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**Effects of citrus overwintering predators, host plant phenology and environmental variables on aphid infestation dynamics in clementine citrus**

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## **Abstract**

The Spirea citrus aphid, *Aphis spiraecola* Patch and cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) are key pests of clementine mandarines in the Mediterranean basin. Severity of aphid infestations is determined by environmental variables, host plant phenology patterns and the biological control exerted by their associated natural enemies. However, there is no information about the role these limiting and regulating factors play. Aphid densities, citrus phenology and associated predators that overwinter in the crop were monitored weekly throughout 2 shooting periods (February to July) in 4 clementine mandarin groves. Relationships between these parameters and environmental variables (temperature and precipitation) were studied. Our results show exponential increase in aphid infestation levels to coincide with citrus phenological stages B3 and B4; shoots offer more space and nutritional resources for colony growth at these stages. Duration of these phenological stages, which was mediated by mean temperature, seems to importantly determine the severity of aphid infestations in the groves. Micro-coccinellids, mostly *Scymnus* species, were the only group of studied predators with the ability to efficiently regulate aphid populations. These natural enemies showed the highest temporal and spatial demographic stability. Aphid regulation success was only achieved through their early presence in the grove; at the aphid colonization phase. Our results suggest that conservation strategies aimed at preserving and enhancing *Scymnus* sp. populations may make an important contribution to the future success of the biological control of these key citrus pests.

**Keywords:** Conservation biological control, phenology, temperature, predator, *Scymnus*

## **Introduction**

The Spirea citrus aphid, *Aphis spiraecola* Patch, and the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) are major pests of clementine mandarin crops (*Citrus clementina* Hort. ex Tan) in the Mediterranean basin (Marroquín et al. 2004; Franco et al. 2006; Yahiaoui et al. 2009; Hermoso de Mendoza et al. 2012; Vacante and Gerson 2012; Belati and Belabed 2014). Both aphid species use fresh, un-hardened plant tissue (shoots) for development and reproduction on citrus. Infestations consequently take place during the main sprouting periods of the season and, under the Mediterranean growing conditions, are most important in spring when citrus trees are at the height of their shoot growth (Llorens 1990; Hermoso de Mendoza et al. 2006; Lebbal and Laamari 2016).

Sap removal during aphid feeding activity causes a general weakening of the plant. At high pest densities tree growth is delayed and production reduced (Hermoso de Mendoza et al. 2001). In the case of *A. spiraecola*, the interaction of saliva with plant tissues causes leaf curl deformation (Hermoso de Mendoza et al. 2006). Colonies of this species use this strategy to protect themselves from natural enemies and adverse environmental conditions (Cole 1925; Wang and Tsai 2000; van Emden and Harrington 2007). Development of sooty mould on leaves and fruit as a consequence of the honeydew secreted by aphids also indirectly affects tree growth, production and causes cosmetic damage to fruit. Honeydew also serves as source of carbohydrate for ants that in turn protect aphid colonies from their natural enemies (Calabuig et al. 2015). Both aphid species are known vectors of Citrus Tristeza Virus (CTV); *A. gossypii* is considered to be the most efficient one in the Mediterranean basin (Raccah et al. 1976; Hermoso de Mendoza et al. 1984; Cambra et al. 2000).

Under current crop management conditions, regulation exerted by natural enemies is generally not sufficient to keep aphid densities below their economic threshold in the Mediterranean clementine mandarin agroecosystems during the spring citrus growing period (Hermoso de Mendoza et al. 2001, 2006; Urbaneja et al. 2018). Successful pest control must therefore rely on at least one insecticide application. The extensive use of neonicotinoids, either as a preventive or curative tactic for aphid control in clementine mandarins, greatly hinders the implementation of integrated pest management principles and highlights the urgency to develop less detrimental aphid control alternatives (Wang et al. 2017; Urbaneja et al. 2018).

Aphid densities are known to be strongly modulated by environmental variables such as temperature or precipitation, along with biotic factors associated with their host; among them, are abundance and quality of shoots (Zhou et al. 1995; Awmack and Leather 2002). The temperature range for *A. spiraecola* survival and proliferation is between 2.3°C and 35°C and between 6.2° and 35°C for *A. gossypii*; therefore, temperatures below and above these ranges limit their infestation potential (Kersting et al. 1999; Wang and Tsai 2000). Heavy rain could mechanically disturb aphid colonies. Nevertheless studies that evaluated the effect of rain on aphid populations in perennial crops did not find important demographic reductions even after torrential downpours (Kaakeh and Dutcher 1993; Michaud 1999). Shoots are an ephemeral resource essential to aphid development. Their presence in citrus occurs in specific periods of the year; thus the risk of infestation is confined to those periods of citrus shoot growth. The three major sprouting periods of spring, beginning of summer and mid-autumn have been identified under Mediterranean basin crop conditions; spring being the most important in terms of shoot abundance (Hermoso de Mendoza et al. 2012; Lebbal and Laamari 2016). Cultivars and species of citrus strongly determine plant growing patterns. Crop management practices also have an important

influence on citrus phenology (Iglesias et al. 2007). Abiotic variables such as temperature and precipitation ultimately mediate citrus phenology throughout the season. Nevertheless, there is no information about how all these limiting factors specifically influence the severity of aphid infestations in citrus of western Mediterranean basin.

In the last two decades numerous efforts have been addressed to understand the causes restraining aphid biological control success in citrus. In this search, complex trophic interactions between *A. spiraecola*, its primary parasitoid, *Binodoxys angelicae* (Haliday), and six hyperparasitoids have completely nullified the potential control of this pest by parasitism (Michelena and Sanchis 1997; Gómez-Marco et al. 2015). A rich predatory assemblage has been described to feed on aphid colonies (Hermoso de Mendoza et al. 2012). These authors observed a demographic synchrony between aphid and predator densities with the typical predator-prey response lag. Nevertheless, the high reproductive rate of aphids makes this lag sufficient to escape from an efficient biological control. Aphidophagous predators achieve maximum impact if they arrive early and in sufficient numbers (Michaud 2012). Recent studies aimed at reducing the numeric lag response of predators demonstrated the use of monocotyledon based crop covers help advance the arrival of aphidophagous predators in the beginning of spring. Earlier arrival resulted in a significant reduction of aphid densities (Gómez-Marco et al. 2016a, b). Nevertheless, the components of the predatory complex that functionally contribute to aphid regulation are not yet well known.

