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Role of edaphic arthropods on the biological control of the olive fruit fly (*Bactrocera oleae*)

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Aos meus pais
Ao Luís

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Abstract

The olive fruit fly, *Bactrocera oleae* (Rossi) is a major pest of the olive tree. A great part of its life cycle is spent inside the olive fruit, which hinders the action of natural enemies. However, pupation usually occurs on the ground, which makes this stage more vulnerable to predation by edaphic arthropods. In this context, with the present work, it was studied the role of the edaphic arthropods on the biological control of olive fruit fly. Under laboratory conditions, *Calathus granatensis* Vuillefroy and *Pterostichus globosus* Quensel, two species of carabids abundant in groves of Trás-os-Montes were evaluated as potential predators of olive fruit fly. The food preferences of both carabids were studied as olive fruit fly pupae were offered together with pupae of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) in different proportions. It was also evaluated the functional responses of both carabids on different densities of olive fruit fly pupae. Under field conditions predation by edaphic arthropods on olive fruit fly pupae was evaluated using exposed-exclusion boxes to predators along with pitfall traps for capture of the arthropods active near the boxes. The assay was conducted in two olive groves of the region of Mirandela (northeast of Portugal) between January and May. Biological control provided by edaphic arthropods was measured by calculating biological control services indexes that were further correlated with the abundance of arthropods and functional groups captured in the pitfall traps. The results of the laboratory experiments indicate that both species of carabids studied preyed olive fruit fly pupae, however, *C. granatensis* proved to have more preference for olive fruit fly pupae over the alternative prey independently of the offered ratio whereas *P. globosus* demonstrated no preference for olive fruit fly having consuming the two types of pupae. This species of carabid proved to be more polyphagous and revealed a "switching" behavior. The functional response curves demonstrated that both carabids exhibited a type II functional response in which the number of pupae consumed increased as the density of offered pupae increased until it reached a plateau where the consumption remained constant regardless of the offered density. In the field experiment, it was demonstrated that family Formicidae, the order Araneae and family Forficulidae dominated the arthropod community, wherein family Formicidae dominated during the period between the end of winter and beginning of spring and Forficulidae during the winter period. Concerning functional groups, omnivorous arthropods dominated the community, followed by granivorous and predators. The maximum value of biological control services index was achieved in the period between late winter and early spring, when the abundance of predators and omnivorous arthropods reached its maximum. Relationships between the presence of these two functional groups and the biological service index values were found, especially the presence of omnivorous, in which the family Forficulidae stood out during the winter period and family Formicidae during the spring period. The results demonstrate that important biological control services can be provided by edaphic arthropods against olive fruit fly pupae in olive groves. These services are the result of great complementarity among arthropods groups in the different periods of the year. On one hand, during the fall, groups of arthropods such as carabids can be important predators of olive fruit fly pupae and during the period of winter and beginning of spring, omnivorous arthropods such as insects from families Forficulidae and Formicidae may have higher importance. Therefore, it becomes necessary to conserve these groups of arthropods in olive groves in order to maintain or even increase the biological control services against the olive fruit fly.

Key-words: *Bactrocera oleae*, edaphic arthropods, biological control services index, carabids, food preference, functional responses.

Resumo

A mosca-da-azeitona, *Bactrocera oleae* (Rossi) é uma das principais pragas da oliveira. Grande parte do seu ciclo de vida é passado dentro da azeitona, o que dificulta a ação dos seus inimigos naturais. No entanto geralmente a fase de pupa ocorre no solo, o que torna este estádio mais vulnerável à predação por artrópodes edáficos. Neste contexto, com o presente trabalho, foi estudado o papel dos artrópodes edáficos na limitação natural de mosca-da-azeitona. Em condições laboratoriais, *Calathus granatensis* Vuillefroy e *Pterostichus globosus* Quensel, duas espécies de carabídeos abundantes nos olivais de Trás-os-Montes, foram avaliados como potenciais predadores de mosca-da-azeitona. Foram estudadas as preferências alimentares dos carabídeos quando pupas de mosca-da-azeitona foram fornecidas em conjunto com pupas de mosca-do-Mediterrâneo (*Ceratitis capitata* Wiedemann) em diferentes proporções. Foram também avaliadas as respostas funcionais de ambos os carabídeos a diferentes densidades de pupas de mosca-da-azeitona. Em condições de campo, avaliou-se a predação de pupas de mosca-da-azeitona por artrópodes edáficos recorrendo à instalação de caixas de exposição-exclusão de predadores juntamente com armadilhas de queda para captura dos artrópodes ativos na proximidade das caixas. Este ensaio decorreu em dois olivais da região de Mirandela (nordeste de Portugal) e no período de janeiro a maio de 2014. A luta biológica providenciada pelos artrópodes edáficos foi quantificada através do cálculo de índices de serviços de luta biológica que foram posteriormente relacionados com a abundância de artrópodes e grupos funcionais capturados nas armadilhas de queda. Os resultados das experiências laboratoriais indicam que ambas as espécies de carabídeos estudadas predaram mosca-da-azeitona, no entanto, *C. granatensis* provou ter maior preferência alimentar por mosca-da-azeitona em detrimento da presa alternativa, independentemente da proporção oferecida. *P. globosus* não demonstrou preferência por mosca-da-azeitona, tendo consumido os dois tipos de pupas. Esta espécie de carabídeo demonstrou ser mais polífaga e revelou um comportamento "switching", trocando a sua preferência dependendo da presa disponível em maior abundância. As curvas de respostas funcionais demonstraram que ambas as espécies de carabídeos exibiram uma resposta funcional do tipo II, em que o número consumido de pupas de mosca-da-azeitona aumentou à medida que a sua densidade também aumentou até atingir um plateau em que o consumo se manteve constante, independentemente do aumento da densidade. Na experiência que decorreu em campo, verificou-se que a família Formicidae, a ordem Araneae e a família Forficulidae dominaram a comunidade de artrópodes encontrada no período de amostragem, sendo que a família Formicidae foi a dominante durante o período entre o fim do inverno e início da primavera e a família Forficulidae dominou durante o período de inverno. Em termos de grupos funcionais, os artrópodes omnívoros dominaram a comunidade, seguindo-se os granívoros e os predadores. O valor máximo para o índice de serviços de luta biológica foi atingido no período entre o fim do inverno e início da primavera, quando a abundância de artrópodes predadores e omnívoros atingiu o seu máximo. Foram encontradas correlações entre a presença destes dois grupos funcionais e os valores de índice de serviços de luta biológica, sobretudo a presença de omnívoros, dos quais se destacaram as famílias Forficulidae no período de inverno e Formicidae no período de primavera. Os resultados demonstram que os artrópodes edáficos podem providenciar importantes serviços de luta biológica contra pupas de mosca-da-azeitona nos olivais. Esses serviços são o resultado de uma grande complementaridade entre grupos de artrópodes nos diferentes períodos do ano. Por um lado, durante o outono grupos de artrópodes como os carabídeos podem ser importantes predadores de pupas de mosca-da-azeitona; durante o período de inverno e início da primavera, grupos de artrópodes omnívoros como insetos da família Forficulidae e Formicidae podem ter maior importância. Assim, de forma a manter ou até aumentar os serviços de luta biológica contra mosca-da-azeitona, torna-se necessária a conservação destes grupos de artrópodes nos olivais.

Palavras-chave: *Bactrocera oleae,* artrópodes edáficos, índice de serviços de luta biológica, carabídeos, preferência alimentar, respostas funcionais.

Chapter 1

Introduction and objectives

1.1. General Introduction and objectives

The olive tree (*Olea europaea* L.) is known historically to be a very ancient tree and a classical feature of the Mediterranean landscape (Cherubini *et al*., 2013). Its cultivation exists since the late prehistory, in the early Bronze Age, where it has been grown for its oil-rich fruit (Carrión *et al.*, 2010).

Portugal is the tenth larger producer of olives in the world and the forth larger producer in Europe (FAO, 2014) having a long tradition in the cultivation of this crop, especially in Alentejo, Trás-os-Montes and Beira Interior regions. In Trás-os-Montes (Northeast of Portugal), the olive groves cover an area of more than 75 thousand ha that represent about 22% of the olive grove area in the country (INE, 2011).

The olive tree is susceptible to pests and diseases that have negative effects on its production. Among the pests, the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is considered the major pest of olives around the world (Daane and Johnson, 2010) causing annual losses estimated between 5% and 30% of the total olive production (Katsoyannos, 1992) costing about 800 million US dollars per year (Montiel- Bueno and Jones, 2002).

Chemical treatments are the main control methods for the olive fruit fly. However, populations of the olive fruit fly are susceptible to natural mortality factors in the field such as predation by edaphic arthropods when the pupal stage of the fly occurs in the ground (Neuenschwander *et al.*, 1983; Orsini *et al.*, 2007).

Olive groves of Trás-os-Montes region comprehend a rich and diverse edaphic community of arthropods (Santos *et al*., 2007; Gonçalves and Pereira, 2012) capable of providing important biological control of this pest. Potential predators include insects from the families Carabidae, Formicidae, Staphylinidae and Forficulidae that were reported in studies conducted in Europe and the in the USA as potential predators of pupae of the olive fruit fly (Neuenschwander *et al.*, 1983; Orsini *et al.*, 2007). However, in Portugal, namely in Trás-os-Montes region, the role of edaphic arthropods is still poorly understood and needs to be investigated in order to enhance the valuation of potential predators of pupae of the olive fruit fly in the field. Hereupon, the major objective of this work was to study the role of edaphic arthropods on the biological control of the olive fruit fly. The specific aims were;

i) To study the potential of *Calathus granatensis* and *Pterostichus globosus*, two abundant carabid beetles in the olive groves of Trás-os-Montes region, as predators of pupae of the olive fruit fly under laboratory conditions by measuring their feeding preferences and functional responses (Chapter 2).

ii) To measure biological control of olive fruit fly pupae provided by edaphic predators and relate it with the most abundant arthropods and functional groups (Chapter 3).

This work is divided in four chapters; Chapter 1 – General introduction and objectives; Chapter 2 – Feeding preferences and functional responses of *Calathus granatensis* and *Pterostichus globosus* (Coleoptera: Carabidae) on pupae of *Bactrocera oleae* (Diptera: Tephritidae**)**; Chapter 3 – Predation by edaphic arthropods on pupae of *Bactrocera oleae* (Diptera: Tephritidae) under field conditions; Chapter 4 – General discussion and conclusions.

1.2. The olive fruit fly, *Bactrocera oleae* (Rossi)

1.2.1. Life-cycle and bioecology

The olive fruit fly is a dipteran of the family Tephritidae (Nardi *et al.*, 2003). It is a holometabolic insect, with a life cycle that comprehends four developing stages: egg, larva, pupae and the adult.

