Aphid predators in citrus crops: the least voracious predators are the most effective

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Abstract

Rich and abundant predator complexes are frequently associated with aphids in perennial agroecosystems. The ability of these predators to successfully suppress aphid populations is nevertheless highly variable. The development of operative conservation biological control strategies is mostly hindered by the lack of knowledge of the specific roles of the aphidophagous assemblage components, their intra-guild relationships and the predatory attributes that chiefly determine their effectiveness.

The role of predation in the biological control of aphids in perennial agroecosystems was assessed through exclusion experiments in aphid infested citrus crops. Important predator attributes such as recruitment, aphid consumption rates, and foraging strategies were related to their efficacy.

Predation greatly affected aphid colony phenology as well as size. Predators with lower aphid consumption rates (Micro-coccinellid species and Cecidomyiidae) were revealed to be the most efficient aphidophaga. These predators encountered aphid colonies at earlier colony stages and significantly reduced their population growth rates. Later more voracious aphidophaga groups (Chrysopidae and Macro-coccinellids) did not present effective biological control of the colonies.

Contrarily to what was widely believed, the less voracious aphidophaga groups such as the Micro-coccinellids and Cecidomyiids are probably the groups who are mostly responsible for aphid suppression. Future conservation biological control studies in this crop should therefore chiefly focus on these groups.

Key words

Citrus, conservation biological control, exclusion, intra-guild interactions, agroecosystems

Key Message:

- Little is known about how the specific components of aphid predator gilds contribute to their suppression in citrus crops.
- Effective control was traditionally attributed to voracious species such as those of the Coccinellinae.
- Aphid suppression was nevertheless mainly achieved by the Cecidomyiidae and Scymninae.

- Owing to their low voracity, the Scymninae and Cecidomyiidae could be useful to prevent aphid outbreaks.
- Conservation measures should therefore mostly focus on the Scymninae and Cecidomyiidae instead of the Chrysopidae and Coccinellinae.

1. Introduction

Aphids are one of the most important pest groups in agriculture causing economic damage to many crops worldwide (Van Emden and Harrington 2017). Control measures are consequently required to reduce aphid populations below economic injury levels. Among these measures, biological control has been studied extensively and implemented in the aphid control programs of cereals, vegetables, and perennial crops (Brewer and Elliott 2004; Bugg et al. 2008; Gómez-Marco et al. 2016). Predation is a major regulating factor of aphid demography (Van Emden and Harrington 2017). These herbivorous pests have very diversified guilds of aphidophagous species, thus, their biological control outcome is the result of complex interactions within those guilds (Lucas 2005).

The ability of predators to change their predation and reproductive rates in response to varying prey densities along with their functional and numerical responses have traditionally been considered key components to determine their efficiency as biological control agents (Hassell 1978). In this sense, specialist predators were, in the past, considered to be superior natural enemies owing to their faster functional and numerical responses to the changing densities of a target prey (Hassell and May 1986; Van Driesche et al. 2010). However, these responses are spatially and temporally affected by multiple factors such as intraspecific and intraguild interactions or the utilization of alternative prey species (Lester and Harmsen 2002). Conclusions from studies that simplify trophic interactions to the predator-prey relationship may therefore be of limited use. Generalist and stenophagous predators can, on the other hand, offer satisfactory biological control through complex interactions at different trophic levels (Bouvet et al. 2019a). In fact, this type of predator is able to keep prey densities at stable equilibriums in non-density-dependent ways (Symondson et al. 2002, Harwood and Obrycki 2005).

Citrus are perennial crops that shape highly stable agroecosystems with the potential to harbour rich complexes of natural enemies (Bouvet et al. 2019a). The characteristics of this crop have made it paradigmatic in respect to biological control and applied ecological research (DeBach and Rosen 1991; Tena et al. 2015). Aphids are also important pests of citrus crops. Their feeding behaviour causes a general weakening of the plant. The most important threat associated with aphid damage is, nevertheless, their role as potential vectors of diseases such as Citrus Tristeza Virus (Hermoso de Mendoza et al. 1984; Cambra et al. 2000). Aphids feed exclusively on tender plant tissue, shoots, and use them to develop and reproduce (Komazaki 1982). Their presence is therefore closely associated with the growth periods of the tree. Under Mediterranean growing conditions these pests are most important in the spring when citrus trees have their main sprouting activity. The spirea citrus aphid, *Aphis spiraecola* Patch and the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) are the species most frequently found in clementine mandarin crops (*Citrus clementina* Hort. ex Tan) of the Mediterranean basin (Yahiaoui et al. 2009; Bouvet et al. 2019a). Unlike *A. gossypii*, the interaction of *A. spiraecola* saliva with plant tissues causes leaf curl deformation (Hermoso de Mendoza et al. 2006). The colonies of this species are therefore better protected against natural enemies and adverse environmental conditions.

The complex of aphid natural enemies in citrus agroecosystems is very extensive, but, in most cases insufficient to prevent the populations of this pest group from exceeding the economic threshold (Hermoso de Mendoza et al., 2006). In the Mediterranean basin, the role of parasitoids has been observed to be marginal due to the high rates of hyperparasitism of the primary parasitoid species (Gómez-Marco et al. 2015). Nevertheless, aphidophagous predators are abundant and diverse but usually arrive late to the colonies (Gómez-Marco et al. 2016). The Micro-coccinellids *Scymnus interruptus* Goeze and *Scymnus subvillosus* Goeze (Coleoptera: Coccinellidae) were the only predators found in sufficient numbers at the end of winter to provide effective aphid regulation at early infestation stages (Bouvet et al. 2019b). However, aphid suppression by the aphidophagous predators has not been quantified. Neither the specific role of the main components of this guild, nor the predator-prey and predator-predator relationships that determine their relevance are well understood. We hypothesize that although the whole complex of aphid predators may contribute to its regulation, effective aphid suppression is mainly driven by specific components of the

guild; the biological and ecological predator attributes that mostly contribute to their efficacy may not be related with their consumption rates.

Thus the objectives of this research were to i) quantify the role of predation as a regulating factor of aphid populations in an undisturbed perennial agroecosystem; ii) rank the importance of aphidophagous predators for the development of future biological control strategies; and iii) explore predator-prey relationships that influence their biological control outcome.

2. Material and methods

2.1. Location and experimental conditions

Experiments were conducted in four clementine mandarin (*Citrus clementina*) plots in the Valencia Region of Spain during the spring of 2015 and 2016. This region has a Mediterranean climate with mild (mean temperature of 12° C) and relatively humid winters and hot (mean temperature of 26° C) and dry summers that limit citrus growth in that period of the year (Bouvet et al. 2019b). The two experimental plots employed were as follows: Moncada (39°35'17.43"N/0°23'53.28"O) (0.40 ha) and Castellon (39°59'29.92"N/0°4'12.77"O) (0.11 ha) belonging to the Instituto Valenciano de Investigaciones Agrarias and to the Jaume I University respectively. The Moncada plot was surrounded by other experimental citrus plots of the research station. The Castellon plot at the Jaume I University campus was located 930 m from the commercial citrus groves of that area. The other two plots were commercial groves located in Algimia (39°42'55.11"N/0°18'57.46"O) (0.31 ha) and Ribesalbes (40°0'53.30"N/0°8'48.21"O) (0.76 ha). The Algimia plot was surrounded by other citrus groves and patches of Mediterranean forest. The Ribesalbes plot was part of a 18.75 ha organic agribusiness citrus operation. Trees in the selected plots were approximately 15 years old; they were drip-irrigated and had not been treated with insecticides neither during the experiments nor for at least two years before the experiments were started. Weeds were mowed three times between January and July and once at the end of September in the Algimia, Castellon and Ribesalbes plots; whereas the weeds in Moncada were treated with glyphosate every three months.

2.2. Exclusion studies

The contribution of predation to aphid density regulation in citrus was assessed through exclusion techniques (Monzó et al. 2014). The exclusion study was repeated four times; two per year and aphid

120species. Predation on *A. spiraecola* colonies was evaluated during the spring seasons of 2015 and 2016 in
121the Moncada plot; while predation on *A. gossypii* colonies was evaluated in the Algimia (spring 2015) and
122Ribesalbes (spring 2016) plots. Plot selection was based on observed specific aphid infestation levels.

123 Experiments were started when approximately 5% of citrus shoots in the plots were occupied with
124aphid infestations (beginning of exponential growth of plot infestation (Bouvet et al. 2019b)). A randomize
125complete design was used in each exclusion experiment. Approximately 60 recently established aphid
126colonies per experiment (i.e., one shoot with only one or two apterous and/or alate adults and <30 aphid
127nymphs) were selected in random trees of each plot. Only one colony per tree was used in the studies. The
128selected colonies were randomly assigned to the following treatments: i) 'predation' (colonies exposed to
129natural enemies) (n_{total} = 83 colonies), ii) 'exclusion' (colonies protected from natural enemies with cages)
130(n_{total} = 81 colonies) and iii) 'semi-exclusion' (colonies protected with cages open at one end to allow access
131of natural enemies; thus the same conditions as the exclusion treatment were mimicked) (n_{total} = 83
132colonies). The goal of the 'semi-exclusion' treatment was to discern any potential negative effect of the
133exclusion cages on the natural growth of the aphid colonies.

134 The cages were built with 45 x 20 cm muslin bags, with a mesh size of 0.2 x 0.2 mm, covering
135bamboo rods (60 cm long and 1 cm diameter) with 10 cm diameter wire rings attached to the middle of the
136rods. Rods were used to hold the cages in the branches hosting the infested shoots. The wire rings were
137designed to create a room within the cages that allowed the free growth of the shoot and the normal
138development of the colonies. Aphid colonies were carefully inspected for the presence of predators prior to
139caging and during the study. If any predator was observed within the exclusion cages, the colony was
140discarded from the study.

141 Aphid colonies were inspected with a hand held magnifying glass twice a week from April 16 to May
14218, 2015 in Moncada and Algimia; from April 7 to June 16, 2016 in Moncada; and from May 2 to June 17,
1432016 in Ribesalbes. On each sampling date, the total number of aphids per colony was counted. Parasitized
144specimens were also recorded. Counts were stopped once no more live aphids were observed (dead colony).

145 *2.3. Aphid predators*

146 The presence of predators in the 'predation' treatment of study was also recorded through the
147 course of the study. Predators were observed in the field and classified to the most specific taxonomical
148 level possible.

