

## ORIGINAL CONTRIBUTION

**Suppression of *Bactrocera oleae* (Diptera: Tephritidae) pupae by soil arthropods in the olive grove**A. M. Dinis<sup>1</sup>, J. A. Pereira<sup>1</sup>, M. C. Pimenta<sup>1</sup>, J. Oliveira<sup>1</sup>, J. Benhadi-Marín<sup>1,2</sup> & S. A. P. Santos<sup>1,a</sup><sup>1</sup> Mountain Research Center, CIMO, School of Agriculture, Polytechnic Institute of Bragança, Bragança, Portugal<sup>2</sup> Department of Life Sciences, University of Coimbra, Coimbra, Portugal**Keywords**

Forficulidae, olive fruit fly, omnivorous, predators, pupae suppression

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**Abstract**

Soil arthropods can provide ecosystem services, such as biological control of crop pests that spend part of their life cycle in the soil. This is the case of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), one of the most important pests of olives. The impact of edaphic arthropods on the abundance of *B. oleae* pupae was evaluated and their contribution for biological control of the pest was quantified. Exclusion and exposed boxes with *B. oleae* pupae were installed in olive groves in parallel with pitfall traps used for sampling arthropods and the percentage of pupae suppression was evaluated from January to May 2014. Forficulidae dominated the community during the winter period while Formicidae dominated in spring. Pupae suppression reached the maximum value in the beginning of spring and these results indicate that soil arthropods have strong impact in the decline of *B. oleae* pupae in olive groves.

**Introduction**

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is the key pest of commercial olive production, surviving and developing in any area where olive tree is cultivated (Daane and Johnson 2010; Matallanas et al. 2013). Losses caused by this insect include the premature fall of infested fruits, pulp consumption due to the larvae development inside the fruit and, ultimately, the general reduction in olive oil quality (Pereira et al. 2004). Control measures for this pest have been based on the use of organophosphate insecticides cover sprays, which have led to the development of pesticide resistance and enhancement of the risk of pest outbreaks (Hawkes et al. 2005; Kakani and Mathiopoulos 2008). Furthermore, insecticide applications have both ecological and toxicological side effects, such as environmental pollution, destruction of beneficial arthropods and contamination of olive products (Ruano et al. 2004; Santos et al.

2007a; Daane and Johnson 2010). Thus, environmentally friendly methods to control this pest have been developed in the context of integrated pest management programs, such as the use of kaolin (Saour and Makee 2004), insecticide bait sprays (Ruiz-Torres et al. 2004; Gonçalves et al. 2012), mass trapping (Haniotakis et al. 1986; Broumas et al. 2002) and lure and kill (Mazomenos et al. 2002). Overall, these methods provided divergent results, showing limited efficacy mainly at high pest population levels and side effects on the community of natural enemies (Broumas et al. 2002; Mazomenos et al. 2002; Pascual et al. 2010; Gonçalves et al. 2012).

Considering the use of arthropods as biological control agents, this has mainly been focused on parasitoids, such as *Psytalia concolor* (Szepligeti) (Hymenoptera: Braconidae), that revealed low effectiveness and low rate of establishment and persistence (Kapatos et al. 1977; Delrio et al. 2005; Wang et al. 2012). The main reasons for this can be related with the

availability of host flies throughout the year, overwintering success, or searching efficiency at low host densities (Wang et al. 2012).

Regarding these constraints, other approaches are needed, such as exploring the impact of soil arthropods as predators of *B. oleae*, since this pest overwinters as pupae in the soil starting to emerge in spring (Collier and Van Steenwyk 2003). During the overwintering period, the olive fruit fly is more exposed and vulnerable to the attack of different predaceous species (Dimou et al. 2003).

Olive groves comprise complex soil arthropod communities composed mainly by carabids, staphylinids, ants, spiders, opiliones, centipedes and earwigs (Santos et al. 2007b; Gonçalves and Pereira 2012). Some studies conducted in Europe and in the USA (California) indicate that some carabids, staphylinids, centipedes and ants can be potential predators of pupae (Neuenschwander et al. 1983; Orsini et al. 2007; Odo-guardi et al. 2008), although the impact of these groups on olive fruit fly populations is poorly known. Under field conditions, there are some methods that can be used to demonstrate this impact (e.g. Gardiner et al. 2009). Among these, exclusion methods can offer valuable clues to examine linkages between arthropod communities and pest suppression by comparing prey population from which natural enemies have been excluded, with population to which natural enemies are allowed to access (Gardiner et al. 2009; Chisholm et al. 2014). Thus, the main objective of this work was to evaluate the potential of soil arthropods as biological control agents of olive fruit fly pupae, using a paired exposed-exclusion method.