This research thus seeks to understand how environmental and host plant related biotic factors influence the infestation potential of *A. gossypii* and *A. spiraecola* in clementine mandarins and how biotic community factors regulate their populations during the spring sprouting period. The specific objectives were i) understand the dynamics response of aphid populations in clementine mandarin groves to temperature, precipitation and the phenology of its host; and ii) analyse the relationship between aphid infestation rates and the aphid predator groups that overwinter in the crop.

## **2. Material and methods**

### **2.1. Location and experimental conditions**

The studies were conducted during the spring seasons of 2015 and 2016 in four clementine mandarins (*Citrus clementina* Hort. ex Tan) plots containing approximately 15 year-old trees at the Valencian citrus growing region. Two of them were experimental plots belonging to the Valencian Institute of Agricultural Research (IVIA) in Moncada (39°35'17.43"N / 0°23'53.28"O) [Valencia Province] and the Jaume I University (UJI) in Castellón (39°59'29.92"N / 0° 4'12.77"O) [Castellón Province]. The other two were commercial plots

located in Algimia (39°42'55.11"N / 0°18'57.46"O) [Valencia Province] and Ribesalbes (40° 0'53.30"N / 0° 8'48.21"O) [Castellon Province]. All plots were drip-irrigated and had not been treated with insecticides for at least two years before the onset of the study and during the course of it.

## 2.2. Meteorological data

Daily mean temperatures and rainfall from June 21, 2014 to June 20, 2016 were obtained from the 'Sistema de Información Agroclimática para el Regadío' (SIAR, [www.siar.es](http://www.siar.es)) database, using daily summaries from two meteorological stations: Moncada IVIA (V101) at Valencia Province, and Castellón Benadresa (CS05) at Castellón Province. The greatest distance between meteorological stations and study plots was 15 km.

## 2.3. Temporal sampling of aphids, citrus phenology and overwintering predators

From February to July in 2015 and 2016, aphid populations were monitored weekly on 10 trees haphazardly selected in a diagonal transect in each plot. A 0.25 m<sup>2</sup> ring was placed on two points of the surface of the canopy of each tree. The number of suitable aphid-infested and un-infested shoots within the ring area was counted (Hermoso de Mendoza et al. 2001, 2006).

The dominant phenological stage of clementine shoots in each ring was also recorded. A simplified BBCH phenological scale for citrus was used (Garrán et al. 1993; Agustí et al. 1997). Specifically, buds from the beginning of lengthening until shoots reach 20% of their final size were assigned to the **B1** stage (corresponding to the values of the BBCH scale: 31-32); we assigned shoots between 20% and 40% of their final size to the **B2** stage (BBCH: 32-34); **B3** was defined as shoots with 40% - 70% of their final size (BBCH: 34-37); **B4**, shoots with 70% -90% of their final size (BBCH: 37-39); **B5**, tender shoots with final size (BBCH: 19); and **B6**, shoots with final size and leaves totally mature and hardened (BBCH: 91).

In the same trees selected for phenology and aphid surveys, potential predators of aphids were also monitored at the same time by stem-tap sampling. Two haphazardly selected branches per tree were struck three times with a 40 cm-length of PVC pipe. A plastic tray (45 x 30 cm) was placed under each branch to capture the dislodged arthropods. Predators were identified *in-situ*. In the cases in which determinations to species level were not possible, specimens were collected with an entomological aspirator and determined in the laboratory under stereoscope microscope (Smirnoff 1973; Alvis 2003; Montoya and José 2016).

## 2.4. Spatial sampling of aphids and predators

Using the same methodologies previously described to measure aphid and predator populations, an extensive sampling was carried out through the main citrus growing areas of the Valencia region, representing an area of approximately 240,000 ha. From April 22 to May 31, 2016, at the end of the spring major citrus

sprouting period, 60 commercial citrus plots containing aphid infestations and with trees at **B3** to **B5** phenological stages were randomly selected. The minimum distance between plots was 0.5 km.

## 2.5. Statistical analysis

Meteorological data were grouped per month. Differences in mean temperatures between meteorological stations, years, and months were studied using general linear model analysis (Wolfinger and O'connell 1993). Normal distribution of the variable was tested prior to the model selection.

Differences in citrus shooting patterns between years were studied by comparing the following response variables: greatest shoot density, day when greatest shoot density was reached, day of the beginning of B2 phenological phase (upon which aphid colonies start to develop), and days of duration of B3 and B4 phenological phases (upon which aphid colonies can exhibit their highest growth potential). Student's *t*-test was used in all the cases and 'year' was used as predictor variable. Prior to model selection, data were tested for normality. Pearson Correlation was used to study potential relationships between duration of B3 and B4 phenological stages and mean temperatures during these phenological phases.

Differences in aphid infestation patterns between years were studied by comparing the following response variables: beginning of the first colonies and day of highest infestation rate. Student's *t*-test for two samples was used and 'year' was selected as the predictor variable. Percentage of shoots infested by *A. gossypii* and *A. spiraecola* in the spatial sampling was also compared using a Student's *t*-test for two samples.

Temporal and spatial variability of the activity-density of the most important predator groups associated to aphids in citrus was also compared. Coefficients of Variation (ratio of the standard deviation  $\sigma$  to the mean  $\mu$ ) were calculated for the data collected by stem-taps of each predatory group (micro-coccinellids, macro-coccinellids, neuropterans, hemipterans and dermapterans) in each sampling date and citrus grove. Differences in temporal variability between groups was studied through general linear model analysis. Differences between predator groups in the beginning of their seasonal activity (after the winter period) was studied by comparing the date for which 10% of all captured individuals was reached each year. General linear model analysis was used wherein 'date of 10% of all captures' was the response variable and 'predator groups' the explanatory variable. Post-hoc *t*-test (Tukey) comparisons were made in each case having a significant effect ( $P < 0.05$ ) for the two analyses.