In what concerns morphology; the eggs are whitish and elongated (Alvarado *et al.*, 2008). Larvae are apodal, of yellowish-white color, cylindrical shape and go through three development stages (Neuenshwander *et al.*, 1986; Cantero, 1997 in Torres, 2007). Pupae are elliptical (Figure 1.1A) and have a color that ranges from yellow-ocher to yellowish-white (Cantero, 1997; López-Villalta 1999 in Torres, 2007).

The adults are flies of about 4 to 5 mm of length (Alvarado *et al.*, 2008). Their head is yellow-reddish and features two large compound eyes and a pair of short antennae (Neuenschwander *et al.*, 1986 in Torres, 2007). The thorax is yellow with four greyish stripes (Alvarado *et al.*, 2008) and between the head and the thorax it has an ivory scutellum (Alvarado *et al.*, 2008). Wings are hyaline and iridescent, with brown ribs and the abdomen is reddish-brown, with two black lateral spots (Neuenschwander *et al*. 1986 in Torres, 2007). Female flies can be distinguished from males by the ovipositor, a pointed structure at the end of the female's abdomen (Figure 1.1B), absent in males (Collier and Van Steenwyk, 2003).

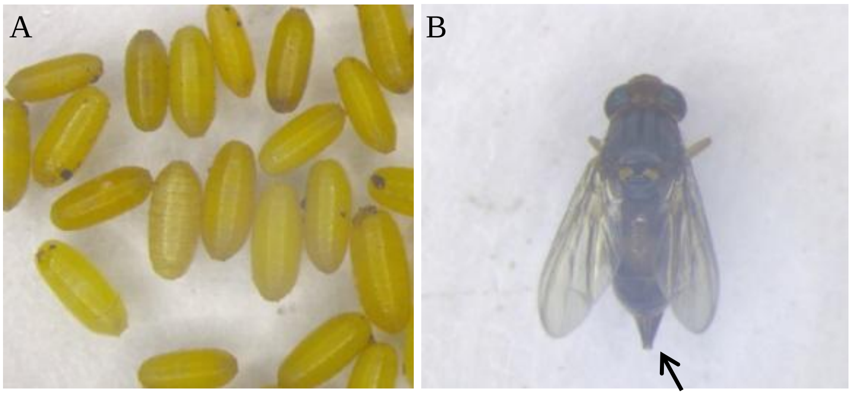

Figure 1.1. Two of the four development stages of the olive fruit fly; A - pupae, B - adult female fly showing the ovipositor (pointed arrow).

In what concerns its origin, the olive fruit fly was first believed to be originated in the Mediterranean (Daane and Johnson, 2010), but recently, molecular, phylogenetic and ecological studies indicate that the most probable origin of *B. oleae* is Africa (Nardi *et al*., 2005) where this pest has its closest relatives and where the greatest assemblage of specialized natural enemies have been reported (Daane and Johnson, 2010). Nevertheless, details of the history of olive fly populations are still incomplete and relationships of olive fly to other *Bactrocera* species are not well understood but it is possible that the historical range expansion of the species is tightly linked to the evolution and distribution of the olive tree (Nardi *et al*., 2005).

Nowadays, its distribution follows closely the distribution of the olive tree (Augustinos *et al*., 2002) and is primarily limited to regions where cultivated and wild trees are found (Daane and Johnson, 2010). It extends its range to the east, as far as India, and to the west, as far as the Canary Islands (Augustinos *et al*., 2002), being reported throughout the Mediterranean basin, South and Central Africa, the Near and Middle East, California, and Central America (Daane and Johnson, 2010).

Adult olive fruit flies first emerge in the spring (Collier and Van Steenwyk, 2003) and begin their activity, seeking sugary substances such as nectar or honeydew to feed themselves (Torres, 2007). During this period the adults usually attack olives that remained on the trees in the previous season (Collier and Van Steenwyk, 2003). However, in the early summer, when temperatures become higher and there are no mature olives to attack in the trees, the ovarian maturation in females is inhibited (Fletcher *et al*. 1978). This period is thought to be time of adult dispersal (Collier and Van Steenwyk, 2003) in which the flies can travel great distances, spread and colonize new groves (Torres, 2007).

As the new crop of olives develops throughout the summer, females interrupt their reproductive diapause and begin to produce eggs (Collier and Van Steenwyk, 2003) initiating their postures, once the fruits reach the proper development (Torres, 2007) Usually, each female lays a single egg in the susceptible olive fruit, when the pits begin to harden (Collier and Van Steenwyk, 2003). When larvae are produced during the summer and early fall, the development of the fly can be completed within the olive fruit (Dimou *et al*., 2003), with pupation occurring inside it and adult flies emerging later in the season (Collier and Van Steenwyk, 2003). However, from mid-autumn onwards an increasing number of third instar larvae leave the olive fruit to pupate in the soil (Dimou *et al*., 2003) where they overwinter and do not emerge until the following spring (Collier and Van Steenwyk, 2003). Olive fruit flies can also overwinter as adults in areas of mild climate (Torres, 2007) and also, although less commonly, as eggs and larvae in unharvested fruit (Kapatos and Fletcher, 1984).

The average duration of the life cycle of the olive fruit fly greatly depends on climatic conditions, varying from 30 or 80 days during the summer or cooler areas to 130 days in the winter or colder areas (Alvarado *et al*., 2008). Climatic conditions also affect the phenology of the fly. Hot and dry summers cause a delay in the increase of *B. oleae* population while on the other hand, humid and warm summers frequently allow an early infestation of olive fruit (Tsolakis *et al*., 2011). The number of generations of the olive fruit fly is variable and depends on climatic and agronomic conditions (Bento *et al*., 1999a; Torres, 2007; Alvarado *et al*., 2008) in areas with continental climate it usually has 2 or 3 generations per year while in Mediterranean coastal areas it usually has about 3 or 4 (Alvarado *et al*., 2008).

1.2.2. Damages and losses

The olive fruit fly is considered the key pest of olives in countries of the Mediterranean basin where it is of tremendous economic importance due to the losses that it causes in commercial olive production (Haniotakis, 2005). Females are attracted to the host plant when the olives are suitable for oviposition and lay their eggs inside the fruit (Augustinos *et al*., 2002) where the newly hatched larvae feed upon the pulp (Figure 1.2), resulting in a significant quantitative and qualitative loss in the production of table olives and oil (Daane and Johnson, 2010; Nardi *et al*., 2005).

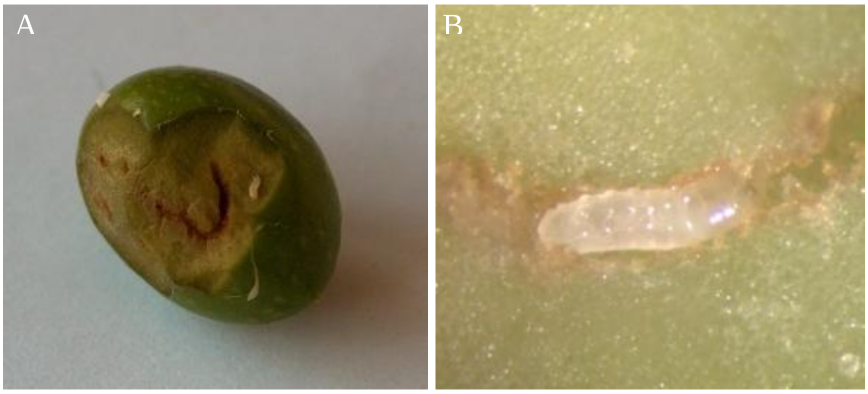

Figure 1.2. Damages caused by the olive fruit fly; A – destruction of the pulp by the feeding larvae, B – Larvae inside a mine.

The importance of the damages caused by this insect varies considerably between locations and years, and depends on the purpose olives are intended to (Pereira *et al.*, 2004; Torres, 2007). If the intention is the production of olive oil, as a result of the development of the larvae inside the fruit, the destruction of the pulp causes its premature fall as well as a decrease in the quality of the oil produced (Pereira *et al.*, 2004). If the fruit is intended for table use, the posture stings result on its complete commercial devaluation (Broumas *et al*., 2002; Torres *et al*., 2009).

1.2.3. Control methods

Over the last forty years, control of the olive fruit fly has relied mainly of chemical treatments, such as organophosphate insecticides (Kakani and Mathiopoulos, 2008; Matallanas *et al*., 2013). However, their continue use led to the development of resistance in olive fruit fly populations (Kakani and Mathiopoulos, 2008) and had ecological and toxicological side effects such as environmental pollution, contamination of olive oils and the destruction of natural enemies (Daane and Johnson, 2010).

More recently, pyrethroid insecticides have been referred as a valuable alternative tool to control the olive fruit fly (Margaritopoulos *et al.*, 2008) as well as the macrocyclic lactone spinosad (Kakani *et al.*, 2010). However, in trials, their use provided evidence that olive fruit fly field populations are capable of developing levels of resistance once this insecticides start to be commonly used.

Alternative methods that don´t involve insecticides were also developed and include: mass trapping (Broumas *et al*., 2002; Bento *et al*., 2003), attract and kill (Bento *et al*., 1999b; Mazomenos *et al*., 2002; Torres *et al*., 2002), the use of kaolin-based particle film (Saour and Makee, 2004), sterile insect technique and biological control.

From the above referred alternative methods, biological control has long been the most desired way to suppress olive fruit fly populations (Daane and Johnson, 2010) and the focus has been mainly devoted to parasitic wasps. In fact, recent surveys have suggested that a small group of braconids of the subfamilies Opiinae and Braconidae from sub-Saharan Africa best represent the primary natural enemies attacking olive fruit fly in its native range (Daane and Johnson, 2010). Among them, *Psyttalia concolor* (Szepligeti) (Hymenoptera: Braconidae) has been the most studied (Daane and Johnson, 2010; Wang *et al*., 2011) in part due to its successful mass-rearing using the Mediterranean fruit fly (*Ceratitis capitata* Weidemann) as a host (Sime *et al*., 2006; Wang *et al*., 2011). *Psytallia concolor* was widely released in the olive-growing regions of southern Europe since it was first identified, however few of these introductions resulted in establishment, and where it has established, inundative releases were required to boost parasitism rates (Sime *et al*., 2006). Besides that, both *B. oleae* and *C. capitata* are known as typical hosts of *P. concolor* in its native range and there is evidence that it may have a wider host range under laboratory conditions, which makes this parasitoid a potential risk for non-target host species (Sime *et al*., 2006).

