



Resource-dependent mutual association with sap-feeders and a high predation rate in the ant *Crematogaster scutellaris*: help or harm in olive pest control?

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Abstract *Crematogaster scutellaris* is a common tree-nesting ant in Mediterranean olive groves, where it acts as both predator and soft scale insect tender. We aimed to quantify the predatory action of this species and assess whether it influences the distribution of soft scale insects. Predation was investigated through experiments using live prey. The mutualistic relationship between ants and soft scales was assessed by supplementing sugar, amino acids or water to ant colonies, then monitoring for changes in scale tending activity. The probability of soft scale infestation on trees hosting or not hosting ant nests was also estimated. Predation rates on trees hosting a *C. scutellaris* nest were significantly higher than on trees without nests. Tending activity was affected by carbohydrate and, surprisingly, water availability. While the presence of *C. scutellaris* was significantly correlated to soft scale infestation, it also favoured predation towards other insects, suggesting a potential role in influencing different insect pests.

Keywords Ants · Biological control · Olive pests · Soft scale insects · Carbohydrates · Water loss

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Introduction

Agriculture is one of the most relevant forms of land use on a worldwide scale. The impact of the widespread use of agrochemicals on the environment has been a matter of interest for decades, and eco-friendly management techniques for reducing this impact are strongly recommended by ecologists (Gomiero et al. 2011; Koohafkan et al. 2012; Reganold and Wachter 2016). Nonetheless, the extensive use of agrochemicals is still practiced in almost every country. One possible way to reduce the use of these pollutants is to employ pests' natural enemies as biological control agents (Heimpel and Mills 2017). In many cases, specialised predators or parasitoids are used against specific pests (e.g. Chailleux et al. 2013; Yang et al. 2014; Gontijo et al. 2015). This method, however, relies on identifying and rearing specific and highly specialised predators or parasitoids and may have a non-negligible initial investment (Barrat et al. 2018). Another possibility is to rely on the services provided by naturally occurring generalist predators, provided that both the cultivated areas and the surrounding landscapes are not species-impooverished (Symondson et al. 2002; Hajek and Eilenberg 2018; Paredes et al. 2019). This option is often put forward as one of the first and most relevant advantages of organic agriculture (Birkhofer et al. 2008; Crowder et al. 2010; Rusch et al. 2017).

Among generalist predators, ants possess many features that make them ideal candidates for being

agents of biological control. Unsurprisingly, ants have been used as biological control agents in many different agroecosystems, particularly in the tropics (Way and Khoo 1992; Offenberg 2015). Though a few species have highly specialised feeding habits (e.g. leaf-cutter or harvester ants), the majority of ants are generalist feeders and may predate a wide array of herbivorous arthropods (Hölldobler and Wilson 1990). Additionally, ants are abundant in both number and biomass in most natural habitats and are widespread in human-modified sites, including many different types of agroecosystems (Philpott et al. 2010). Finally, the social behaviour of ants—which leads to the formation of large colonies that may amount to hundreds or even thousands of individuals, coupled with an efficient recruitment system—makes them particularly effective as pest predators (Way and Khoo 1992).

There is, however, a peculiar feature that can make the contribution of ants in biological control ambiguous. They can potentially interfere with other pest enemies (as parasitoids) via intraguild predation (Rosenheim et al. 1995; Sanders and Platner 2007), and they may actively tend several plant parasites, such as soft scale insects and aphids (e.g. Stadler and Dixon 2005; Styrsky and Eubanks 2006; Oliver et al. 2008; Ness et al. 2010). Owing to this association, ants can disrupt (directly or indirectly) the biological control of these pests and have sometimes been associated with population outbreaks of their honeydew-producing, mutualistic partners (Bartlett 1961; Itioka and Inoue 1996; James et al. 1999). Thus, ants may be either beneficial or detrimental to crops, but the net balance depends on the species involved, the features of the agroecosystem, and other less predictable or local factors (Tillberg 2004; Philpott et al. 2010). To ascertain whether ants can be employed as biological control agents, it is necessary to understand the details of their association with other organisms within an agroecosystem.

The olive tree (*Olea europaea*) is a highly valuable crop that represents a significant source of income for many Mediterranean countries, with more than 700 million trees mainly concentrated in the Mediterranean basin (Uyulaşer and Yildiz 2014). Thus, identifying management practices that could reduce the use of agrochemicals in this region is essential to the pursuit of sustainable agriculture. In the past 15 years, researchers have begun investigating the structure and function of arthropod communities in olive orchards

by examining how they are affected by agricultural practices (e.g. Campos et al. 2011; Cotes et al. 2011; Jerez-Valle et al. 2014). Ants are the most abundant group of arthropods in olive groves and are involved in many different ecologically important trophic relationships (Santini et al. 2007; Ottonetti et al. 2008; Gonçalves et al. 2012). They are known to prey on the larvae of critical olive pests, such as the olive moth *Prays oleae* (Arambourg 1986; Morris et al. 2002), while also potentially suppressing useful insects used as biological control agents (e.g. Morris et al. 1998; Pereira et al. 2004). Furthermore, ants establish mutualistic interactions with several soft scales and their presence is sometimes seen as a nuisance (Daane and Caltagirone 1989; Tena et al. 2007; Ottonetti et al. 2008) as they may disrupt Integrated Pest Management programmes by preying or deterring soft scale parasites or parasitoids (Martínez-Ferrer et al. 2017).

The ant *Crematogaster scutellaris* (Olivier, 1792) is a dominant Myrmicine widespread throughout the western part of the Mediterranean basin. Several of its biological traits suggest that it could be useful as a biological control agent. This species is a top competitor and can be found in natural, semi-natural and urban areas (Morris et al. 1998; Schatz and Hossaert-McKey 2003; Santini et al. 2007; Gramigni et al. 2013; Frizzi et al. 2015, 2017). *C. scutellaris* is a predator that forms large colonies in tree trunks, and there is some evidence that it can impact several herbivorous arthropods (e.g. Villagrán et al. 1992; Lopez-Sebastian et al. 2004; Radeghieri 2004; Schatz et al. 2006; Gallardo et al. 2012). Despite this evidence, there is still a lack of quantitative data that could allow researchers to understand the impact of *C. scutellaris* on agroecosystems.

