This study was developed in five commercial strawberry plots located in the La Plata horticultural belt (central plot GPS coordinates: S34°57′2.7″, W58°04′55.9″) of Buenos Aires, Argentina. Fields were between 5 and 10 km distant from each other. Horticultural farms of the region have several seasonal crops (tomato, sweet pepper, eggplant, artichoke, leaf vegetables, and strawberry) that are cultivated throughout the year under open field or greenhouse conditions. Strawberry plots are approximately 0.25 ha each, under low plastic tunnels and irrigation and soil management standard for the region. In sampled strawberry plots, granulated fertilizer composed of total nitrogen (N) 15.0%, ammoniacal nitrogen (N) 8.89%, nitrate nitrogen (N) 6.11%, assimilable phosphorus 127 (P_2O_5) 15.0% and water soluble potassium (K_2O) 15.0% was applied to the soil 20 days before planting. Methyl bromide was used to disinfect the soil. The beds were covered with black polyethylene mulch and irrigated by drip. The fungicide Benosem 50 PM® (benomyl 50%) and the acaricide New Mectin® (abamectin 1.8%), were applied weekly throughout the season. Every 20 days, from May 2012 until October 2014, sample units were taken randomly in each strawberry plot. Plants were identified with a number in a xy Cartesian coordinates system, and 25 sample units were taken randomly. Each sample unit consisted of different habitat units of the aerial part of the plant (young leaves, mature leaves, buds, and flowers) (5 plots × 4 plant parts × 25 samples × 45 dates = 22,500 samples). Young and mature leaves are easily distinguishable. The young leaves are lighter green, and leaflets are more "jagged" and look more closed than mature leaves. Aphid species were identified and counted under binocular microscope in the laboratory, distinguishing between mummified and not mummified individuals. The mummified individuals were kept separately in Petri dishes until the emergence of primary or secondary parasitoids. As mummification becomes evident in approximately 8 days after parasitism occurs, aphids not mummified when the sample was taken, were kept separately in Petri dishes covered with plastic film for 10 days to corroborate the absence of parasitism. The number of parasitized aphids was obtained from the mummified aphids present when samples were taken, plus mummies originated in a range of up to 10 days. Parasitism proportion was estimated as the number of parasitized aphids/total aphids collected (mummified and living individuals). The identification of all species (aphids, primary and secondary parasitoids) was performed using taxonomic keys (Nieto Nafría 1976; Simbaqueda et al. 2014; Starý et al. 1991; Zumoffen et al. 2015) and consulting specialists. The parasitism caused by each species was not quantified so parasitism was estimated considering all species together.

We performed a χ^2 test of independence, for analyzing aphid distribution on different habitat units and seasons of the year (i.e. winter: June–August, spring: September–November, summer: December–February, and autumn: March–May). Then, we performed a generalized linear model (GLM) analysis of deviance, assuming Poisson errors and a log link function for the number of aphids of different species present in each habitat unit and seasons. Finally, we estimated relative abundances of each aphid species in different habitat units and seasons.

We also performed a generalized linear model (GLM) analysis of deviance, assuming binomial errors and a logit link function for parasitism rate in different habitat units and seasons. We grouped data from two seasons when there were not enough to analyze seasons separately. In addition, we estimated the odds ratios (OR) that quantify how many times an event is more (or less) likely to occur relative to the other (Agresti 2015; Kutner et al. 2005). The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. For example, for a given aphid species in a given habitat unit and season, the odds for the variable “aphid parasitism” is the probability that the aphid will parasitize divided by the probability of that aphid will not parasitize. The odds ratios (OR) were estimated as the ratio of the odds of an event for one group to the odds of the same event for another group (for example, ratio of the odds of aphid species A parasitism to the odds of aphid species B parasitism). A confidence interval of 95% was used to compare two OR, considering that the difference was significant when the interval did not include 1. Since the same data set was used to make multiple comparisons, the level of each individual comparison was adjusted to obtain an experimentwise error rate lesser than 5% (Lymann Ott and Longnecker 2010).

We performed a regression analysis between *A. gossypii* density and parasitism proportion caused by all parasitoid species emerged from this aphid. We previously achieved a Box Cox analysis to find the accurate transformation of the dependent variable.

All analyses were performed with the statistical package R, version 3.2.1.