## Material and Methods

### Rearing of *B. oleae*

*Bactrocera oleae* pupae were obtained from field-collected infested olive fruits in several olive groves in the region of Mirandela (north-eastern Portugal) in October/November 2013 and kept under controlled conditions at  $21 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  relative humidity (RH) and a photoperiod of 16:8 (L:D) h. Pupae were collected and transferred to poly(methyl methacrylate) cages ( $40 \times 30 \times 30$  cm). Fifty to 100 emerged adult flies were kept per cage and fed *ad libitum* on water, a mixture of sucrose and brewer's yeast at a ratio 4:1 (based on dry weight) and they were being provided with 100 healthy olive fruits every 2 days as oviposition places. At the fourth generation, 900 pupae were gradually separated for 5 days, stored at  $5^\circ\text{C}$ , and used in field assays after 1 week.

### Exposed and exclusion boxes

Potential predation exerted by natural control agents on olive fruit fly pupae was tested using exposed and exclusion boxes. These boxes were plastic Petri dishes (6.0 cm diameter and 1.0 cm height), which were modified so that the bottom was removed and replaced by a permeable piece of cloth (1.0 mm mesh), to let the rain water pass through. Each box was filled with sterilized sand and five pupae of olive fruit flies per box were buried at about 0.5 cm depth. A total number of 180 boxes were used, with 90 of those covered by a fine mesh piece of cloth (1.0 mm), glued to the walls, to prevent access of edaphic arthropods to pupae – exclusion boxes – and the other 90 remained uncovered and served as exposed boxes.

### Study areas

The study areas are located in two olive groves near Mirandela (north-eastern Portugal), respectively, in Valbom dos Figos ( $41^\circ 33' 00.58''\text{N}$ ,  $7^\circ 08' 39.92''\text{W}$ ) and Cedães ( $41^\circ 29' 16.86''\text{N}$ ,  $7^\circ 07' 34.02''\text{W}$ ). Valbom dos Figos grove has been conducted according to organic growing guidelines since 1991. The grove covers an area of 5 ha and was planted with trees between 70 and 100 years old, spaced  $10 \times 10$  m apart. The predominant cultivars were Cobrançosa and Verdeal Transmontana. Insecticides were not sprayed during the assay. An application of copper was sprayed in February. Considering soil coverage, a mixture of leguminous plants (*Trifolium repens* L., *Trifolium fragiferum* L., *Trifolium incarnatum* L.) was sown in 2008 and it is regularly grazed by sheep. The grove in Cedães is being treated according to the principles of Integrated Pest Management since 2003. This grove covers an area of 4 ha, with trees of approximately 20 years old; plants are spaced  $7 \times 7$  m apart and the dominant cultivar is Cobrançosa. Pesticides were not sprayed during the assay. Soil was covered by spontaneous plants. Both groves were rain-fed and no vegetation cuttings occurred during the field assay.

### Field assay

A field assay was carried out between January and May 2014. In each olive grove, a central area was selected and nine sets were installed in the south side of the canopy at about 50 cm from the base of the trunk. Each set consisted of five exposed boxes, five exclusion boxes and a pitfall trap that were dug into the ground and levelled with the soil surface. Each pitfall trap was placed in the centre of the set and both

exposed and exclusion boxes were arranged around it at a distance of about 20 cm. Sets were placed in an arrangement of  $3 \times 3$  and spaced 45–50 m from one another. Pitfall traps (plastic cups with a top diameter of 115 mm and 130 mm height) were filled with 250 ml of ethylene glycol (antifreeze liquid) and a lid supported by iron wires was placed to exclude rain, debris and small vertebrates. Pitfall traps were used to assess soil arthropod activity density near exposed and exclusion boxes. Every 3 weeks, for a total of five sampling periods, one exposed box and one exclusion box were taken from each set and were carried out to the laboratory and the content of each pitfall trap was collected. The five sampling periods corresponded to 22, 42, 63, 84 and 105 days after the installation of the experiment on the 21st of January 2014, and represented, respectively, the winter period (day 22 till day 42) and the spring period (day 63 till day 105).

In the laboratory, sand was removed from each box, spread on the bottom of a container ( $15 \times 7 \times 5$  cm) and covered with water. This mixture was shaken and all floating pupae or pupae remains were recovered and examined under a binocular stereomicroscope for signs of predation (i.e. traces of pupae cuticle, or pupae with holes or pierced). Apparently intact pupae were placed under controlled conditions at  $21 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  relative humidity (RH) and a photoperiod of 16:8 (L:D) h for evaluating emergence rates.