149 To better understand the recruitment process of predators to aphid colonies, in parallel to the
150 exclusion study, clementine shoots infested with aphid colonies under the same phenological stage as those
151 previously identified in the caged colonies of the exclusion experiments, were extensively sampled. In 2015,
152 Moncada, Algimia and Castellon orchards, and in 2016 Moncada, Ribesalbes and Castellón orchards were
153 sampled during five consecutive weeks. In each orchard, five shoots (one per canopy orientation and one
154 from inside of the canopy) were collected from 20 randomly selected trees (100 shoots per orchard and
155 sampling date). Shoots were individually isolated in zipper storage bags, transported to the IVIA entomology
156 laboratory, and examined under a stereoscope. The total number of aphids per shoot along with their
157 developmental stages were recorded. The phenological stage of each shoot was also registered using the
158 scale proposed by Bouvet et al. (2019b): **B1**: buds from the beginning of lengthening to 20% of their final
159 size; **B2**: shoots between 20% and 40% of their final size; **B3**: shoots with 40% - 70% of their final size; **B4**:
160 shoots with 70% - 90% of their final size; **B5**: tender shoots with final size; **B6**: shoots with final size and
161 leaves completely mature and hardened. Predator species found on the shoots were also counted and
162 taxonomically identified. The developmental stages of the natural enemies were also recorded.

163 *2.4. Relevance of aphid predators*

164 A deterministic model (1) was formulated to rank the relative relevance (**RR**) as biological control
165 agents of the following predator groups associated with the aphid colonies: 1) Syrphidae, 2) Cecidomyiidae,
166 3) Chrysopidae and 4) Coccinellidae. The Coccinellidae were in turn divided into two groups based on
167 biological and ecological characteristics (Bouvet el al. 2019b, c): 4a) Micro-coccinellids (species measuring
168 less than 3 mm in length), and 4b) Macro-coccinellids (species measuring more than 3 mm). Micro-
169 coccinellids were represented by two species of the Scymninae subfamily whereas the Macro-coccinellid
170 group contained several species of the Coccinellinae subfamily.

171 $$(1)\ RR = r_i \times c_i \times t_i \times rt_i \times rv_i \times f_i \times pa_i$$

The model was built based on the following assumptions: **1)** *conceptual assumptions*: The response variable (the relative relevance (**RR**) of each predator group) is categorized by seven variables; each of them represents a biological or ecological attribute of aphid predators, previously studied in this or other projects, that are expected to have an influence on their biological control effectiveness: predator recruitment rates in aphid colonies (i.e. the amount of predators arriving to feed on the colonies), ($\mathbf{r}_i$); prey consumption ($\mathbf{c}_i$); sequence of arrival of predators to the aphid colonies ($\mathbf{t}_i$); time of maximum predator recruitment ($\mathbf{rt}_i$); predator recruitment rate variability (i.e. differences in the amount of predators arriving to the colonies between plots and years), ($\mathbf{rv}_i$); foraging ability of predators ($\mathbf{f}_i$); and ability of predator adult stages to prey ($\mathbf{pa}_i$); **2)** *mathematical assumptions*: the response variable (**RR**) is mathematically represented as the product of the seven explanatory variables. These variables are therefore considered independent; **3)** *numerical assumptions*: the explanatory variables are categorical and they all have similar weight in the model. For each variable a rank value from 1 to 5 is assigned to each predator group; higher values are indicative of greater suitability for biological control. When distinct predator groups have similar characteristics for a given attribute, the assigned rank value represents the mean value of their corresponding ranks. For the five predator groups, higher **RR** values would be indicative of greater efficacy of aphid biological control in citrus.

The recruitment rate of each group ($\mathbf{r}_i$), was measured as the mean number of specimens per shoot collected during the shoot sampling. Prey consumption ($\mathbf{c}_i$) ranks were chosen according to the results of previous research with the predator groups found in our study (Zollner and Poehling 1994; Chen and Liu 2001; Zarpas et al. 2007; Sobhani et al. 2013; Bouvet et al. 2019c). Sequence of arrival to aphid colonies ($\mathbf{t}_i$) was determined as the average time from the beginning of aphid colonization phase (first sampling date) until the day of first predator detection in the non-protected colonies of the exclusion studies. Time of maximum predator recruitment ($\mathbf{rt}_i$) was measured as the time from the beginning of aphid colonization phase until the sampling date for which each predator group reached its highest recruitment rate in the shoot sampling. Predator recruitment rate variability ($\mathbf{rv}_i$**)** was measured as the coefficient of variation of the recruitment rates of each predator group in the shoot sampling. Foraging ability of predators ($\mathbf{f}_i$) was divided into two categories: 1) low foraging activity (i.e. Diptera larvae lacking appendages with reduced mobility (Raki et al. 2009; Boulanger et al. 2019)) and 2) high foraging activity (predator species whose larvae had

appendages that allow them to move between aphid colonies (Coleoptera and Neuroptera)). The ability of adult stage predators to prey ($\mathbf{pa}_i$) was also a binomial categorical variable: 1) predator groups in which the adult stage feeds on plant resources (Diptera and Neuroptera) and 2) the adult stage feeds on arthropods (Coccinellidae).

2.5. Statistical analysis

The effect of predation on the phenology of aphid colonies as well as their size was studied by evaluating how treatments ('predation' and 'exclusion') affected the three variables: i) maximum aphid colony density, ii) day when the peak colony density was reached, and iii) cumulative number of aphids in the colonies from the beginning until colony elimination. Differences in maximum aphid colony density between treatments ('predation' and 'exclusion') were analysed using generalized linear mixed model (GLMM) (Wolfinger and O'connell 1993). Different error distributions of the response variable were tested and the Negative binomial was selected since its ratio of the Pearson Chi-Square to its degrees of freedom was the closest to 1 (Schabenberger and Pierce 2002). Aphid Species ('*A. gossypii*' and '*A. spiraecola*'), Year, as well as all the interactions between the three explanatory variables were also included in the model as fixed effects. Location of the plot in which each experiment was conducted was included as random factor. In the event that the covariance estimate of the random factor was zero, this was removed from the model. Those fixed factors and interactions without a significant effect in the full model were sequentially excluded. Model selection between the full and reduced models was done based on Akaike (AIC) information criterion (Anderson and Burnham 2002). A close to 1 ratio of the Pearson Chi-Square to its degrees of freedom was used to discard the presence of overdispersion in the selected models (Schabenberger and Pierce 2002). Differences between treatments at the day when the peak colony density was reached and in the cumulative number of aphids found in the colonies were also analyzed using GLMM. The error distribution of the response variable was selected following the same criterion as explained above. Aphid Species, Year, as well as the interaction between the three explanatory variables were also included as fixed effects in the two initial full models as well as the plot location as random effect. Model selection between the full and reduced models was done following the same criteria as explained above. Prior to concluding any effects due to treatment, the nature (quantitative or qualitative) of all significant interactions of the three models

was studied. Kenward and Roger Satterthwaite approximation for degrees of freedom was applied in all three models.

A Cox proportional hazard model was used to evaluate differences between the survival probabilities of *A. gossypii* and *A. spiraecola* colonies exposed to predators ('predation') and protected from them ('exclusion') in the two years of study (Liu et al. 2009). Aphid species ('*A. gossypii*' and '*A. spiraecola*'), Year, as well as the interactions between the three explanatory variables were also included in the model. Prior to concluding any effects due to treatment, the nature (quantitative or qualitative) of all significant interactions of the three models was studied.

Differences in recruitment rates between the predatory groups were studied using general linear model analysis. Year, along with the interaction between Predatory Group and Year were also included in the model. Because the interaction was significant and qualitative, data was analysed separately by year and predatory group. Post-hoc t-test (Tukey) comparisons were made in each case with a significant effect ($P < 0.05$ and $P < 0.01$). The coefficient of variation (CV) of the recruitment rate of each predatory group, defined as the ratio of the standard deviation to the mean, was used as an estimation of their spatial (variability between plots) and temporal (variability between years) recruitment variability.

Differences in the arrival times of the distinct groups of predators to the aphid colonies were studied using GLMM analysis. Negative binomial error distribution of the variable was selected. Both year and location were included as random factors.

The relationship between the arrival time of each predatory group and the maximum aphid colony size was studied using linear regression analysis. 'Predator arrival day to the colony' was defined as the explanatory variable and 'Maximum number of aphids in the colony' as the response variable. In the case of Cecidomyiidae the relationship between these two variables was exponential. Non-linear least-squares regression analysis was used to explain this relationship. Data was fitted to an exponential equation ($y=a \cdot e^{bx}$) and the two parameters 'a' and 'b' were estimated by using the Newton-Raphson iterative estimation procedure (SAS/STAT 15.1 User's Guide). All the analyses were done using SAS® University Edition software.

3. Results

3.1. Exclusion studies

Overall, 247 aphid colonies (121 *A. gossypii* and 126 *A. spiraecola*) were included in this study; 81 in the 'exclusion' treatment, 83 in the 'semi-exclusion' treatment, and 83 in the 'predation' treatment. Predation importantly affected the size and dynamics of aphid colonies, in both aphid species, although differences between aphid species and years were observed (Fig. 1). In the 'semi-exclusion' treatment the average colony size was between that of the 'predation' and 'control' treatments for *A. gossypii* and similar to the 'predation' treatment for *A. spiraecola* (Fig. 1). Maximum aphid colony density was reduced in colonies exposed to predators with respect to those that were protected (Treatment: $F = 78.45$; df = 1, 153; $P < 0.001$). Differences between species were also observed (Species x Treatment: $F = 31.85$; df = 1, 153; $P < 0.001$) with the highest reductions found in *A. gossypii* colonies (Table 1). No differences between years were found (Year: $F = 1.1$; df = 1, 153; $P = 0.295$). Differences in peak colony density between treatments and aphid species changed quantitatively depending on the year (Treatment x Species x Year: $F = 4.48$; df = 3, 153; $P = 0.005$); no differences in peak colony densities were observed for *A. spiraecola* between colonies exposed to predators and those protected in the second year (Table 1). The day in which the peak colony density was reached was affected by treatment (Treatment: $F = 71.6$; df = 1, 158; $P < 0.001$); colonies exposed to predators reached their highest aphid density 9.98 days earlier on average than protected colonies in the two years of the study (Year: $F = 2.25$; df = 1, 158; $P = 0.136$). No other effects were observed for this variable. The cumulative number of aphids in the colonies, from the beginning until the end, was significantly reduced in colonies exposed to predators with respected to the protected colonies (Treatment: $F = 85.15$; df = 1, 155; $P < 0.001$). This reduction was more important in *A. gossypii* than *A. spiraecola* colonies (Species x Treatment: $F = 27.73$; df = 1, 155; $P < 0.001$). The percentage of parasitized aphids in the colonies exposed to natural enemies was 0.81% throughout the experiment.