The primary aim of this paper was to increase the understanding of the ecology of this species by evaluating its predatory activity and the strength of its mutualistic relationship with a common pest in Mediterranean olive groves, the black soft scale *Saissetia oleae* (Paraskakis et al. 1980; Raspi 1988; Haber and Mifsud 2007), which can cause contamination of the fruits by a sooty mould that grows on the secreted honeydew (Ebeling 1950; Lampson and Morse 1992). While predation by ants may be useful to trees by diminishing herbivorous pests, tending activity can have negative effects by promoting soft scale infestations. To investigate this further, we set up a three-part experimental study. First, we assessed the

predatory impact of *C. scutellaris* by comparing the survival of living baits on the trunk and in the canopy of trees housing or not housing an ant nest. Second, to investigate the association between ant nests and soft scale infestations, we mapped all trees in an olive orchard, noting the presence or absence of each. Third, we evaluated how resource availability affects soft scale attendance by ants. It is known that ant colonies artificially supplied with sugary resources reduce their defence of aphids, given their diminished need for carbohydrates, thus exposing aphids to their natural enemies and aiding their control (Wäckers et al. 2017). Here, we supplemented soft scale-tending ant colonies not only with sugary resources, but also with amino acids and water, and we evaluated the change in attendance behaviour by assessing the variation in the number of tending ants over a three-day period.

Materials and methods

Study area and species

The study was conducted in an olive orchard near Florence (northern Tuscany, Italy, 43° 53' 8" N, 11° 9' 40" E) during June and July. The plantation covers approximately 40 hectares on the slopes of a hill with a maximum elevation of 140 m a.s.l. The climate in this location is typical of the Mediterranean region, with hot, dry summers and mild, wet winters.

Predation experiment

To assess short-term predation rates on trees hosting and not hosting a *C. scutellaris* nest, we carried out a predation experiment aiming to address the following topics: (1) differing predation rates on trees hosting or not hosting *C. scutellaris* colonies, (2) variation in predation rates based on the time of day, and (3) differences in predation rates on the trunk and in the canopy of the tree. To answer these questions, the following experimental protocol was carried out. A total of 80 trees were selected—40 hosting an ant colony and 40 not hosting a colony. In all trees, *S. oleae* were occasionally present, but no tree was infested. For each of the two groups, 20 trees were randomly selected and used to assess predation rates on the trunk, while the remaining 20 were used to assess predation rates in the canopy. Finally, ten trees

from each of the above groups were randomly chosen for trials during daylight and ten during the night.

Treatment order was randomised to minimise confounding effects. On each tree, a plastic cup (diameter = 3 cm, height = 3 cm) was pinned to either the trunk or the leaves, and three live *Musca domestica* (housefly) larvae were placed inside each cup. Instead of focusing on a specific parasite or herbivore, we staged the experiment using the larvae of a neutral prey species. Fly larvae were chosen as a generalised model prey and were preferred over olive-specific pests in order to obtain general insights on the predatory activity of the ants. The cups were covered with mesh (mesh size = 9 × 9 mm) to prevent vertebrate predators from entering. Three larvae were used in each cup to trigger mass recruitment and to maximise the likelihood of observing a predation event. A cup was scored as 'preyed' when the first larva disappeared from the cup or when it was attacked by ants. If other animals were found to predate the larvae, that data was excluded from the analysis. Experiments were repeated during both day and night. Daylight trials were run between 9h00 AM and 11h00 AM while night trials were conducted between 2:00 a.m. and 4:00 a.m. The contents of the cups were checked 30, 60, 90, 120 and 270 min after prey placement. The data were fitted using Kaplan–Meier survival curves (Bland and Altman 1998). The difference between bait survival in the course of time in the different groups was tested using the log-rank test, as implemented in the R 3.5.2 'survival' package (Therneau 2015).

Co-occurrence of ant nests and soft scale infestations

In order to map the distribution of ants and soft scales, a total of 531 trees were surveyed. The presence of *C. scutellaris* nests was assessed by hammering on the trunks to elicit a defensive response (Santini et al. 2011). If at least 20 individuals swarmed out, the tree was noted as housing a nest. We also noted the presence of the soft scale *S. oleae*: a tree was scored as 'infested' when at least one branch bore the presence of at least 50 clumped soft scales. We applied a χ^2 test and calculated the odds ratio for assessing the strength of association between ant nests and soft scale infestations.

Effect of resource availability on soft scale attendance

To assess the influence of previous short-term resource availability on soft scale attendance, 20 trees hosting both a *Crematogaster* colony and a large tended soft scale clump (> 100 *S. oleae* on a single branch of the tree hosting the colony) were chosen. To minimise the possible influence of colony size on recruitment (Dornhaus and Franks 2006), only mature nests were selected for the subsequent trials. Mature nests were distinguished by more than 100 ants swarming out of nest holes in the first four minutes after hammering on the trunk. Each tree was randomly assigned to one of the following groups: (1) controls, (2) water supplemented, (3) sucrose supplemented or (4) amino acid supplemented. Thus, each group included five different colonies. Three plastic containers (diameter = 4.5 cm, height = 2 cm) were pinned to the trunk of each tree close to the nest entrance and filled with the corresponding solution. Cups on the control trees were left empty. For both sucrose and amino acids, supersaturated solutions (50% weight per volume, e.g. 50 g sugar per 100 ml water) were used. Throughout the duration of the trials, the cups were checked twice a day and refilled when necessary to compensate for evaporation or consumption by ants. Each day, the cups were also checked to be sure that ants used them. In all cases except the controls, groups of ants were observed feeding in the cups.

To obtain an index for the number of ants tending the soft scales, all ants moving across a marked section (10 cm length) of the proximal part of a branch hosting a clump were counted for three minutes. Counting of ants took place at the start of the trial (before filling the cups) and again after one, two and three days. Surveys were conducted between 9h00 AM and 11h00 AM and each colony was inspected five times at 30 min intervals. For each day, the average number of ants visiting the clump during the five inspections was used in the subsequent analysis. We calculated the ratio between the average number of ants visiting a clump each day (N_t) and the number of ants observed on the same branch at day zero (N_0), here called ant ratio ($AR = N_t/N_0$). Temporal changes in AR-values across treatments were compared using Linear Mixed Models as implemented in the 'lme4' package (Bates et al. 2015) in R version 3.5.2 excluding time 0 from the data (Zuur et al. 2009).