### Arthropod identification

All individuals captured with the pitfall traps were sorted, counted, identified using a binocular stereomicroscope and preserved in ethanol 70%. Araneae, Formicidae, Carabidae and Staphylinidae were identified to order, family or species according to Roberts (1985, 1987), Collingwood and Price (1998), Aguiar and Serrano (2012), and Outerelo and Gamarra (1985), respectively. Each taxon was further classified by their trophic guild based on personal observations and literature review. Arthropods were classified as predators (P) that actively pursue their prey, mainly predators (MP) that complement their diets with other type of foods, or have scavenger or opportunistic habits, omnivorous (OM) that have different food sources, granivorous (G) that eat seeds, or are seed harvesters, saprophagous/fungivorous (SFA) that feed on organic matter, or microorganisms. Specimens not belonging to any of these groups were labelled as non-identified (NI) and were not included in the analysis.

### Data analysis

Data analyses were performed for comparing the abundance of arthropods and trophic guilds collected in pitfall traps in both olive groves and over the sampling period. First, the normal distribution of the residuals and the homogeneity of variance were evaluated by means of the Kolmogorov–Smirnov test and Levene's tests, respectively. General linear models were used to test the effect of olive grove and sampling date followed by the Tukey–Kramer HSD test. The olive grove was included in the analyses as random factor and data values were transformed as  $\log(x + 1)$  to normalize the data.

A Wilcoxon paired signed-rank test was used to compare the average number of *B. oleae* pupae found in the exclusion and exposed boxes for the five sampling dates and each olive grove. These statistical analyses were performed with IBM-SPSS statistics, version 19.0.0 (SPSS Inc. IBM Company, 2010).

Data from exposed and exclusion boxes were used to calculate the percentage of pupae suppression that expresses the change in the number of *B. oleae* pupae in the presence of edaphic arthropods (Eq. 1).

$$\text{Pupae suppression (\%)} = \frac{(B_{\text{ex}} - B_{\text{e}})}{B_{\text{ex}}} \times 100 \quad (1)$$

Pupae suppression was calculated for each pair of exclusion/exposed boxes taken from each set and counts of *B. oleae* pupae on the exclusion box ( $B_{\text{ex}}$ ) were compared with counts of *B. oleae* pupae on the exposed box ( $B_{\text{e}}$ ). Mean and standard error of the mean (SE) were calculated for each sampling period. Only pupae recovered with no signs of predation were counted.

## Results

### Composition of the community of edaphic arthropods

A full list of the abundance, relative abundance (%), mean  $\pm$  standard error (SE) and trophic guilds of captured taxa in total pitfall traps in the two olive groves is provided in Appendix and summarized in table 1. A total of 6967 arthropods were captured in both olive groves (table 1).

Captures were numerically dominated by the class Insecta, followed by Arachnida and Chilopoda, representing, respectively, 75.9%, 23.9% and 0.2% of the total captures. Among the class Insecta, the family Formicidae was the most abundant, representing 43.3% of the total captures, followed by the family

Group	N (n <sup>1</sup> = 90)	Relative abundance (%)	Mean ± SE	F <sub>4, 84</sub>	P
Insecta					
Formicidae	3014	43.3	33.49 ± 9.28	8.36	<0.001
Forficulidae	987	14.2	10.97 ± 1.68	9.37	<0.001
Staphylinidae	586	8.4	6.51 ± 2.31	2.13	0.08
Carabidae	79	1.1	0.88 ± 0.18	4.80	0.09
Other Coleoptera	621	8.9	6.90 ± 1.47	10.80	<0.001
Subtotal	5287	75.9			
Arachnida					
Araneae	1361	19.5	15.12 ± 1.26	5.61	<0.001
Acari	307	4.4	3.41 ± 1.48	7.95	<0.001
Subtotal	1668	23.9			
Chilopoda					
Scolopendromorpha	12	0.2	0.13 ± 0.08	–	–
Total arthropods	6967		43.68 ± 6.06	2.75	0.101
Trophic Guilds					
Predators	1541	22.1	17.10 ± 1.29	4.80	0.002
Mainly Predators	120	1.7	1.33 ± 0.20	4.14	0.004
Omnivorous	2272	32.6	25.24 ± 5.68	1.44	0.227
Granivorous	1704	24.5	18.93 ± 7.20	8.25	<0.001
Saprophagous/Fungivorous	404	5.8	4.49 ± 7.20	3.53	0.010
Non-identified	928	13.3	10.31 ± 2.28	8.01	<0.001

<sup>1</sup>Total number of samples. F and P are statistical results for comparisons of abundance between sampling dates.

Forficulidae (14.2%), Staphylinidae (8.4%) and Carabidae (1.1%). Class Arachnida was dominated by the order Araneae with 19.5% of relative abundance, followed by Acari with 4.4%. The most abundant trophic guild of arthropods captured in pitfall traps were omnivorous, representing 32.6% of total captures, followed by granivorous representing 24.5% and predators, representing 22.1%.