To study the potential relationship between peak aphid infestation levels and the presence of natural enemies early in the season and throughout the spring aphid infestation period, the cumulative number of individuals captured by stem-tap sampling of each predatory group, i) from the beginning of the sampling until

the beginning of aphid infestations and ii) throughout the study period, was related through linear regression to the maximum aphid infestation rates measured in each grove and year.

### 3. Results

#### 3.1. Meteorological data

The average temperatures in the study period varied between 10.5 °C and 22.7 °C in the months of January and June, respectively (**Figure 1**).

There were no differences in mean temperatures between years ( $t = 1.15$ ,  $df = 1$ , 1460,  $P = 0.28$ ) for the whole study period. Nevertheless, winter months (December-January-February) were significantly colder in year 1 than year 2 ( $F = 65.77$ ,  $df = 1$ , 360,  $P < 0.0001$ ); early spring (March-April) had similar mean temperatures between years ( $F = 0.20$ ,  $df = 1$ , 242,  $P = 0.66$ ) whereas mean temperatures in late spring (May-June) were significantly higher in year 1 than year 2 at the two weather stations ( $F = 9.38$ ,  $df = 1$ , 242,  $P = 0.002$ ).

Accumulated rainfall throughout the months of the study was higher in both stations in year 1 than year 2, and higher in the Benadresa than in the Moncada weather station. Temporal rainfall distribution throughout the study period was similar in the two weather stations with the greatest precipitation in December, March and June in year 1 and in December, April and May in year 2.

#### 3.2. Citrus phenology

Clementine shooting patterns were similar between plots, but differed between years of study (**Figure 2**). Highest shoot density occurred in year 1 ( $t = 66.69$ ,  $df = 1$ , 6,  $P < 0.0001$ ) but appeared earlier in year 2 ( $t = 20.06$ ,  $df = 1$ , 6,  $P = 0.004$ ) (**table 1**). Similarly, the beginning of B2 phenological phase, upon which aphid colonies start to develop, occurred approximately 25 days earlier in year 2 than year 1 ( $t = 67.00$ ,  $df = 1$ , 6,  $P = 0.0002$ ). Duration of B3 and B4 phenological phases, upon which aphid colonies can exhibit their highest growth potential, was nevertheless significantly longer in year 2 than year 1 ( $t = 23.57$ ,  $df = 1$ , 5,  $P = 0.005$ ) (**Table 1**). Duration of these phases was strongly correlated with temperatures during that period of time ( $\rho = -0.94$ ,  $P = 0.0017$ ).

#### 3.3. Aphid phenology

Aphid infestation patterns were closely related to citrus shooting patterns. Aphid colonies were first found on average  $10.78 \pm 4.64$  days later than the beginning of B2 phase with no differences between years ( $t = 1.123$ ,  $df = 1$ , 6,  $P = 0.330$ ). Exponential growth of aphid infestations in groves coincided with B3 and B4 phenological phases (**Figure 2**). Peak infestation rates observed were  $35.14 \pm 3.18$  days after the beginning of



aphid infestations with no differences between years ( $t = 1.58$ ,  $df = 1, 5$ ,  $P = 0.175$ ). Proportion of infested shoots exceeded the economic threshold of 25% of infested shoots, above which insecticide treatments are recommended (Hermonso de Mendoza et al. 2001, 2006), in two groves in year 1 (Algimia and Castellón) and in all groves in year 2 (**Figure 2**). This threshold was exceeded two weeks earlier in year 2 than in year 1.

#### 3.4. Citrus aphid species

In 2016 *Aphis spiraecola* and *A. gossypii* were the only two aphid species found that were associated with citrus groves in the spatial sampling across the Valencia region. *Aphis spiraecola* was present in all the groves whereas *A. gossypii* was found in only 62% of the groves. In all cases, infestations with *A. gossypii* were associated to *A. spiraecola*; whereas in 38% of all groves sampled *A. spiraecola* was the only species found. Average infestation rate was  $50.0 \pm 5.5\%$  of infested shoots. Percentage of total shoots infested by *A. spiraecola* ( $45.5 \pm 5.4\%$ ) was significantly higher than those infested by *A. gossypii* ( $12.3 \pm 3.5\%$ ) ( $t = 7.92$ ,  $df = 1, 118$ ,  $P < 0.0001$ ). *Aphis spiraecola* colonies were mostly pure colonies (84% of the observed colonies) whereas most of *A. gossypii* colonies (63%) were mixed with *A. spiraecola* specimens.

#### 3.5. Overwintering aphid predators

Sixteen species of predators commonly associated with aphid species were found by the stem-tap sampling in the four study sites and in both of two years of data collection (**Table 2**). Coccinellidae was the group with the greatest abundance and species richness. Within this family, micro-coccinellid species measuring less than 3 mm long were the most abundant with more than 1,600 specimens collected belonging to 4 species. *Scymnus subvillosus* (Goeze) and *Scymnus interruptus* (Goeze) were the most captured species, whereas *Rhyzobius litura* (Fabricius) and *Scymnus mediterraneus* Khnzorian were captured less frequently. Among the macro-coccinellid species measuring more than 3 mm long *Propylea quatuordecimpunctata* (L.) was the most captured. Neuroptera was the second group of predators in terms of captures being *Chrysoperla carnea* (Stephens) the dominant species within this group with 83.7% of all captures. Only one Hemipteran species, *Pilophorus cf. gallicus* (Hemiptera: Miridae), was found with most of its captures occurring in year 2 in Moncada. *Forficula auricularia* L. (Dermaptera: Forficulidae) was found in three of the plots sampled but in low abundance.

