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Life history traits of the coccinellids *Scymnus subvillosus* and *S. interruptus* on their prey *Aphis spiraecola* and *A. gossypii*: implications for biological control of aphids in clementine citrus

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21 **Highlights**

- 22 - Two *Scymnus* species are associated with the two key citrus aphids in the Mediterranean basin.
- 23 - *Scymnus interruptus* performed better when fed *Aphis gossypii*.
- 24 - *Scymnus subvillosus* had no preference between *Aphis gossypii* and *Aphis spiraecola*.
- 25 - Both coccinellids performed much better when preying on a combination of both aphids.
- 26 - Prey suitability differences could explain the *Scymnus* spp abundances naturally found.

Abstract

Predator-prey interactions are not static, but spatially and temporally dynamic. In addition to the climatic conditions and the prey density, the dynamics of predator populations may be influenced by the suitability of their diet. Therefore, to better understand aphid predator-prey relationships within food webs, it is necessary to know how their life history traits are affected by diet quality. In this research, under laboratory conditions, the suitability of the two most abundant aphid species in citrus agroecosystems of the Western Mediterranean basin, *Aphis gossypii* and *A. spiraecola* were evaluated for two of their principle natural enemies, the coccinellid predators *Scymnus subvillosus* and *S. interruptus*. The intrinsic rate of increase of *S. subvillosus* was found to be higher than that of *S. interruptus* regardless of the type of prey consumed. Some biological parameters of *S. interruptus* were lower when they were exclusively fed *A. spiraecola*; as opposed to when only fed *A. gossypii*. These differences were not found with *S. subvillosus*. When a mixed diet of both aphids was offered, the fitness of both predators was higher than when they were each fed only a single aphid species. These laboratory observations were further confirmed under field conditions, wherein *S. subvillosus* abundance was greater in those colonies where *A. spiraecola* was predominant. On the other hand both, *S. subvillosus* and *S. interruptus* were found equally in *A. gossypii* colonies. Implications of these results for the biological control of aphids in this crop are discussed.

Key words: Conservation biological control, intrinsic rate of increase, predator-prey interactions, prey suitability, diet

1. Introduction

Ladybird predators (Coleoptera: Coccinellidae) rank among the most important biological control agents in agriculture due to their polyphagy, high voracity of both larval and adult stages, and rapid numerical and aggregative responses to pest densities (Dixon, 2000; Obrycki et al., 2009; Hodek et al., 2012). Numerous species of this family are commonly used to control a wide range of agricultural pest including whiteflies, aphids, mealybugs, scales and psyllids in many crops and cultivation systems; both outdoors and in greenhouses (Cabral et al., 2009; Obrycki et al., 2009; Hodek et al., 2012; van Lenteren et al., 2018).

The quality and type of prey plays a key role in predator optimal foraging strategies such as prey selection and consumption, or trophic transfer efficiencies (Kalushkov and Hodek, 2005; Keshavarz et al., 2015). The study of the interaction between these natural enemies and their prey from a nutritive point of view will reveal valuable information for the development of more effective biological control tactics (Li et al., 2015). Most Coccinellidae species have a mixed diet composed of essential and alternative prey species (Evans et al., 1999; Nielsen et al., 2002). The former support development and reproduction, whereas the alternative prey enables adult survival when primary prey is scarce or absent (Lucas, 2005; Omkar et al., 2009). One of the most important essential prey species for coccinellids are the aphids (Soares et al., 2004; Weber and Lundgren, 2009; Obrycki et al., 2009; Hodek et al., 2012).

Aphids are considered to be major pests in citrus crops. The two most important species associated with Mediterranean citrus agroecosystems are *Aphis spiraecola* Patch and *A. gossypii* Glover (Hemiptera: Aphididae) (Zappalà, 2010; Tena and Garcia-Marí, 2011; Hermoso de Mendoza et al., 2012; Lebbal and Laamari, 2016). Both species develop and feed exclusively on young, tender citrus vegetation (flush shoots). Feeding activity directly affects tree growth but indirect damage is also produced with the large amounts of honeydew that these phytophagous arthropods excrete. *Aphis spiraecola* and *A. gossypii* have also been described as efficient vectors of *Citrus tristeza* virus (Cambra et al., 2000). Clementine cultivars are the most vulnerable and suffer the most damage by aphids; they have an abundant and prolonged flushing period in spring which offers aphids almost unlimited resources for rapid development. Under conventional growing conditions, aphid populations in Mediterranean clementine agroecosystems frequently exceed their economic thresholds (Wang et al., 2000; Hermoso de Mendoza et al., 2001; Hermoso de Mendoza et al., 2006).