Other parasitoids that recently brought attention were *Psittalia lounsburyi* (Szepligeti), *Psyttalia ponerophaga* (Silvestri) and *Bracon celer* Szepligeti. The first two species, considered highly specialists on olive fruit fly (Daane and Johnson, 2010; Wang *et al*., 2011), could be advantageous in classical biological control programs by not attacking non-target host species (Wang *et al*., 2011). However, besides being very difficult to rear (Daane *et al*., 2008; Wang *et al*., 2011) proved to be efficient as larval parasitoids only in small fruit cultivars because the ovipositor length wasn´t long enough to reach the larvae inside large fruits (Sime *et al*., 2007; Wang *et al*., 2011). The last one, received attention because of it high parasitism rates on *B. oleae* (Daane and Johnson, 2010). However, in a study of non-target risk for future release of this species in a classical biological control program against olive fruit fly in California it proved to have a broad physiological host range, having a strong potential to negatively impact non-target species (Nadel *et al*., 2009).

The results provided so far on biological control with the use of parasitic wasps make necessary further investigation on the subject (Daane and Johnson, 2010) and other kind of approaches concerning its natural enemies. Besides parasitoids, olive groves provide a complex of ground-dwelling generalist predators (Neuenschwander *et al*., 1983) and since the immature stages of the olive fruit fly spent almost entirely inside the olive fruit, a window of exposure to predators is often created when the fly pupates (Daane and Johnson, 2010).

1.3. The community of edaphic arthropods in olive groves

The soil biota is extremely rich and comprises a high biodiversity (Bezemer *et al.*, 2010). Within it, arthropod communities are highly rich in species, immensely diverse and contribute to fundamental ecosystem services (Santorufo *et al.*, 2012) such as biological control of crop pests.

In what concerns olive groves, the composition and structure of the arthropod fauna of the soil is less known in comparison to the one that inhabits the olive tree canopy (Santos *et al.,* 2007), studies however have shown that it is rich and diverse, composed by several orders of arachnids, chilopods and insects, including the classes Chilopoda, Malacostraca, Entognatha, Insecta, and Arachnida (Lasinio and Zapparoli, 1993; Gonçalves and Pereira, 2012) in which Formicidae, Coleoptera, Araneae, Acari, Collembola, Hemiptera, Chilopoda, Diplopoda, Dermaptera, Isopoda, and Orthoptera represent the true soil inhabitants (Santos *et al.*, 2007).

Within this diversity, potential predators represent a high portion of the edaphic fauna. Groups such as carabid beetles (Neuenschwander *et al*., 1983; Lasinio and Zapparoli, 1993; Morris and Campos, 1999; Gonçalves and Pereira, 2012); ants (Morris and Campos, 1999; Santos *et al*, 2007; Gonçalves and Pereira, 2012), staphylinids, elaterids, spiders and opiliones as well as centipedes and earwings (Gonçalves and Pereira, 2012)

(Figure 1.3) are common groups in the Mediterranean olive groves and some of them have been referred as having importance in the suppression of the olive fruit fly, especially in its pupal stage under laboratory and field conditions (Neuenschwander *et al.,* 1983; Orsini *et al.,* 2007; Odoguardi *et al*., 2008).

Figure 1.3. Examples of individuals belonging to the groups of edaphic arthropods reported predating olive fruit fly pupae; A – earwigs; B – staphylinids; C – carabid beetles, D – ants.

Most soil-dwelling ant species are referred to be predators (or scavengers), feeding on invertebrates or arthropods such as earthworms, acarids, isopods, different kinds of myriapods, collembolans, termites, other ants, and other insects (Cerdá and Dejean, 2011). Earwings are considered key generalist predators to a variety of orchard pests (Gobin *et al*., 2008) such as aphids, spider mites and psyllids (Phillips, 1981). Adults and larvae of most species of staphylinids are facultative predators of other arthropods (Frank and Thomas, 2012) and most species of carabid beetles are generalist predators, feeding on diverse types of prey (Lövei and Sunderland, 1996; Kromp, 1999) such as molluscs, ants, millipedes and collembolans (Wallace, 2004).

1.4. The importance of generalist predators

Generalist beneficial arthropods long have been assumed to be more inefficient in biological control of pests than specialist natural enemies (Hassell and May, 1986; Stiling and Cornelissen, 2005). In fact it was common to refer that to be effective, a biocontrol agent should be highly specific (Stiling and Cornelissen, 2005).

This assumption came mainly because specific natural enemies have evolved to seek out their specific targets (Symondson *et al.*, 2002) and their efficacy being not compromised by the presence of alternative hosts and non-target effects being minimized (Stiling and Cornelissen, 2005). Specific natural enemies have closer interrelationships with and specific adaptations to the pest whereas generalist haven´t (Gurr *et al.*, 2012), often possessing much more complex trophic relations than specialists, which are usually interpreted as a lower capacity in suppressing crop pests (Monzó, 2010).

Although that, recent studies have modified the idea that only specialists are efficient biological control agents. In a revision about generalist predator efficiency in biological control based on 181 studies, it was concluded that in about 75% of the cases generalist predators significantly decreased pest abundance (Symondson *et al.*, 2002) and in a meta-analysis of biological control agent performance it was concluded that biocontrol efficacy tended to be higher when agents were generalists (Stiling and Cornelissen, 2005).

Generalist natural enemies exhibit some characteristics that make them suitable biocontrol agents. They can more readily adjust to the conditions that the environment provides them with and take advantage of whatever prey or food resources are available (Gurr *et al.*, 2012), subsisting on non-pest prey, having the capacity to locally drive pests to extinction without necessarily declining in number and efficacy (Stiling and Cornelissen, 2005).

On the other hand, specialist natural enemies have the weakness of inflexibility; because the adaptation to a specific prey often entails adaptation to a specific habitat, life cycle or other conditions that maximizes the ability to exploit that specific pest as a prey (Gurr *et al.*, 2012) and thus, they are more likely to go locally extinct (Symondson *et* *al*., 2002) because agronomic practices are not always conducive to such rigid ecological requirements (Gurr *et al*., 2012).

Agroecosystems are habitats of transitory nature; periodically disrupted by cultivation, pesticides, and crop rotations and any event that happens to be detrimental to the prey even if not to the predator is likely to decline its number as total prey abundance declines (Symondson *et al*., 2002). The capacity that generalist predators have on feeding on a wide range of prey enables their survivorship in the field independently from the presence of specific prey (Lövei and Sunderland, 1996) and thus their populations can persist even when populations of pest are unavailable (Symondson *et al*. 2002) which makes them better suited than specialists in such environments and indicates their potential in conservation biological control (Tscharntke *et al*., 2002).

1.5. Measuring predation

There are some techniques that can be applied to field, and in some cases laboratory, to demonstrate that natural enemies have a significant impact upon prey populations and to measure rates as well as to provide predation indices (Kidd and Jervis, 2005).

Under field conditions, predation of insect natural enemies on prey populations can be assessed by means of exclusion methods. The principle behind their use is the comparison between prey populations in plots from which natural enemies have been excluded with populations in plots to which natural enemies are allowed access (Kidd and Jervis, 2005). These methods typically begin with an equal number of prey placed in a natural-enemy accessible treatment which is compared to survivorship and/or population growth in one or more exclusion treatments (Chisholm *et al*., 2014) using devices depending on the natural enemies being investigated and whether the aim is to exclude all natural enemies or a particular group (Kidd and Jervis, 2005).

Exclusion methods need to account for both the effectiveness of the device and the impact on the survivorship or population growth of the focal prey. For example, mesh cages can alter microclimatic factors such as light intensity, humidity, and temperature (Chisholm *et al*., 2014) and in order to separate these effects from natural enemy exclusion upon prey populations, exclusion devices must be similar as possible in construction, or very different in construction but nevertheless providing similar microclimatic conditions in their interiors (Kidd and Jervis, 2005).

Data provided by exclusion methods can be used to measure biological control by means of a Biological Control Services Index (BSI) (Gardiner *et al.*, 2009) by comparing counts of prey in the exclusion of predators with counts of prey in the presence of predators a given number of days following the initiation of the experiment.

Predation can also be measured in laboratory conditions by means of feeding assays in which individual predators are confined with prey in small containers in order to determine their predatory potential (Greenstone, 1999). Although such methods do not provide realistic means of assessing predation that may occur in a field situation, they have important advantages since they provide information relative to prey preferences by a specific predator, prey range of a predator, searching abilities, feeding behavior, attack behavior and functional responses (Grant and Sheppard, 1985).

The functional response of a predator is a key factor in the population dynamics of predator-prey systems (Murdoch and Oaten, 1975; Schenk and Bacher, 2002) and an important factor in biological control studies. A predator`s functional response is its per capita feeding rate as a function of prey abundance (Holling, 1959; Skalski and Gillian, 2001). It describes the rate at which a predator kills its prey at different densities and can determine if a predator is able to regulate the density of its prey (Murdoch and Oaten, 1975).

There are three main types of functional response; type I, type II and type III (Holling, 1959) that represent respectively an increasing linear relationship, a decelerating curve or a sigmoidal relationship (Pervez and Omkar, 2005). The ‘type I’ functional response describes that consumption rate rises linearly with prey density, Type II functional response describes that consumption rate rises with prey density, but gradually decelerates until a plateau is reached at which consumption rate remains constant irrespective of prey density while type III functional response at high prey densities is similar to a type II response, while at low prey densities, has an accelerating phase where an increase in density leads to a more than linear increase in consumption rate, resulting in a ‘S-shaped’ or ‘sigmoidal’ curve (Begon *et al.*, 2006).

The major advantage in estimating the functional response in biological control studies is that it can describe the potentiality of a predator to regulate the density of its prey and thus, its efficiency as a biocontrol agent. For this to be the case, the predator has to impose a type III functional response curve (Fernández-Arhex and Corley, 2003), or a functional response that shows density dependence, which means the predator must respond to higher prey densities by consuming an increasing proportion of the available prey over a range of prey densities (Schenk and Bacher, 2002). However, it must be referred that certain predators exhibiting Type II response have been successfully established and succeed in managing prey populations (Hughes *et al*., 1992; Fernández-Arhex and Corley, 2003).

1.6. References

Alvarado M., Civitanos, M., Durán, J.M. 2008. Plagas. In: Barranco, D., Férnandez-Escobar, R. And Rallo, L. 2008. El Cultivo del Olivo 6th edition. Ediciones Mundi Prensa and Junta de Andalucia. Madrid (Spain). 509-594 pp.

Augustinos, A.A., Stratikopoulos, E.E., Zacharopoulou, A., Mathiopoulos, K.D. 2002. Polymorphic microsatellite markers in the olive fly, *Bactrocera oleae*. Molecular Ecology Notes. 2(3): 278-280.

Begon, M., Townsend, C.R., Hayer, J.L. 2006. Ecology: From from individuals to ecosystems. 4th edition. Wiley-Blackwell, Oxford, U.K.