AR-values were normally distributed (Shapiro–Wilk normality test: $W = 0.98$, $p = 0.313$). We fitted four different models of increasing complexity, including the 'time' factor (continuous variable), the 'treatment' factor (categorical with four levels) and their interaction as fixed effects. We also added colony identity as a random effect to manage repeated measures.

Results

Predation experiment

Temporal variation in the survival of larvae (i.e. the number of cups not visited by ants as a function of time) and the significance of log-rank tests are reported in Fig. 1, showing the Kaplan-Mayer survival curves for bait in the different experimental groups. Overall, baits on trees hosting a colony suffered greater mortality over time than on unoccupied trees. Moreover, predation over time was greater on trunks than in the canopy and higher during the night than the day. The maximal predation probability was observed during the night on trunks (Fig. 1c), when survival probability was ~ 0.6 and ~ 0.4 on trees not hosting and hosting a *C. scutellaris* nest, respectively. The lowest risk was faced during daytime in the canopy (Fig. 1b), when survival probability was nearly 1.0 and 0.9 on trees not hosting and hosting *C. scutellaris*, respectively.

Co-occurrence of ant nests and soft scale infestations

Of the 531 trees surveyed, 129 hosted a *C. scutellaris* colony. The presence of soft scale clumps in the trees was clearly associated with the presence of *C. scutellaris* nests. There were 77 total 'infested' trees, 54 of which also hosted a *C. scutellaris* nest. Therefore, 41.9% of the trees hosting a *C. scutellaris* nest were also infested by soft scales, while only 5.7% of the other trees were infested by soft scales. This difference was significant ($\chi^2_1 = 99.98$, $p < 0.001$). The odds ratio for a tree hosting a *C. scutellaris* colony to also be infested by soft scales was 11.86, meaning that an infestation was over 11 times more likely to occur on a tree with a nest than on a tree without. Furthermore, only 8 out of 23 trees (infested, without *C. scutellaris*) were apparently free of any ant species.

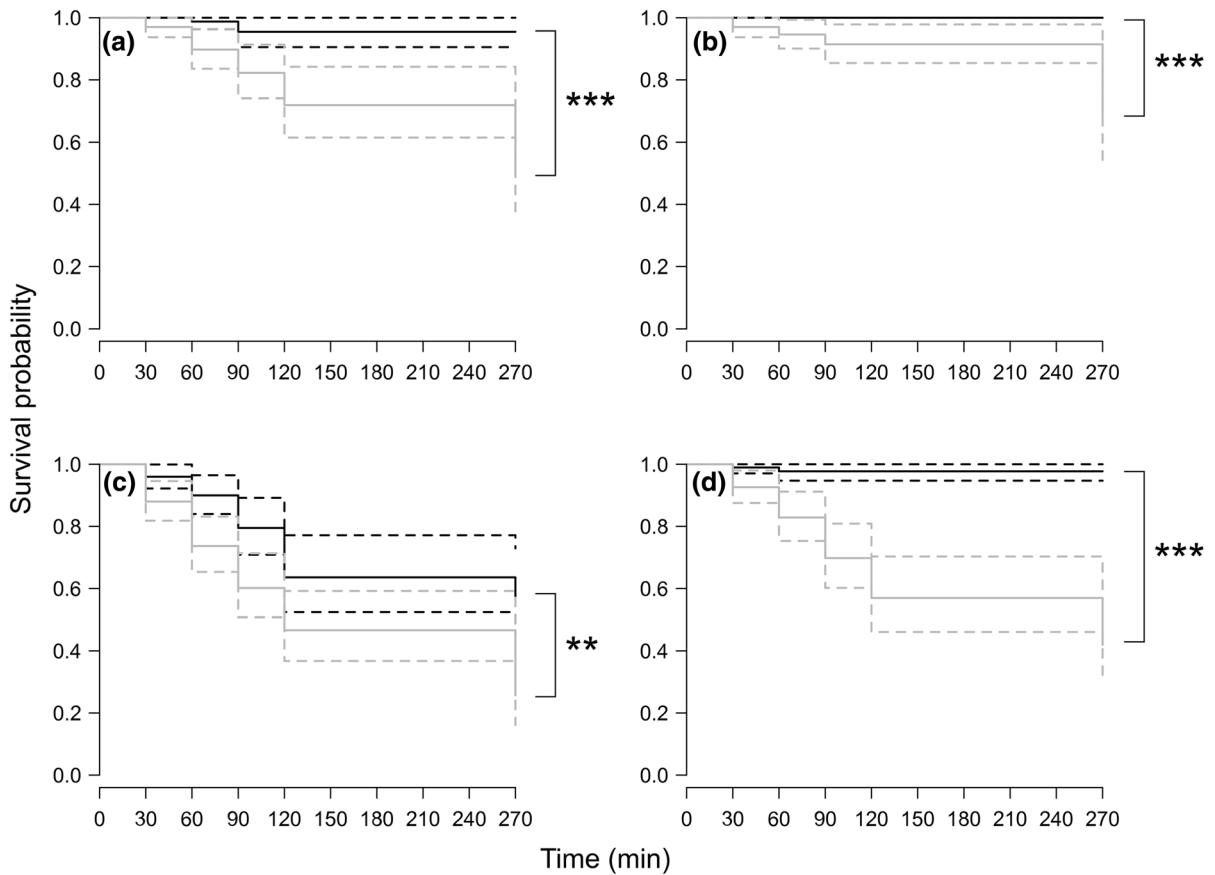


Fig. 1 Temporal variation in the survival (solid lines, \pm 95% CI dashed lines) of larvae. Trees hosting a *C. scutellaris* colony are shown in grey, and trees without nests are in black. Asterisks

indicate the significance of the log-rank test between the two curves. Significance: ** < 0.01; *** < 0.001. **a** night/canopy; **b** day/canopy; **c** night/trunk; **d** day/trunk

In the remaining 15 trees, the soft scale clumps were attended by other species: nine by *Camponotus aethiops*, five by *Lasius lasioides* and one by *Camponotus vagus*, although their nests were not located on the trees. No colonies of other ant species were found in the checked trees.