Biological control of aphid populations in citrus is provided primarily by an assemblage of cecidomid, syrphid, and most importantly, coccinellid species (Michaud, 2000; Brown, 2004; Michaud, 2005; Obrycki et al., 2009; Hodek et al., 2012; Gómez-Marco et al., 2016a). In the Mediterranean basin, the most abundant species of aphidophagous coccinellids associated with citrus are *Scymnus (Pullus) subvillosus* (Goeze) and *Scymnus (Scymnus) interruptus* (Goeze). These species are cosmopolitan; they are found in Europe, Asia Minor, and Northern Africa (Tawfik et al., 1973; Raimundo and Alves, 1986). They are common in arboreal agroecosystems, including apple, citrus, peach, plum, walnut, oak and natural habitats (Raimundo and Alves, 1986; Atlihan and Güldal, 2009; Sebastião et al., 2015). Few studies examine their relevance and role as biological control agents in these agroecosystems (Alvis, 2003; Kavallieratos et al., 2004). *Scymnus subvillosus* and *S. interruptus* are mainly predators of aphids even though they can feed on other prey such as scales, mealybugs and spider mites (Tawfik et al., 1973; Magro et al., 1999; Hodek et al., 2012; Bouvet et al., 2019). The smaller size and lower voracity of *Scymnus* species compared to other larger coccinellid species presume them to be less competitive and less efficient biological control agents. Notwithstanding, their high abundance in citrus agroecosystems, capacity to persist at low prey densities and great longevities give these small ladybirds the ability to exploit aphid colonies in both earlier and later plant phenological stages and for longer periods of time than the larger ladybirds (Tawfik et al., 1973; Borges et al., 2013; Sebastião et al., 2015).

Scymnus interruptus and *S. subvillosus* apparently have overlapping niches in citrus agroecosystems. Both predators are frequently found coexisting and competing for the same food resources: *A. spiraecola* and *A. gossypii* (Zappalà, 2010; Tena and Garcia-Marí, 2011; Hermoso de Mendoza et al., 2012). The competitive exclusion principle would nevertheless suggest that one of these predators would displace the other one (Hardin, 1960). The causes preventing this displacement in the citrus agroecosystem have never been studied. Thus, in this research we hypothesized that resource partitioning through the development of a feeding preference of either of the two predators for either of the two aphid species may explain their coexistence. Dietary self-selection behaviors have already been described for other coccinellid species (Soares et al., 2004). A better understanding of this phenomenon will help to develop new conservation biological control strategies for these natural enemies.

The objectives of this research were therefore: i) to know the suitability of *A. spiraecola* and *A. gossypii* as prey for *S. interruptus* and *S. subvillosus*; for this, the life history traits of both predators when raised on *A. spiraecola*, *A. gossypii* or a mixture in equal amounts of the two aphid species were studied in the laboratory; and ii) to relate the information obtained in the laboratory studies with field observations of the presence and abundance of the two *Scymnus* species in three scenarios: citrus trees with aphid colonies dominated by a) *A. spiraecola*, b) *A. gossypii*, and c) with colonies in which both aphid species were found in similar abundances.

2. Materials and Methods

2.1. Stock cultures

Laboratory colonies of *A. spiraecola* and *A. gossypii* were initiated by collecting specimens on clementine trees [*Citrus clementina* Hort. ex Tan. (Geraniales: Rutaceae)] located in orchards belonging to the Instituto Valenciano de Investigaciones Agrícolas (IVIA) in Moncada, Valencian Community, Spain (39°35'17.43"N / 0°23'53.28"O). Aphids were reared on two-year old, potted, clementine plants (*Citrus reticulata* Blanco [Clementina de Nules cultivar Iniasel 22] grafted onto *Citrango* Carrizo rootstock [*Poncirus trifoliata* (L.) Rafinesque-Schmaltz x *Citrus sinensis* (L.) Osbeck]) and kept in a heated chamber of a greenhouse at $24 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity with the natural photoperiod.

A colony of *Myzus persicae* (Sulzer) (green phenotype) was also set up to provide a source of food for coccinellid rearing. This aphid colony was established on sweet pepper plants (*Capsicum annuum* L.; Solanales: Solanaceae); it was started from a stock colony maintained on potted broad bean plants (*Vicia faba* L.; Fabales: Fabaceae) at the IVIA since 2004. Colonies of *M. persicae* on pepper plants were selected for their practicality; the aphids are easy to rear and manipulate. This colony was kept in growth chambers of a greenhouse at $24 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity with the natural photoperiod.

To establish the *S. subvillosus* and *S. interruptus* colonies, specimens were collected from clementine trees in the IVIA orchard (as above), by stem-tap sampling. Around fifty insects were kept in Petri dishes (9.0 cm in diameter x 1.5 cm in height) with a muslin covered hole in the lid (4 x 4 cm) to avoid excess humidity. In each Petri dish pepper leaves with *M. persicae* were provided every two days, as well as pollen, honey, and water on a filter paper. Petri dishes were kept isolated in a growth chamber (SANYO MLR-350; Sanyo, Japan) at $25 \pm 1^\circ\text{C}$, 60-70% RH with a photoperiod of 14:10 h (L:D).