Bento A., Torres L., Lopes J., Sismeiro, R. 1999a. A contribution to the knowledge of *Bactrocera oleae* (Gmel.) in Tráas-os-Montes region (Northeastern Portugal): phenology, losses and control. Acta Horticulturae. 474: 541-544.

Bento, A., Pereira, J.A., Cabanas, J.E., Pinto, A., Torres, L. 2003. Economic injury levels for the olive fly, *Bactrocera oleae* (Gmel.), in Trás-os-Montes region (Northeast of Portugal). Proceedings of 1st European Meeting of the IOBC/WPRS Study Group "Integrated Control in Olives", 29-31 May 2003 Chania, Crete, Hellas: 27.

Bento, A., Torres, L., Lopes, J. 1999b. Studies on the control of the olive fruit fly, *Bactrocera oleae* (Gmel.) by mass trapping. *XIV lnternational Plant Protection congress (IPPC),* Jerusalem, Israel, July 25-30, 1999, 104.

Bezemer, T.M., Fountain, M.T., Barea, J.M., Christensen, S., Dekker, S.C., Duyts, H., van-Hal, R., Harvey, J.A., Hedlund, K., Maraun, M. 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. Ecology. 91(10): 3027-3036.

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. Journal of Applied Entomology. 126(5): 217-223.

Carrión, Y., Ntinou, M., Badal, E. 2010. *Olea europaea* L. in the North Mediterranean basin during the Pleniglacial and the Early–Middle Holocene. Quaternary Science Reviews. 29: 952-968.

Cerdá, X., Dejean, A. 2011. Predation by ants on arthropods and other animals. Predation in the Hymenoptera: An Evolutionary Perspective. National Academy of Sciences (US). 39-78 pp.

Cherubini, P., Humbel, T., Beeckman, H., Gärtner, H., Mannes, D. 2013. Olive tree-ring problematic dating: a comparative analysis on Santorini (Greece). PLoS ONE 8(1): e54730.

Chisholm, P., Gardiner, M., Moon, E., Crowder, D. W. 2014. Tools and techniques for investigating impacts of habitat complexity on biological control. Biological Control. 75: 48-57.

Collier, T., Van Steenwyk, R. 2003. Prospects for integrated control of olive fruit fly are promising in California. California Agriculture. 57(1): 28-32.

Daane, K. M., Johnson, M. W. 2010. Olive Fruit Fly: Managing managing an Ancient ancient Pest pest in Modern modern Timestimes. Annual Review of Entomology. 55: 151-169.

Daane, K.H., Sime, K.R., Wang, X-G, Nadel, H., Johnson, M.W., Walton, V.M. 2008. Psyttalia lounsburyi (Himenoptera: Braconidae), potential biological control agent for the olive fruit fly in California. Biological Control. 44: 78–89.

Dimou, I., Koutsikopoulos, C., Economopoulos, A. P., Lykakis, J. 2003. Depth of pupation of the wild olive fruit fly, *Bactrocera (Dacus) oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. Journal of Applied Entomology. 127(1): 12-17.

FAO 2014. FAO Statistics 2012. http://faostat.fao.org/

Fernández-Arhex, V., Corley, J.C. 2003. The functional response of parasitoids and its implications for biological control. Biocontrol Science and Technology. 13: 403−413.

Fletcher, B.S., Pappas, S. and Kapatos, E. 1978. Changes in the ovaries of olive flies (*Dacus oleae* (Gmelin)) during the summer, and their relationship to temperature, humidity and fruit availability. Ecological Entomology. 3:99–107.

Frank, J. H., Thomas, M. C. 2012. *Rove Beetles of Florida, Staphylinidae (Insecta: Coleoptera: Staphylinidae)*. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

Gardiner, M.M., Landis, D.A., Gratton, C., DiFonzo, C.D., O'Neal, M., Chacon, J.M., Wayo, M.T., Schmidt, N.P., Mueller, E.E., Heimpel, G.E. 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecological Applications. 19: 143-154.

Gobin, B., Moerkens, R., Herwig Leirs, H., Peusens, G. 2008. Earwigs in fruit orchards: phenology predicts predation effect and vulnerability to side-effects of orchard management. IOBC/wprs Bulletin. 35: 35-39.

Gonçalves, M.F., Pereira, J.A. 2012. Abundance and diversity of soil arthropods in the olive grove ecosystem. Journal of Insect Science. 12(20): 1-14.

Grant, J. F., Shepard, M. 1985. Technics for evaluating predators for control of insect pests. Journal of Agricultural Entomology. 2: 99·116.

Greenstone, M.H. 1999. Spider predation: how and why we study it. Journal of Arachnology. 27: 333-342.

Gurr, G.M., Wratten, S.D., Snyder, W.E. 2012. Biodiversity and insect pests: key issues for sustainable management. John Wiley & Sons. Oxford, U.K.

Haniotakis, G.E. 2005. Olive pest control: present status and prospects. IOBC/wprs Bulletin. 28(9): 1-9.

Hassell, M.P., May, R.M. 1986. Generalist and specialist natural enemies in insect predator-prey interactions. Journal of Animal Ecology. 55: 923-940.

Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. The Canadian Entomologist. 91(07): 385-398.

Hughes, R.D., Woolcok, L.T., Hughes, M.A. 1992. Laboratory evaluation of the parasitic Hymenoptera used in attempts to biologically control aphid pests of crops in Australia. Entomologia Experimentalis et Applicata. 63:177–185.

INE. 2011. Recenseamento Geral agrícola 2009. Instituto Nacional de Estatística, Lisboa.

Kakani, E.G., Mathiopoulos, K.D. 2008. Organophosphate resistance-related mutations in the acetylcholinesterase gene of Tephritidae. Journal of Applied Entomology. 132: 762-771.

Kakani, E.G., Zygouridis, N.E., Tsoumani, K.T., Seraphides, N., Zalom, F.G., Mathiopoulos, K.D. 2010. Spinosad resistance development in wild olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) populations in California. Pest Management Science. 66(4): 447-453.

Kapatos, E.T, Fletcher, B.S. 1984. The phenology of the olive fly, *Dacus oleae* (Gmel.) (Diptera, Tephritidae), in Corfu. Zeitschrift für Angewandte Entomologie. 97(1-5): 360-370.

Katsoyannos, P. 1992. Olive pests and their control in the Near East. FAO Plant Production and Protection. Paper 115. FAO, Rome.

Kidd, N.A., Jervis, M.A. 2005. Population dynamics. In *Insects as natural enemies* (435-523 pp). Springer, Netherlands.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture, Ecosystems and Environment. 74: 187-228.

Lasinio, J.P, Zapparoli, M. 1993. First data on the soil arthropoda community in an olive-grove in central Italy. In Soil biota, nutrient cycling and farming systems (eds W. Paoletti, W. Foissner and D. Coleman). , 113-121 pp. Lewis Publisher. Boca Raton.

Lövei G., Sunderland, K.D. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology. 41: 231-256.

Margaritopoulos, J.T., Skavdis, G., Kalogiannis, N., Nikou, D., Morou, E., Skouras, P. J., Tsitsipis, J.A., Vontas, J. 2008. Efficacy of the pyrethroid alpha-cypermethrin against *Bactrocera oleae* populations from Greece, and improved diagnostic for an iAChE mutation. Pest Management Science. 64: 900-908.

Matallanas, B., Lantero, E., M'Saad, M., Callejas, C., Ochando, M. D. 2013. Genetic polymorphism at the cytochrome oxidase I gene in mMediterranean populations of *Bactrocera Ooleae* (Diptera: Tephritidae). Journal of Applied Entomology. 137:(8) 624-630.

Mazomenos, B.E., Pantazi-Mazomenou, A., Stefanou, D. 2002. Attract and kill of the olive fruit fly *Bactrocera oleae* in Greece as a part of an integrated control system. Use of pheromones and other semiochemicals in integrated production. IOBC/wprs Bulletin. 25: 137-146.

Montiel Bueno, A., Jones, O. 2002. Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. IOBC/wprs Bulletin. 25: 1-11.

Monzó Ferrer, C. 2010. Artrópodos depredadores potenciales de *Ceratitis capitata* (Wiedermann) presentes en el suelo de cítricos. Tésis doctoral. Universidad Politécnica de Valencia. España.

Morris, T., Campos, M. 1999. Predatory insects in olive-grove soil. Zoologica Baetica 10: 149-160.

Murdoch, W.W., Oaten, A. 1975. Predation and population stability. Advances in Ecological Research. 9: 1-131.

Nadel, H., Daane, K.M., Hoelmer, K.A., Pickett, C.H., Johnson, M.W. 2009. Non-target host risk assessment of the idiobiont parasitoid *Bracon celer* (Hymenoptera: Braconidae) for biological control of olive fruit fly in California. Biocontrol Science and Technology. 19(7): 701-715.

Nardi, F., Carapelli, A., Dallai, R., Frati, F. 2003. The mitochondrial genome of the olive fly *Bactrocera oleae*: two haplotypes from distant geographical locations. Insect Molecular Biology. 12(6): 605-611.

Nardi, F., Carapelli, A., Dallai, R., Roderick, G.K.. and Frati, F. 2005. Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). Molecular. Ecology. 14: 2729-2738.

Neuenschwander, P., Bigler, F., Delucchi, V., Michelakis, S.E. 1983. Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in Western Crete. I. Bionomics and phonologies. *Bollettino del Laboratorio di Entomologia Agraria. “Filippo Silvestri*”. 40: 3-32.

Odoguardi, R., Bonnacci, T., Bruno, L., BrandMayr, P., Zetto, T. 2008. Carabid beetles as potential natural predators of olive fly pupae. Young Ideias in Insect Science. 1st meeting of PhD students and Post-Doctoral Fellows. Florença.

Orsini, M.M., Daane, K.M., Sime, K.R., Nelson, E.H. 2007. Mortality of olive fruit fly pupae in California. Biocontrol Science and Technology. 17: 797-807.

Pereira, J.A., Alves, R., Casal, S., Oliveira, M.B.P.P. 2004., Effect of olive fruit fly infestation on the quality of olive oil from cultivars Cobrançosa, Madural and Verdeal Transmontana. ltalian Journal of Food Science. 16: 355-365.

Pervez, A. Omkar 2005. Functional responses of coccinellid predators: An illustration of a logistic approach. Journal of Insect Science. 5:5.

Phillips, M.L. 1981. The ecology of the common earwig *Forficula auricularia* in apple orchards. PhD thesis, University of Bristol.

Santorufo, L., Van Gestel, C.A., Rocco, A., Maisto, G. 2012. Soil invertebrates as bioindicators of urban soil quality. Environmental Pollution. 161: 57-63.

Santos, S.A.P., Cabanas, J.E., Pereira, J.A., 2007. Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. European Journal of Soil Biology. 43: 77-83.