Results

We identified seven aphid species: *Chaetosiphon fragaefolii*, *Aphis gossypii*, *Macrosiphum euphorbiae*, *Myzus persicae*, *Rhodobium porosum*, *Myzus ornatus* and *Aulacorthum solani* (Hemiptera: Aphididae) among which the first three species were the most abundant. Indeed the total aphid density was low (*C. fragaefolii* = 0.165 ± 1.867; *A. gossypii* = 0.092 ± 0.869; *M. euphorbiae* = 0.027 ± 0.280; *M. persicae* = 0.002 ± 0.046; *R. porosum* = 0.001 ± 0.018 aphids/sample unit; only one individual of *M. ornatus* and one of *A. solani* were recovered from the total sample).

The distribution of *C. fragaefolii*, *A. gossypii* and *M. euphorbiae* was not independent either of habitat units (χ^2 = 449.68, df = 6, p < 2.2e^{-16}) or of seasons (Winter: χ^2 = 156.52, df = 6, p < 2.2e^{-16}; Spring: χ^2 = 511.41, df = 6, p < 2.2e^{-16}) (Fig. 1). It was not possible to find a suitable model including all combinations of habitat units, species and seasons, given the scarcity of individuals in some of them. During the autumn, *C. fragaefolli* and *M. euphorbiae* were very scarce, and *A. gossypii* was more abundant on mature leaves, while during the summer *M. euphorbiae* was not detected. Nonetheless, the low abundance of aphids in these seasons did not allow us to perform a statistical analysis of these trends.

For that reason, three different models were used: (1) to describe *C. fragaefolii*, *A. gossypii* and *M. euporbiae* abundance in different habitat units during the winter, (2) to describe *C. fragaefolii*, *A. gossypii* and *M. euporbiae* abundance in different habitat units during the spring, (3) to describe *A. gossypii* abundance in all habitat units throughout the year.

During the winter (model 1: Residual deviance = 3825.3, df = 324, p < 2.2e^{-16}), on buds *C. fragaefolii* was six times more abundant than *A. gossypii* and 12 times more than *M. euporbiae* (Table 1). *Chaetosiphon fragaefolii* predominated also on young leaves being twice more abundant than *A. gossypii*, while *M. euphorbiae* was insignificant. In turn, *A. gossypii* was the most numerous species on flowers, whereas the mature leaves were colonized most often by both *A. gossypii* and *C. fragaefolii* (Fig. 1).

Regarding aphid locations, more than half of the *C. fragaefolii* individuals collected in winter were distributed on buds (55.33%), and the rest were on young and mature leaves. Only an insignificant number of individuals were found on flowers; for example, they were 22 times fewer on flowers than on young leaves (Table 2; Fig. 1). With respect to *A. gossypii*, 90% of total individuals collected in winter were distributed on buds (23.85%), young (28.46%) and mature leaves (38.08%), and only about 10% of individuals were collected from flowers. No significant differences were found between young and mature leaves, as well as between