2.2. Developmental parameters

Every two days *S. subvillosus* and *S. interruptus* eggs were selected and taken from the colonies. They were divided into three groups and placed in Petri dishes (5.5 cm diameter and 1.0 cm tall) with a muslin covered hole in the lid (2 × 2 cm), to allow gas exchange, and a plaster ball (5 mm) at the base to maintain the humidity constant within the experimental units.

Newly emerged coccinellid larvae (< 24 h old) were individually transferred to numbered Petri dishes similar to the ones described above. The larvae were reared with the aphid species corresponding to one of the three experimental diet treatments: 1) *A. spiraecola*, 2) *A. gossypii* and 3) *A. spiraecola* and *A. gossypii* in equal proportions. Each larva was provided aphids *ad libitum* and water in the plaster every day until larvae either pupated or died. Experimental arenas were kept in climatic chambers in the same conditions as those for *Scymnus* spp. colonies.

Larval (the presence of exuviate was used as evidence of molting) and pupal development and survival were checked daily under a stereoscope until death or adulthood. After adult emergence, they were measured (photographed with a camera mounted stereoscope using the software package Leica Application Suite, LAS version 4.6.2), weighed (precision scale) and sexed. These adults were used to assess the reproductive parameters. For each *Scymnus* species and prey diet treatment, 30 replicates were made; each replicate consisted of one female and one male adult.

2.3. Reproductive parameters

Following adult emergence, males and females were kept individually with the corresponding diet during 5-7 days (pre-breeding period) (Tawfik et al., 1973; Sebastião et al., 2015). After that, all the adult coccinellids of the same species and treatment were put together in an empty Petri dish (9.0 x 1.5 cm) and visually observed. Every time a couple was formed it was isolated in a Petri dish (similar to the development experimental units described above) for 24 h to allow mating. Males were later removed and gravid females were studied for 6 weeks. During this period, on a fresh piece of excised plant tissue females were provided a mixture of all nymphal stages of the corresponding aphid species *ad libitum* according to treatment. A small piece of corrugated cardboard (2 x 2 cm) was placed in the arena as oviposition substrate. Eggs were counted and removed daily. Water was supplied daily with a pipette as well as new aphids of the corresponding treatment. Experimental arenas were kept in the same climatic conditions as described above.

2.4. Demographic growth indexes

The intrinsic rate of increase (r_m) was computed using the Euler equation,

$$\sum e^{-r_m x} l_x m_x$$

where l_x is survivorship of the original cohort over the age interval from day $x - 1$ to day x , and m_x is the mean number of female offspring produced per surviving female during the age interval x (Birch, 1948). The sex ratio of 0.5 based on other studies was used to calculate the statistics (Tawfik et al., 1973; Sebastião et al., 2015). Values of m_x for the population were calculated from the mean number of eggs laid per female per day. Other parameters, including reproductive rate (R_0) and generation time (T) were calculated as described by Birch (1948) using jackknife (Maia et al., 2000). The finite rate of increase ($\lambda_m = e^{r_m}$) and doubling time ($DT = \ln 2 / r_m$) were also calculated (Mackauer, 1983).

2.5. Spatial sampling

To further explore the relationships between the populations of the citrus aphid species, *A. spiraecola* and *A. gossypii*, and their predators, *S. subvillosus* and *S. interruptus*, sixty clementine orchards separated by a minimum distance of 1 km were sampled throughout the Valencian Community, covering an area of 240,000 hectares within the provinces of Castellón and Valencia (Figure 1). From April 22 to May 31, 2016, in the full citrus sprouting stage, 10 random aphid infested trees per orchard were sampled for *Aphis* spp. and *Scymnus* spp. populations.

For aphid sampling, in each tree a PVC (Polyvinyl chloride) ring delineating an area of 0.25 m² was randomly placed twice on the canopy. Within the ring area, the numbers of suitable aphid-infested and non-infested shoots were observed (Hermoso de Mendoza et al., 2001, 2006). Trees were classified according to the predominant aphid species (more than 70% of shoots) as i) dominated by *A. gossypii*, ii) dominated by *A. spiraecola* or iii) presenting mixed colonies in the case where neither of the two aphid species was present in more than 70% of the shoots.

At the same time, by stem tap sampling, in the same trees used for the aphid surveys, *Scymnus* spp populations were also sampled. For this, we used a plastic tray (45 x 30 cm) and a PVC pipe. The species of *Scymnus* (larvae and adults) dislodged from the stricken branches by the stem-tapping were identified inside the plastic tray and then released.

2.6. Data analysis

The effect of the prey diet on the life history traits of *S. interruptus* and *S. subvillosus* (developmental durations of the distinct stages, and development and reproductive parameters) were analyzed by one way ANOVA and subsequent Tukey's Honestly Significant Difference test (HSD). R Studio 1.1.383 software was used for the statistical analysis.