Aphid colony survival time differed between species ($\chi^2 = 14.40$; df = 1; $P < 0.001$) and years ($\chi^2 = 37.39$; df = 1; $P < 0.001$). The interaction between these two variables was significant (Species x Year: $\chi^2 = 12.14$; df = 1; $P < 0.001$) due to the important increase of *A. spiraecola* survival time in the second year which was not so in the *A. gossypii* colonies (Fig. 2). Survival time was also affected by predation ($\chi^2 = 13.37$; df = 1; $P < 0.001$). This effect was similar for the two years of study (Year x Treatment: $\chi^2 = 1.59$; df = 1; $P = 0.208$) although it changed depending on the aphid species (Species x Treatment: $\chi^2 = 5.18$; df = 1; $P =$

280 0.023); predation reduced survival time more importantly in *A. gossypii* than *A. spiraecola* colonies (Fig. 2).
281 The triple interaction between the 3 explanatory variables was not significant (Treatment x Species x Year
282 interaction: χ^2 = 1.62; df = 1; P < 0.203).

283 *3.2. Aphid predators*

284 Five predator groups were found to be associated with citrus aphid colonies in the 3,000 citrus
285 shoots sampled: larvae of Cecidomyiidae and Syrphidae (Diptera), Chrysopidae larvae (Neuroptera) and both
286 adults and larvae of Micro-coccinellids (Coleoptera: Scymninae) and Macro-coccinellids (Coleoptera:
287 Coccinellidae). Differences in recruitment levels between predator groups changed depending on the year of
288 study (Interaction: F = 48.9; df = 4, 14915; P < 0.001). In year 1, predator recruitment in aphid colonies
289 differed between groups (F = 47.7; df = 4, 7420; P < 0.001); Cecidomyiidae was the most frequently recruited
290 predator group followed by Micro-coccinellids, Macro-coccinellids, and Chrysopidae, which all had similar
291 recruitment levels; Syrphidae was the least recruited predator group (Fig. 3). In year 2, although recruitment
292 was also different between predator groups (F = 30.4; df = 4, 7495; P < 0.001), the Micro-coccinellid group
293 was the most frequently recruited predator, whereas the other groups presented similar recruitment levels.
294 All the predator groups except the Micro-coccinellids had greater recruitment in year 1 than year 2
295 (Cecidomyiidae: F = 80.0; df = 1, 2983; P < 0.001; Micro-coccinellids: F = 0.00; df = 1, 2983; P = 0.944; Macro-
296 coccinellids: F = 31.5; df = 1, 2983; P < 0.001; Chrysopidae: F = 11.8; df = 1, 2983; P = 0.001; Syrphidae: F =
297 14.1; df = 1, 2983; P < 0.001;). This was especially important for Cecidomyiidae (Fig. 3). Micro-coccinellids
298 presented the lowest spatial and temporal recruitment variability (CV = 428.7) followed by Cecidomyiidae
299 (CV = 543.0), Macro-coccinellids (CV = 562.0), Chrysopidae (CV = 686.4) and finally, Syrphidae (CV = 778.3).

300 Overall, *Aphidoletes aphidimyza* Rondani larvae (Diptera: Cecidomyiidae) was the most frequent
301 predator species associated with aphid colonies in citrus, followed by the Micro-coccinellids, *S. subvillosus*
302 and *S. interruptus* larvae (Coleoptera: Coccinellidae). These species where the most abundant predators
303 during the aphid exponential growth phase (2) and their highest recruitment was associated with the peak
304 phase of the colonies (3) (Table 2). The highest recruitment of Syrphidae was associated with the decline in
305 the aphid colony phase (4), whereas the greatest Chrysopidae and Macro-coccinellid larvae recruitment
306 coincided with the aphid colony death phase (5).

307 The average arrival time of predator groups associated with citrus aphids varied (F = 43.8; df = 4,
308856.5; P < 0.001). The Syrphid larvae were the first predators to reach the aphid colonies followed by the
309Cecidomyiid larvae (3.8 days later) and then the Micro-coccinellids (5.7 days later). On average the
310Chrysopidae and Macro-coccinellid larvae arrived to the aphid colonies last (9.4 days later) (Fig. 4).

311 Maximum aphid colony size was significantly affected by the arrival time of the Cecidomyiid and
312Micro-coccinellid larvae (Table 3). A positive exponential function explained this relationship for
313Cecidomyiidae, whereas, in the case of the Micro-coccinellids this relationship was linear (Fig. 5). No
314significant relationships were found for the rest of predator groups. Of all the predators, the Cecidomyiidae
315group had the strongest ability to reduce the maximum aphid colony size according to its estimated mean
316colony arrival time (50.8 CLM95: 5.3–96.4) (Table 3).

317 *3.3. Relevance of aphid predators*

318 Micro-coccinellids (*S. interruptus* and *S. subvillosus*) were predicted to be the most relevant
319predators of *A. spiraecola* and *A. gossypii* in citrus agroecosystems (Table 4). Macro-coccinellid species and
320Cecidomyiidae (*A. aphidimyza*) were the second most important groups of citrus aphid predators having
321similar estimated relevance, whereas Chrysopidae and Syrphidae had the least relevant role in aphid
322predation in citrus.

323
324**4. Discussion**

325 The present study demonstrates the importance of predation in the biological control of *A. gossypii*
326and *A. spiraecola* colonies in western Mediterranean citrus agroecosystems. In plots without pesticide use,
327predators were able to significantly reduce the colony size (73.9% on average), as well as the average colony
328lifetime.

329 The exclusion experimental units seem to lack a measurable micro-environmental effect on the
330aphid colonies and the shoots on which they were established that could distort the measurements of
331predation. The observed aphid colony size in the ‘semi-exclusion’, similar to the ‘predation’ treatment or
332between that of either ‘exclusion’ or ‘predation’, suggests that the open experimental units partially

restricted the access of predators but any altered micro-environment did not significantly affect the development of the colonies.

The effect of predation on the biological control was greater in *A. gossypii* than in *A. spiraecola*. This can clearly be observed by the greater and faster reduction of colony size as well as in the lower survival time of the *A. gossypii* colonies exposed to predators. The physical protection that confers the leaf deformation from *A. spiraecola* colonies may partially explain this difference (Van Emden and Harrington 2017). The distinct prey preferences of the predators may also lead to differential control efficacies. In fact, previous research showed that the two species of Scymninae had different preferences for *A. gossypii* and *A. spiraecola* (Bouvet et al., 2019c). The species of aphid has also been described as a factor influencing the foraging and oviposition behaviours of aphidophagous hoverflies and gall midges (Raki et al. 2009; Boulanger et al., 2019).

Predation of *A. spiraecola* colonies was not relevant during the second year. Favourable environmental conditions for large aphid infestations were described in the study area during that year (Bouvet al. 2019b). Large infestation rates cause apterae migration from mature colonies to uninfested or recently infested shoots in search of higher quality food resources (Michaud 1999). The continuous recruitment of multiple foundresses during the second year in addition to the observed less efficient control by predators in the naturally protected *A. spiraecola* colonies could explain this.

The predator groups associated with aphid colonies in citrus presented important differences in recruitment rates; some groups were found more frequently associated with the colonies than others. There were also differences in recruitment variability; recruited rates between years and plots varied more for some groups than others. Larvae of *A. aphidimyza* were the predators most frequently found in aphid colonies (Table 2) and had the second lowest spatial and temporal recruitment variability (CV). The differences in the number of specimens recruited in aphid infested shoots were observed between years in this and previous studies (Gómez-Marco et al. 2016); thus, more effective conservation strategies need to be developed for this group of predators. Larvae of the micro-coccinellids, *S. interruptus* and *S. subvillosus*, were also frequently associated with aphid colonies; their recruitment rates seem to be relatively stable

(lowest CV) in well preserved citrus agroecosystems. Their low food consumption rates, their ability to successfully exploit alternative food resources associated with citrus when their preferential prey (aphids) is not present, and their ability to overwinter in the agroecosystem probably explain this stability (Bouvet et al. 2019a, b, c).

The short generation time and exponential growth of aphids make the colony arrival time of predators a key attribute for effective biological control (Gómez-Marco et al. 2016). Micro-coccinellid and Cecidomyiid larvae were most frequently found before and during the peak aphid colony phase whereas the highest abundance of Syrphid, Macro-coccinellid and Chrysopid larvae was associated with the oldest phenological phases (decline and colony death) (Table 2 and Fig. 4**)**. Aphidophagous female oviposition is preceded by an evaluation of the food resource quality for its offspring to maximize fitness (Hodek and Honêk 2013; Seagraves 2009; Kindlmann and Dixon 1999). Syrphid, Macro-coccinellid and Chrysopid larvae are very voracious (Chen and Liu 2001; Zarpas et al. 2007; Sobhani et al. 2013) and mothers preferentially select well-developed aphid colonies where food is abundant enough to guarantee completion of larval development. Micro-coccinellid and especially Cecidomid larvae are less voracious (Bouvet et al. 2019a; Boulanger et al. 2019) which enables adult females to forage for oviposition patches even in recently founded aphid colonies. The selection of this type of colony for oviposition could be a strategy to reduce the predation risk to their offspring from more voracious predators (Lucas et al. 1998).

An early presence of Cecidomyiidae larvae importantly reduced the maximum aphid colony size (Fig. 5). Its effectiveness nonetheless strongly decreased when these predators were recruited later, once colonies reached their exponential growth phase (Fig. 5). This phenomenon could be an indicative of low functional response to the prey. Further research focusing on this predator-prey interaction may help to better understand how and when Cecidomyiidae can be an effective aphid predator in citrus. Early presence of *S. interruptus* and *S. subvillosus* larvae also resulted in smaller aphid colony sizes. The suppression ability of these predators was not so drastically reduced when their presence was first detected in mature aphid colonies (Fig. 5). *Scymnus* larvae are more voracious and less sessile than *A. aphidimyza* larvae and are therefore expected to have better searching and prey handling abilities. This would ultimately translate into

a more efficient control at higher aphid densities. No clear relationship between recruitment time and maximum aphid colony size was observed neither for Syrphids, Macro-coccinellids nor Chrysopids. The observed low recruitment frequency of Syrphids did not allow the illustration of any pattern between these two variables. Macro-coccinellid and Chrysopid larvae were rarely present during the earlier stages of aphid colonies. Despite the fact that these two groups of predators are known have great responses to increasing prey densities (Athian et al. 2004; Xia et al. 2003), their recruitment at mature aphid colony phases seems to prevent them from successful aphid suppression.