Saour, G., Makee, H. 2004. A kaolin-based particle film for suppression of the olive fruit fly *Bactrocera oleae* Gmelin (Dip., Tephritidae) in olive groves. Journal of Applied Entomology. 128(1): 28-31.

Schenk, D., Bacher, S. 2002. Functional response of a generalist insect predator to one of its prey species in the field. Journal of Animal Ecology. 71(3): 524-531.

Sime, K.R., Daane, K.M., Kirk, A., Andrews, J.W., Johnson, M.W., and Messing, R. H. 2007. *Psyttalia ponerophaga* (Hymenoptera: Braconidae) as a potential biological control agent of olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) in California. Bulletin of Entomological Research. 97(03): 233-242.

Sime, K.R., Daane, K.M., Messing, R.H., Johnson, M.W. 2006. Comparison of two laboratory cultures of *Psyttalia concolor* (Hymenoptera: Braconidae), as a parasitoid of the olive fruit fly. Biological Control. 39(2): 248-255.

Sime, K.R., Daane, K.M., Messing, R.H., Johnson, M.W. 2006. Comparison of two laboratory cultures of *Psyttalia concolor* (Hymenoptera: Braconidae), as a parasitoid of the olive fruit fly. Biological Control. 39: 248-255.

Skalski, G.T., Gilliam, J.F. 2001. Functional responses with predator interference: viable alternatives to the Holling type II model. Ecology. 82(11): 3083-3092.

Stiling, P., Cornelissen, T. 2005. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. Biological Control. 34(3): 236-246.

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H. 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology. 47: 561–594.

Torres, L. 2007. Manual de protecção integrada do olival. João Azevedo Editores. Mirandela.

Torres, L.M., Pereira, J.A., Bento, A. 2009. Protecção contra a mosca-da-azeitona – Bactrocera oleae (Gmel.) em olivicultura biológica: situação actual e perspectivas. Actas Portuguesas de Horticultura. 116–125.

Torres, L.M., Pereira J.A., Bento, A., Torres, R. 2002. Experiments to control the olive fly, *Bactrocera oleae* (Gmel.) in north-eastern Portugal. VIIth European Congress of Entomology. Athens, Greece. 155.

Tscharntke, T., Steffan-Dewenter, I., Kruess, A., Thies, C. 2002. Contribution of small habitat fragments to conservation of insect communities of grassland-cropland landscapes. Ecological. Applications. 12: 354–363.

Tsolakis, H., Ragusa, E., and Tarantino, P. 2011. Control of *Bactrocera oleae* by low environmental impact methods: NPC methodology to evaluate the efficacy of lure-and-kill method and copper hydroxide treatments. Bulletin of Insectology. 64(1): 1-8.

Wallace, S.K. 2004. Molecular gut analysis of carabids (Coleoptera: Carabidae) using aphid primers. MSc Thesis, Montana State University, Bozeman.

Wang, X.G., Johnson, M.W., Yokoyama, V.Y., Pickett, C.H., and Daane, K.M. 2011. Comparative evaluation of two olive fruit fly parasitoids under varying abiotic conditions. BioControl. 56(3): 283-293.

Chapter 2

Feeding preferences and functional responses of *Calathus granatensis* and *Pterostichus globosus* (Coleoptera: Carabidae) on pupae of *Bactrocera oleae* (Diptera: Tephritidae)

Feeding preferences and functional responses of *Calathus granatensis* and *Pterostichus globosus* (Coleoptera: Carabidae) on pupae of *Bactrocera oleae* (Diptera: Tephritidae)

Abstract

The olive fruit fly, *Bactrocera oleae* (Rossi) is one of the most serious pests of olives. Biological control could represent a valuable alternative method to reduce the attack of this pest in the pupal stage. Carabids can be potential predators of *B. oleae* pupae. In this context, the feeding preferences and the functional responses of two carabid species, *Calathus granatensis* (Vuillefroy) and *Pterostichus globosus* (Fabricius), were studied under laboratory conditions. Feeding preferences assays consisted on exposing carabids to olive fruit fly pupae in the presence of an alternative prey, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), over different ratios while functional response curves were estimated on different densities of *B. oleae* pupae. Both predators revealed to predate olive fruit fly pupae, however, *C. granatensis* proved to have a strong preference for *B. oleae* pupae whereas *P. globosus* revealed to be more polyphagous, switching its preference depending on the prey density. Both predators demonstrated a type II functional response; however *P. globosus* demonstrated shorter handling time and attack rate on *B. oleae* pupae and a higher consumption of pupae before satiation. The results seems to demonstrate that both carabids may be important natural enemies of this pest in the field, *C. granatensis* due to its preference may be more efficient in the moment of pest outbreak whereas that *P. globosus* may be more efficient in prolonging the time between pest outbreaks.

Key-words: *Calathus granatensis*, *Pterostichus globosus*, olive fruit fly, food preference, functional response, predators.

2.1. Introduction

Carabid beetles are important polyphagous predators in agroecosystems (Crowson, 1981; Lövei and Sunderland, 1996; Lövei, 2008) that feed on diverse types of prey ranging from arthropods, slugs and a varying degree of plant matter (Lövei and Sunderland, 1996; Kromp, 1999; Symondson *et al*., 1999).

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is considered the major pest of olives in most commercial olive growing regions worldwide (Nardi *et al*., 2005; Daane and Johnson, 2010). Although control options for this pest are still based on insecticides (Kakani and Mathiopoulos, 2008) recent efforts intend to promote biological control. So far, the use of natural enemies, mainly parasitoids, is still unsuccessful (Daane and Jonhson, 2010) and the action of predators in the larvae is difficult because this pest spends this stage inside the olive fruit. However, pupation occurs in the soil making this developmental stage the most susceptible to the attack of edaphic predators (Civantos, 1999; Orsini *et al*., 2007).

Predation is a biotic interaction that can alter the distribution and abundance of both organisms involved in the relationship (Begon *et al*., 2006) and should be promoted in integrated pest management programs as a mortality factor for reducing pest populations (DeBach and Rosen, 1991). Such programs have been receiving increased attention because of the current need to reduce the use of synthetic insecticides for pest control (Directive 2009/128/EC). Although, successful biocontrol is critically dependent on the consumption rate of the predator in order to maintain pest density at a low level, which can vary with preferences and availability of alternative prey (Sengonca *et al*., 2005).

Polyphagous predators usually show some degree of preference for a particular type of prey which is evident when the proportion of that prey in the diet of predators is higher than its proportion in the environment (Begon *et al*., 2006). Thus, the simultaneous occurrence of potential preys can decrease the efficacy of predators.

Another important factor regulating population dynamics of predator-prey systems is the functional response of a predator. It represents the relationship between prey density and the number of prey consumed by an individual predator (Solomon, 1949) and an accurate description is important for practical and applied aspects of biological control (van Leeuwen *et al*., 2007). Holling's functional responses describe three types of curves dependent on prey density. Thus, for type I, type II and type III functional responses, the number of prey consumed increases linearly, asymptotically to a plateau and sigmoidally with increasing prey density, respectively (Holling, 1966).

Due to their predatory behavior, carabids are considered important natural control agents of crop pests (Kromp, 1999) and they can have an important role in the suppression of olive fruit fly populations. Previous studies showed that they are abundant insects among the edaphic arthropod community of the olive grove (Santos *et al.*, 2007; Gonçalves and Pereira, 2012), mainly in autumn (Oliveira, 2013) coinciding with the increase of pupae on the soil. Moreover, carabids (i.e., species such as *Carabus banonii* Dejean and *Pterostichus creticus* (Frivaldsky) were referred to predate pupae of the olive fruit fly in laboratory as well as in field experiments (Neuenschwander *et al.*, 1983; Orsini *et al.*, 2007; Odoguardi *et al.*, 2008). However, no studies were performed in order to understand the potential of carabids as natural control agents of the olive fruit fly. Thus, the main objective of this work was to evaluate the feeding preference and functional responses of two carabid species, *Calathus granatensis* (Vuillefroy) and *Pterostichus globosus* (Fabricius), fed on pupae of *B. oleae* in laboratory conditions. *C. granatensis* and *P. globosus* were dominant species in olive groves, mainly in northeastern Portugal (Oliveira, 2013), representing interesting species for evaluating predation on pupae of the olive fruit fly under laboratory conditions.

2.2 Material and Methods

2.2.1. Test organisms

Bactrocera oleae pupae were obtained from field-collected infested olive fruits in several olive groves in the region of Mirandela (Northeastern Portugal) in October/November 2013 and kept under controlled conditions at 21 ± 1 ºC, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. Adult flies were kept in poly-methyl-methacrylate cages (40 x 30 x 30 cm) (Figure 1A) and every two days, around 100 healthy olive fruits were provided as oviposition places. Larvae were collected daily from the infested olives and stored in plastic boxes to pupate.

Ceratitis capitata (Wiedemann) pupae were originally collected from the stock colony in the Unidad de Protección de Cultivos of Technical University of Madrid, and have been maintained in the School of Agriculture of Polytechnic Institute of Bragança since September 2012. Adult flies were kept in poly-methyl-methacrylate cages (40 x 30 x 30 cm) (Figure 1B) under controlled conditions at 24 ± 2ºC; 60 ± 5% relative humidity (RH) and a photoperiod of 16:8 (L:D) h. Larvae were reared on an artificial diet according to González-Núñez (1998). Both adults of *B. oleae* and *C. capitata* were fed ad libitum with water and an artificial diet composed by a mixture of sucrose and brewer's yeast at a ratio 4:1 (based on dry weight) according to Kendra *et al.* (2006). Pupae of *C. capitata* were used as alternative prey in preference experiments and were selected due to its similarity with *B. oleae*.

Adult specimens of *C. granatensis* and *P. globosus* were collected by hand in an organic, rain-fed olive grove in the region of Mirandela (Northeastern Portugal) between May of 2013 and May of 2014. Each species of carabid were kept in plastic boxes (15 x 37 x 53 cm) containing dry natural soil (Figures 2.1C and 2.1D) and several stones to provide shelter. They were fed every five days with different food items such as *C. capitata* larvae and dead adults, *B. oleae* dead adults, and cat food; water was provided in wet acrylate spheres.