Considering the family Formicidae, 3014 individuals were captured in both olive groves belonging to 22 species from 13 genera. The most abundant species was *Messor barbarus* (L.), representing 55.8% of total captured individuals, followed by *Tapinoma nigerrinum* (Nylander) with 29.5%, *Crematogaster auberti* Emery with 4.8% and *Cataglyphis hispanicus* (Emery) with 2.2% (Appendix 1). For the family Formicidae, granivorous was the dominant functional group (56.5%) followed by omnivorous (42.4%) and mainly predators (1.1%) (Appendix 1). Forficulidae was represented by a single species, *Forficula auricularia* (L.), included in the omnivorous guild, with 987 individuals captured in both groves.

In the family Staphylinidae, 586 individuals were captured, belonging to five different subfamilies that were identified in 16 genera. The most abundant genera were *Anotylus* (subfamily Oxytelinae) with 64.3%

of relative abundance, followed by *Ocyopus* (subfamily Staphylininae) with 13.0%, *Mycetoporus* (subfamily Tachyporinae) with 8.7% and *Quedius* (subfamily Staphylininae) with 4.6%. In Staphylinidae, saprophagous/fungivorous represented the dominant functional group followed by predators (68.9% and 19.5% respectively) and mainly predators (11.6%) (Appendix 1).

For the family Carabidae, 79 individuals were captured in both olive groves belonging to 16 species and 11 genera. The most abundant species were *Calathus granatensis* Vuillefroy, representing 33% of the total captures, *Pterostichus globosus* (Quensel in Schonherr) representing 20.3%, *Licinus punctatulus* (Fabricius) representing 10.1% and *Amara aenea* (De Geer) representing 7.6%. Within Carabidae, predators were the most abundant functional group (65.8%) followed by species that are mainly predators (25.3%) and omnivorous species (8.9%) (Appendix 1).

In the order Araneae, 1361 individuals were captured in both olive groves belonging to 13 different families. The most abundant families were Gnaphosidae representing 47.1% of the total captures, followed by Lycosidae with 19.1%, Zodariidae with 9.7% and Thomisidae with 9.4% (Appendix 1).

**Table 1** Abundance (N), relative abundance (%) and mean ± standard error (SE) of taxa and trophic guilds captured in pitfall traps (n = 90) in two olive groves, January–May 2014

The abundance of the different groups of soil arthropods varied through the sampling period (fig. 1), and statistical analyses showed significant differences for Araneae, Formicidae, Forficulidae, Coleoptera and Acari (table 1). During the winter period (days 22 and 42 after the installation), soil community was dominated by Forficulidae, followed by Araneae and Formicidae. During the spring period (days 63 to 105), the community was dominated by Formicidae followed by Araneae and Staphylinidae. The dynamics of abundance of different functional groups through sampling time is shown in fig. 2. In the winter period and in the first sampling date of spring (day 63 after installation), the community was dominated by omnivorous and predators. In the two last sampling dates, granivorous dominated the community followed by omnivorous and predators (fig. 2).

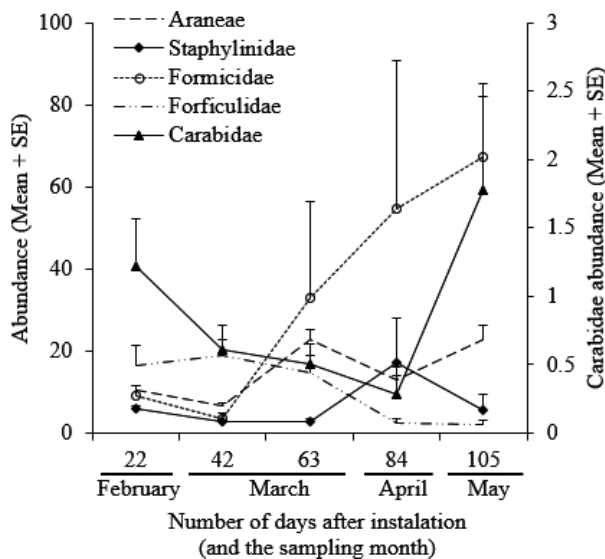
**Exposed vs. exclusion boxes and pupae suppression**

The Wilcoxon test showed that the number of *B. oleae* pupae found in exposed boxes (median [quartiles] of 1 [0, 2]) was significantly different from exclusion boxes (median of 5). The percentage of pupae suppression was calculated for each sampling time and varied between 62.39% in the first date and 100% in the last date (table 2).