The ability of the Micro-coccinellids, *S. interruptus* and *S. subvillosus* and the Cecidomyiid, *A. aphidimyza* to importantly reduce the maximum aphid colony size when they are recruited in freshly established colonies would give these predators a competitive advantage over the most voracious heterospecific aphidophagous competitors (Lucas et al. 1998). Significant reductions in colony size would prevent Chrysopid and Macro-coccinellid females from selecting those colonies as oviposition sites as those sites would compromise successful development of their offspring.

Citrus aphidophaga could be classified into three groups according to their expected relative relevance as biological control agents obtained with the proposed deterministic model. *Scymnus interruptus* and *S. subvillosus* are probably the best candidates to develop effective biological control strategies. These Micro-coccinellids have relatively high, stable, recruitment rates in aphid colonies as has been revealed in this study. Both adult and larvae are aphid predators; additionally, the former also have the ability to exploit alternative prey (Bouvet et al. 2019a). Their stenophagia could therefore be used as a way to manipulate the population densities of this species through the provision of alternative food sources. Cecidomyiidae larvae and Macro-coccinellid species could be categorized as having a secondary role for different reasons. *Aphidoletes aphidimyza* is probably the most efficient aphid predator and the best competitor due to its early recruitment in aphid colonies and to its ability to significantly reduce aphid colony size at the colonization phase (Table 2 and Fig. 5, 6). However, the high variability of their abundance between years found in this and other studies (Gómez-Marco et al. 2016) hinders, at present, the use of them as an effective aphid biological control agent in this system. Macro-coccinellid species are highly voracious; a trait

411which is probably their main disadvantage. Although they can be abundant, their highest densities are
412related to the more mature colony phases when food resources are abundant enough to guarantee
413preimaginal development. Finally, Chrysopidae and Syrphidae larvae would be ranked as the two least
414relevant aphid predators in citrus. The two groups are considered to be highly voracious, they were not so
415frequently found in aphid colonies (Table 2), and their recruitment rates were also highly variable.
416Differences in predation efficiency between species within the Chrysopidae, Syrphidae and Macro-
417coccinellid groups could also be observed. Further research should be addressed to identify the most
418relevant predator species within those groups.

419 Other groups of predators that were associated with aphid colonies in citrus in previous research,
420such as earwigs (Dermaptera) or mirid bugs (Hemiptera: Miridae) (Piñol et al. 2009; Bouvet et al. 2019b)
421were not found in the present study. Although earwigs are known to be efficient predators of aphids in this
422crop (Piñol et al. 2009) the diurnal sampling conducted in our experiments probably disregarded their role
423being that they mainly present nocturnal activity (Moerkens et al. 2008). *Pilophorus* cf *gallicus* (Hemiptera:
424Miridae) was also found to be associated with aphids in the same citrus growing area. Its presence is
425nevertheless documented to be highly variable (Bouvet et al. 2019b) which could be attributed to its
426sensitivity to some insecticides used in this crop (Monzo et al. 2019).

427 In conclusion, the present research demonstrates that predation can be a major cause of aphid
428colony reduction in citrus agroecosystems. The least voracious predators were found to be the most efficient
429ones at controlling population densities. This is due to the ability of their immature stages to successfully
430develop at low aphid densities, at the early stages of patch infestation. Their earlier arrival to the colonies
431also confers them an ecological advantage over the most voracious aphidophagous predators competing for
432the same resource. The demographic stability of *S. interruptus* and *S. subvillosus* makes these predators
433outstanding candidates for the development of conservation biological control strategies.

434**Author contribution statement**

435JPRB, CM, and AU designed the research, participated in data analyses, and wrote the manuscript.

436**Acknowledgments**

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Conflict of Interest: Juan Pedro Bouvet declares that he has no conflict of interest. Alberto Urbaneja declares that he has no conflict of interest. Cesar Monzo declares that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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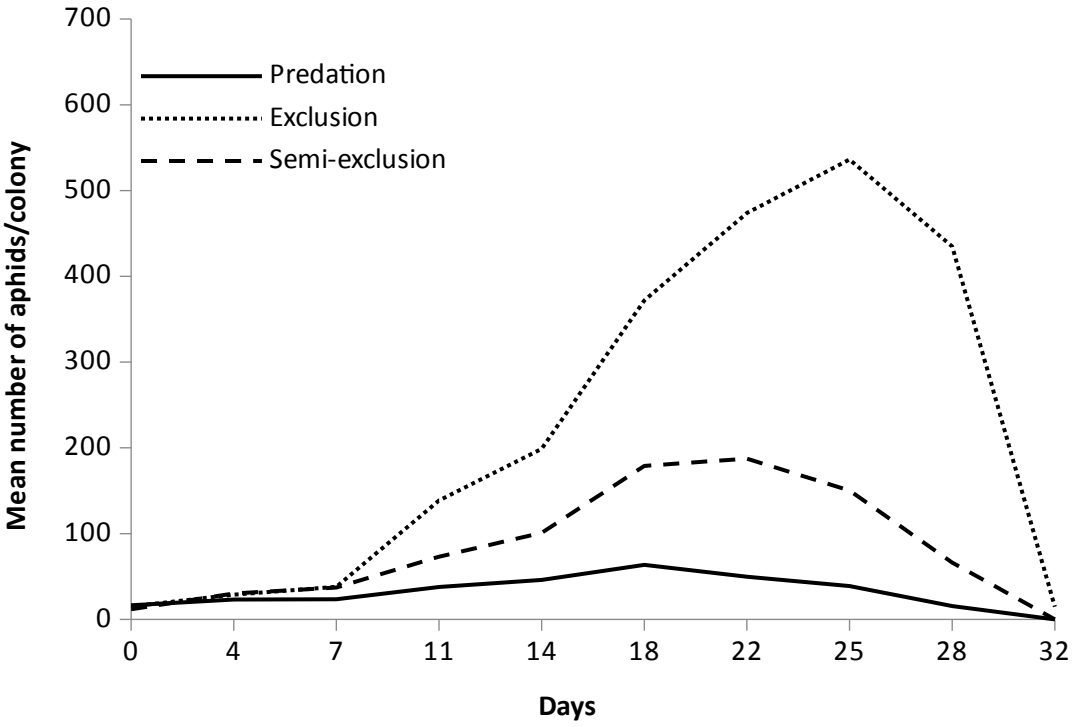
Figure captions

Fig. 1. Number of aphids per colony (mean± SE) in the three treatments: colonies exposed to predators, 'Predation'; colonies protected with exclusion cages, 'Exclusion'; and colonies protected with open cages, 'Semi-exclusion'. Data from the beginning until the end of *Aphis gossypii* and *Aphis spiraecola* infestation in 2015 and 2016: **a)** *A. gossypii* colonies in 2015, **b)** *A. gossypii* colonies in 2016, **c)** *A. spiraecola* colonies in 2015, and **d)** *A. spiraecola* colonies in 2016.

a)



552**b)**
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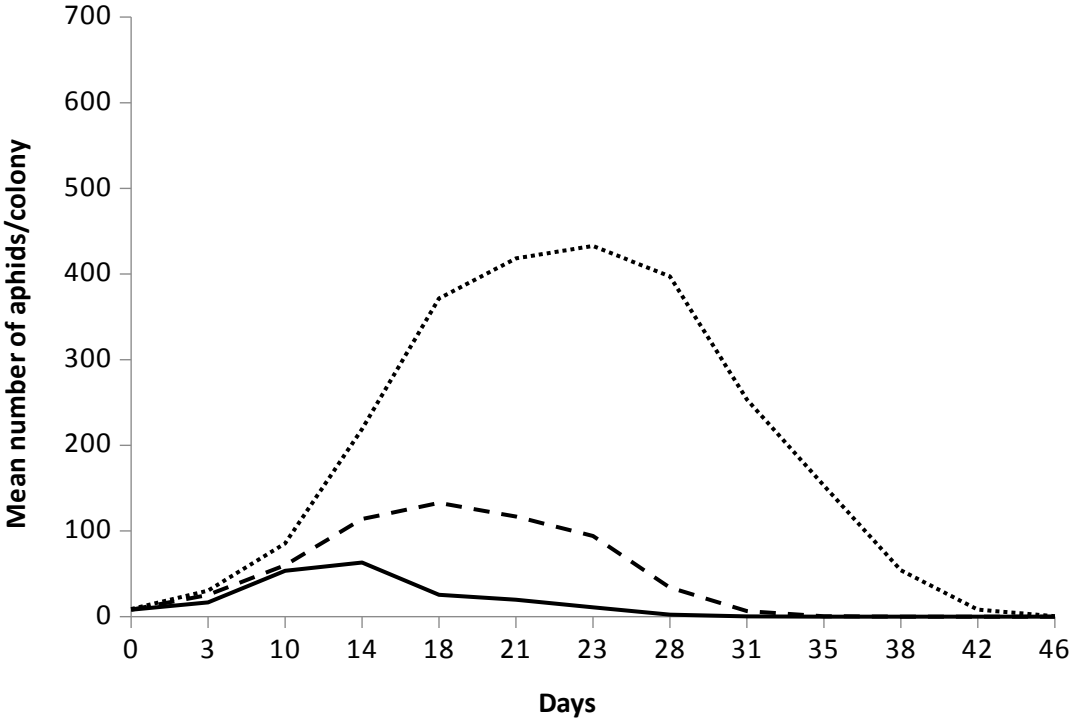
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Mean number of aphids/colony
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Days