The Cox proportional hazards model was used to evaluate differences between the survival probabilities of *S. interruptus* and *S. subvillosus* among the three types of diets. Significant differences of the life table parameter means were determined using Student's *t* test. R Studio 1.1.383 software was used for the statistical analysis.

To describe the potential demographic spatial relationships between the two dominant citrus aphid species and their predators, *S. interruptus* and *S. subvillosus*, the number of *Scymnus* specimens captured by stem-tap sampling in each tree throughout the spatial sampling (dependent variable) was related to the *Scymnus* species dominance (*S. interruptus* or *S. subvillosus*) along with the aphid dominance (*A. spiraecola*, *A. gossypii* or mixture of the two aphid species in the colonies). The interaction between these two fixed effects was also included in the model. Generalized linear mixed model (GLMM) analysis was used for this purpose. Negative binomial distribution of the variable was assumed based on Akaike and Bayesian information criteria. Tukey's HSD test was used to investigate differences in the abundance of the two *Scymnus* species depending on aphid dominance.

3. Results

3.1. Developmental parameters

Duration of the different immature stages of *S. subvillosus* and *S. interruptus* when reared on *A. spiraecola* and *A. gossypii* and on the mix of both, are shown in Table 1. Total developmental time for both coccinellids when reared exclusively on *A. spiraecola* was significantly longer than when fed with *A. gossypii*, either alone or in a mixture with *A. spiraecola*.

Immature survival of *S. subvillosus* gradually decreased to between 65 and 80% throughout development until adult emergence. Whereas immature survival of *S. interruptus* was higher than 90%. When comparing the probability of survival of each coccinellid species fed with the three diets, no statistical differences were found (*S. interruptus*: $\chi^2 = 1.9300$, *df* = 2, *P* = 0.381; *S. subvillosus*: $\chi^2 = 3.5222$, *df* = 2, *P* = 0.17; Cox proportional hazards model) (Figure 2). The weight and size of the emerged adults

(males and females) was not significantly different for any of the coccinellid species when fed the three diets except one (Table 2). The only significant difference observed was the size of the *S. interruptus* males that were only fed *A. spiraecola*; they were smaller than those raised on the other two diets.

3.2. Reproductive parameters

Diet had a significant effect on the reproductive parameters of both of the *Scymnus* species when raised on the three diets (Table 3). Fecundity of both predators was significantly higher when fed the mixed diet than when fed the *A. gossypii* diet and lowest with the *A. spiraecola* diet. Daily fecundity (eggs/day) was higher for both coccinellids with the mixed diet. No significant differences in fertility of *S. interruptus* were found among the three diets; however, in the case of *S. subvillosus*, fertility was lower when raised on *A. spiraecola* alone. The egg incubation period was similar among diets for *S. interruptus*. However, *S. subvillosus* eggs hatched significantly sooner with the *A. gossypii* diet than with both the *A. spiraecola* and the mixed diet.

3.3. Demographic growth indexes

Effects of diets on selected life history parameters of both coccinellids are presented in Table 4. When fed the mixed diet, the net reproductive rate (R_0), the intrinsic rate of increase (r_m) and the finite rate of increase (λ_m) were significantly higher in both *Scymnus* species. The second highest rates were found with the *A. gossypii* diet. Generation time (T) was significantly longer for *S. interruptus* when raised on *A. gossypii*. *Scymnus subvillosus*, on the other hand, presented the longest generation times with the *A. gossypii* and mixed diets. *Scymnus interruptus* doubled its population sooner when fed *A. gossypii* as opposed to when raised on *A. spiraecola*; the contrary was observed for *S. subvillosus*.

3.4. Spatial sampling

Approximately 7,300 citrus shoots containing aphid colonies were observed. *Aphis spiraecola* was the dominant colony species in 75% of the trees whereas only 10% of them presented dominance of *A. gossypii* colonies. The remaining 15% of the observed trees showed mixed colonies of both aphid species.

Scymnus spp. Abundance, monitored through stem-taps in the regional sampling, was significantly affected by the type of dominant aphid colony in the trees ($F = 4.20$, $df = 2$, 1126 , $P = 0.015$). Coccinellids were more abundant in trees presenting dominant *A. gossypii* colonies than those with *A. spiraecola* ($t = 2.75$, $df = 1126$, $P = 0.006$) or mixed colonies of the two species ($t = 2.61$, $df = 1126$, $P =$

0.009). No differences in *Scymnus* captures were found between trees mainly infested by *A. spiraeicola* colonies and trees presenting mixed colonies ($t = 0.64$, $df = 1126$, $P = 0.521$). *Scymnus subvillosus* was the most abundant coccinellid species found by stem-taps throughout the regional sampling ($F = 15.67$, $df = 1$, 1126 , $P < 0.0001$). This species was found in 80% of the plots with an average of 13.33 (± 2.05) specimens per plot. *Scymnus interruptus* was observed in 55% of the plots sampled with an abundance of less than 5.02 (± 1.09) specimens per plot. Marginal differences were found in the abundance of the two *Scymnus* species depending on the dominant type of aphid colonies (Interaction: $F = 2.23$, $df = 1$, 1126 , $P = 0.108$): *Scymnus subvillosus* was the most abundant species in trees dominated both by *A. spiraeicola* colonies ($t = -7.66$, $df = 1126$, $P < 0.0001$) and mixed colonies ($t = -3.29$, $df = 1126$, $P = 0.001$) whereas no differences between the two *Scymnus* species were found in trees dominated by *A. gossypii* colonies ($t = -0.25$, $df = 1126$, $P = 0.806$) (figure 3).