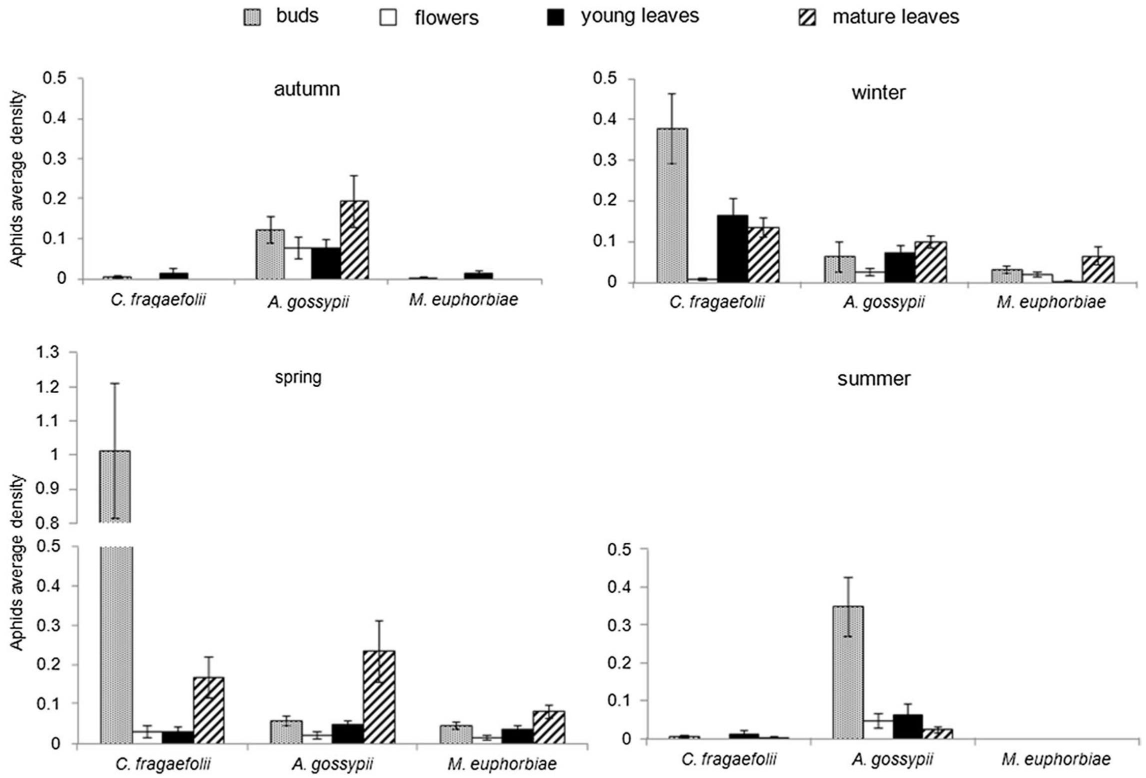

Fig. 1 Aphids average density on different strawberry plant organs (habitat units) throughout the year. Bars indicate standard error

Table 1 Mean relative abundance (confidence interval at 95%) of aphid species on different strawberry plant organs (habitat units) in winter

Aphid species	Plant organ			
	Buds	Flowers	Young leaves	Mature leaves
(Cf/Ag)	6.10* (4.49–8.17)	0.30* (0.10–0.93)	2.24* (1.53–3.27)	1.35 (0.94–1.94)
(Me/Ag)	0.50* (0.32–0.83)	0.75 (0.33–1.72)	0.03* (0.01–0.19)	0.66 (0.43–1.02)
(Cf/Me)	11.70* (7.08–19.15)	0.40 (0.12–1.29)	66.00* (11–373)	2.10* (1.36–3.10)

Cf C. fragaefolii, *Ag A. gossypii*, *Me M. euphorbiae*

*Significant differences between species by GLM ($p < 0.005$)

Table 2 Mean relative abundance (confidence interval at 95%) on different strawberry plant organs (habitat units) of each aphid species in winter

Plant organs	Aphid species		
	Gossypii	*C. fragaefolii*	*M. euphorbiae*
(Flowers/buds)	0.40* (0.24–0.67)	0.02* (0.01–0.05)	0.58 (0.26–1.27)
(Young leaves/buds)	1.20 (0.81–1.72)	0.44* (0.34–0.56)	0.08* (0.01–0.46)
(Mature leaves/buds)	1.60* (1.11–2.25)	0.35* (0.27–0.46)	2.00* (1.12–3.59)
(Young leaves/flowers)	3.00* (1.50–5.70)	22.00* (7.00–64.00)	0.14* (0.01–0.90)
(Mature leaves/flowers)	4.10* (2.10–7.50)	17.50* (6.10–52.50)	3.40* (1.60–7.40)
(Mature leaves/young leaves)	1.30 (0.86–2.10)	0.80 (0.60–1.13)	25.00* (4.10–166)

*Significant differences between plant organs by GLM ($p < 0.05$)

young leaves and buds, while the number of individuals was always lower on flowers than in the other habitat units (e.g. the number of individuals was 4.10 times greater on mature leaves than on flowers) (Table 2). Finally, mature leaves were the preferred habitat unit for *M. euphorbiae* in the winter (55% of individuals), and only a low number of these aphids were found on young leaves, being 25 times more abundant on mature leaves than on young leaves (Table 2).

In the spring (model 2: Residual deviance = 782.5, $df = 360$, $p < 2.2\mathrm{e}^{-16}$), on buds the prevalence of *C. fragaefolii* was even greater, being 17 times more abundant than *A. gossypii* and 21 times more than *M. euporbiae* (Table 3). On mature leaves, *A. gossypii* was the predominant species, and aphid abundance was not significantly different between flowers and young leaves (Fig. 1).