Effect of resource availability on soft scale attendance

Results of soft scale feeding trials are summarised in Fig. 2. The best model explaining the observed variation involved the treatment factor only (AIC = 71.23, lowest Δ AIC = 8.27), suggesting that the number of ants visiting the soft scale clumps only changed with treatments, but not with time (ANOVA with Satterthwaite method: $F_{3,16} = 10.82, p < 0.001$). The effect of amino acid supplementation was not

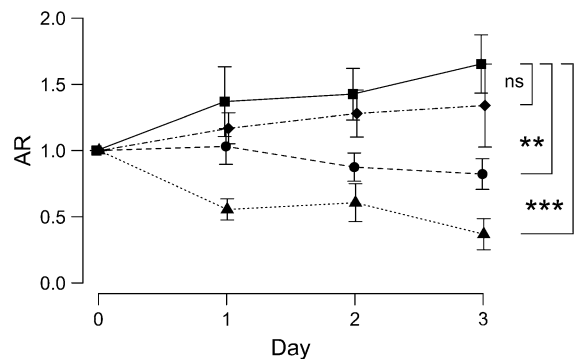


Fig. 2 Effect of supplementation on soft scale attendance by ants. Average ant ratio (AR \pm SE) for the different treatment types and for all observation days. Squares/solid line = controls, circles/dashed line = water, triangles/dotted line = sugar, rhombuses/dot-dash lines = amino acids. Results of Dunnett contrasts are reported on the right side of the graph, ns, not significant, ** < 0.01, *** < 0.001

different from that observed in controls (Dunnett contrast: $z = -1.207$, $p = 0.481$). In contrast, a significant difference was observed both in sucrose and water-supplemented colonies (Dunnett contrasts: $z = -5.317$, $p < 0.001$ and $z = -2.723$, $p = 0.005$, respectively).

Discussion

The results of this study demonstrated that *C. scutellaris* can play a key role in the functioning of arthropod assemblages in olive orchards, both as a predator and as a tender of sap-feeders. *C. scutellaris* was confirmed to be an effective predator, as prey were discovered and consumed much faster on trees hosting an ant colony than on unoccupied trees. That predation is an important part of *C. scutellaris* trophic ecology was somewhat expected. In a study of trophic dynamics in this same ecosystem using stable isotope analysis, Ottonetti et al. (2008) demonstrated that this species had a $\delta N15$ signature comparable to that of spiders, indicating a high trophic level and a prevalence of predation over tending of sap-feeders. Similarly, Blüthgen et al. (2003) reported a comparable situation for another *Crematogaster* species in a tropical rainforest food web. Day/night changes can be explained by variation in ant activity based on the time of the day, though this species is known to have a wide thermal niche and is able to maintain elevated activity levels during even the hottest part of the day (Santini et al. 2007; Arnan and Blüthgen 2015). The difference between predation rates on the trunk and in the canopy could be a result of the greater spatial complexity and total surface area of branches and leaves as compared to trunks. Overall, these results confirm that this species has potential for use in the control of harmful pest populations and that any potential prey would be at risk when landing or feeding on a tree hosting a nest. Other ants, such as *Oecophylla smaragdina* and *O. longinoda*, are known to protect several types of crops against various insect pests, including several *Bactrocera* species (Way and Khoo 1992; Vayssieres et al. 2016; Abdulla et al. 2017). Interestingly, the protection is not limited to direct control through predation but is also achieved through trait-mediated interactions (Dáttilo et al. 2016). There is a growing body of evidence supporting the ability of ant pheromones to deter herbivorous

pests, even in the absence of ants (Adandonon et al. 2009; Offenberg 2015). In this way, the positive action of *C. scutellaris* may not be solely limited to the predation of certain prey stages.

Another important finding was that the risk of a tree incurring a soft scale infestation was almost 12 times higher if it harboured a *C. scutellaris* nest. Although other ant species can trigger soft scale infestations, their influence appeared to be lower in the present study system. Many studies have previously documented the relationship between soft scale populations and the presence of ants. Hence, this association was not surprising, although no specific assessment of the risk of infestation incurred by an olive tree exists (e.g. Bishop and Bristow 2003; Dao et al. 2014). Although soft scale infestation in olive groves can be slightly detrimental, ant association with sap-feeders may not be entirely negative and may have indirect positive cascade effects. First, a lack of tending ants can increase the presence of non-honeydew producing pests (Pekas et al. 2011). The carbohydrates provided by soft scales might fuel colony growth and are often associated with an increase in colony activity (Grover et al. 2007; Dussutour and Simpson 2008; Kay et al. 2010). This, in turn, could increase tree-patrolling and predation by ants, which is also stimulated by a diet strongly unbalanced towards carbohydrates (Davidson 1997; Ness et al. 2009; Pringle et al. 2011; but see Rudolph and Palmer 2013). Moreover, Clark et al. (2016) demonstrated in a forest system that ant mutualism with sap-feeders can strengthen top-down effects of ants on caterpillars under some conditions. Therefore, their association with soft scales could in some way enhance predatory behaviour towards other harmful pests. On the other hand, if ants reduce their defence of soft scales, they are more exposed to their natural enemies, such as parasites or parasitoids (Tena et al. 2016). The presence of ants on trees may deter natural pest enemies, as described for the Opiine parasitoid *Fopius arisanus*—a natural enemy of the invasive fly *Bactrocera invadens* in African mango cultivations—which perform fewer attacks on the fly when the ant *Oecophylla longinoda* is present (Appiah et al. 2014). Furthermore, honeydew can be an important food resource for natural enemies of flying pests. In California, it has been demonstrated that a parasitoid of *B. oleae*, *Psytalia humilis*, avoids feeding on the lethal Spinosad-based GF-120 baits when honeydew is available (Wang et al. 2011). The

patrolling carried out by ants might prevent parasitoids of flying pests from feeding on honeydew, increasing the probability that they will move towards different food sources away from olives.