4. Discussion

For the two *Scymnus* species studied, our results presented shorter developmental periods when offered diets that included *A. gossypii* alone and in combination with *A. spiraeicola*, as opposed to those of *A. spiraeicola* alone. This would indicate that *A. gossypii* is more suitable for larval development, probably because of the presence of essential growth nutrients. Shortening of developmental periods leads to quicker multiplication of predator populations and therefore makes them more effective at controlling pests. Mixed diets have been shown to be the most favorable for the development of coccinellids (Evans et al., 1999; Nielsen et al., 2002; Schuldiner and Coll, 2017). Nevertheless, in our study this was not observed probably because both prey species were reared on the same host plant which would not provide such a noticeable advantage as observed when coccinellids are provided diets of mixed aphid species which had been reared on distinct hosts (Nielsen et al., 2002). Similar results were obtained by Sebastião et al., (2015) who did not find differences in *S. subvillosus* survival in any of the stages studied when fed with three different species of aphids reared on the same host plant species (*Vicia faba* L.; Fabales: Fabaceae).

When we used the Cox proportional hazards model, the probability of survival during the larval developmental period was not affected by the type of diet on which they were raised, as has been observed in other research (Omkar et al., 2009; Schuldiner and Coll, 2017). Again, this could be

attributed to the use of the same aphid host plant species in all the treatments. On the other hand, the differences in survival rates observed among *Scymnus* species could possibly be attributed to intrinsic factors of each particular species (Tawfik et al., 1973; Sebastião et al., 2015).

The size and weight of males and females of the two *Scymnus* species were not significantly affected by any of the three diets provided. Only a reduction in the male size of *S. interruptus* fed with *A. spiraecola* was observed; which supports the hypothesis that *A. spiraecola* does not meet the predator's needs as well as *A. gossypii* as previously observed by Kalushkov and Hodek (2005). Low quality diets consumed during the larval period are thought to negatively influence the size of adults (Michaud, 2000).

The reproductive parameters for both predators were importantly influenced by the diet consumed. The *Scymnus* females raised on the aphid mixture diet had a significantly greater total and daily fecundity, followed by those whom had been fed the diet composed exclusively of *A. gossypii*. Omkar and James (2004) found a relatively higher fecundity in ladybugs raised on *A. gossypii*; this was associated with the impact of early ovariole maturity, which depends on the prey species consumed. On the other hand, a diet composed only of *A. spiraecola* seemed to be unsuitable since lower fecundity values were produced in both species of predators. In addition, *S. subvillosus* fed only *A. spiraecola* presented lower fertility rates. The data clearly support the idea of diet distinctly influencing predators according to the specific nutritional requirements of each species for its development and reproduction.

Differences in biological responses of *Scymnus* species under distinct diets can be illustrated with their estimated life history parameters. The net reproductive rate (R_0) in both *Scymnus* species presented higher values when fed the mixed diet. Thus mixed diets benefit the populations of stenophagous predators, probably because they provide a greater diversity of nutrients (Nielsen et al., 2002; Schuldiner and Coll, 2017). On the contrary, the R_0 values, when the two ladybird species were fed *A. spiraecola* alone were the lowest; again supporting the idea that this species of aphid is of lower nutritional value than *A. gossypii* for these predators.

Estimated R_0 values for *S. subvillosus* were similar to those obtained in other studies where the specimens were raised with only one type of prey, *Hyalopterus pruni* (Geoffroy) (Hemiptera: Aphididae) or *A. gossypii* (Atlihan and Güldal, 2009; Satar and Uygun, 2012). The R_0 values obtained with a mixed diet, on the other hand, were higher than when only one prey was offered; also similar to those

observed by Gibson et al. (1992) for *Scymnus frontalis* (Fabricius). In contrast, estimated R_0 values for *S. interruptus* were lower than for *S. subvillosus*, independent of diet type.

The high R_0 values obtained for the two *Scymnus* species may be an indication of why these predators are so abundant in the Mediterranean region and particularly in citrus, where both predators are thought to play a primary role in aphid control (Alvis, 2003; Soler et al., 2006; Santos et al., 2012).