Seasonal and spatial patterns of the predator groups found associated with aphids in the four study sites strongly varied (**Figure 3** and **Table 3**). Micro-coccinellids presented the lowest temporal and spatial variability being present throughout all the study period in all the study sites. Macro-coccinellids on the contrary, presented an important spatial variability, along with the highest temporal variability, with marked seasonal-activity peaks

after aphid infestations. The Neuropteran group was the second less variable in terms of captures throughout the study period and the third one as for spatial variability. Hemipterans also presented a marked seasonal-activity with a well-defined activity peak after aphid infestations, and the highest spatial variability with most of the captures originating from only one of the sampled groves. The micro-coccinellids group was detected earlier in the season (**Table 3**), with 10% of all their captures obtained just two weeks after the beginning of sampling. Neuroptera was the second group with early seasonal-activity. 10% of all their captures were obtained approximately 30 days after the beginning of sampling, whereas this threshold was reached 40-45 days after the beginning of sampling for the rest of the groups.

### 3.6. Regional distribution of Coccinellidae

The micro-coccinellid *S. subvillosus* was the most captured species in the 60 sampled citrus groves through Valencia region; it was present in 80% of them (**Table 4**). The macro-coccinellid *P. quatuordecimpunctata*, was the second most captured species and was also widely distributed in the region being present in more than 70% of the plots sampled. *Scymnus interruptus* was the third species in terms of captures and was found in more than 50% of the sampled plots. The other species of coccinellids had a marginal role in terms of captures and distribution in the Valencia region.

### 3.7. Aphid-predator relationship

Maximum aphid infestation rates in the study sites were negatively correlated to the cumulative number of *Scymnus* spp. measured from the beginning of the sampling until the beginning of B2 (**Figure 4** and **Table 5**). Nevertheless, no relationship was found between the maximum aphid infestation rates and the cumulative number of *Scymnus* spp. measured throughout the study period. No relationships were found between maximum aphid infestation rates and the cumulative number of predators measured, for the rest of aphid predator groups (macro-coccinellids, neuropterans and hemipterans), neither at the beginning of aphid infestations (B2 phenological state) nor throughout the study period.

## **4. Discussion**

*Aphis gossypii* and *A. spiraecola* were the only two aphid species found associated with clementine citrus crops in our study; the former being the dominant one. Eight species of aphids have been previously described to be associated with citrus crops in Spain but only *A. gossypii* and *A. spiraecola* are considered as being of economic importance in the crop (Hermoso de Mendoza et al. 2012). Similarly, *A. spiraecola* is the most abundant aphid species in citrus on the Mediterranean Basin (Algeria, Morocco, Tunisia, Italy, Spain) and in other countries such as the USA and Japan (Marroquín et al. 2004; Franco et al. 2006; Yahiaoui et al. 2009;

Hermoso de Mendoza et al. 2012; Vacante and Gerson 2012; Belati and Belabed 2014). The relative dominance of *A. spiraecola* over *A. gossypii* nevertheless may vary according to specific environmental conditions or crop management factors (Hermoso de Mendoza et al. 1997). Several studies sought to explain this phenomenon. The deformation produced by *A. spiraecola* saliva in the citrus shoot is thought to be a key factor since it confers an ecological advantage by protecting its colonies against adverse environmental conditions and natural enemies (Cole 1925; Kadioglu et al. 2012). *Aphis gossypii*, unlike *A. spiraecola*, is also known to be more sensitive to the detrimental effects of high concentrations of some plant metabolites such as free proline which is produced in larger quantities in response to physiological stresses common under the Mediterranean growing conditions, such as water deficits (Mostefaoui et al. 2014).

Temperature and precipitation do not seem to directly limit the development of *A. gossypii* and *A. spiraecola* in western Mediterranean citrus agroecosystems (Ro et al. 1998; Alyokhin et al. 2011; Lu et al. 2015; Holloway et al. 2018). Both the lower and higher thermal developmental thresholds for the two aphid species (*A. spiraecola*: 2.3 °C and 35 °C; *A. gossypii*: 6.2 °C and 35 °C) (Wang and Tsai 2000; Kersting et al. 1999) were never reached throughout our study period. Heavy rain has been previously shown not to importantly affect the development of aphid colonies in citrus of growing regions where the precipitation regime is characterized by the frequency of torrential rain (Michaud 1999).

Our results nevertheless proved the great influence of these two environmental variables on aphid infestation dynamics through their effects on citrus plant phenology. Both temperature and precipitation importantly affect the abundance and frequency of citrus sprouting, its timing throughout the season as well as the duration of each citrus vegetative and reproductive stage (Iglesias et al. 2007; Udell et al. 2017). Growth stages are accelerated by higher temperatures but low temperatures during the dormant season are required to release the dormancy of the buds in citrus trees and also influence the intensity of spring sprouting (Iglesias et al. 2007). This was clearly observed in our research; differences in the winter temperatures between years resulted in distinct sprouting behaviours at the beginning of the growing season. Lower winter temperatures of the first year caused a greater but also later sprouting as well as a prolongation of the initial phenological stages of the clementine flushes. The greater amount of rainfall accumulated during winter and early spring of the first year probably also explained the greater sprouting activity in all the study groves.

Aphid infestations in citrus are closely related to plant phenology since these insects require tender growing tissues to develop (Zhou et al. 1995; Awmack and Leather 2002). In the present study we were able to parameterize this association. Foundational aphid colonies were observed to be formed upon B2 phenological

stage (shoots with 20% - 40% of their final size, corresponding to BBCH: 32-34). The exponential increase in aphid infestation levels coincided in most cases with phenological stage B3 in clementines (shoots with 40% - 70% of their final size, corresponding to BBCH: 34-37), when shoots offer more space and nutritional resources for colony growth. The maximum outbreak infestation rate occurred when the shoots reached stage B4 (shoots with 70% -90% of their final size, corresponding to BBCH: 37-39); probably due to the fact that at this phase shoots are sufficiently developed and big enough to accommodate large aphid colonies and still offer high quality food resources (Sétamou et al. 2016). Infestation rates rapidly declined once B5 phase was reached; which is probably associated with the rapid reduction on the concentrations of total and essential amino acids in phloem sap once flush shoots mature, and with the increasing rigidity of leaves in response to calcium supply at this phase (Sétamou et al. 2016; Cifuentes-Arenas et al. 2018).