We found that tending behaviour towards soft scales was influenced by the short-term availability of carbohydrates, as a few days of sucrose supplementation was effective in reducing ant attendance. Reduced tending has also been shown, for example, by Offenberg (2001) for *Lasius niger* and by Wäckers et al. (2017) for *Lasius grandis*. The rapidity of this response indicates that this species can opportunistically modulate its behaviour as resource availability changes over time (Frizzi et al. 2016). However, we did not observe any direct predation attempts on soft scales by ants, as happens in other species that switch from tending to predation when colonies are supplied with abundant carbohydrates and are short on proteins (Sakata 1995; Offenberg 2001). In our specific case, the duration of supplementation was likely too short to trigger such a response. Amino acid supplementation did not have any influence on ant behaviour, confirming the results obtained by Frizzi et al. (2016) on the foraging choices of this species. The most exciting and unexpected result of this study was the effect of water supplementation, which elicited a significant decrease in attendance, although slower and less marked than with sucrose. This effect indicates that during the summer, ants may seek out soft scales as not only a source of carbohydrates, but also of water. This finding reinforces the importance of water regulation during the summer by ant colonies. In a recent study on the feeding preferences in this species, Frizzi et al. (2016) demonstrated that when facing a water shortage during the summer, the ants include low-value carbohydrate solutions in their diet, which were not so actively sought when water was not limiting. An interesting point that deserves further investigation is whether these findings can have some practical utility in, for example, the planning of colony supplementation with carbohydrates during critical stages for the soft scales (Wäckers et al. 2017) or with water during the summer, both of which should reduce tending and soft scale survival. Nevertheless, it must be recalled that seasonality can significantly affect the nutritional needs of the colonies (see Csata and Dussutour 2019 for a review). For example, during the early phase of the development of the larvae, the colony usually tends to utilise proteins (Wheeler and Buck 1995),

whereas during the peak of activity, carbohydrates are preferred (Cook et al. 2011). The two summer months when our experiments were carried out are months in which *C. scutellaris* is nearly at the peak of its activity (Santini et al. 2007), so a considerable need for carbohydrates was expected. This may partially explain the low interest shown by the ants for protein solutions. In general, however, previous investigations on the same species have demonstrated a higher preference for sugar resources than for protein resources, independent of season (Frizzi et al. 2016). From a practical point of view, as ants significantly reduce their attendance despite their higher need for carbohydrates, it is suggested that supplementation with sugar could have detrimental effects on soft scale pests, at least during the period of maximum activity of the ants.

A final important point worth examining is the spatial distribution of the observed effects. *C. scutellaris* is known to be a polydomous species, with colonies often subdivided among several interconnected nests. This, of course, is the norm in the studied system (Santini et al. 2011). Additionally, this species shows a clear preference for large, old trunks in which a nest is easier to excavate, and the spatial distribution of these trunks drives the spatial arrangement of colonies (Santini et al. 2011). Under some circumstances, this leads to a mosaic-like system characterised by patches of trees dominated by this species intermixed with other patches in which this species is not present. Therefore, both predation and soft scale infestation will also have a patchy distribution, with *Crematogaster*-dominated areas having higher predation rates and higher soft scale infestation risk and *Crematogaster*-free areas having less predation but lower soft scale densities. Beyond the many interesting ecological consequences that this spatial pattern may have (Massol et al. 2011; Tilman and Kareiva 1997), it is important to understand whether this knowledge could be of any management utility. Both surveillance and the treatments could be targeted to a reduced number of selected trees in the orchard, although the trees to be treated would be different depending on the type of pest considered. Any action aimed at the control of sap-feeders would preferentially be addressed towards trees hosting a nest, while any action against other pests should be preferentially targeted towards *Crematogaster*-free trees.

By hypothetically transposing the results of our predation trials onto specific olive pests other than soft scales, in our opinion, the final balance may be more in favour of positive over negative effects. The impact of sap-feeders such as *S. oleae* is usually less harmful than other pests, such as *P. oleae* or the olive fruit fly, *Bactrocera oleae*, because the only damage they typically cause is limited to the contamination of a small portion of the olives by sooty mould without impairing olive production (Lampson and Morse 1992). On the other hand, massive outbreaks of the latter species can extend over large spatial scales and can put the entire yearly production of a country at risk. Damage caused by this pest amounts to several hundreds of thousands of US\$ annually (Montiel-Bueno and Jones 2002; Tzanakakis 2003). In parts of the Mediterranean basin, where most of the olive production takes place, the losses can reach 80–100% of the total harvest in some years (e.g. Katsoyannos 1992; Broumas et al. 2002). This situation is in danger of worsening after the European Regulation 2019/1090 of 26 June 2019 which banned the use of dimethoate, a widely used organophosphate insecticide, in all of the European Union starting in the 2020 olive crop season (Official Journal of the European Union L 173/39, 27 June 2019). *B. oleae* is also considered the primary pest for olive production in the USA (Burrack et al. 2008; Daane et al. 2005). The same is not true for soft scale infestations, whose impact is considerably lower and usually more spatially restricted (Delrio and Foxi 2010). However, it should be considered that such a parallel between the model prey we used in our experiment (housefly) and other potential olive pests might not be that simple. First, since we used fly larvae, the predation rate we recorded should, in theory, be applied only to larval stages of the two main insect olive pests, *P. oleae* and *B. oleae*, but not to their adults. From this perspective, our conclusions likely fit better with *P. oleae* than with *B. oleae* since the cryptic developmental stages of the latter within olive drupes (Dominici et al. 1986) might hamper ant predation. Conversely, the pupal developmental stage of *P. oleae* is more exposed to predation, as pupation can take place in the soil at the base of the tree at the end of the season (Dimou et al. 2003; Picchi et al. 2017). Second, pest species may have evolved escape strategies or other protection measures to deal with canopy ants that probably never evolved in housefly larvae. These points should be considered

when speculating on the usefulness of the presence of a *C. scutellaris* nest in olive trees, since its predatory ability might be only partially effective in the control of such olive pests.

In conclusion, this study provided some evidence that even though the occurrence of *C. scutellaris* is related to sap-feeding soft scale infestation in olive groves, it may also act as a biological control agent against larval stages of other herbivorous pests such as the olive moth and, to a lesser extent, the olive fly, similar to what occurs with *Oecophylla* ants in tropical Africa and Asia. Future studies aiming to test the effective usefulness of this feature in biological control are recommended.