During the spring, the abundance of *C. fragaefolii* on buds was even greater than during the winter, with more than 80% of individuals in this type of habitat unit. Regarding the distribution of *A. gossypii* among different habitat units in spring, more than half of the sampled individuals were found on mature leaves (Fig. 1), being for example 10.7 times more abundant in this habitat unit than on flowers (Table 4). Finally, *M. euphorbiae* was found mainly on mature leaves as in winter, although comparing both seasons, in the spring more *M. euphorbiae* were found on young leaves (Tables 4; Fig. 1).

Aphis gossypii abundance (model 3: residual deviance = 2899.8, $df = 356$, $p < 2.2\mathrm{e}^{-16}$) was not independent of habitat units or seasons ($\chi^2 = 252.53$, $df = 9$, $p < 2.2\mathrm{e}^{-16}$).

The higher seasonal abundance of *A. gossypii* on buds was registered in summer (0.35 aphids/bud) in comparison to the other study seasons (0.123, 0.062 and 0.059 aphids/bud in autumn, winter and spring, respectively). There were no significant differences in abundance of *A. gossypii* on buds between winter and spring (Table 5). On flowers, this aphid was over four times more abundant in the summer than in the spring and over three times more abundant in the summer than in the winter. On young leaves, this aphid was less abundant in the spring and no differences were recorded among the other seasons. Finally, on mature leaves it was more abundant in autumn and spring.

Regarding seasons, in the autumn *A. gossypii* predominated on mature leaves (Table 6), and a similar trend was found in the winter, although on young leaves a greater quantity of this aphid was present. In the spring the preference of this aphid for mature leaves was more evident. In

Table 3 Mean relative abundance (confidence interval at 95%) of aphid species on different strawberry plant organs (habitat units) in spring

Aphid species	Plant organs			
	Buds	Flowers	Young leaves	Mature leaves
(Cf/Ag)	17.10* (12.70–23.00)	1.41 (0.65–3.05)	0.66 (0.35–1.23)	0.72* (0.54–0.95)
(Me/Ag)	0.80 (0.52–1.24)	0.76 (0.31–1.87)	0.76 (0.42–1.39)	0.35* (0.25–0.50)
(Cf/Me)	21.20* (14.10–32.00)	1.90 (0.80–4.30)	0.87 (0.44–1.67)	2.10 (1.41–2.96)

Cf C. fragaefolii, *Ag A. gossypii*, *Me M. euphorbiae*

*Significant differences between species by GLM ($p < 0.05$)

Table 4 Mean relative abundance (confidence interval at 95%) on different strawberry plant organs (habitat units) of each aphid species in spring

Plant organs	Aphid species		
	A. gossypii	*C. fragaefolii*	*M. euphorbiae*
(Flowers/buds)	0.37* (0.21–0.65)	0.03* (0.02–0.05)	0.35* (0.16–0.77)
(Young leaves/buds)	0.83 (0.54–1.27)	0.03* (0.02–0.05)	0.78 (0.43–1.43)
(Mature leaves/buds)	3.96* (2.86–5.47)	0.17* (0.13–0.21)	1.73* (1.05–2.86)
(Young leaves/flowers)	2.24* (1.04–4.74)	1.00 (0.49–2.17)	2.23 (0.94–5.27)
(Mature leaves/flowers)	10.7* (5.60–20.60)	5.70* (3.10–9.60)	4.90* (2.30–10.80)
(Mature leaves/young leaves)	4.80* (3.00–7.60)	5.70* (3.00–9.20)	2.20* (1.20–4.00)

*Significant differences between plant organs by GLM ($p < 0.05$)

Table 5 Mean relative abundance (confidence interval at 95%) of *A. gossypii* in each season, in different plant organs

Seasons	Plant organs			
	Buds	Flowers	Young leaves	Mature leaves
(Winter/autumn)	0.48* (0.32–0.72)	0.31* (0.15–0.64)	0.90 (0.51–1.58)	0.48* (0.32–0.72)
(Spring/autumn)	0.36* (0.24–0.55)	0.21* (0.10–0.45)	0.47* (0. 25–0.87)	0.90 (0.64–1.27)
(Summer/autumn)	4.12* (2.93–5.80)	0.94 (0.46–1.93)	1.22 (0.63–2.38)	0.19* (0.08–0.43)
(Spring/winter)	0.75 (0.46–1.23)	0.69 (0.31–1.55)	0.53* (0.32–0.87)	1.88* (1.35–2.61)
(Summer/winter)	8.57* (5.74–12.80)	3.06* (1.42–6.61)	1.35 (0.76–2.39)	0.39* (0.17–0.88)
(Summer/spring)	11.42* (7.56–17.27)	4.42* (1.98–9.85)	2.57* (1.38–4.77)	0.21* (0.09–0.45)