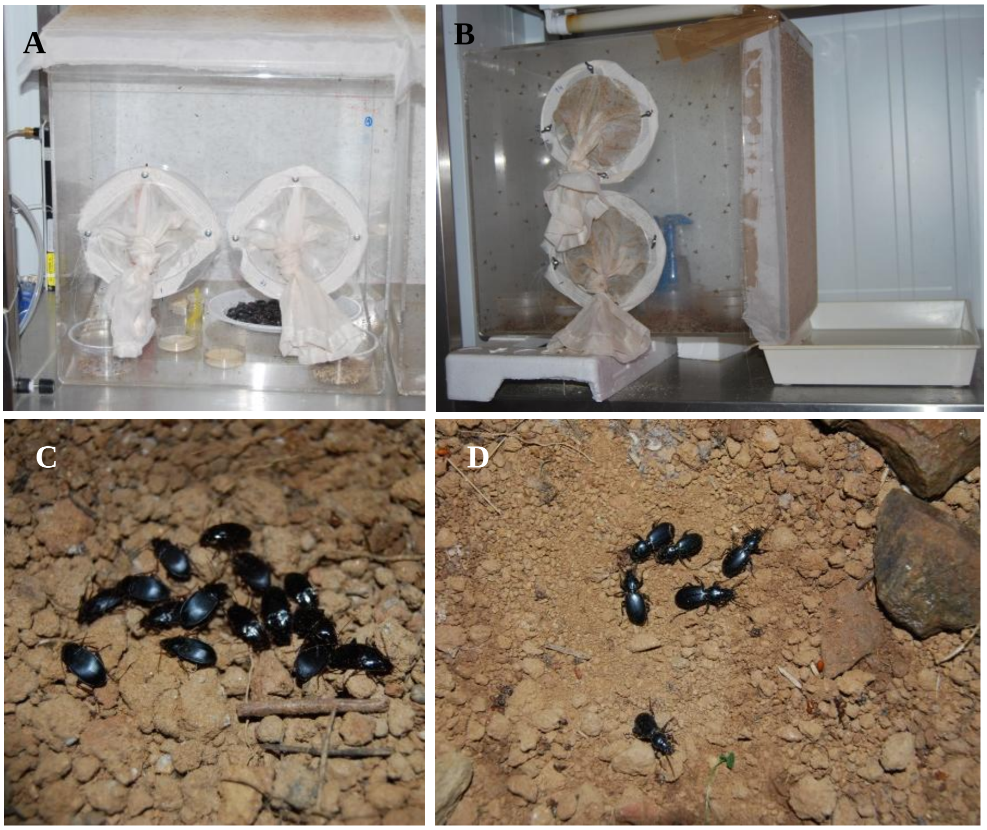

Figure 2.1. Rearing of *Bactrocera oleae* (A), *Ceratitis capitata* (B), *Calathus granatensis* (C) and *Pterostichus globosus* (D).

2.2.2. Feeding preferences, predation efficiency and functional responses

Specimens of *C. granatensis* and *P. globosus* were placed individually in plastic boxes (10.7 cm diameter and 4.0 cm height) with a layer of dry natural soil, a small stone for sheltering and one wet acrylate sphere for water supplying (Figure 1B, C). The lids of boxes contained a hole with a permeable piece of cloth (1.0 mm mesh) to ensure ventilation (Figure 2A). Experiments were performed under controlled conditions at 21 ± 1 ºC, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. Carabids were starved for 48 hours prior to the start of each food experiment, Feeding preferences - 25 individuals of each carabid species were tested in each treatment and eight treatments, corresponding to eight different ratios of prey, were offered to each species of carabid. Each replicate contained the following treatments: (1) 20 *B. oleae* pupae, (2) 18 *B. oleae* pupae and 2 *C. capitata* pupae, (3) 15 *B. oleae* pupae and 5 *C. capitata* pupae, (4) 12 *B. oleae* pupae and 8 *C. capitata* pupae, (5) 10 *B. oleae* and 10 *C. capitata* pupae, (6) 8 *B. oleae* pupae and 12 *C. capitata* pupae, (7) 5 *B. oleae* pupae and 15 *C. capitata* pupae and (8) 2 *B. oleae* pupae and 18 *C. capitata* pupae. Pupae were mixed and offered to each individual in a Petri dish (6.0 cm diameter and 1.0 cm height) (Figure 2B). The amount of consumed pupae was recorded after 24 hours.

Predation efficiency - The weight of 37 individuals of *C. granatensis* and *P. globosus* was recorded in order to calculate the average weight of each species. Individuals were starved for 5 days to guarantee equal conditions; they were cleaned with a moisten paint-brush to remove soil particles and weighted individually in plastic tubes. The weights of 50 pupae of *B. oleae* and *C. capitata* were also recorded to calculate the average weight of each prey. Data obtained was used to evaluate the biomass of prey consumed by each predator, by multiplying the average weight of pupae by the number of pupae consumed by each individual and was also used to measure a predator weight/prey weight ratio.

Functional responses - Experiments were conducted using 10 adult specimens of each species of carabid as replicates in each density (Figure 2.2C). Different densities of the prey (pupae of *B. oleae*) were offered to each species of carabid. Thus, *C. granatensis* were exposed to seven densities (2, 5, 10, 15, 20, 25 and 30 pupae of *B. oleae*) whereas *P. globosus* were exposed to 11 densities due to their bigger size (2, 3, 5, 8, 10, 15, 20, 25, 30, 40 and 50 pupae of *B. oleae*). After 24 hours, the number of pupae consumed was recorded.

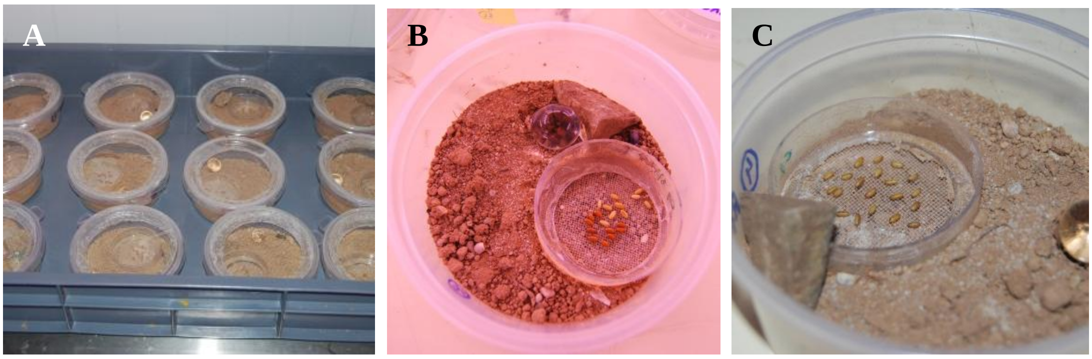

Figure 2.2. Aspects of preference and functional responses experiments. A – Boxes used in the experiments B – A box showing a carabid beetle below the stone, the acrylate sphere for water supply and the Petri dish containing *Bactrocera oleae* pupae together with *Ceratitis capitata* pupae in a ratio of 10/10; C – Detail of the box (density 20 pupae).

2.2.3. Data analysis

Statistical analyses were performed with IBM-SPSS statistics, version 19.0.0 (SPSS Inc. IBM Company 2010).

The consumed ratios of *B. oleae* pupae were calculated by dividing the number of *B. oleae* pupae by the total number of pupae consumed.

Feeding preferences of *C. granatensis* and *P. globosus* were accessed using Manly's preference index (Manly *et al.*, 1972), which is a method to evaluate preference that takes into account the prey densities depletion by predation during experiments (Cock, 1978) as following:

$$a = (r_1/n_1) / (r_1/n_1 + r_2/n_2) \qquad \text{Eq. 1}$$

Where r_1 represents the proportion of prey 1 in the predator diet (*B. oleae* pupae), and n_1 the proportion of prey 1 available (0.9, 0.75, 0.6, 0.5, 0.4, 0.25, 0.1); r_2 represents the proportion of prey 2 in the predator diet (*C. capitata* pupae) and n_2 the proportion of prey 2 available (0.1, 0.25, 0.4, 0.5, 0.6, 0.75, 0.9).

The predation efficiency was evaluated using the total number of pupae consumed, the total biomass of pupae consumed (calculated as the weight of the pupae x number of pupae consumed) and the percentage of pupae biomass consumed (calculated as the total biomass consumed/total biomass offered in percentage).

The consumed ratios of pupae of *B. oleae,* the Manly´s preference index values, the total number, the total biomass and the percentage of biomass of pupae consumed were compared between species of carabids and between the offered ratios of pupae of *B. oleae* using one-way ANOVA.

Functional response - To determine the shape of the functional response, it was used a logistic regression analysis of proportion of predated pupae versus initial density of pupae (Texler *et al.*, 1988). In the regression, it was fitted a polynomial function (Juliano, 2001) using R (R Core Team, 2014) as following:

$$N_e/N_0 = \exp(\beta_0 + \beta_1 N_0 + \beta_2 N_0^2 + \beta_3 N_0^3)/1+\exp(\beta_0 + \beta_1 N_0 + \beta_2 N_0^2 + \beta_3 N_0^3) \qquad \text{Eq. 2}$$

Where N_e represents the number of pupae of *B. oleae* consumed, N_0 is the initial density of pupae of *B. olea*e, β_0, β_1, β_2 and β_3 are, respectively, the constant, linear, quadratic and cubic parameters related to the slope of the curve that were estimated using the method of maximum likehood (Juliano, 2001).

Data indicated type II functional responses for both species of carabids and were fitted to Holling's disc equation (Holling, 1959) as following:

$$N_e = a\,T\,N_0 / 1 + a\,T_h\,N_0 \qquad \text{Eq. 3}$$

where a represents the attack rate, T_h the handling time and, T the time of the experiment (24 h). Fitting was performed using a non-linear least squares approach (Livdahl and Stiven, 1983). Estimated T_h were used to calculate maximum attack rates T/T_h, which is the maximum number of prey that can be attacked by a predator during the time interval considered. The functional responses of both carabid species were compared using a non-linear least squared regression as described in Juliano (2001).

All statistical tests were performed at 5 % significance level. Data are presented as mean values ± 1 standard error (SE).

2.3. Results

2.3.1. Feeding Preferences

The consumed ratio of pupae of *B. oleae* was significantly different over the offered ratio of pupae of *B. oleae* pupae for each carabid species ($F_{7, 192} = 9.91$, $P < 0.001$ for *C. granatensis* and $F_{7, 192} = 207.04$, $P < 0.001$ for *P. globosus*) (Figure 2.3). For *C. granatensis*, the consumed ratio decreased when the offered ratio of *B. oleae* decreased but was always superior to the offered ratio of *B. oleae* (Figure 2.3). For *P. globosus*, the consumed ratio was superior to the offered only when the number of *B. oleae* available was higher than the number of *C. capitata*. For this species, the consumed ratio of pupae of *B. oleae* decreased with the decrease of the offered ratio of pupae of *B. oleae* (Figure 2.3).