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Compliance with ethical standards

Conflicts of interest Authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

- Abdulla NR, Rwegasira GM, Jensen KMV, Mwatawala MW, Offenberg J (2017) Potential of *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) in managing major insect pests in organic cashew production systems. *Organic agriculture* 7:95–104
- Adandonon A, Vayssieres JF, Sinzogan A, van Mele P (2009) Density of pheromone sources of the weaver ant *Oecophylla longinoda* affects oviposition behaviour and damage by mango fruit flies (Diptera: Tephritidae). *Int J Pest Manage* 55:285–292
- Appiah EF, Ekesi S, Afreh-Nuamah K, Obeng-Ofori D, Mohamed SA (2014) African weaver ant-produced semiochemicals impact on foraging behaviour and parasitism by the opiine parasitoid, *Fopius arisanus* on *Bactrocera invadens* (Diptera: Tephritidae). *Biol Control* 79:49–57
- Arambourg Y (1986) Pyralidae. *Margaronia unionalis* Hübn. In: Arambourg Y (ed) *Traité d'entomologie oléicole*. International Olive Oil Council, Madrid, Spain, pp 75–80
- Arnan X, Blüthgen N (2015) Using ecophysiological traits to predict climatic and activity niches: lethal temperature and water loss in Mediterranean ants. *Global Ecol Biogeogr* 24:1454–1464

- Barratt B, Cock M, Oberprieler R (2018) Weevils as targets for biological control, and the importance of taxonomy and phylogeny for efficacy and biosafety. *Diversity* 10:73
- Bartlett BR (1961) The influence of ants upon parasites, predators, and soft scale insects. *Ann Entomol Soc Am* 54:543–551
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Birkhofer K, Fließbach A, Wise DH, Scheu S (2008) Generalist predators in organically and conventionally managed grass-clover fields: implications for conservation biological control. *Ann Appl Biol* 153:271–280
- Bishop DB, Bristow CM (2003) Effects of the presence of the allegheny mound ant (Hymenoptera: Formicidae) in providing enemy-free space to myrmecophilous aphid and soft scale populations. *Ann Entomol Soc Am* 96:202–210
- Bland JM, Altman DG (1998) Survival probabilities (the Kaplan-Meier method). *BMJ* 317:1572–1580
- Blüthgen N, Gebauer G, Fiedler K (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia* 137:426–435
- Broumas T, Haniotakis G, Liaropoulos C, Tomazou T, Ragoussis N (2002) The efficacy of an improved form of the mass-trapping method, for the control of the olive fruit fly, *Bactrocera oleae* (Gmelin) (Dipt., Tephritidae): pilot-scale feasibility studies. *J Appl Entomol* 126:217–223
- Burrack HJ, Connell JH, Zalom FG (2008) Comparison of olive fruit fly (*Bactrocera oleae* (Gmelin)) (Diptera: Tephritidae) captures in several commercial traps in California. *Int J Pest Manage* 54:227–234
- Campos MR, Picanço MC, Martins JC, Tomaz AC, Guedes RNC (2011) Insecticide selectivity and behavioral response of the earwig *Doru luteipes*. *Crop Prot* 30:1535–1540
- Chailleux A, Bearez P, Pizzol J, Amiens-Desneux E, Ramirez-Romero R, Desneux N (2013) Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*. *J Pest Sci* 86:533–541
- Clark RE, Farkas TE, Lichter-Marck I, Johnson ER, Singer MS (2016) Multiple interaction types determine the impact of ant predation of caterpillars in a forest community. *Ecology* 97:3379–3388
- Cook SC, Eubanks MD, Gold RE, Behmer ST (2011) Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. *PLoS ONE* 6(9):e25407
- Cotes B, Campos M, García PA, Pascual F, Ruano F (2011) Testing the suitability of insect orders as indicators for olive farming systems. *Agric Forest Entomol* 13:357–364
- Crowder DW, Northfield TD, Strand MR, Snyder WE (2010) Organic agriculture promotes evenness and natural pest control. *Nature* 466:109–112
- Csata E, Dussutour A (2019) Nutrient regulation in ants (Hymenoptera: Formicidae): a review. *Myrmecol News* 29:111–124
- Daane K, Caltagirone L (1989) Biological control of black soft scale in olives. *Calif Agr* 43:9–11
- Daane KM, Rice RE, Zalom FG, Barnett WW, Johnson MW (2005) Arthropod pests of olive. In: Sibbett GS, Ferguson L (eds) *Olive production manual*, 2nd edn. University of California, Agriculture and Natural Resources, Berkeley, pp 105–114
- Dao HT, Meats A, Beattie GAC, Spooner-Hart R (2014) Ant-coccid mutualism in citrus canopies and its effect on natural enemies of red soft scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). *B Entomol Res* 104:137–142
- Dáttilo W, Aguirre A, De la Torre PL, Kaminski LA, García-Chávez J, Rico-Gray V (2016) Trait-mediated indirect interactions of ant shape on the attack of caterpillars and fruits. *Biol Letters* 12:20160401
- Davidson DW (1997) The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biol J Linn Soc* 61:153–181
- Delrio G, Foxi C (2010) Current status of *Saissetia oleae* biological control in Sardinia (Italy). *IOBC/WPRS Bulletin* 59:171–176
- Dimou I, Koutsikopoulos C, Economopoulos AP, Lykakis J (2003) Depth of pupation of the wild olive fruit fly, *Bactrocera* (*Dacus*) *oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. *J Appl Entomol* 127:12–17
- Dominici M, Pucci C, Montanari GE (1986) *Dacus oleae* (Gmel.) ovipositing in olive drupes (Diptera, Tephritidae). *J Appl Entomol* 101:111–120
- Dornhaus A, Franks NR (2006) Colony size affects collective decision-making in the ant *Temnothorax albipennis*. *Insect Soc* 53:420–427
- Dussutour A, Simpson SJ (2008) Carbohydrate regulation in relation to colony growth in ants. *J Exp Biol* 211:2224–2232
- Ebeling W (1950) *Subtropical entomology*. Lithotype Process Co, San Francisco
- Frizzi F, Bartalesi V, Santini G (2017) Combined effects of temperature and interspecific competition on the mortality of the invasive garden ant, *Lasius neglectus*: a laboratory study. *Journal Therm Biol* 65:76–81
- Frizzi F, Rispoli A, Chelazzi G, Santini G (2016) Effect of water and resource availability on ant feeding preferences: a field experiment on the Mediterranean ant *Crematogaster scutellaris*. *Insect Societia* 63:565–574
- Frizzi F, Ciofi C, Dapporto L, Natali C, Chelazzi G, Turillazzi S, Santini G (2015) The rules of aggression: how genetic, chemical and spatial factors affect intercolony fights in a dominant species, the Mediterranean acrobat ant *Crematogaster scutellaris*. *PLoS ONE* 10(10):e0137919
- Gallardo A, Jiménez A, Antonietty CA, Villagrán M, Ocete ME, Soria FJ (2012) Forecasting infestation by *Coraebus undatus* (Coleoptera, Buprestidae) in cork oak forests. *Int J Pest Manage* 58:275–280
- Gomiero T, Pimentel D, Paoletti MG (2011) Environmental impact of different agricultural management practices: conventional vs. organic agriculture. *Cr Rev Plant Sci* 30:95–124
- Gonçalves MF, Pereira JA, Liu TX (2012) Abundance and diversity of soil arthropods in the olive grove ecosystem. *J Insect Sci* 12:20
- Gontijo LM, Beers EH, Snyder WE (2015) Complementary suppression of aphids by predators and parasitoids. *Biol Control* 90:83–91
- Gramigni E, Calusi S, Gelli N, Giuntini L, Massi M, Delfino G, Chelazzi G, Baracchi D, Frizzi F, Santini G (2013) Ants as