*Significant differences among seasons by GLM ($p < 0.05$)

Table 6 Mean relative abundance (confidence interval at 95%) of *A. gossypii* in different plant organs, in each season

Plant organs	Seasons			
	Autumn	Winter	Spring	Summer
(Flowers/buds)	0.63 (0.39–1.02)	0.40* (0.21–0.76)	0.37* (0.19–0.74)	0.14* (0.08–0.25)
(Young leaves/buds)	0.63 (0.39–1.02)	1.18 (0.74–1.88)	0.83 (0. 49–1.41)	0.19* (0.11–0.32)
(Mature leaves/buds)	1.58* (1.08–2.32)	1.58* (1.02–2.45)	3.96* (2.65–5.92)	0.07* (0.03–0.16)
(Young leaves/flowers)	1.00 (0.52–1.94)	2.95* (1.57–5.54)	2.24* (1.10–4.55)	1.30 (0.63–2.68)
(Mature leaves/flowers)	2.52* (1.45–4.38)	3.95* (2.15–7.26)	10.71* (5.78–19.84)	0.50 (0.19–1.28)
(Mature leaves/young leaves)	2.52* (1.45–4.38)	1.34 (0.88–2.03)	4.79* (3.10–7.39)	0.38* (0.16–0.95)

*Significant differences ($p < 0.05$)

the summer, mature leaves were less preferred, and most of aphids were found on buds and young leaves.

Regarding parasitism, parasitoids were obtained from *A. gossypii*, *M. persicae* and *M. euphorbiae*, but not from *C. fragaefolii*, *R. porosum*, *M. ornatus* and *A. solani*.

Three species of the genus *Aphidius* and two undetermined species of the genus *Aphelinus* were recovered from aphids. The all parasitoid species emerged from *A. gossypii*, but we only found a single parasitoid species associated to each one of the other parasitized aphids. Moreover, *A. gossypii* was the only aphid species from which secondary parasitoids were obtained (Table 7). We recovered only two individuals belonging to two genera, and both were found in summer.

Average parasitism of *A. gossypii* was 7.3%. For temporal analysis of the parasitism of *A. gossypii* we grouped data into: (1) Spring, (2) Summer, (3) Autumn and (4) Winter. As data on parasitism were scarce on young leaves, buds and flowers, they were grouped in: (1) mature leaves and (2) other organs (i.e. young leaves, buds and flowers all together). Parasitism proportion on mature leaves was more than eight times higher in winter [OR (Winter/Autumn) = 8.31; 95% confidence interval, CI 2.73–25.26] and almost seven times higher in the summer [OR (Summer/Autumn) = 6.86; CI 1.27–37.09] than in the autumn. Parasitism rate in the spring was similar to that registered in the autumn [OR (Spring/Autumn) = 1.23; CI 0.39–3.91] (Fig. 2).

Parasitism of *A. gossypii* was independent of its density, and the number of parasitized aphids was never higher than 6 (Fig. 3). A nonlinear inverse relationship was found when analyzing only parasitized aphid data (i.e., we removed cases where the number of parasitized aphids was 0). We

Table 7 Aphid species and their primary and secondary parasitoids, found on strawberry fields in La Plata (Buenos Aires, Argentina)