The important differences in the duration of B3 and B4 phenological stages (when aphid colonies are expected to exhibit their highest potential growth) observed between the two years may be the reason behind the distinct infestation rates observed. The period covered by these two phenological stages was almost three times shorter the first year than in the second one. The duration of B3 and B4 was on the other hand strongly correlated to the mean temperatures during that period of time. According to Wang and Tsai (2000) and Kersting et al. (1999), at the measured temperatures in that period and the duration of these two phenological phases, both *A. spiraecola* and *A. gossypii* could complete just one generation during the first year (18.8 days at 19.27°C) yet three generations during the second year (47.3 days at 15.05°C). This may explain the higher infestation rates observed in the second year (83.2% on average) than the first year (48.8% on average).

During the two years of study, peak infestation rates of aphids were registered at the end of April in Valencia province and mid-May in Castellon province study sites. This is approximately one month earlier to what was previously recorded in the same region 20 years ago (Meliá 1995; Hermoso de Mendoza et al. 1997). Further research would therefore be needed to elucidate how global climatic change may be influencing on the phenology of these citrus key pests and which will be the consequences for their management.

Citrus aphids in the Western Mediterranean region have been previously associated with a rich guild of predators belonging to several taxonomical orders (Michelena and Sanchis 1997; Alvis 2003; Hermoso de Mendoza et al. 2012). The most frequently captured predator species in our study that are already present in citrus groves before aphid infestations begin, coincided with what was previously observed: the micro-coccinellids *S. subvillosus* and *S. interruptus* and the macro-coccinellid *P. quatuordecimpunctata* were the dominant aphidophagous predators. Of all the predator groups found in this study, micro-coccinellids had the

most homogenous populations throughout the season but also across the study sites. The macro-coccinellids showed the highest seasonality with peak populations strongly associated to the spring aphid infestation. Differences in demographic seasonal patterns between the two groups of coccinellids could be associated with the behavior of gravid females; oviposition is influenced by the existence of sufficient resources for their offspring (Hodek et al. 2012). Models of reproductive behavior of predators, usually assume egg production to be directly regulated by the rate of food consumption (Kindlmann and Dixon 1999, 2001). Resources required by micro-coccinellids are expected to be lower than those for macro-coccinellids. In addition, recent research demonstrates *Scymnus* species to frequently exploit alternative food resources such as nectar, pollen and other arthropods when its primary prey (aphids) is scarce (Hodek et al. 2012; Bouvet et al. 2019). The other groups of predators registered in this study (neuropterans, mirids, and dermapterans) did not present a defined pattern in their activity-density. Their more erratic activity makes their effective management for biological purposes a future challenge for integrate pest management strategies in this crop. Further studies will be required to understand the factors that modulate their populations. The predatory dipterans *Aphidoletes aphidimiza* (Diptera: Cecidomyiidae) and hoverflies (Diptera: Syrphidae) were not included in this study. Although larvae of these predators are commonly associated to citrus aphids colonies in spring (Hermoso de Mendoza et al. 2012), they were not found in the crop at the end of the winter when our sampling began. *Aphidoletes aphidimiza* (Diptera: Cecidomyiidae) overwinters as diapausing cocooned larvae underground (Kostal and Havelka, 2000). Hoverflies have strong flight and foraging abilities. Adults are able to migrate long distances (Odermatt et al. 2017) and they probably only visit citrus trees when searching for oviposition sites once aphids are already present (Almohamad et al. 2009). Further research would nevertheless be needed to understand the role of predatory dipterans in citrus aphid control.

The complex of aphidophagous predators is usually considered inefficient to control the populations of these phytophagous pests. Aphid colonies are characterized by their exponential growth as well as their further rapid decline (Kindlmann et al. 2010). Predators arriving to aphid colonies once they are established are therefore not able to respond quickly enough to the growth of the colonies (Brown 2004; van Emden and Harrington 2017). The success of aphid biological control by predators is attributed to effective control in two periods (Michaud 2012): i) the one carried out in the colonies before winter, which will form new colonies in the spring and ii) the one carried out early in spring, which will prevent the initial development of the colonies thus reducing further infestation rates in the growing season (Carroll and Hoyt 1984). Winter control is not considered to be a key factor in Mediterranean citrus agroecosystems since under those conditions both A.

*spiraecola* and *A. gossypii* are anholocyclic and females initiating spring infestations mostly come from non-citrus hosts (Barbagallo et al. 1986). A previous study in the region nevertheless showed that the early arrival of aphidophagous and polyphagous predators to the colonies of *A. spiraecola* in citrus clementine in spring was able to reduce their populations to levels below their pre-established economic thresholds (Gómez-Marco et al. 2015). The authors however, did not identify the predator groups that successfully controlled *A. spiraecola*. Our study demonstrates a successful reduction in aphid infestation levels in citrus to be strongly correlated to the early presence of micro-coccinellids (mostly *Scymnus* species). On the other hand, a high density of predators once the colonies are formed was not a guarantee of successful aphid biological control. The ability of *Scymnus* species to stay at a more homogenous population level throughout the season as well as their high frequency of captures throughout the extensive citrus growing region in which this study was conducted makes these natural enemies potential key predators of aphids in Mediterranean citrus agroecosystems. Further studies will be required to better understand the ecological and biological traits of these coccinellid species that currently limit their role as aphid biological control agents.

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## **Author contribution statement**

JPRB, CM and AU conceived and designed the research. JPRB and CM participated in data collection and analyses. JPRB and CM wrote the manuscript. All authors read and approved the manuscript.