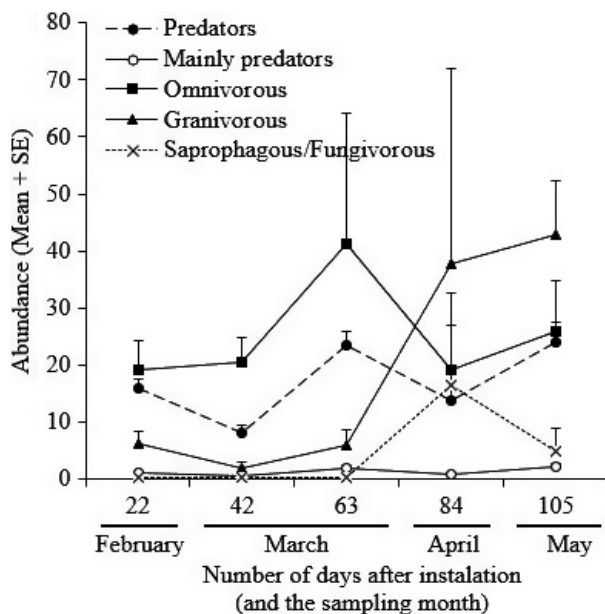
During the field assay, from a total of 450 pupae placed in 90 exposed boxes (i.e. five pupae per box), only 41 pupae were recovered; in the first sampling time, 31 of 90 pupae (34.4%) were recovered from exposed boxes, from which 13 pupae (41.9%) had signs of predation and three adults (9.7%) emerged from pupae in laboratory conditions. In the second sampling date, 10 of 90 pupae (11.1%) were recovered, from which eight pupae (80.0%) had signs of predation and no adults emerged from pupae in laboratory conditions. In the three last sampling dates, no pupae were recovered from exposed boxes. The initial number of pupae placed in each exclusion box was recovered at the end of the sampling time, but only five adults (1.1% of the total) emerged from pupae collected in the first date.

**Discussion**

During this study, there were several evidences that soil arthropods could have impact on the abundance of *B. oleae* pupae. These evidences were mainly supported by the functional composition of soil arthropod community, by the decrease in the number of pupae in the exposed boxes when compared to the



**Fig. 1** Dynamics of the abundance (mean + standard error - SE) of edaphic arthropods captured in pitfall traps in two olive groves, Mirandela, Portugal. The x-axis represents the number of days after the installation of pitfall traps on the 21st of January 2014 and the sampling month. Note different scales of right and left y-axes. n = 18 for each sampling period.



**Fig. 2** Abundance (mean + standard error - SE) of trophic groups captured in pitfall traps in two olive groves, Mirandela, Portugal. The x-axis represents the number of days after the installation of pitfall traps on the 21st of January 2014 and the sampling month. n = 18 for each sampling period.

exclusion boxes and by the remains of pupae recovered from exposed boxes with signs of predation. The percentage of pupae suppression ranged from 62.39%

**Table 2** Percentage of pupae suppression, cumulative abundance of predators and cumulative abundance of omnivorous (mean  $\pm$  SE) during five sampling periods

Number of days after box installation	Pupae suppression (%)	Cumulative abundance of predators	Cumulative abundance of omnivorous
22	62.39 $\pm$ 6.24	17.22 $\pm$ 1.47	19.22 $\pm$ 5.16
42	86.88 $\pm$ 3.95	26.00 $\pm$ 2.17	39.89 $\pm$ 6.31
63	100.00 $\pm$ 0.00	51.56 $\pm$ 3.75	81.28 $\pm$ 24.58
84	100.00 $\pm$ 0.00	66.11 $\pm$ 4.63	100.33 $\pm$ 37.65
105	100.00 $\pm$ 0.00	92.17 $\pm$ 5.25	126.22 $\pm$ 44.87

to 100% indicating that soil arthropods can actively reduce *B. oleae* in its pupal stage.

This study took place during winter to early spring, which is a period that has been less considered regarding the study of composition of soil arthropod communities in olive groves and their relevance for suppressing *B. oleae*. The community of arthropods that was active in this period was mainly composed by Formicidae, Forficulidae, Araneae, Staphylinidae, Carabidae and Scolopendromorpha. In general, these soil arthropods have been commonly found in olive groves throughout the year and in several countries of the Mediterranean region (Neuenschwander et al. 1983; Morris and Campos 1999; Ruano et al. 2004; Santos et al. 2007b; Gonçalves and Pereira 2012).