Aphid	Primary/secondary parasitoids	
Aphis gossypii ($n = 852$)	Primary parasitoids ($n' = 69$)	*Aphidius colemani* (Hymenoptera: Braconidae)
		Aphidius matricariae (Hymenoptera: Braconidae)
		Aphidius ervi (Hymenoptera: Braconidae)
		Aphelinus sp1. Hymenoptera: Aphelinidae)
		Aphelinus sp2. (Hymenoptera: Aphelinidae)
	Secondary parasitoids ($n' = 2$)	*Syrphophagus* sp. (Hymenoptera: Encyrtidae)
		Asaphes sp. (Hymenoptera: Pteromalidae)
Myzus persicae ($n = 20$)	Primary parasitoids ($n' = 8$)	*Aphidius colemani* (Hymenoptera: Braconidae)
Macrosiphum euphorbiae ($n = 248$)	Primary parasitoids ($n' = 7$)	*Aphidius ervi* (Hymenoptera: Braconidae)
Chaetosiphon fragaefolii ($n = 1532$)		–
Rhodobium porosum ($n = 8$)		–
Myzus ornatus ($n = 1$)		–
Aulacorthum solani ($n = 1$)		–

n denotes the total number of aphids collected; n' denotes the number of mummies (those obtained at the moment the samples were taken plus those obtained 10 days after that, in laboratory)

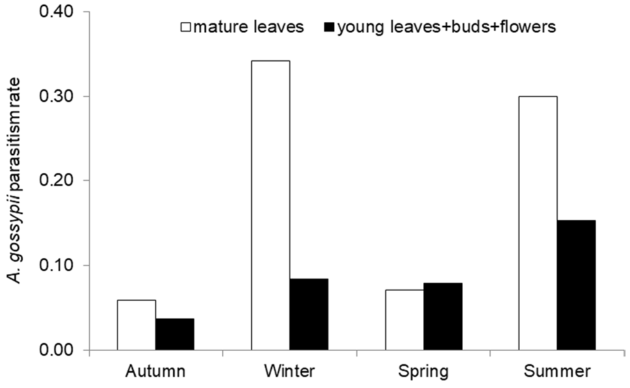

Fig. 2 *Aphis gossypii* parasitism rate of all individuals of this species on different strawberry plant organs over seasons

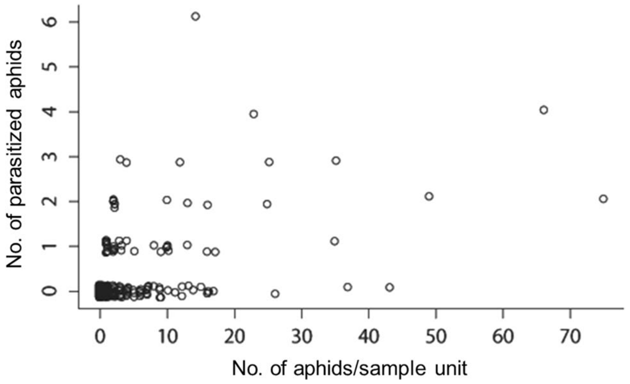

Fig. 3 Number of parasitized *A. gossypii* in relation to aphids density. Overlapping data points were separated by adding jitter to improve the interpretation of the figure

performed a Box Cox analysis to find the accurate transformation of the dependent variable, and we found the following relationship between variables ($F = 286.8$; $df = 1$ and 49; $p < 2.2\mathrm{e}^{-16}$; $R^2 = 0.851$) (Fig. 4):

$$A.gossypiiparasitism^{\lambda} = 1.004 - 0.083\ln(aphidsabundance)$$

where $\lambda = 0.1186869$ (box cox transformation of the dependent variable).

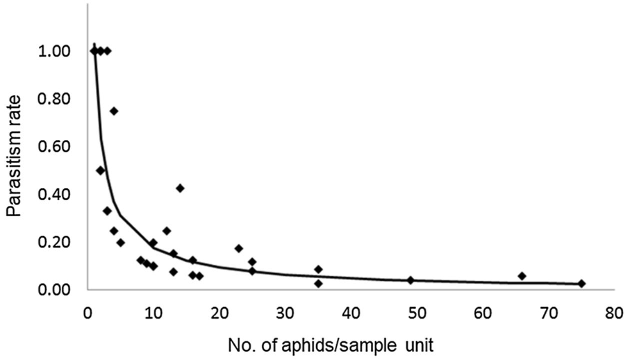

Fig. 4 Relationship between *A. gossypii* abundance and the rate of parasitized aphids. Points represent observed parasitism proportion and line represents the relationship estimated by Box Cox analysis

Myzus persicae showed relatively high parasitism percentage, 40% of collected individuals, although this aphid was very scarce. Parasitism of *M. euphorbiae* was very low (3%), and independent of seasons (Spring–Summer, Autumn–Winter) (Fisher exact test $p = 1$).