As expected, the intrinsic growth rate (r_m) and the finite rate of increase (λ_m) showed the same pattern as R_0 . These parameters were highest in the populations reared on the mixed diet and lowest in those fed only the *A. spiraecola* diet. Similar to these rates were those observed in other studies with *S. subvillosus*, *S. levaillanti* Mulsant, *S. scyriacus* (Marseul), *S. nubilus* (Mocquard) and *S. frontalis* (Gibson et al., 1992; Uygun and Atlihan, 2000; Soroushmehr et al., 2008; Atlihan and Güldal, 2009; Satar and Uygun, 2012; Borges et al., 2013). The intrinsic growth rate quantifies the potential population increase of a certain species under specific ecological conditions. The low effectiveness of both predators in the control of aphids observed in some field studies (Brown, 2004; Hermoso de Mendoza et al., 2012), could be partially explained by the fact that *A. spiraecola* and *A. gossypii* present r_m values three times greater than those of *S. subvillosus* and *S. interruptus* (Kersting et al., 1999; Wang and Tsai, 2000). The higher intrinsic rate of increase of these aphid species compared to their predators may impair an effective numerical response of the later. Therefore, the potential of these predator species should be exploited through other means, such as early arrival to aphid colonies through the use of banker plants or the management of cover crops (Hodek et al., 2012; Gómez-Marco et al., 2016a, b). Bouvet et al. (Unpublished) recently found aphid biological control success in western Mediterranean citrus clementine crops to be achieved only through early presence of *Scymnus* species in the orchards. Gómez-Marco et al., (2016a, b) demonstrated how a sown grass cover enriched with wild forbs improved the biological control of aphids in citrus since it promoted the early presence of predators, including *Scymnus* species, in citrus canopies before aphid exponential increase.

At a regional scale, either by the samplings made in this study or by those previously carried out by other researchers in the Mediterranean region (Hermoso de Mendoza et al., 2012; Mostefaoui et al., 2014; Lebbal and Laamari, 2016), the most abundant aphid species in citrus crops has been identified to be *A. spiraecola*. However, although both *Scymnus* species are the most abundant predators recorded in the region, there is some controversy about which of the two could be the most important based on

their demography. Some authors reported greater *S. interruptus* abundance in citrus crops (Magro et al., 1999; Alvis, 2003), while others cited *S. subvillosus* as the dominant species (Hermoso de Mendoza et al., 2012), as observed in the present study. The greater abundance of *S. subvillosus* in the field could be explained by their estimated life history parameters. On one hand, estimated R_0 , r_m and λ_m for *S. subvillosus* with all of the three diets were much higher than those obtained for *S. interruptus*. Nevertheless, developmental time (T) of *S. subvillosus* was reduced when it was only fed *A. spiraecola*. The developmental time of these aphid species is particularly short. A reduction of T would therefore give *S. subvillosus* a considerable selective advantage over *S. interruptus* making the former especially more effective in the control of *A. spiraecola* (Hemptinne and Dixon, 1997).

Due to coexistence of the two predator and two prey species at different relative abundances in the citrus agroecosystems of the studied region (Alvis, 2003) we initially hypothesized there to be some type of diet preference by the predators since females, as is well known, choose the best diet to achieve maximum fitness. The field sampling revealed the abundance of *S. interruptus* to be higher in shoots occupied by *A. gossypii*. In contrast, *S. subvillosus* abundance could not be associated with any of the aphid species occupying the citrus shoots. Therefore, our results, from both the laboratory and field support our initial hypothesis. In addition, one of the causes explaining the low rates of *A. gossypii* in the field compared to *A. spiraecola*, could be the higher abundance of *S. interruptus* in trees dominated by *A. gossypii* colonies together with the fact that the life history parameters of this predator are better suited to the diets that included this aphid species. On the other hand, the better performance of *S. interruptus* when fed *A. gossypii* leads us to believe a competitive pressure between the *Scymnus* species could be higher in shoots with this aphid species. The fact of finding greater abundance of *S. interruptus* in the shoots with greater competition indicates that this species of predator would be a better competitor than *S. subvillosus* in this situation. Studies on these aspects of competition must be carried out to clarify how these two closely related species of predators can coexist in the citrus agroecosystem without one displacing the other.

5. Conclusion

This research provides novel information about *Scymnus* species and their relationships with the aphid species found in citrus crops in the Mediterranean basin. Our hypothesis on the quality of the

366 diet influencing both predators' development and reproductive parameters, and consequently, their
367 field abundance and field distribution was confirmed. Even though the mixed diet that included *A.*
368 *gossypii* and *A. spiraecola* was the most suitable for the development of both predator species, in the
369 laboratory and field *S. interruptus*, in particular, demonstrated better performance when preying on *A.*
370 *gossypii*. This may be one of the main factors determining the distribution and dynamics of the citrus
371 aphid complex and its natural enemies in the Mediterranean basin.