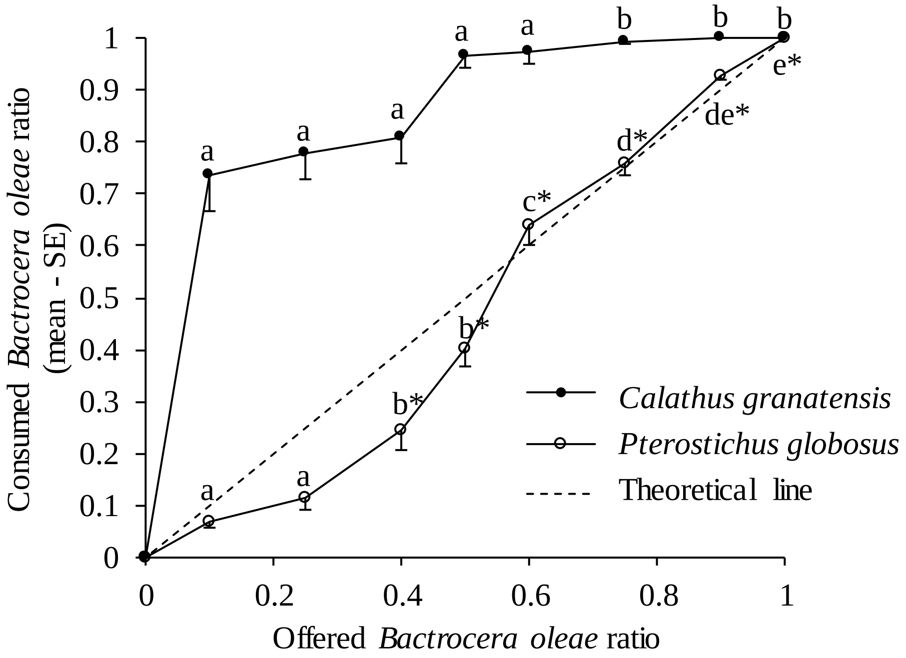

Figure 2.3. Consumed ratio of pupae of *Bactrocera oleae* (Mean - standard error of the mean - SE) for *Calathus granatensis* and *Pterostichus globosus*. Means with different letters for each carabid species were significantly different at $P < 0.05$. The asterisks (*) mean that carabid species were significantly different for the same ratio of pupae of *B. oleae* at $P < 0.05$.

Manly´s preference index was significantly different over the offered ratio of pupae of *B. oleae* for each carabid species ($F_{6, 168} = 5.38$, $P < 0.001$, for *C. granatensis* and $F_{6, 168} = 12.37$, $P < 0.001$ for *P. globosus*). Comparing both carabid species, *C. granatensis* showed significantly higher Manly's preference indexes than *P. globosus* for all offered ratios of pupae of *B. oleae* ($F_{1, 336} = 750.52$, $P < 0.001$) (Table 2.1). When all ratios were pooled, *C. granatensis* showed a mean Manly's preference index of 94.1% for *B oleae* while *P. globosus* showed 42.8% of preference for *B. oleae.*

For *C. granatensis*, the Manly´s preference index increased as the offered ratio of pupae of *B. oleae* also increased, being higher than 0.80 (80%) for all the offered ratios. On the other hand, for *P. globosus*, the Manly's preference index decreased when the offered ratio of *B. oleae* decreased. When the offered ratio was superior than 0.5, this species showed a preference above 50%, however, when the offered ratio reached 0.5 the index decreased rapidly reaching 24%.

Table 2.1. Manly's preference indexes (Mean ± SE) for different ratios of offered pupae of *Bactrocera oleae* for *Calathus granatensis* and *Pterostichus globosus*.

Offered ratio of pupae of *Bactrocera oleae*	Manly´s Preference Indexes	
	Calathus granatensis	*Pterostichus globosus*
0.9 (18/20)	1.000 ± 0.000 (a)	0.646 ± 0.046 (a)*
0.75 (15/20)	0.988 ± 0.012 (ab)	0.510 ± 0.040 (ab)*
0.6 (12/20)	0.972 ± 0.027 (ab)	0.556 ± 0.040 (ab)*
0.5 (10/20)	0.966 ± 0.022 (ab)	0.402 ± 0.034 (bc)*
0.4 (8/20)	0.848 ± 0.039 (c)	0.306 ± 0.047 (c)*
0.25 (5/20)	0.886 ± 0.029 (bc)	0.240 ± 0.044 (c)*
0.1 (2/20)	0.924 ± 0.021 (abc)	0.333 ± 0.050 (c)*
All ratios pooled	0.941 ± 0.021	0.428 ± 0.060*

SE = standard error of the mean.

Means within a column with different letters were significantly different at $P < 0.05$.

The asterisks (*) mean that, within the row, carabid species were significantly different for the same ratio of pupae of *B. oleae* at $P < 0.05$.

Figure 2.4 shows carabid species feeding on *B. oleae*.

Figure 2.4. Consumption of pupae of *Bactrocera oleae* by *Calathus granatensis* (A) and *Pterostichus globosus* (B). Detail; (C) *Calathus granatensis,* (D) *Pterostichus globosus.*

2.3.2. Predation efficiency

The average weight of *C. granatensis* was 47.03 ± 1.78 mg (n = 37) and the average weight of *P. globosus* was 248.05 ± 7.05 mg (n = 37). For the prey, the average weight of pupae of *B. oleae* was 5.0 mg (n = 50), whereas that of *C. capitata* was 9.0 mg (n = 50). Predator/*B. oleae* weight ratio was of 9.6 for *C. granatensis* whereas for *P. globosus* was of 49.6.

The total number of consumed pupae was statistical significantly different for both carabid species over the offered ratio of pupae of *B. oleae* ($F_{6, 168}$ = 2.04; P = 0.063 for *C. granatensis* and $F_{6, 168}$ = 10.02, P < 0.001 for *P. globosus*) and also between species for each offered ratio of pupae of *B. oleae* ($F_{1, 336}$ = 411.03, P < 0.001) (Table 2.2). *C. granatensis* consumed a significantly higher number of pupae in the 0.9 ratio of pupae of *B. oleae* when compared with the 0.1 ratio of pupae of *B. oleae* while *P. globosus* consumed significantly fewer pupae in the 0.9 ratio of *B. oleae* than in the other ratios studied.

The percentage of biomass consumed over the offered ratio of pupae of *B. oleae* differed significantly for *C. granatensis* ($F_{6, 168}$ = 3.20, P = 0.005) but was not different for *P. globosus* ($F_{6, 168}$ = 0.399, P = 0.879). There were significant differences between the percentage of biomass consumed by both species ($F_{1, 336}$ = 619.66, P < 0.001) (Table 2.2). The number of pupae and the percentage of total biomass consumed by *P. globosus* were about three times higher than that consumed by *C. granatensis* (Table 2.2).

Table 2.2. Total number of consumed pupae (Mean ± SE) and percentage of total biomass consumed in 24 h (Mean ± SE) by *Calathus granatensis* and *Pterostichus globosus* for different offered ratios of pupae of *Bactrocera oleae*.

Offered ratio of pupae of *Bactrocera oleae*	Number of consumed pupae	% of total biomass consumed
Calathus granatensis		
0.9 (18/20)	5.88 ± 0.659 (a)	27.22 ± 3.051 (a)*
0.75 (15/20)	4.88 ± 0.681 (ab)*	20.47 ± 2.868 (ab)*
0.6 (12/20)	4.36 ± 0.635 (ab)*	16.88 ± 2.447 (ab)*
0.5 (10/20)	4.44 ± 0.507 (ab)*	16.43 ± 1.946 (b)*
0.4 (8/20)	5.04 ± 0.599 (ab)*	20.27 ± 2.696 (ab)*
0.25 (5/20)	4.68 ± 0.515 (ab)*	18.03 ± 2.382 (ab)*
0.1 (2/20)	3.12 ± 0.463 (b)*	12.51 ± 2.331 (b)*
Pterostichus globosus		
0.9 (18/20)	6.52 ± 0.798 (a)	74.48 ± 7.355 (a)
0.75 (15/20)	15.16 ± 1.053 (b)	75.57 ± 5.345 (a)
0.6 (12/20)	14.08 ± 1.150 (b)	69.21 ± 5.973 (a)
0.5 (10/20)	13.80 ± 0.926 (b)	72.03 ± 4.428 (a)
0.4 (8/20)	14.80 ± 0.938 (b)	78.43 ± 4.066 (a)
0.25 (5/20)	13.36 ± 0.914 (b)	70.35 ± 4.256 (a)
0.1 (2/20)	15.04 ± 0.908 (b)	76.00 ± 4.473 (a)

SE = standard error of the mean.

For each carabid species, means within a column with different letters were significantly different at $P < 0.05$.

The asterisks (*) mean that, within a column, carabid species were significantly different for the same ratio of pupae of *B. oleae* at $P < 0.05$.

The total biomass consumed over the offered ratio of pupae of *B. oleae* differed significantly for *P. globosus* ($F_{6, 168} = 6.16$, $P < 0.001$), increasing the total biomass of prey consumed as the offered ratio of pupae of *B. oleae* decreased. However, for *C. granatensis* the total biomass consumed did not differ significantly with the decrease of the offered ratio of pupae of *B. oleae* ($F_{6, 168} = 1.07$, $P = 0.383$). The total biomass consumed was significantly different between the two carabid species ($F_{1, 336} = 680.05$, $P < 0.001$) (Table 2.3).

Table 2.3. Total biomass of prey consumed in 24 h (Mean ± SE) by *Calathus granatensis* and *Pterostichus globosus* for different ratios of pupae of *Bactrocera oleae*.

Offered ratio of pupae of *Bactrocera oleae*	Total biomass consumed (mg)	
	Calathus granatensis	*Pterostichus globosus*
0.9 (18/20)	29.4 ± 3.3 (a)	80.4 ± 7.9 (a)*
0.75 (15/20)	24.6 ± 3.4 (a)	90.7 ± 6.4 (ab)*
0.6 (12/20)	22.3 ± 3.2 (a)	91.4 ± 7.9 (ab)*
0.5 (10/20)	23.0 ± 2.7 (a)	100.8 ± 6.2 (ab)*
0.4 (8/20)	30.0 ± 4.0 (a)	116.1 ± 6.0 (bc)*
0.25 (5/20)	28.8 ± 3.8 (a)	112.6 ± 6.8 (bc)*
0.1 (2/20)	21.5 ± 4.0 (a)	130.7 ± 7.7 (c)*

SE = standard error of the mean.

Means within a column with different letters were significantly different at $P < 0.05$.

The asterisk (*) means that, within the row, carabid species were significantly different for the same ratio of pupae of *B. oleae* at $P < 0.05$.

2.3.3. Functional responses

The estimated parameters from the logistic regression analysis of the proportion of *B. oleae* pupae consumed by *C. granatensis* and *P. globosus* indicated that the linear coefficient $\beta 1$ is significantly negative ($P < 0.001$) and a type II functional response for both species (Table 2.4).