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555**c)**
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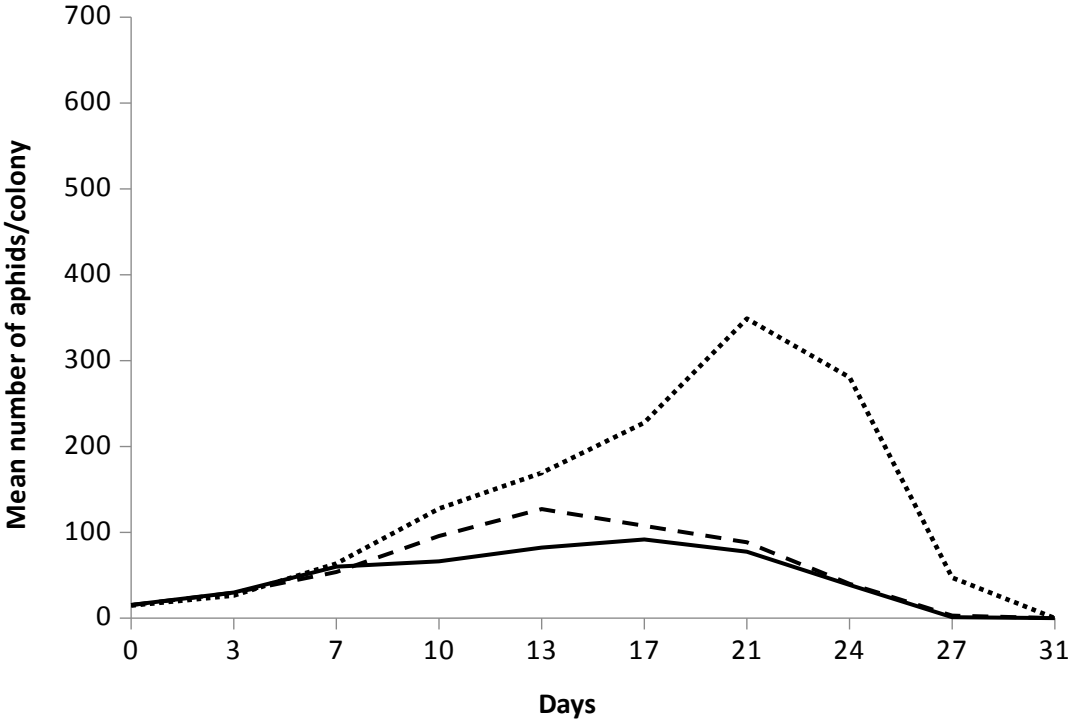
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558**d)**
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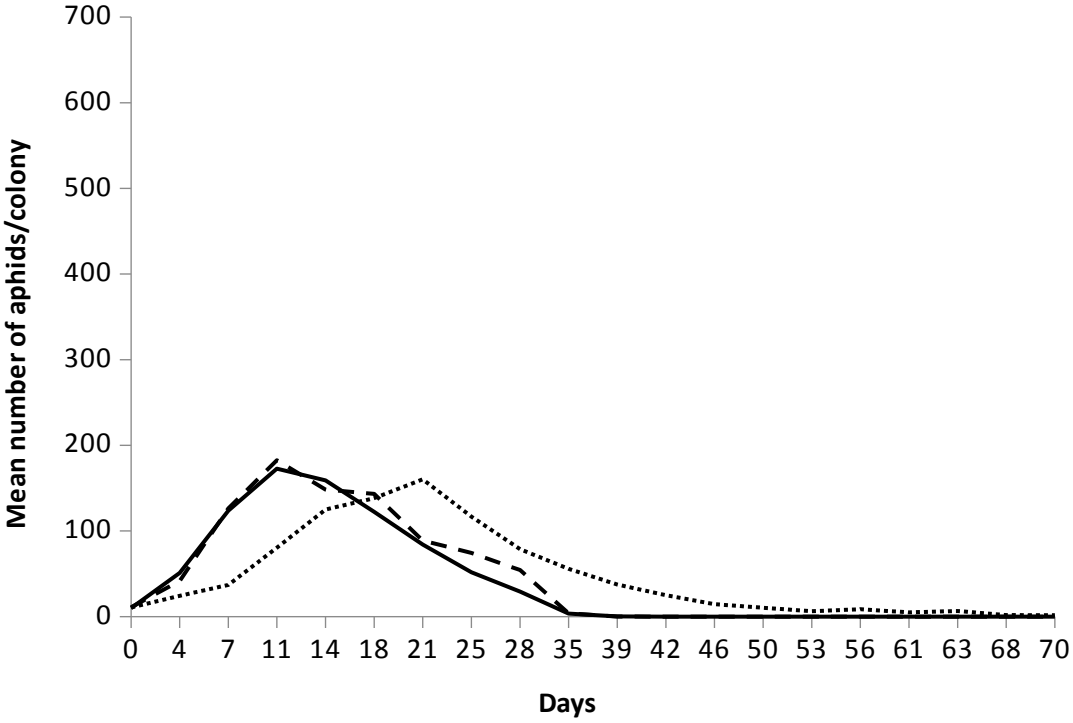

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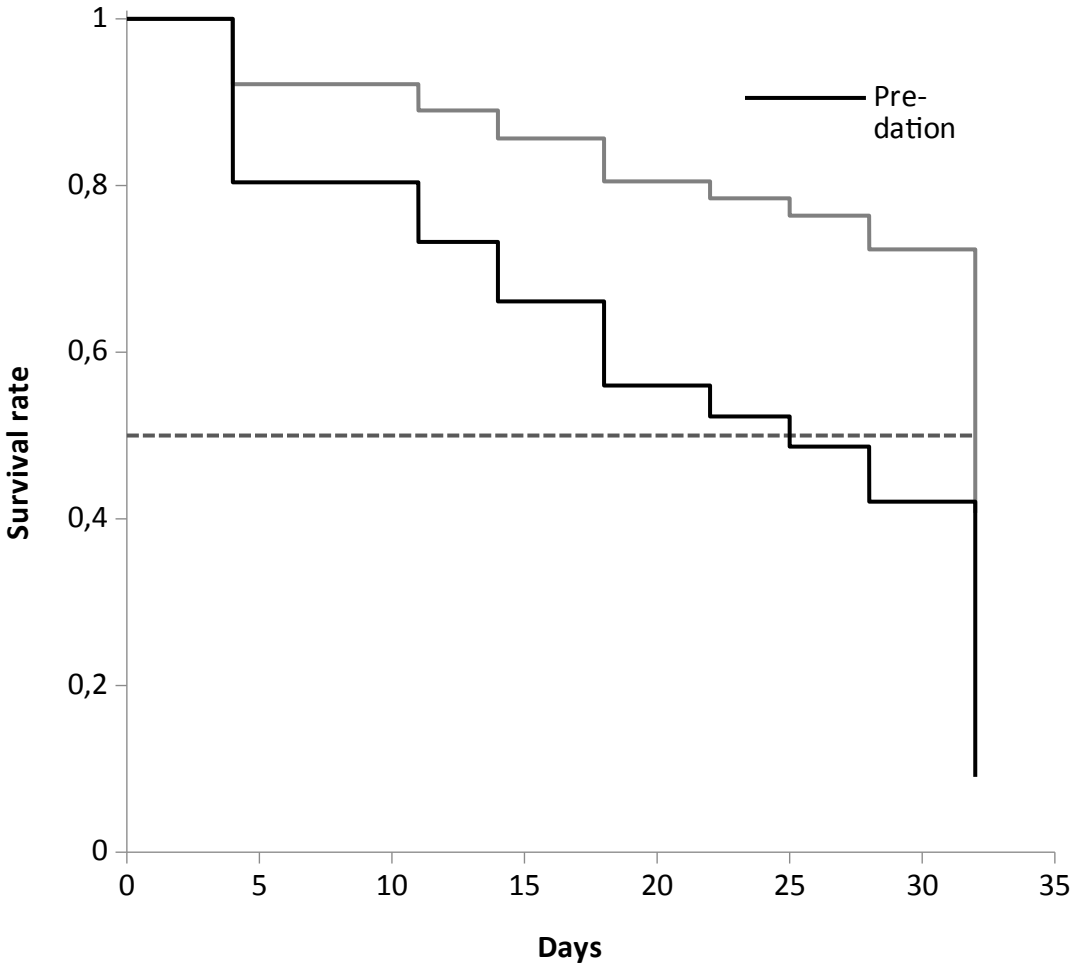
562**Fig. 2.** Survival rates of aphid colonies exposed to predators 'Predation' and protected from them with
563exclusion cages 'Exclusion' in citrus orchards. **a)** *A. gossypii* in 2015; **b)** *A. gossypii* in 2016; **c)** *A. spiraecola* in
5642015; and **d)** *A. spiraecola* in 2016. For each aphid species and year, different letters indicate significant
565differences in survival rates between exposed and protected colonies (P < 0.05). The dotted line marks the
566median survival time. The x-axis ranges from 0 to 35 days in 2015 and from 0 to 70 days in 2016.
567

568**a)**



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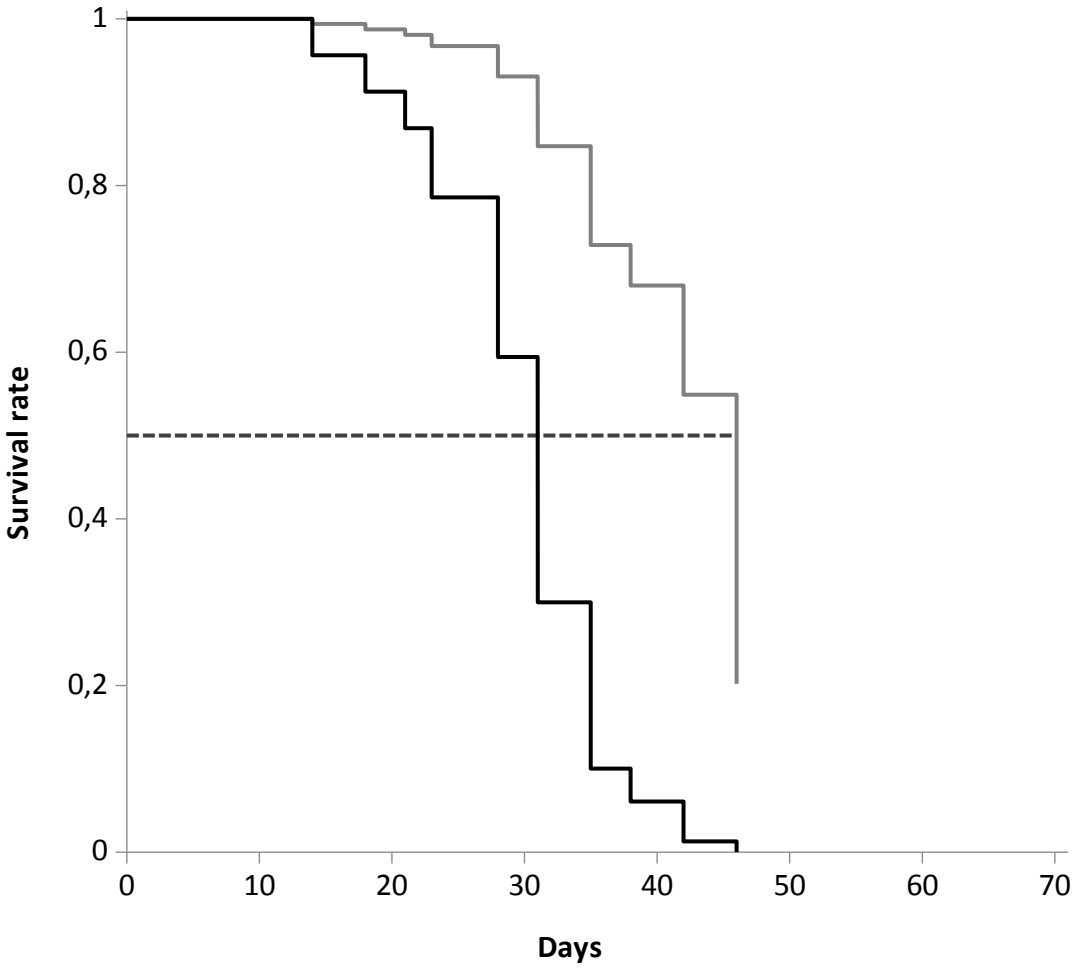
571**b)**