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528

529 **Figure captions**

530 **Figure 1.** Location of sampling sites in the Valencian region (Comunidad Valenciana, Spain).

Figure 2. Immature survival curves of a) *S. subvillosus* and b) *S. interruptus* when reared on 1) *A. spiraecola*, 2) *A. gossypii* and 3) *A. spiraecola* + *A. gossypii* (df = 2). Survivorship curves followed by the same letter are not significantly different ($P < 0.05$; Cox regression model).

Figure 3. Mean number ($\pm$ SE) of specimens of *S. subvillosus* and *S. interruptus* on trees with a predominant abundance of 1) *A. spiraecola*, 2) *A. gossypii* or 3) both species are found in similar proportions. Same letters between columns of the same predominance grouping indicate no significant differences ($P < 0.05$; Tukey test).

Tables

Table 1. Mean developmental times (days $\pm$ SE) of *S. subvillosus* and *S. interruptus* when reared on *A. spiraecola*, *A. gossypii* and *A. spiraecola* + *A. gossypii* (df = 2, 150). Within rows means followed by different letters are significantly different ($P < 0.05$; Tukey test).

Species	Stage	Diet			F value	P value
		<i>A. spiraecola</i>	<i>A. gossypii</i>	<i>A. spiraecola</i> + <i>A. gossypii</i>		
<i>S. interruptus</i>	First instar	2.52 $\pm$ 0.08a	2.20 $\pm$ 0.06b	2.23 $\pm$ 0.08b	6.02	<u>0.003</u>
	Second instar	1.68 $\pm$ 0.08	1.57 $\pm$ 0.07	1.58 $\pm$ 0.08	0.63	0.53
	Third instar	1.98 $\pm$ 0.06a	1.76 $\pm$ 0.09ab	1.71 $\pm$ 0.07b	3.76	<u>0.025</u>
	Fourth instar	3.32 $\pm$ 0.10	3.29 $\pm$ 0.09	3.27 $\pm$ 0.08	0.08	0.93
	Pre-pupae	2.16 $\pm$ 0.09a	2.06 $\pm$ 0.08ab	1.85 $\pm$ 0.07b	3.91	<u>0.022</u>
	Pupae	5.92 $\pm$ 0.08	5.94 $\pm$ 0.07	6.13 $\pm$ 0.06	2.95	0.06
	Total	17.78 $\pm$ 0.18a	16.82 $\pm$ 0.16b	16.77 $\pm$ 0.12b	8.61	<u>< 0.001</u>
<i>S. subvillosus</i>	First instar	2.22 $\pm$ 0.09b	2.62 $\pm$ 0.09a	2.10 $\pm$ 0.10b	8.39	<u>< 0.001</u>
	Second instar	1.90 $\pm$ 0.11a	1.50 $\pm$ 0.07b	1.61 $\pm$ 0.09ab	5.28	<u>0.006</u>
	Third instar	2.00 $\pm$ 0.09a	1.48 $\pm$ 0.08b	1.59 $\pm$ 0.09b	9.62	<u>< 0.001</u>
	Fourth instar	3.24 $\pm$ 0.13a	2.77 $\pm$ 0.12b	2.98 $\pm$ 0.12ab	3.62	<u>0.03</u>
	Pre-pupae	2.44 $\pm$ 0.11	2.29 $\pm$ 0.09	2.59 $\pm$ 0.10	2.30	0.100
	Pupae	5.62 $\pm$ 0.11	5.48 $\pm$ 0.08	5.65 $\pm$ 0.07	1.09	0.34
	Total	17.42 $\pm$ 0.23a	16.13 $\pm$ 0.16b	16.51 $\pm$ 0.19b	11.42	<u>< 0.001</u>

Table 2. Mean weight (mg $\pm$ SE) and area (mm² $\pm$ SE) of *S. subvillosus* and *S. interruptus* when reared on *A. spiraecola*, *A. gossypii* and *A. spiraecola* + *A. gossypii* (df = 2, 150). Within rows means followed by different letters are significantly different ($P < 0.05$; Tukey test).

Species		Diet			F value	P value
		<i>A. spiraecola</i>	<i>A. gossypii</i>	<i>A. spiraecola</i> + <i>A. gossypii</i>		
<i>S. interruptus</i>	Pupation (%)	100	100	100	1.04	0.36
	Male weight	0.97 $\pm$ 0.07	0.88 $\pm$ 0.05	0.95 $\pm$ 0.06	0.46	0.63
	Female weight	1.08 $\pm$ 0.06	1.21 $\pm$ 0.10	1.33 $\pm$ 0.07	2.38	0.10
	Male area	7.30 $\pm$ 0.11b	7.74 $\pm$ 0.10a	7.94 $\pm$ 0.14a	7.92	<u>< 0.001</u>
	Female area	9.61 $\pm$ 0.26	9.65 $\pm$ 0.27	9.83 $\pm$ 0.26	0.17	0.85
<i>S. subvillosus</i>	Pupation (%)	96.49 $\pm$ 1.96	97.01 $\pm$ 1.68	98.46 $\pm$ 1.34	0.25	0.78
	Male weight	1.38 $\pm$ 0.07	1.45 $\pm$ 0.06	1.45 $\pm$ 0.07	0.35	0.71
	Female weight	1.52 $\pm$ 0.05	1.53 $\pm$ 0.06	1.56 $\pm$ 0.06	0.12	0.89
	Male area	10.30 $\pm$ 0.25	10.31 $\pm$ 0.27	10.27 $\pm$ 0.30	0.006	0.99
	Female area	10.76 $\pm$ 0.25	11.11 $\pm$ 0.29	10.62 $\pm$ 0.35	0.64	0.53