Discussion

In strawberry fields in La Plata *C. fragaefolii, A. gossypii*, *M. euphorbiae* and *M. persicae* are commonly found (Cédola and Greco 2010). Regarding the other aphid species that were identified in this work, *R. porosum* is practically cosmopolitan and is limited to the rose (Remaudière and Remaudière 1997). Eastop (1958) recorded this aphid on *Fragaria* in America. *Myzus ornatus* occurs throughout the world and is extremely polyphagous and has been recorded from nearly 80 plant families. Lastly, *A. solani* distribution is virtually cosmopolitan having one of the broadest host ranges in comparison to other aphids (Remaudière and Remaudière 1997). However, so far, this is the first time that the last three mentioned species are cited for the strawberry crop in La Plata horticultural belt. La Plata biotypes of these species could have a more restricted host range, or maybe these aphids were not taken into account in other field studies.

From all the seven aphid species found in this study, *C. fragaefolii* and *A. gossypii* were the most important. The former lives specifically on strawberry, while the latter is a polyphagous species, with strawberry as a secondary host plant (Blackman and Eastop 2000). Both species are vectors of virus. When *A. gossypii* is the dominant species the problem is not serious as this aphid transmits mainly SMoV, which strawberry cultivars are tolerant to, whereas *C. fragaefolii* transmits other viruses causing mixed virus infections (Martin and Tzanetakis 2006); therefore, this aphid species affects crop yield more severely. Some cultivars are susceptible, but tolerant to some of these viruses and remain symptomless when infected, making it difficult to diagnose virus presence. The key of virus management is the implementation of aphid management programs to reduce the aphid and virus pressure.

The settling and feeding location of ambulatory aphid populations within a given host plant seems to be nonrandom and based, in part, on a preference for palatable leaves of a certain developmental stage or physiological age (Gould et al. 2007). *Chaetosiphon fragaefolii* was recovered mainly from buds while *M. euphorbiae* was found mainly on mature leaves. In the same way, Rondon et al. (2005) found that adults of *C. fragaefolii* predominated on plant buds. It has been documented that plant nutritional quality influences insect herbivores (Perrenoud 1990) and many of them, such as phloem feeding aphids and others, are sensitive to changes in macronutrients such as nitrogen or potassium (Petitt et al. 1994). As it is known, younger leaves usually have a higher concentration of primary metabolites, e.g. soluble nitrogen, than mature ones (Dixon 1998, Merritt 1996). Gould et al. (2007) found that for *Chaitophorous populicola* (Hemiptera: Aphididae) feeding on leaves of eastern cottonwood, *Populus deltoids* (Malpighiales: Salicaceae), phytochemical and physiological differences associated with leaf developmental stage may result in differential suitability of feeding sites within the host plant. Concentrations of gamma-aminobutyric acid (GABA) and aspartic acid, as well as the phenolic glycoside salicin, varies in different developmental stages of plant leaves and may be used by *C. populicola* to determine leaf age. They found also that the distance to the vascular bundles and lignifications varied significantly with leaf developmental stage. Chemical and physiological characteristic of citrus leaves also influence feeding by psyllids (Ammar et al. 2013; Killiny 2017).

The prevalence of *C. fragaefolii* on buds showed a temporal trend, because this type of habitat unit was more preferred in the spring than in the winter, and in the summer all individuals of this species were located on it. Temperature is a key abiotic factor influencing the development and reproduction of aphids, so buds may represent a shelter to mitigate high temperatures. Moreover, *M. euphorbiae* was found mainly on mature leaves and it was not collected during the summer. De Conti et al. (2011) found that *M. euphorbiae* showed lower rates of survival at high temperatures, and at 31 °C no aphids reached adulthood. Spatial and temporal trend was also observed for *A. gossypii* since it was more abundant on buds than on other plant organs in the summer. Hosseini-Tabesh et al. (2015) found that *A. gossypii* developed in the field at temperatures and relative humidities ranging from 23 to 43 °C and from 27 to 95%, respectively. This species may be more tolerant to high temperatures and to a wide relative humidity range, like those registered in the study region (14.3–28.8 °C as maximum temperatures and 70–83% relative humidity) (Servicio Meteorológico Nacional Argentina 2017).