Usually, Formicidae was the dominant group in studies developed in spring (in particular, in late spring) and summer (Morris and Campos 1999; Santos et al. 2007b) but, during winter, its activity was reduced, remaining in nests, due to low temperatures. Gonçalves and Pereira (2012) also observed lower numbers of Formicidae in early spring. The community of formicids was mainly composed by the species *M. barbarus* and *T. nigerrimum* that have been previously referred in other works concerning the same ecosystem (Morris and Campos 1999; Santos et al. 2007b; Gonçalves and Pereira 2012). The former was the dominant species in the winter period while the latter dominated in the beginning of spring, and previous studies also indicated that both species were highly abundant in late spring and summer (Morris and Campos 1999; Santos et al. 2007b). *M. barbarus* is a seed harvester species that prefers open areas and *T. nigerrimum* is an aggressive omnivorous species that consumes honeydew and animal items (Cerdá et al. 1989; Azcárate and Peco 2003). In the olive grove, it can be an important predator of the olive moth, *Prays oleae* (Bern.) (Morris and Campos 1999). In this study, formicids could have important predatory action on

the olive fruit fly between the second and the third sampling periods as it was in this period that their activity (mainly *T. nigerrimum* activity) increased significantly and pupae suppression reached 100%. This corresponds to the rise of temperatures that can also promote *B. oleae* pupae emergence. The teneral stage may be more susceptible of being predated by formicids due to its reduced mobility as it was reported by several authors for other fruit flies (Wong and Wong 1988; Eskafi and Kolbe 1990; Hodgson et al. 1998). On the other hand, *M. barbarus* seems an unlikely predator of *B. oleae* pupae due to its granivorous habits, although, in a laboratory experiment, Neuenschwander et al. (1983) observed this species carrying pupae into the nest. Thus, it is possible that this behaviour could also occur in the field and contribute to the decline of pupae in exposed boxes as well as to bury the pupae in deeper layers of the soil, hindering emergence.

The order Araneae was also abundant in this study and was mainly composed by the families Gnaphosidae, Lycosidae, Zodariidae and Thomisidae, which is similar to the results obtained by Cárdenas et al. (2012) in Spain and Thaler and Zapparoli (1993) in Italy. Gnaphosidae dominated in all sampling dates, except in the last date, where Lycosidae were more abundant. Gnaphosidae is a typical family in Mediterranean habitats (Cardoso et al. 2007), represented essentially by nocturnal hunters that move very fast on the ground and that were reported to forage actively for larvae and eggs of Diptera, other spiders, Thysanoptera, Hemiptera and Coleoptera (Richman et al. 1980; Chatzaki 2008). Lycosidae includes both diurnal and nocturnal active hunters with a wide range of prey in their diet such as dipterans and collembolans (Nyffeler and Benz 1988; Allen and Hagley 1990) and that rely on vibratory and visual stimuli to locate and detect prey (Rovner 1991; Persons and Uetz 1996). There are no references about consumption of *B. oleae* pupae by Gnaphosidae or Lycosidae families. However, Monzó et al. (2009) observed that *Pardosa cribata* Simon, an abundant lycosid spider in citrus orchards in Spain, fed on both larval and adult stages but not on pupae of *Ceratitis capitata* (Wiedemann). Thus, due to the immobility of pupae on the ground, it seems unlikely that spiders could act as active predators of *B. oleae* pupae, although some predation can occur on teneral flies.

Forficulidae was composed by a single species, *F. auricularia* that dominated the community of arthropods in winter period, decreasing its abundance in spring. In winter period, captures were mainly composed by nymphal stages. In spring, nymphs of

the third instar migrate from the soil to the tree motivated by the increase of the temperature (Gobin et al. 2008) which can explain the decrease of the abundance on soil. *F. auricularia* is an omnivorous species, feeding on a high variety of food items such as soft-fleshed fruit and plant material as well as a wide range of arthropods (Shaw and Wallis 2010), and is referred as an important generalist predator (Gobin et al. 2008). In Crete, Neuenschwander et al. (1983) observed *F. aetolica* Brunner preying on *B. oleae* pupae in laboratory experiments. In this study, *F. auricularia* could be one of the most active predators of pupae, mainly in the first three sampling dates (winter and early spring), since its abundance was high in that period and they were frequently found in exposed boxes.

Considering Staphylinidae, the community was dominated by *Ocypus* sp. that was mainly abundant in winter and *Anotylus* sp. that was abundant in spring. Neuenschwander et al. (1983) also reported the occurrence of *Ocypus* sp. in olive groves in Crete (Greece). Staphylinids have been referred as predators of buried pupae such as *C. capitata* in coffee and orange orchards in Guatemala (Eskafi and Kolbe 1990), *Rhagoletis pomonella* (Walsh) in apple orchards in Southern Ontario (Allen and Hagley 1990) and *B. oleae* in laboratory experiments (Neuenschwander et al. 1983).