Table 3. Reproductive parameters of *S. subvillosus* and *S. interruptus* when reared on *A. spiraecola*, *A. gossypii* and *A. spiraecola* + *A. gossypii*. Within rows means followed by different letters are significantly different ($P < 0.05$; Tukey test).

Species	Reproductive parameters	Diet			F-value	df	P-value
		<i>A. spiraecola</i>	<i>A. gossypii</i>	<i>A. spiraecola</i> + <i>A. gossypii</i>			
<i>S. interruptus</i>	Fecundity (total eggs)	115.69 ± 1.62c	158.69 ± 1.91b	174.00 ± 2.21a	326.30	2, 41	< 0.001
	Fertility (%) (hatched eggs)	81.44 ± 2.52	86.32 ± 2.26	84.89 ± 2.57	1.37	2, 901	0.26
	Daily fecundity (eggs/day)	2.57 ± 0.12b	3.53 ± 0.12a	3.87 ± 0.15a	30.88	2, 1977	< 0.001
	Incubation period (days)	4.63 ± 0.05	4.54 ± 0.03	4.51 ± 0.04	2.28	2, 760	0.10
<i>S. subvillosus</i>	Fecundity (total eggs)	261.29 ± 4.02c	272.86 ± 3.37b	361.82 ± 3.22a	384.71	2, 42	< 0.001
	Fertility (%) (hatched eggs)	79.78 ± 7.89b	91.54 ± 5.45a	89.95 ± 4.07a	11.40	2, 993	< 0.001
	Daily fecundity (eggs/day)	5.81 ± 0.25b	6.06 ± 0.20b	8.04 ± 0.23a	24.38	2, 2022	< 0.001
	Incubation period (days)	3.27 ± 0.04b	3.08 ± 0.03c	3.41 ± 0.03a	24.84	2, 870	< 0.001

Table 4. Life table parameters of *S. subvillosus* and *S. interruptus* females when reared on 1) *A. spiraecola*, 2) *A. gossypii* and 3) *A. spiraecola* + *A. gossypii* (df = 2, 41 for *S. interruptus* and df = 2, 42 for *S. subvillosus*). Within rows means followed by different letters are significantly different ($P < 0.05$; Tukey test).

Species	Life table parameters	Diets			F-value	P-value
		<i>A. spiraecola</i>	<i>A. gossypii</i>	<i>A. spiraecola</i> + <i>A. gossypii</i>		
<i>S. interruptus</i>	Net reproductive rate, R_0 (♀/♀)	51.82 ± 0.79c	73.79 ± 0.91b	83.52 ± 1.06a	422.54	< 0.001
	Intrinsic rate of increase, r_m (♀/♀/day)	0.11 ± 0.0006c	0.12 ± 0.0003b	0.13 ± 0.0005a	629.33	< 0.001
	Finite rate of increase, λ_m (♀/♀/day)	1.12 ± 0.0006c	1.12 ± 0.0004b	1.14 ± 0.0005a	630.69	< 0.001
	Generation time, T (days)	40.90 ± 0.40a	43.63 ± 0.12b	40.41 ± 0.17a	87.569	< 0.001
	Doubling time, DT (days)	7.18 ± 0.06a	7.03 ± 0.02b	6.33 ± 0.03c	207.62	< 0.001
<i>S. subvillosus</i>	Net reproductive rate, R_0 (♀/♀)	82.31 ± 1.27c	87.31 ± 1.08b	137.86 ± 1.28a	1009.06	< 0.001
	Intrinsic rate of increase, r_m (♀/♀/day)	0.14 ± 0.0004c	0.14 ± 0.0003b	0.16 ± 0.0002a	1477.10	< 0.001
	Finite rate of increase, λ_m (♀/♀/day)	1.15 ± 0.0005c	1.15 ± 0.0003b	1.17 ± 0.0003a	1492.00	< 0.001
	Generation time, T (days)	35.31 ± 0.14b	37.23 ± 0.11a	37.44 ± 0.18a	92.51	< 0.001
	Doubling time, DT (days)	5.55 ± 0.02b	5.77 ± 0.01a	5.27 ± 0.02c	326.07	< 0.001