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490

## Tables

	Maximum number of shoots	Days to peak	Days to B2	Days B3 to B4
<b>Year 1</b>	213.27 ± 9.50a	80.8 ± 1.8a	97.8 ± 3.1a	18.8 ± 1.9a
<b>Year 2</b>	89.07 ± 2.27b	68.5 ± 2.1b	72.0 ± 0.6b	47.3 ± 5.8b

**Table 1** Mean of maximum number of shoots per square meter ( $\pm$  SE) on tree canopy surface; days from December 21<sup>th</sup> to the peak of shoots, days to the beginning of B2 and days of duration of B3 to B4.

			Castellon		Moncada		Ribesalbes		Algimia		TOTAL	
Order	Family	Species	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2		
Coleoptera	Coccinellidae (Micro-coccinellid)	<i>Scymnus subvillosus</i> (Goeze, 1777)	95	299	79	221	125	62	72	3	956	
		<i>Scymnus interruptus</i> (Goeze, 1777)	17	88	64	156	3	1	200	6	535	
		<i>Rhyzobius litura</i> (Fabricius, 1787)	53	44	0	0	4	1	3	0	105	
		<i>Scymnus mediterraneus</i> Khnzorian, 1972	2	8	0	1	0	0	1	0	12	
	Coccinellidae (Macro-coccinellid)	<i>Propylea quatuordecimpunctata</i> (Linnaeus, 1758)	8	40	3	33	4	20	171	8	287	
		<i>Coccinella septempunctata</i> (Linnaeus, 1758)	7	0	32	0	0	0	14	0	53	
		<i>Adalia decempunctata</i> (Linnaeus, 1758)	12	36	0	0	1	1	1	0	51	
		<i>Hippodamia variegata</i> (Goeze, 1777)	20	3	1	0	0	0	2	0	26	
		<i>Adalia bipunctata</i> (Linnaeus, 1758)	1	0	1	0	0	0	2	0	4	
		<i>Cryptolaemus montrouzieri</i> Mulsant, 1850	0	0	0	0	0	0	0	2	2	
		Total Coleoptera		215	518	180	411	137	85	466	19	2031
		Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i> (Stephens, 1836)	689	137	8	30	3	52	102	100
	Coniopterygidae		<i>Semidalis aleyrodiformis</i> (Stephens, 1836)	4	20	1	10	3	32	15	28	113
			<i>Conwentzia psociformis</i> (Curtis, 1834)	0	0	10	2	2	1	3	20	38
Hemerobiidae	Brown lacewing spp		1	52	0	2	1	5	2	4	67	
Total Neuroptera			694	209	19	44	9	90	122	152	1339	
Hemiptera	Miridae	<i>Pilophorus cf gallicus</i> Remane, 1954	0	5	26	680	0	94	0	8	813	
		Total Hemiptera		0	5	26	680	0	94	0	8	813
Dermaptera	Forficulidae	<i>Forficula auricularia</i> Linnaeus, 1758	17	18	0	13	0	0	20	16	84	
		Total Dermaptera		17	18	0	13	0	0	20	16	84
		TOTAL SPECIMENS										4267

497 **Table 2** Predator abundance found by stem-taps; grouped by taxonomic groups (Order, Family and  
498 Specie), site and season.

Predator group	Temporal variability	Spatial variability	Day with 10% to abundance
Micro-coccinellid	1,08 ± 0.2c	0,75	15.8 ± 8.0b
Macro-coccinellid	1,67 ± 0.2a	1,90	41.2 ± 10.3a
Neuropteran	1,21 ± 0.2bc	1,22	31.5 ± 8.0ab
Hemipteran	1,61 ± 0.2a	2,32	44.2 ± 10.9a
Dermapteran	1,42 ± 0.2ab	0,85	45.6 ± 11.8a

**Table 3** Coefficients of variation of the different groups of predators associated with aphids in citrus; throughout all the sampling period (Temporal variability), between sampled plots (Spatial variability), and days from the first sampling date until 10% of all captures are obtained for each predator group.

Group	Species	Abundance	Mean	Number of plots present	% of plots present
Micro-coccinellid	<i>Scymnus subvillosus</i> (Goeze, 1777)	650	0.63 ± 0.04	42	81 %
	<i>Scymnus interruptus</i> (Goeze, 1777)	213	0.20 ± 0.02	28	54 %
	<i>Rhyzobius litura</i> (Fabricius, 1787)	6	0.01 ± 0.003	1	2 %
	<i>Scymnus rufipes</i> (Fabricius, 1798)	2	0.002 ± 0.001	1	2 %
	<b>Total</b>	<b>871</b>	<b>0,84</b>	<b>47</b>	<b>90 %</b>
Macro-coccinellid	<i>Propylea quatuordecimpunctata</i> (Linnaeus, 1758)	565	0,54 ± 0.04	38	73 %
	<i>Hippodamia variegata</i> (Goeze, 1777)	4	0,004 ± 0.002	2	4 %
	<i>Coccinella septempunctata</i> (Linnaeus, 1758)	3	0,003 ± 0.002	2	4 %
	Coccinellid larvae	48	0,05 ± 0.009	11	21 %
	<b>Total</b>	<b>620</b>	<b>0,60</b>	<b>42</b>	<b>81 %</b>