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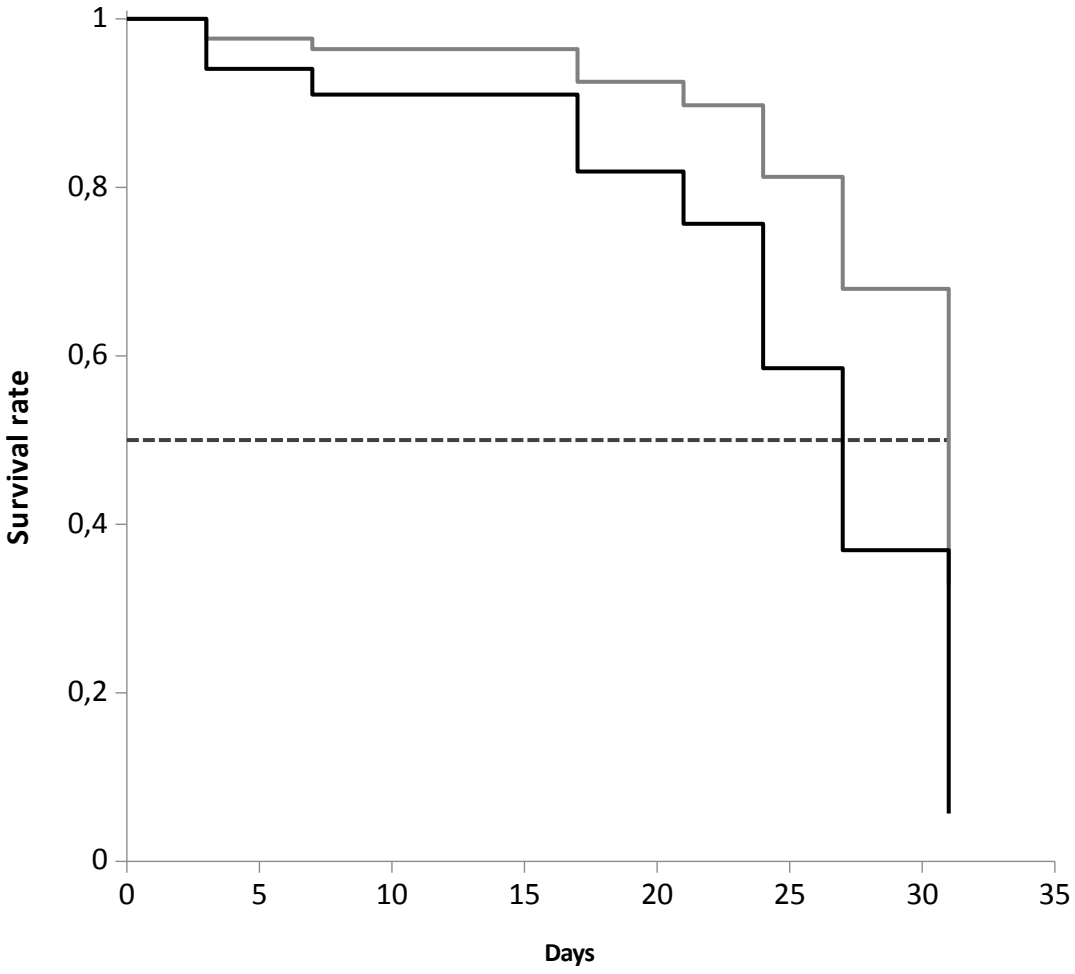

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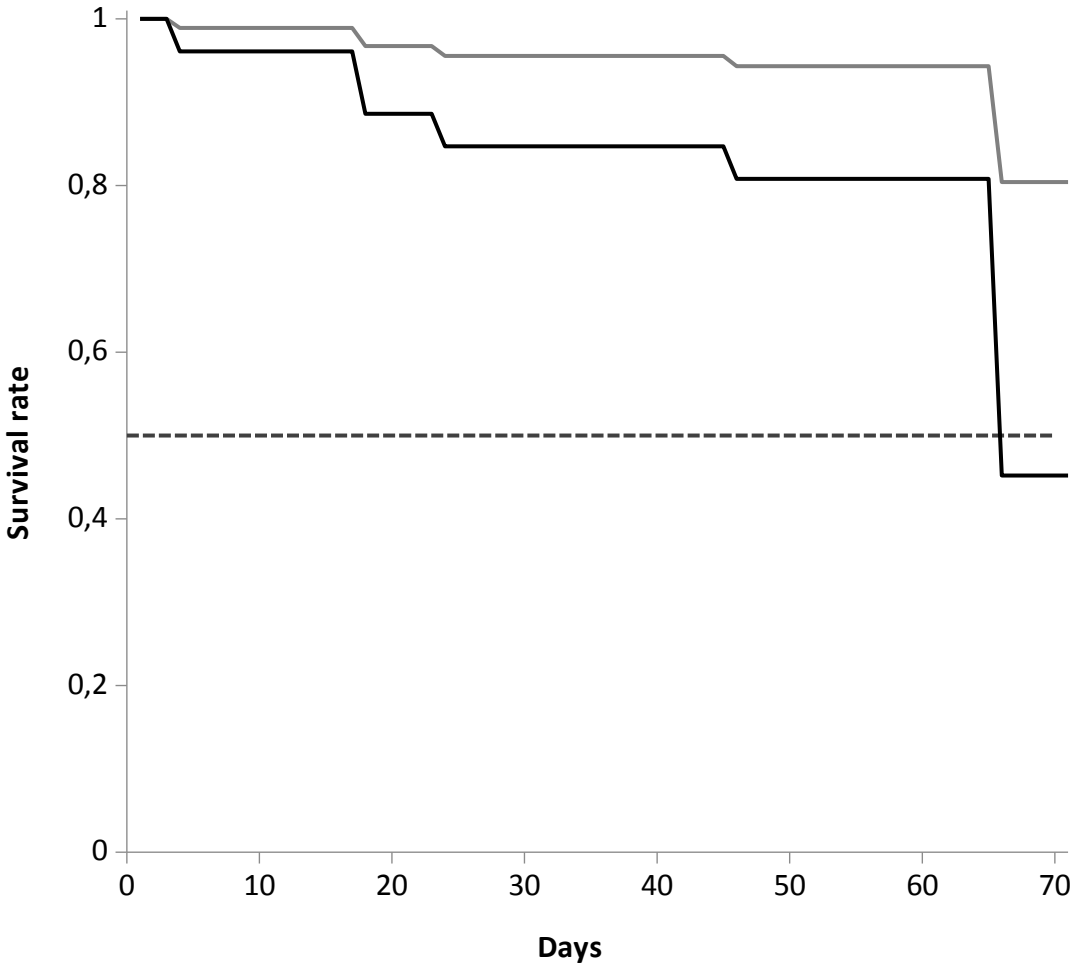