Several parasitoid species, such as those belonging to *Aphelinus* (Hymenoptera: Aphelinidae), *Aphidius*, *Praon* and *Lysiphlebus* (Hymenoptera: Braconidae) genera, are able to parasitize most of the aphid species collected in this study. The absence of parasitism on *R. porosum*, *M. ornatus* and *A. solani* could be explained by the very low abundance of these species. However, this would not be a plausible explanation for *C. fragaefolii*, here one of the most abundant species. No parasitism was registered for this aphid species in other countries where *C. fragaefolii* is present (Rondon and Cantliffe 2004). These authors suggested that *C. fragaefolli* capitate hairs constitute a barrier that prevents parasitism. The susceptibility of herbivores to parasitoids depends on many factors related to the biology and ecology of both, including parasitoid searching ability and the capacity of the host to escape. The first is related to chemical and physical characteristics of plants, anti-predator behavior, physical host defenses and shelter for the host. In the same sense, Mace and Mills (2016) found a decline in mummies produced on aphids on seedlings with higher chlorophyll content in response to added nitrogen. They suggested that the lower parasitism may have resulted from reduced oviposition by the female wasps due to a perceived reduction in the quality of aphids. Although they did not detect an effect of nitrogen treatment on aphid size, there may have been an effect on aphid quality for parasitoid development. Also, aphids have been shown to develop greater immune responses to parasitism on higher-quality plants (Gerardo et al. 2010). Similarly, although *A. gossypii* showed a greater diversity of parasitoids species, parasitism of this aphid was lower on young leaves, buds and flowers than on mature leaves. These could be a shelter structure due to chemical or physical differences in relation to mature leaves of the strawberry plant.

Regarding the temporal pattern of parasitism, the highest proportion recorded in the winter and the summer suggests a broad tolerance of parasitoids to extreme abiotic conditions.

Although in this study the proportion of parasitism of *A. gossypii* was related to density without discrimination by species of parasitoid, the results suggest a Type II functional response. This type of response was found in several *Aphidius* species, such as *A. ervi* (He et al. 2006), *A. smithi* (Mackauer 1983) and *A. sonchi* (Liu 1985), *A. colemani* and *A. matricariae* (Zamani et al. 2006). The number of fertile eggs/female/day of *A. colemani* and *A. matricariae* that was reported ranged between 1.6 and 12.9 depending on the temperature. This range contains the maximum number of parasitized aphids per sample unit found in this study (six aphids parasitized per sample unit), while the other mentioned species showed a higher fertility. The same type of functional response was found for *Aphelinus certus* (Frewin et al. 2010) and *Aphelinus albipodus* (Lester and Holtzer 2002). A slight increase in parasitism rate at medium *A. gossypii* density could be interpreted as a mixed Type II and III response (Byeon et al. 2011).

The two secondary parasitoids found in this study belong to two genera usually considered as obligate secondary parasitoids of aphids, *Syrphophagus* and *Asaphes*. Three species of *Syrphophagus* (*S. bacchae*, *S. flavitibiae*, *S. nubeculus*) and two *Asaphes* species (*A. suspensus* and *A. vulgaris*) were cited for Argentina (De Santis 1967). Some studies have been made in other countries about *Syrphophagus africanus* (Hymenoptera: Encyrtidae) on *Lysiphlebus fabarum* and *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) (Ganyo et al. 2012), and of *Syrphophagus aphidivorus* (Hymenoptera: Encyrtidae) on *A. ervi* (Iemma et al. 2016). *Asaphes* is a worldwide genus, as the most frequent secondary parasitoids of aphids through Aphidiinae (Bouçek et al. 1978). Brodeur and McNeil (1994) suggested that high fecundity and longevity of *A. vulgaris* might play a role in reducing the impact of the primary parasitoid *Aphidius nigripes* on *M. euphorbiae*.

Buds and mature leaves seem to be the most appropriate sample unit to estimate *C. fragaefolii* and *M. euphorbiae* density, respectively. Mature leaves would be also a good sample unit to estimate *A. gossypii* density in autumn, winter and spring. This is a base knowledge to develop aphids sampling programs in strawberry. This study also provides information about the seasons of the year when natural parasitism is the highest. However, low average parasitism found in this study (7.3%) indicates the parasitoids as not relevant to aphid management unless there are other non-pesticide tactics. The combined effects of parasitoids and predators, which also reduce aphid population growth rates, could provide a stable management solution.

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