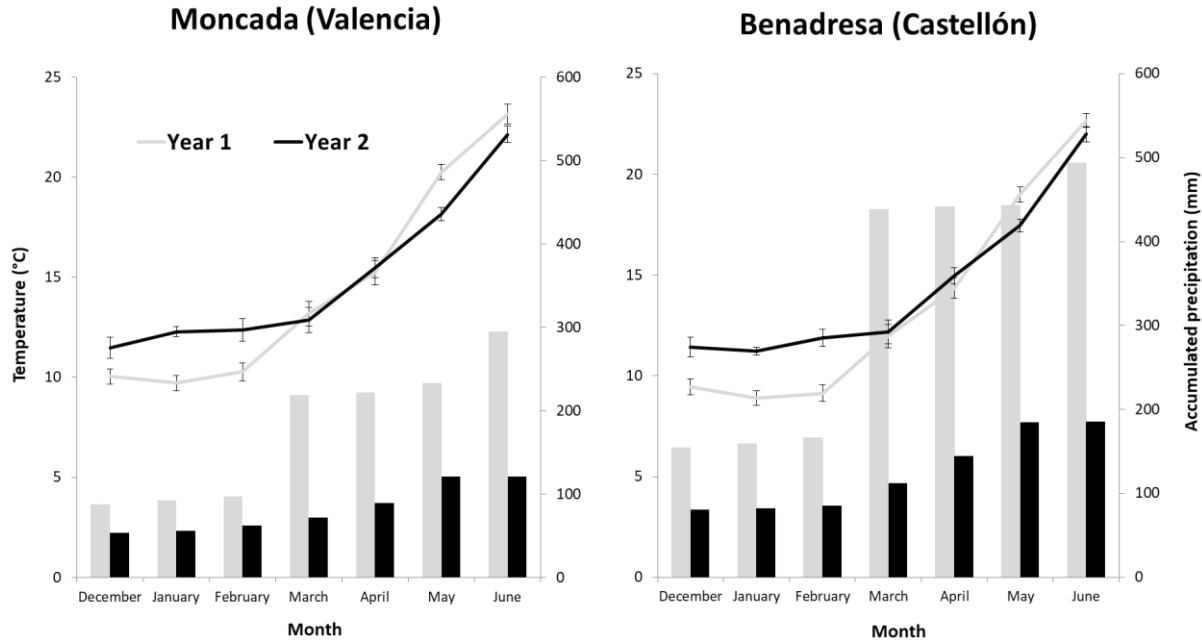
**Table 4** Coccinellid abundance found by stem-taps; mean per plots, presence of predators in number and percentage of plots. Specimens grouped in micro-coccinellids and macro-coccinellids according to the size (more or less than 3 mm long).

Group	Accumulated until	df <sub>error</sub>	F	P	r <sup>2</sup>	a	b	b, SE
<i>Scymnus spp</i>	B2	6	39,72	0,0007439	0,8469	41,54364	-0,41503	0,06585
<i>Scymnus spp</i>	B6	6	0,6445	0,4527	-0,0535	54,8337	0,9247	1,1518
Macro-coccinellid	B2	6	1,013	0,3531	0,001842	1,165065	-0,009479	0,009418
Macro-coccinellid	B6	6	0,5101	0,5019	-0,07525	-3,876	1,592	2,228
Neuropteran	B2	6	3,46E-02	0,9955	-0,1667	5,2388663	-0,0008533	0,145106
Neuropteran	B6	6	0,5641	0,481	-0,0664	32,394	1,157	1,54
Hemipteran	B2	6	NaN	NA	NaN	0	0	0
Hemipteran	B6	6	0,3365	0,583	-0,1047	-9,619	1,151	1,984

**Table 5** Estimated parameters of the relationship between the maximum aphid infestation rates measured in the study sites and the cumulative number of predators from the beginning of the sampling until two important phenological moments of clementine trees (B2 and B6) for aphid infestations.