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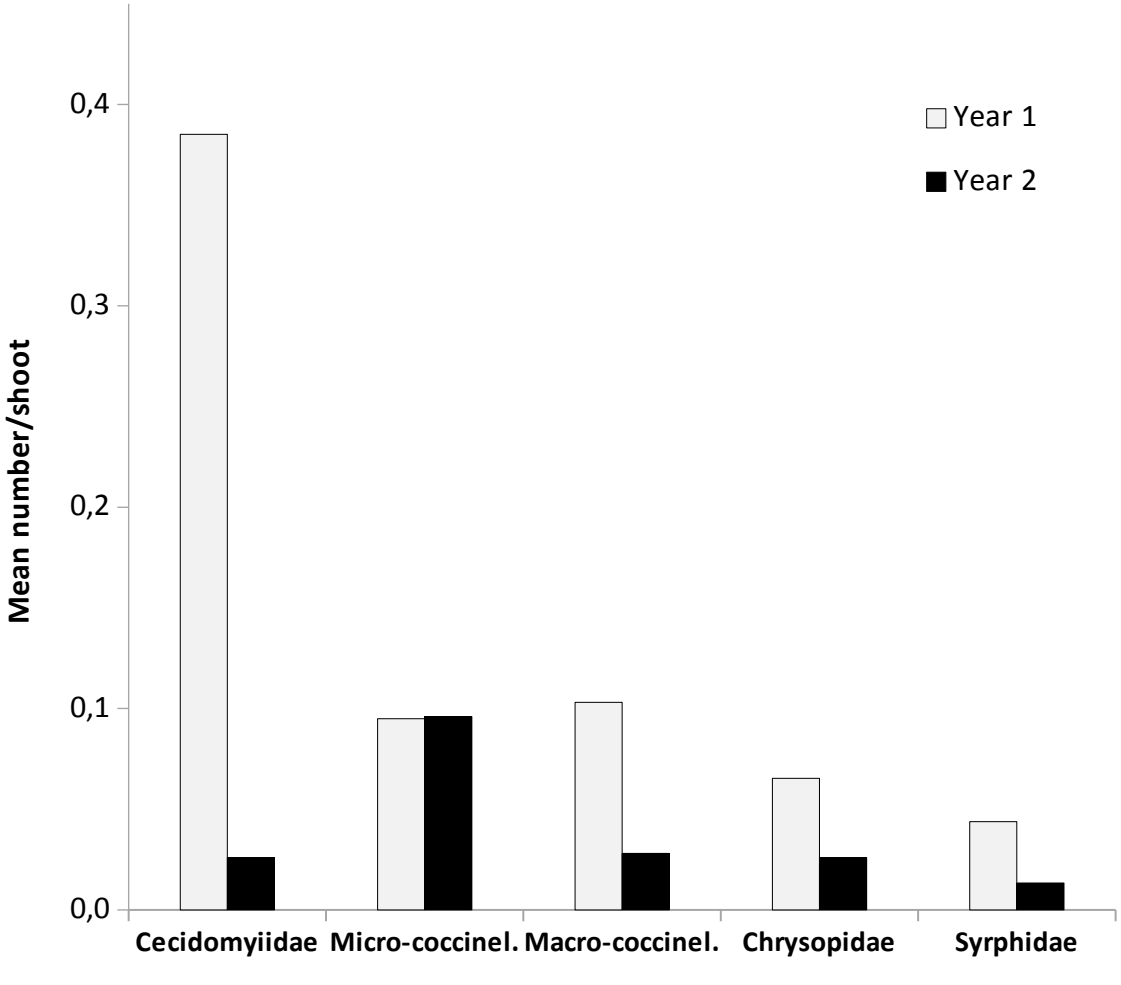
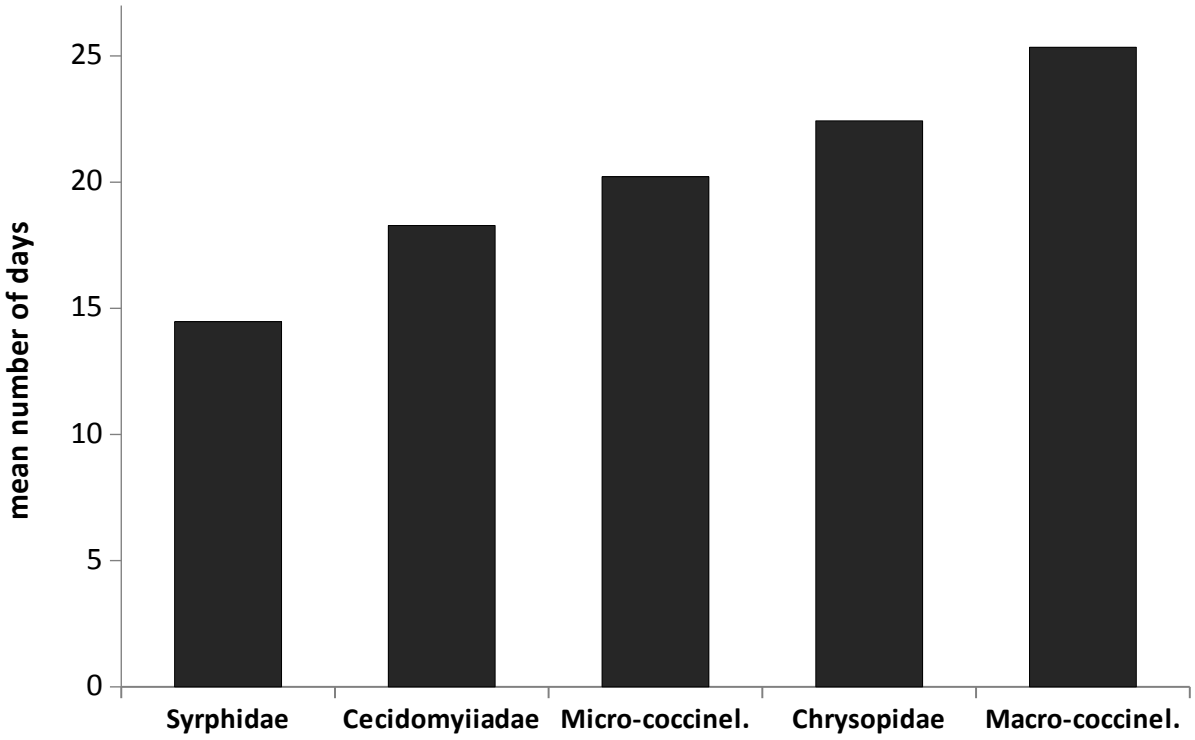
Fig. 3. Mean number (±SE) of predator specimens of citrus aphid predator groups recruited in aphid infested shoots during the spring shooting period of 2015 (year 1) and 2016 (year 2). Different capital letters indicate significant differences of predator recruitment between groups in year 1 and different lower case letters indicate significant differences in year 2 (Tukey's test, $P < 0.05$). Asterisks within the grey column indicate differences between years of predator recruitment within groups ($P < 0.05$).



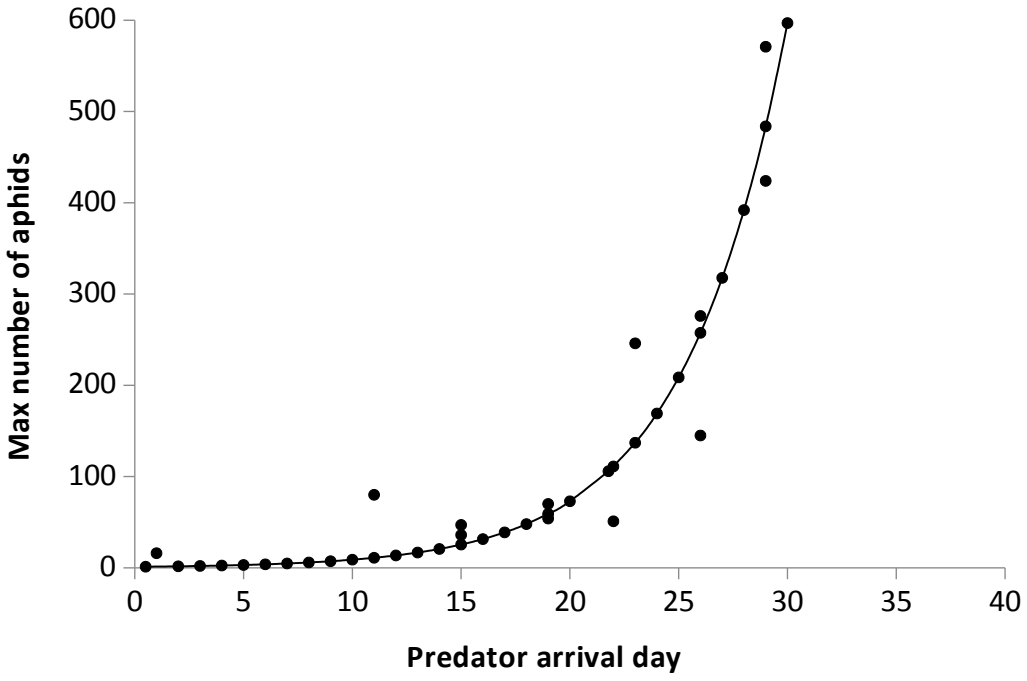
591**Fig. 4.** Arrival time of predators to citrus aphid colonies (mean ±SE) measured from the beginning of the
592aphid colonization phase in two citrus orchards and two years of study (2015 and 2016). Different letters
593indicate significant differences between predator groups (Tukey's test, $P < 0.05$).
594



595

596**Fig. 5.** Relationships between the arrival time of different aphid predator groups and the maximum aphid
597colony size measured in the 'Predator' treatment conducted in three citrus orchards in 2015 and 2016. **a)**
598Cecidomyiidae, **b)** Micro-coccinellids, **c)** Macro-coccinellids, **d)** Chrysopidae, and **e)** Syrphidae. Statistics from
599the regressions are displayed in Table 3.
600

601 **a)**



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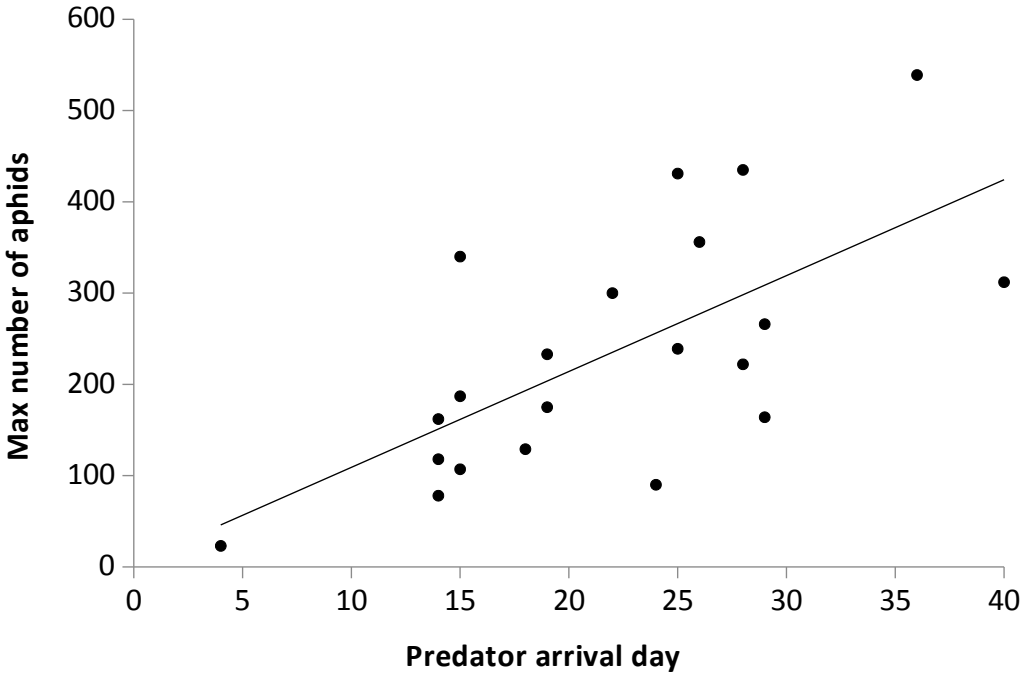
604**b)**


Max number of aphids
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30
35
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Predator arrival day

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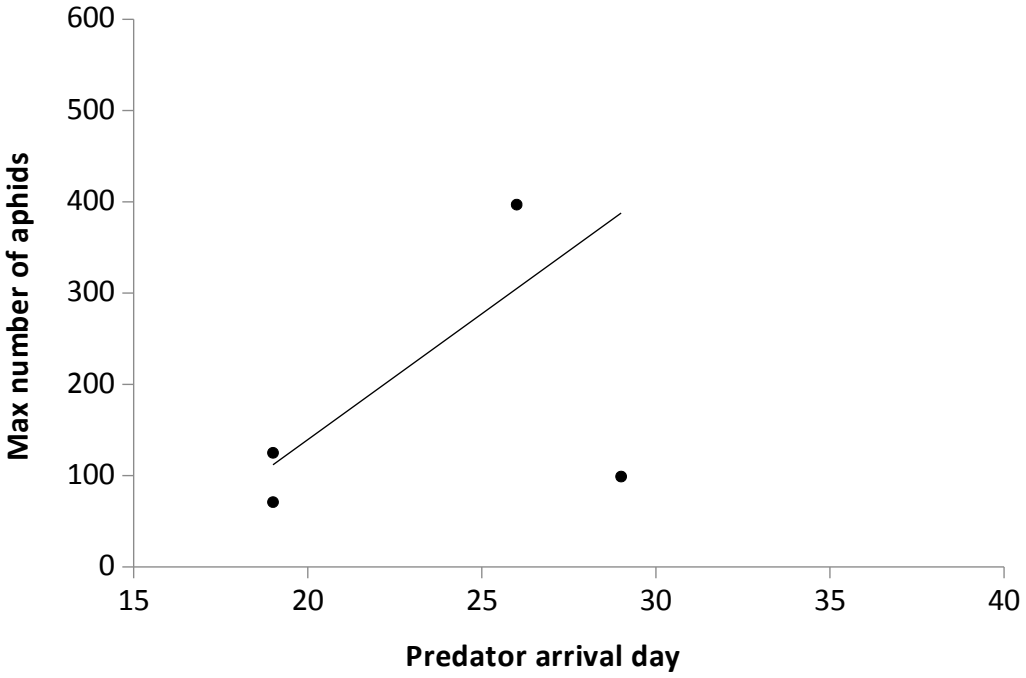
606