- bioaccumulators of metals from soils: body content and tissue-specific distribution of metals in the ant *Crematogaster scutellaris*. *Eur J Soil Biol* 58:24–31
- Grover CD, Kay AD, Monson JA, Marsh TC, Holway DA (2007) Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. *P Roy Soc B-Biol Sci* 274:2951–2957
- Haber G, Mifsud D (2007) Pests and diseases associated with olive trees in the Maltese Islands (Central Mediterranean). *The Central Mediterranean Naturalist* 4:143–161
- Hajek AE, Eilenberg J (2018) Natural enemies: an introduction to biological control. Cambridge University Press, Cambridge
- Heimpel GE, Mills NJ (2017) Biological control. Cambridge University Press, Cambridge
- Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge
- Itioka T, Inoue T (1996) Density-dependent ant attendance and its effects on the parasitism of a honeydew-producing soft scale insect, *Ceroplastes rubens*. *Oecologia* 106:448–454
- James DG, Stevens MM, O'Malley KJ, Faulder RJ (1999) Ant foraging reduces the abundance of beneficial and incidental arthropods in citrus canopies. *Biol Control* 14:121–126
- Jerez-Valle C, García PA, Campos M, Pascual F (2014) A simple bioindication method to discriminate olive orchard management types using the soil arthropod fauna. *Appl Soil Ecol* 76:42–51
- Katsoyannos P (1992) Olive pests and their control in the Near East. FAO plant production and protection paper, Food and Agriculture Organization (FAO), Rome
- Kay AD, Zumbusch T, Heinen JL, Marsh TC, Holway DA (2010) Nutrition and interference competition have interactive effects on the behavior and performance of Argentine ants. *Ecology* 91:57–64
- Koohafkan P, Altieri MA, Gimenez EH (2012) Green agriculture: foundations for biodiverse, resilient and productive agricultural systems. *Int J Agr Sustain* 10:61–75
- Lampson LJ, Morse JG (1992) Impact of insect growth regulators on black soft scale, *Saissetia oleae* (Olivier) (Homoptera: Coccidae), and inter-tree dispersal. *J Agr Entomol* 9:199–211
- López-Sebastián E, Arlandis JS, Ranera JAT (2004) Acerca de *Crematogaster scutellaris* (Olivier, 1791) (Hymenoptera, Formicidae) como depredador de huevos de la procesionaria del pino. *Boletín de sanidad vegetal Plagas* 30:699–702
- Martínez-Ferrer MT, Campos-Rivela JM (2017) Diversity, spatial distribution, and sampling for ant management decision-making in integrated pest management programs in citrus groves. *Entomol Exp Appl* 162:251–260
- Massol F, Gravel D, Mouquet N, Cadotte MW, Fukami T, Leibold MA (2011) Linking community and ecosystem dynamics through spatial ecology. *Ecol Lett* 14:313–323
- Montiel-Bueno A, Jones O (2002) Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. *IOBC/WPRS Bulletin* 25:147–155
- Morris TI, Symondson WOC, Kidd NAC, Campos M (2002) The effect of different ant species on the olive moth, *Prays oleae* (Bern.), in Spanish olive orchard. *J Appl Entomol* 126:224–230
- Morris TI, Campos M, Jervis MA, McEwen PK, Kidd NAC (1998) Potential effects of various ant species on green lacewing, *Chrysoperla carnea* egg numbers. *J Appl Entomol* 122:401–403
- Ness JH, Morris WF, Bronstein JL (2009) For ant-protected plants, the best defence is a hungry offense. *Ecology* 90:2823–2831
- Ness J, Mooney K, Lach L (2010) Ants as mutualists. In: Lach L, Parr C, Abbott K (eds) *Ant ecology*. Oxford University Press, Oxford, pp 97–114
- Offenberg J (2015) Ants as tools in sustainable agriculture. *J Appl Ecol* 52:1197–1205
- Offenberg J (2001) Balancing between mutualism and exploitation: the symbiotic interaction between *Lasius* ants and aphids. *Behav Ecol Sociobiol* 49:304–310
- Oliver TH, Leather SR, Cook JM (2008) Macroevolutionary patterns in the origin of mutualisms involving ants. *J Evolution Biol* 21:1597–1608
- Ottonetti L, Tucci L, Chelazzi G, Santini G (2008) Stable isotopes analysis to assess the trophic role of ants in a Mediterranean agroecosystem. *Agr Forest Entomol* 10:29–36
- Paraskakis M, Neuenschwander P, Michelakis S (1980) *Saissetia oleae* (Oliv.) (Hom., Coccidae) and its parasites on olive trees in Crete, Greece. *Zeitschrift für angewandte Entomologie* 90:450–464
- Paredes D, Karp DS, Chaplin-Kramer R, Benítez E, Campos M (2019) Natural habitat increases natural pest control in olive groves: economic implications. *J Pest Sci* 92:1111–1121
- Pekas A, Tena A, Aguilar A, Garcia-Marí F (2011) Spatio-temporal patterns and interactions with honeydew-producing Hemiptera of ants in a Mediterranean citrus orchard. *Agr Forest Entomol* 13:89–97
- Pereira JA, Bento A, Cabanas JE, Torres LM, Herz A, Hassan SA (2004) Ants as predators of the egg parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) applied for biological control of the olive moth, *Prays oleae* (Lepidoptera: Plutellidae) in Portugal. *Biocontrol Sci Techn* 14:653–664
- Philpott SM, Perfecto I, Armbrrecht I, Parr C (2010) Ant diversity and function in disturbed and changing habitats. In: Lach L, Parr C, Abbott K (eds) *Ant ecology*. Oxford University Press, Oxford, pp 137–156
- Picchi MS, Marchi S, Albertini A, Petacchi R (2017) Organic management of olive orchards increases the predation rate of overwintering pupae of *Bactrocera oleae* (Diptera: Tephritidae). *Biol Control* 108:9–15
- Pringle EG, Dirzo R, Gordon DM (2011) Indirect benefits of symbiotic coccoids for an ant-defended myrmecophytic tree. *Ecology* 92:37–46
- Radeghieri P (2004) *Cameraria ohridella* (Lepidoptera Gracillariidae) predation by *Crematogaster scutellaris* (Hymenoptera Formicidae) in Northern Italy (Preliminary note). *B Insectol* 57:63–64
- Raspi A (1988) Preliminary notes of the entomophages of *Saissetia oleae* (Oliv.) and *Lichtensia viburni* Sing. in olive groves of coastal Tuscany and western Liguria. *Frustula Entomologica* 11:119–128
- Reganold JP, Wachter JM (2016) Organic agriculture in the twenty-first century. *Nat Plants* 2:15221

- Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA (1995) Intraguild predation among biological-control agents: theory and evidence. *Biol Control* 5:303–335
- Rudolph KP, Palmer TM (2013) Carbohydrate as fuel for foraging, resource defense and colony growth—a long-term experiment with the plant-ant *Crematogaster nigriceps*. *Biotropica* 45:620–627
- Rusch A, Bommarco R, Ekblom B (2017) Conservation biological control in agricultural landscapes. *Adv Bot Res* 81:333–360
- Sakata H (1995) Density-dependent predation of the ant *Lasius niger* (Hymenoptera: Formicidae) on two attended aphids *Lachnus tropicalis* and *Myzocallis kuricola* (Homoptera: Aphididae). *Res Popul Ecol* 37:159–164
- Sanders D, Platner C (2007) Intraguild interactions between spiders and ants and top-down control in a dry grassland. *Oecologia* 150:611–624
- Santini G, Ramsay PM, Tucci L, Ottonetti L, Frizzi F (2011) Spatial patterns of the ant *Crematogaster scutellaris* in a model ecosystem. *Ecol Entomol* 36:625–634
- Santini G, Tucci L, Ottonetti L, Frizzi F (2007) Competition trade-offs in the organisation of a Mediterranean ant assemblage. *Ecol Entomol* 32:319–326
- Schatz B, Hossaert-Mckey M (2003) Interactions of the ant *Crematogaster scutellaris* with the fig/fig wasp mutualism. *Ecol Entomol* 28:359–368
- Schatz B, Proffitt M, Rakhi BV, Borges RM, Hossaert-McKey M (2006) Complex interactions on fig trees: ants capturing parasitic wasps as possible indirect mutualists of the fig–fig wasp interaction. *Oikos* 113:344–352
- Stadler B, Dixon AF (2005) Ecology and evolution of aphid-ant interactions. *Annu Rev Ecol Evol Syst* 36:345–372
- Styrsky JD, Eubanks MD (2006) Ecological consequences of interactions between ants and honeydew-producing insects. *P Roy Soc B-Biol Sci* 274:151–164
- Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? *Annu Rev Entomol* 47:561–594
- Tena A, Wäckers FL, Heimpel GE, Urbaneja A, Pekas A (2016) Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control. *Curr Opin Insect Sci* 14:100–104
- Tena A, Soto A, Vercher R, Garcia-Marí F (2007) Density and structure of *Saissetia oleae* (Hemiptera: Coccidae) populations on citrus and olives: relative importance of the two annual generations. *Environ Entomol* 36:700–706
- Therneau T (2015) A package for survival analysis in S. version 2.38, <https://cran.r-project.org/package=survival>
- Tillberg CV (2004) Friend or foe? A behavioral and stable isotopic investigation of an ant–plant symbiosis. *Oecologia* 140:506–515
- Tilman D, Kareiva P (1997) Spatial ecology: the role of space in population dynamics and interspecific interactions. Princeton University Press, Princeton
- Tzanakakis M (2003) Seasonal development and dormancy of insects and mites feeding on olive: a review. *Neth J Zool* 52:87–224
- Uylager V, Yildiz G (2014) The historical development and nutritional importance of olive and olive oil constituted an important part of the Mediterranean diet. *Crit Rev Food Sci* 54:1092–1101
- Vayssières J-F, Offenberg J, Sinzogan A, Adandonon A, Wargui R, Anato F, Hougbo HY, Ouagoussounon I, Diamé L, Quilici S, Rey J-Y, Goergen G, De Meyer M, van Mele P (2016) The use of weaver ants in the management of fruit flies in Africa. In: Ekesi S, Mohamed SA, De Meyer M (eds) Fruit fly research and development in Africa. Springer International Publishing, Basel, pp 389–422
- Villagrán M, Soria FJ, Ocete ME (1992) Estudio del comportamiento alimentario de *Crematogaster scutellaris* Oliv. (Hym. Formicidae) en tres alcornoques del SW español. *Bol San Veg Plagas* 20:637–642
- Yang NW, Zang LS, Wang S, Guo JY, Xu HX, Zhang F, Wan FH (2014) Biological pest management by predators and parasitoids in the greenhouse vegetables in China. *Biol Control* 68:92–102
- Wäckers FL, Alberola JS, Garcia-Marí F, Pekas A (2017) Attract and distract: manipulation of a food-mediated protective mutualism enhances natural pest control. *Agric Ecosyst Environ* 246:168–174
- Wang XG, Johnson MW, Opp SB, Krugner R, Daane KM (2011) Honeydew and insecticide bait as competing food resources for a fruit fly and common natural enemies in the olive agroecosystem. *Entomol Exp Appl* 139:128–137
- Way MJ, Khoo KC (1992) Role of ants in pest management. *Annu Rev Entomol* 37:479–503
- Wheeler DE, Buck NA (1995) Storage proteins in ants during development and colony founding. *J Insect Physiol* 41:885–894
- Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science & Business Media, New York