Carabidae were the least abundant group collected in this study, contrasting with other works conducted in spring and particularly in autumn where they represented one of the most abundant groups of arthropods (Gonçalves and Pereira 2012; Oliveira 2013). Dominant species, *C. granatensis* and *P. globosus*, are predaceous species and both genera were also caught in olive groves in Crete and observed eating *B. oleae* pupae in laboratory experiments (Neuenschwander et al. 1983). In Italy, *Pterostichus melas* (Creutzer), *Calathus fuscipes* (Goeze), *Pseudoophonus rufipes* (De Geer), *Laemostenus cimmerius* (Fischer von Waldheim) and *Distichus planus* (Bonelli) fed regularly on *B. oleae* pupae in a laboratory feeding assay (Odoguardi et al. 2008). Although staphylinids and carabids were not abundant during this sampling period, it seems likely that they could exert predatory action on pupae buried in exposed boxes. Moreover, their high abundance in autumn can be important to reduce *B. oleae* pupae in this season.

Other factors such as abiotic factors (e.g. low temperatures or high soil moisture levels due to rain) can also contribute to pupal mortality (Daane and Johnson 2010) and the low number of adults emerged from pupae recovered in exclusion boxes can indicate this. Both olive groves studied maintained a ground

cover of vegetation which can influence the abundance and the composition of the community of arthropods occurring in olive groves. Usually, ground covers have been related to a high abundance and activity of several species of ants, carabids and spiders (Cotes et al. 2009; Campos et al. 2011; Cárdenas et al. 2012) which can be reflected in a high biological control ecosystem service. It is possible that changes in ground covers, due to tillage or herbicide application, can negatively affect arthropod communities, but further works are required to look at the effect of these actions on biological control of the olive fruit fly.

In conclusion, an abundant and diverse soil arthropod community in olive groves could have impact and provide important suppression of *B. oleae* during its pupal stage.

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**Appendix 1**

Total abundance (N), mean  $\pm$  standard error (SE) and trophic guilds of captured edaphic arthropods in pitfall traps in the two olive groves, January–May 2014

Taxa	N (n = 90)	Mean $\pm$ SE	Trophic Guild
<b>Carabidae</b>			
<i>Calathus granatensis</i> Vuillefroy	26	0.29 $\pm$ 0.13	Predator
<i>Pterostichus globosus</i> (Quensel in Schonherr)	16	0.18 $\pm$ 0.07	Mainly Predator
<i>Calathus mollis</i> (Marsham)	4	0.04 $\pm$ 0.02	Predator
<i>Calathus cinctus</i> Motschulsky	1	0.01 $\pm$ 0.01	Predator
<i>Calathus</i> sp.	1	0.01 $\pm$ 0.01	Predator
<i>Nebria salina</i> Fairmaire & Laboulbene	3	0.03 $\pm$ 0.02	Predator
<i>Amara aenea</i> (De Geer)	6	0.07 $\pm$ 0.03	Omnivorous
<i>Brachinus</i> sp.	1	0.01 $\pm$ 0.01	Mainly Predator
<i>Brachinus explodens</i> Duftschmid	1	0.01 $\pm$ 0.01	Mainly Predator
<i>Brachinus variventris</i> Schaufuss	2	0.02 $\pm$ 0.02	Mainly Predator
<i>Licinus punctatulus</i> (Fabricius)	8	0.09 $\pm$ 0.04	Predator
<i>Anchomenus dorsalis</i> (Pontoppidan)	1	0.01 $\pm$ 0.01	Predator
<i>Olisthopus fuscatus</i> Dejean	1	0.01 $\pm$ 0.01	Predator
<i>Parophonus maculicornis</i> (Duftschmid)	1	0.01 $\pm$ 0.01	Omnivorous
<i>Trechus obtusus</i> Erichson	4	0.04 $\pm$ 0.02	Predator
<i>Poecilus</i> sp.	3	0.03 $\pm$ 0.02	Predator
<b>Staphylinidae</b>			
<i>Ocypus</i> sp.	76	0.84 $\pm$ 0.23	Predator
<i>Quedius</i> sp.	27	0.30 $\pm$ 0.07	Predator
<i>Mycetoporus</i> sp.	51	0.57 $\pm$ 0.14	Mainly Predator
<i>Oxytelus</i> sp.	16	0.18 $\pm$ 0.07	Saprophagous/Fungivorous
<i>Tachyporus</i> sp.	11	0.12 $\pm$ 0.05	Mainly Predator
<i>Thinodromus</i> sp.	7	0.08 $\pm$ 0.07	Saprophagous/Fungivorous
<i>Othius</i> sp.	5	0.06 $\pm$ 0.02	Predator
<i>Gabrius</i> sp.	2	0.02 $\pm$ 0.02	Predator
<i>Anotylus</i> sp.	377	4.19 $\pm$ 2.32	Saprophagous/Fungivorous
<i>Coproporus</i> sp.	4	0.04 $\pm$ 0.03	Mainly Predator
<i>Philonthus</i> sp.	1	0.01 $\pm$ 0.01	Predator
<i>Xantholinus</i> sp.	2	0.02 $\pm$ 0.02	Predator
<i>Astenus</i> sp.	1	0.01 $\pm$ 0.01	Predator
<i>Tachinus</i> sp.	2	0.02 $\pm$ 0.02	Mainly Predator
<i>Carpelinus</i> sp.	2	0.02 $\pm$ 0.02	Saprophagous/Fungivorous
<i>Metopsia</i> sp.	2	0.02 $\pm$ 0.02	Saprophagous/Fungivorous
Other Coleoptera	621	6.90 $\pm$ 1.47	Non-identified
<b>Formicidae</b>			
<i>Messor barbarus</i> (Linnaeus)	1681	18.68 $\pm$ 7.20	Granivorous
<i>Messor bouvieri</i> Bondroit	20	0.22 $\pm$ 0.18	Granivorous
<i>Camponotus pilicornis</i> (Roger)	23	0.26 $\pm$ 0.12	Omnivorous
<i>Camponotus aethiops</i> (Latreille)	3	0.03 $\pm$ 0.03	Omnivorous
<i>Camponotus piceus</i> (Leach)	7	0.08 $\pm$ 0.06	Omnivorous
<i>Camponotus cruentatus</i> (Latreille)	1	0.01 $\pm$ 0.01	Omnivorous
<i>Camponotus lateralis</i> (Olivier)	1	0.01 $\pm$ 0.01	Omnivorous
<i>Camponotus foreli</i> Emery	2	0.02 $\pm$ 0.02	Omnivorous
<i>Tetramorium forte</i> Forel	63	0.70 $\pm$ 0.19	Omnivorous
<i>Tetramorium semilaeve</i> Andre	11	0.12 $\pm$ 0.05	Omnivorous
<i>Tapinoma nigerimum</i> (Nylander)	890	9.89 $\pm$ 5.50	Omnivorous
<i>Crematogaster auberti</i> Emery	144	1.60 $\pm$ 0.32	Omnivorous
<i>Cataglyphis hispanicus</i> (Emery)	66	0.73 $\pm$ 0.27	Omnivorous
<i>Cataglyphis</i> sp.	54	0.60 $\pm$ 0.24	Omnivorous
<i>Plagiolepis pygmaea</i> (Latreille)	29	0.32 $\pm$ 0.13	Mainly Predator
<i>Lasius</i> sp.	1	0.01 $\pm$ 0.01	Mainly Predator