607**c)**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Predator arrival day

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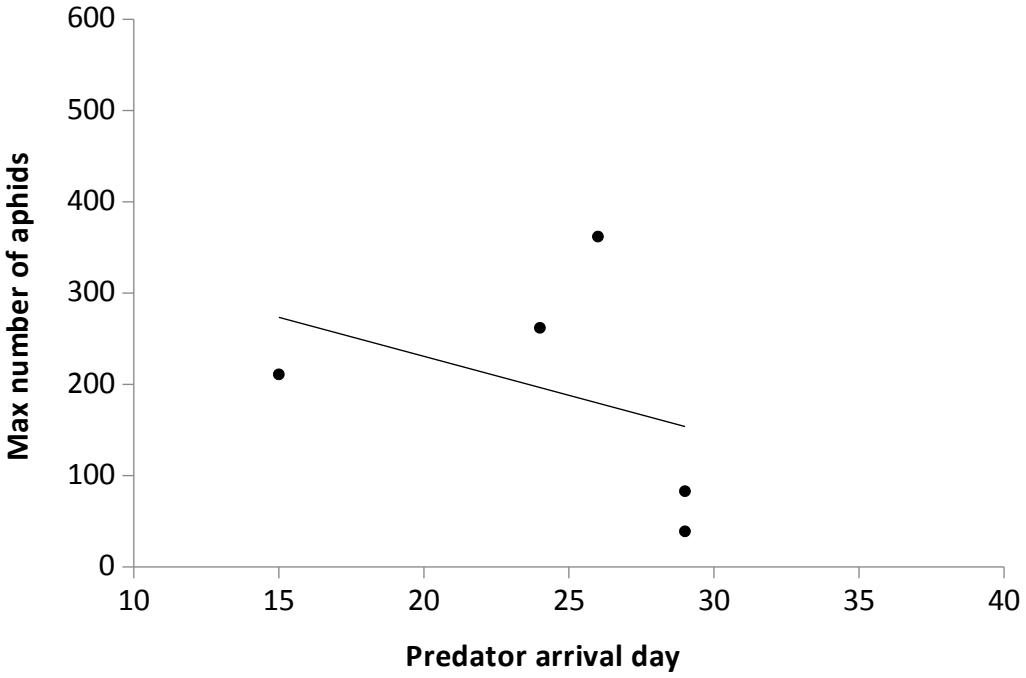
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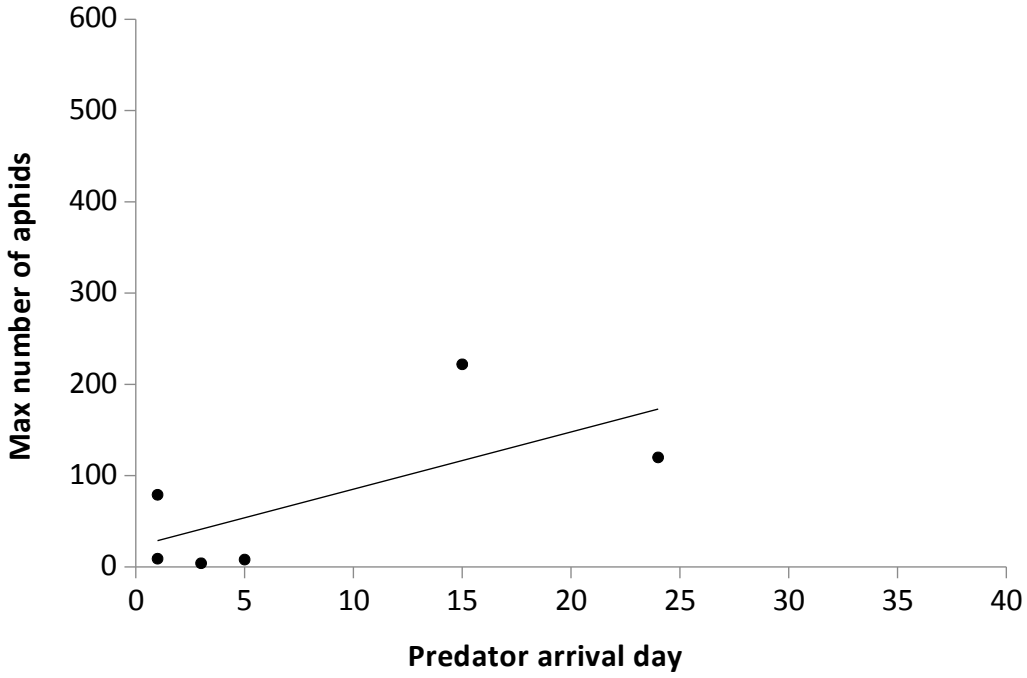
15
20
25
30
35
40
Predator arrival day

611

612

613**e)**


600
500
400
300
200
100
0
Max number of aphids
0
5
10
15
20
25
30
35
40
Predator arrival day

614

615

Tables

Table 1. Day in which aphid colonies reached their highest density, maximum number of aphids in the colony, and cumulative number of aphids (Mean ± SE) throughout the study period in *A. gossypii* and *A. spiraecola* colonies exposed to predators ('Predation') and protected from predators with cages ('Exclusion') for the two years of study.

		A. gossypii			***A. spiraecola***		
		Days to colony peak	**Peak density**	**Aphid cumulative**	**Days to colony peak**	**Peak density**	**Aphid cumulative**
Year 1	**Predation**	**8.6 ± 1.7**	**110.3 ± 30.1**	**1088 ± 293**	**11.3 ± 1.6**	**138.5 ± 25.2**	**1545 ± 276**
	Exclusion	**22.2 ± 1**	**587.8 ± 85.8**	**7945 ± 1021**	**18.8 ± 1**	**390.6 ± 55.5**	**4457 ± 576**
Year 2	**Predation**	**12.6 ± 1**	**74 ± 12.2**	**828 ± 126**	**11.6 ± 1.1**	240.8 ± 37.9	2864 ± 461
	Exclusion	**21.8 ± 1.2**	**567.5 ± 80.1**	**8608 ± 1239**	**21.2 ± 2.2**	194.4 ± 30.5	3525 ± 658

Table 2. Total number of predators recruited at the distinct aphid colony phenological stages during the shoot sampling of the three citrus orchards in 2015 and 2016: 1) colonization phase, 2) exponential growth phase, 3) peak phase, 4) decline phase and 5) colony death. Recruitment values with gray background indicate the highest predator abundance in relation to the aphid colony phases.

	Aphid colony stage					
	1	**2**	**3**	**4**	**5**	**Total**
Aphidoletes aphidimyza	**0**	**36**	**304**	**197**	**76**	**613**
Micro-coccinellidae	**2**	**32**	**113**	**99**	**99**	**345**
Scymnus interruptus	0	9	65	54	51	
Scymnus subvillosus	2	23	48	45	48	
Macro-coccinellidae	**4**	**9**	**12**	**59**	**111**	**195**
Adalia decempunctata	0	2	2	16	3	
Coccinella septempunctata	0	1	2	0	9	
Propylea quatuordecimpunctata	1	1	4	5	1	
Hippodamia variegata	0	0	0	0	2	
Coccinellidae spp.	3	5	4	8	6	
Chrysopidae	**0**	**2**	**5**	**47**	**72**	**126**
Syrphidae	**4**	**10**	**13**	**38**	**2**	**67**

628**Table 3.** Estimated relationship between predator arrival time (x) to citrus aphid colonies and maximum colony size for the different groups of predators associated with this
629phytophage and estimated maximum colony size (mean and 95% confident limits) for each predator group according to their observed mean arrival times. *P*-values marked
630in bold indicated a significant relationship ($P < 0.05$).
631

	Relationship	*F*	df_{error}	*P*	r^2-adjusted	a ± SE	b ± SE	Mean arrival time	Max colony size (95 CLM)
Cecidomyiidae	Exponential: ae^{bx}	70.43	10	**< 0.001**	0.93*	1.09 ± 1.24	0.21 ± 0.04	18.28	50.8 (5.3–96.4)
Micro-coccinellids	Linear: a+bx	15.06	19	**0.001**	0.41	3.9 ± 63.3	10.5 ± 2.8	20.21	216.3 (168.7–263.9)
Macro-coccinellids	Linear: a+bx	1.64	3	0.291	0.14	-412.4 ± 535.2	27.6 ± 21.6	25.34	286.7 (-31.6–605.1)
Chrysopidae	Linear: a+bx	0.49	3	0.534	-0.15	401.8 ± 307.2	-8.6 ± 12.2	22.43	210.0 (-7.8–427.7)
Syrphidae	Linear: a+bx	3.38	4	0.14	0.32	22.5 ± 40.3	6.3 ± 3.4	14.47	113.2 (12.7–213.7)

632*Pseudo-r^2 was calculated for the non-linear regression.

Table 4. Estimated Relative Relevance of the predator groups associated with citrus aphid colonies. A deterministic model was created using seven variables that identify different characteristics of the predators of interest in biological control. For each predator group and variable a rank from 1 to 5 was assigned depending on their relative importance (5: The most important; 1: The least important). Relative Relevance of each predator group (in bold) is the product of the ranks of the seven variables. Higher Relative Relevance values would be indicative of higher efficacy as biological control agents of aphids in citrus.

	Recruitment	Voracity	Arrival time	Time of maximum predator recruitment	Recruitment variability	Foraging ability	Adult predatory ability	Relative Relevance
Micro-coccinellids	4	2	3	4.5	5	4	4.5	**9,720**
Macro-coccinellids	3	3	1.5	1.5	3	4	4.5	**1,094**
Cecidomyiidae	5	1	4	4.5	4	1.5	2	**1,080**
Chrysopidae	2	4	1.5	1.5	2	4	2	**288**
Syrphidae	1	5	5	3	1	1.5	2	**225**