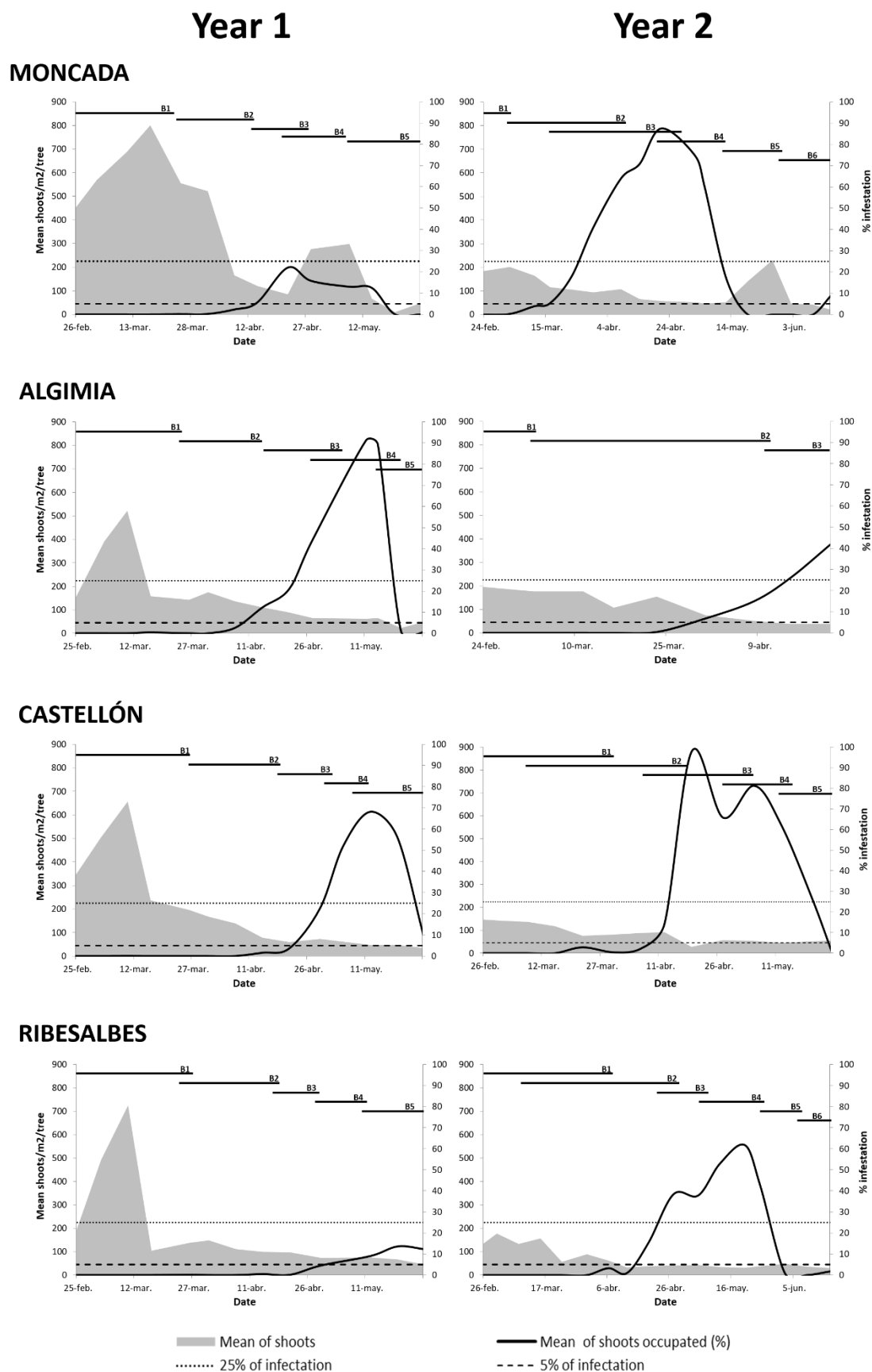
Figure

**Fig. 1** Mean temperature (line) and accumulated precipitation (bars) in two meteorological stations of the Valencian Region: Moncada IVIA (V101) Station in the province of Valencia and Castellón Benadresa (CS05) Station in the province of Castellón.

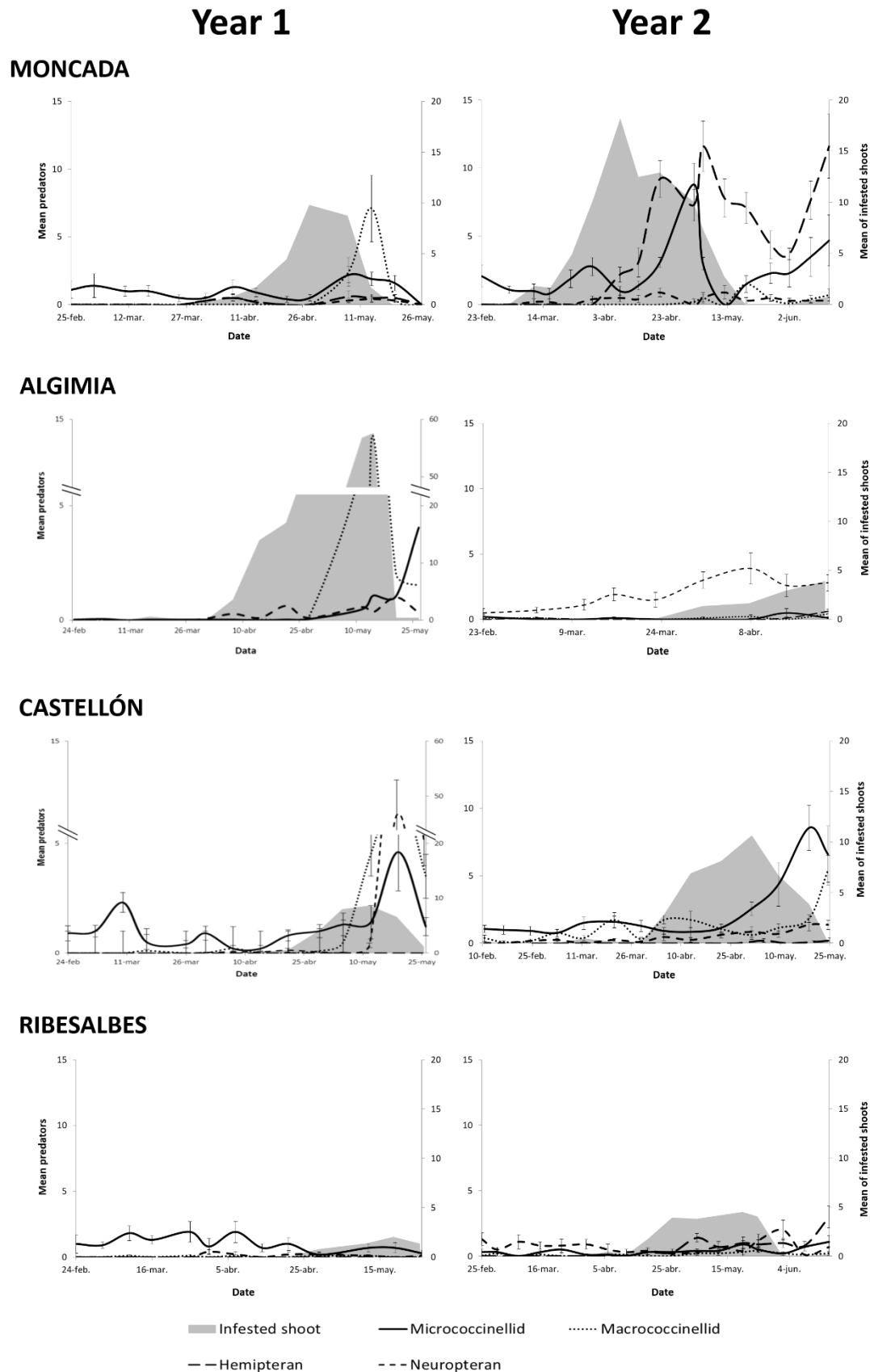




**Fig. 2** Mean number of shoots per square meter on tree canopy surface, percentage of shoots occupied by aphid colonies and type and duration of the dominant clementine tree phenological state measured in four clementine citrus groves from February to June in 2015 and 2016 seasons. Dotted lines represent the thresholds of 5% and 25% of shoots occupied by aphid colonies.



**Fig. 3** Seasonal-activity measured by stem-tap sampling of four groups of predators (*Scymnus* spp., macro-coccinellids, neuropterans and hemipterans) associated with aphid infestations in clementine trees and percentage of shoots occupied by aphid colonies from February to June of the years 2015 and 2016.



**Fig. 4** Linear regressions of the relationship between the maximum aphid infestation rates measured in the study sites and the cumulative number of predators from the beginning of the sampling until two important phenological moments of clementine trees for aphid infestations (B2 and B6).