(continued)

**Appendix 1** (continued)

Taxa	N (n = 90)	Mean $\pm$ SE	Trophic Guild
<i>Goniomma</i> sp.	3	0.03 $\pm$ 0.02	Granivorous
<i>Aphaenogaster gibbosa</i> (Latreille)	4	0.04 $\pm$ 0.03	Omnivorous
<i>Aphaenogaster</i> sp.	8	0.09 $\pm$ 0.09	Omnivorous
<i>Pheidole</i> sp.	1	0.01 $\pm$ 0.01	Omnivorous
<i>Formica subrufa</i> Roger	1	0.01 $\pm$ 0.01	Mainly Predator
<i>Solenopsis</i> sp.	1	0.01 $\pm$ 0.01	Mainly Predator
Forficulidae			
<i>Forficula auricularia</i> Linnaeus	987	10.97 $\pm$ 1.68	Omnivorous
Scolopendromorpha			
Scolopendromorpha	12	0.13 $\pm$ 0.08	Predator
Araneae			
Agelenidae	89	0.99 $\pm$ 0.17	Predator
Dysderidae	1	0.01 $\pm$ 0.01	Predator
Eresidae	1	0.01 $\pm$ 0.01	Predator
Gnaphosidae	641	7.12 $\pm$ 0.71	Predator
Linyphiidae	40	0.44 $\pm$ 0.11	Predator
Lycosidae	260	2.89 $\pm$ 0.64	Predator
Philodromidae	17	0.19 $\pm$ 0.05	Predator
Salticidae	33	0.37 $\pm$ 0.08	Predator
Sparassidae	1	0.01 $\pm$ 0.01	Predator
Tetragnathidae	1	0.01 $\pm$ 0.01	Predator
Theridiidae	17	0.19 $\pm$ 0.06	Predator
Thomisidae	128	1.42 $\pm$ 0.21	Predator
Zodariidae	132	1.47 $\pm$ 0.31	Predator
Acari	307	3.41 $\pm$ 1.48	Non-identified
Total arthropods	6967		