Table 2.4. Estimates of the parameters β_0, β_1, β_2 and β_3 (± SE) of the logistic regression analysis of the proportion of *B. oleae* consumed by *Calathus granatensis* and *Pterostichus globosus*.

Parameter	*Calathus granatensis*	*Pterostichus globosus*
β_0	25.677 ± 5.046	5.759 ± 0.848
β_1	-3.425 ± 0.736*	-0.312 ± 0.090*
β_2	0.1474 ± 0.035	0.004 ± 0.003
β_3	-0.002 ± 0.0005	-0.00001 ± 0.00003

*Significance at $P < 0.001$.

Both carabid species showed an increase in predation with the increase of the density of pupae of *B. oleae*, although *C. granatensis* reached a plateau at lower number of prey density (Figure 2.5). The comparison of both functional responses revealed that the curve of *C. granatensis* was significantly lower than that of *P. globosus* (t_{178} = 2.42, P = 0.016).

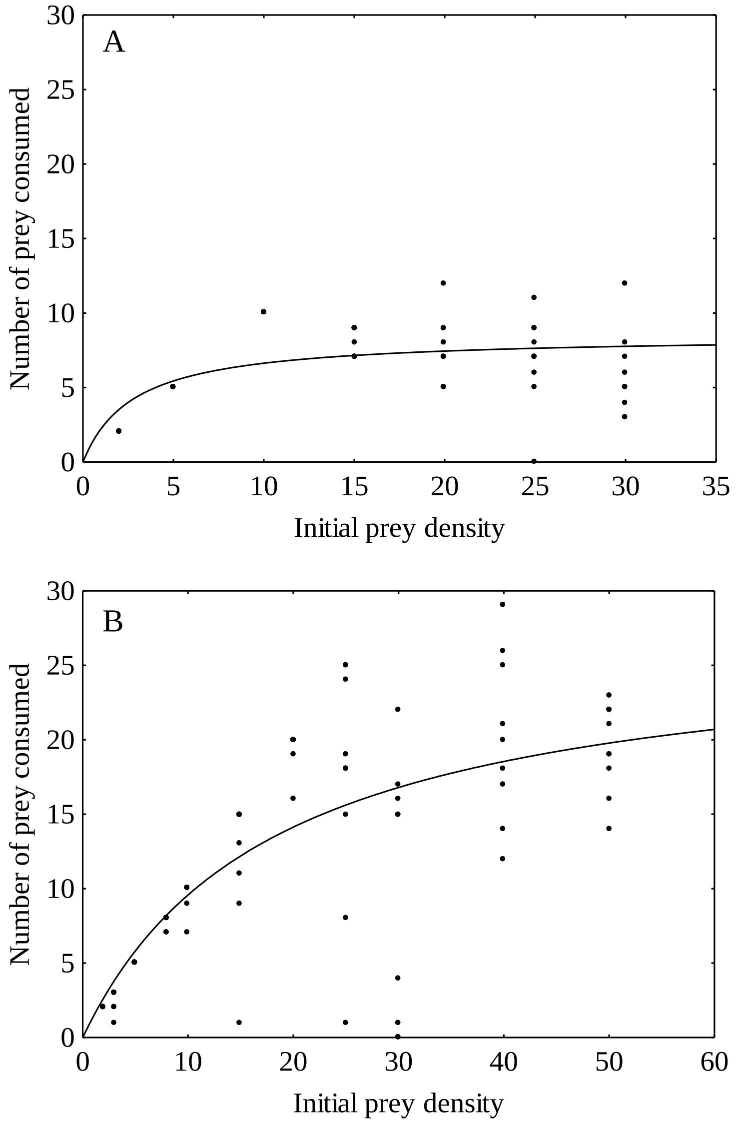

Figure 2.5. Functional responses of *Calathus granatensis* (A) and *Pterostichus globosus* (B) fed on increasing densities of pupae of *Bactrocera oleae*.

The coefficient of attack rates and handling times estimated for each carabid species are shown in Table V. The variation in predation rates over different prey densities increased from *C. granatensis* to *P. globosus*. *P. globosus* had significantly shorter handling time and coefficient of attack rare than *C. granatensis* (Table 2.5). The maximum attack rate (T/T_h) was of 8.49 for *C. granatensis* and 26.95 for *P. globosus*.

Table 2.5. Parameters estimated by the Holling's disc equation.

Species	Attack rate (h^{-1})		Handling time (h)		R^2
	Mean ± S.E.	95% CI	Mean ± S.E.	95% CI	
Calathus granatensis	0.126 ± 0.035	0.056 – 0.197	2.826 ± 0.215	2.397 – 3.256	0.335
Pterostichus globosus	0.062 ± 0.010	0.042 – 0.082	0.890 ± 0.106	0.680 – 1.101	0.577

2.4. Discussion

This study demonstrates that both *C. granatensis* and *P. globosus* were able of predating pupae of *B. oleae*, although they had significantly different feeding preferences and different abilities to respond to increasing prey densities. Thus, *C. granatensis* showed a marked preference for *B. oleae* pupae irrespectively of the offered proportion of prey, and consumed more pupae and more percentage of biomass at high ratios of *B. oleae*.

On the other hand, *P. globosus* preferred the alternative prey and showed some degree of switching since *B. oleae* was disproportionately less eaten when it was present at low ratios. In this context, *P. globosus* seemed to be more polyphagous than *C. granatensis* since the former was able of exploiting both resources. This characteristic was previously noted by Hengeveld (1980) referring that species of the genus *Pterostichus* eat whatever prey they can ingest (Hengeveld, 1980). Diverse prey items, such as slugs (Oberholzer *et al.,* 2003), lepidopterans pests (Suenaga and Hamamura, 1998) and dipterans pupae including *B. oleae* pupae (Neuenshwander *et al*., 1983; Odoguardi *et al*., 2008) are commonly present in the diet of these carabids.

Considering the total biomass consumed by both species, as it would be expected, *P. globosus* consumed significantly more biomass than *C. granatensis* since it is about five times heavier than *C. granatensis* and it is assumed that larger carabids have larger guts and can eat more (Wallace, 2004). Such differences can justify the results obtained as *P. globosus* seemed to select prey items that were most valuable in terms of energy intake per unit handling time. In previous studies conducted to evaluate feeding preferences of carabids on different slug species, the weight of the slug was considered the main factor influencing the predator's choice (Thiele, 1977; Ernsting and Vanderwerf, 1988; Wheater, 1988; Ayre, 2001; McKemey *et al*., 2001) followed by the slug species

(Foltan, 2004). Thus, in our study, *P. globosus* could select *C. capitata* pupae because it is the heaviest prey item representing the most profitable prey in terms of energy gained. Moreover, the apparent switching behavior showed by *P. globosus*, which started when both prey items were equally present, demonstrates that this species can be more opportunistic in its feeding habits, switching to the most abundant prey available, which is a common behavior for carabids (Hengeveld, 1980, Barney and Pass, 1986).

On the other side, the smaller size of *C. granatensis* may determine its ability to efficiently exploit one prey instead of the other. Several morphological constrains, such as the mandible size (Hengeveld, 1980), can limit *C. granatensis* of easily exploiting *C. capitata* pupae that mainly fed on the alternative prey at lower ratios of *B. oleae*. This idea is reinforced by the fact that the total biomass consumed by *C. granatensis* did not differ with the decrease of the offered ratio of *B. oleae* pupae which suggests that the presence of higher densities of the alternative prey did not significantly influence the predator's choice.

As far as we know, there are no other studies considering the feeding preferences and efficiency of these carabid species, although they are quite well distributed in the Iberian Peninsula. *Pterostichus globosus* can be found in many agro-forestry environments (e.g. forests of oaks and pines and olive groves) and grasslands, usually found under stones and in leaf litter (Cárdenas and Bach, 1988; Ortuño, 1990; Oliveira, 2013); *Calathus granatensis* is an Iberian endemism, is also a lapidicolous beetle, commonly found in the olive grove (Cárdenas and Bach, 1993; Cárdenas and Bach, 1985; Zbyšek, 2012; Oliveira, 2013).

Both carabid species exhibited a type II functional response in which the consumption rate of *B. oleae* pupae rises with prey density, but gradually decelerates until a plateau is reached and the consumption rate remains constant with the increase of *B. oleae* pupae density. The plateau in the functional curve was reached at lower numbers of consumed prey for *C. granatensis* than for *P. globosus* meaning that they differ in their maximum consumption rates. This kind of response is the most frequently observed in many arthropod predators (Hassell *et al*., 1977, Sueldo *et al*., 2010) and is characterized by a predation rate that is limited by the handling time that a predator needs to devote to each prey item it consumes (Sueldo *et al*., 2010). Thus, as prey density increases, searching for prey takes shorter time and limits less the predation rate because prey is easier to find, becoming the predation rate affected by the handling time, which causes a decelerating rate of increase in the predation rate (Sueldo *et al*., 2010). The estimated handling time is the cumulative time needed for capturing, killing, subduing, and consuming the prey (Begon *et al*., 2006). For *C. granatensis* the time (in hours) required for handling *B. oleae* pupae was in average three times longer than the time required by *P. globosus* and the coefficient of attack rate (as a fraction of a hour) was in average two times higher than the estimated for *P. globosus*. Thus, although both carabids exhibit the same kind of response in function of *B. oleae* pupae, the values of the parameters used to find out the magnitude of these responses differed significantly between the carabid species indicating different abilities to deal to increasing *B. oleae* densities. Also, the time required for handling *B. oleae* pupae may indicate different levels of satiation, voracity or digestive rates between *P. globosus* and *C. granatensis*. The former can consume higher amount of pupae before satiation and can be more efficient in handling pupae than the latter.

According to these results, both species can be natural control agents of *B. oleae* in the field since both were able to decrease the abundance of pupae. However, the ability of a predator to control pests is dependent on the predator's functional response, on the presence of alternative prey and on the interactions between predator species (Lester and Harmsen, 2002). *C. granatensis* showed higher preference for *B. oleae* pupae in detriment of the alternative prey, thus, for this predator, it is possible that the presence of alternative prey items affect less its functional response on *B. oleae* pupae. However, other studies need to be done using prey items smaller than *B. oleae* pupae in order to better understand the feeding habits of this species. In the case of *P. globosus*, although it consumed more *B. oleae* pupae than *C. granatensis*, the presence of alternative prey items can affect its functional response on *B. oleae* pupae decreasing its consumption due to switching to more energetic prey items and, consequently, to higher levels of satiation given by that prey (Murdoch, 1969; Murdoch and Oaten, 1975), which can be considered a short-term negative impact on biological control of that pest (Settle *et al*., 1996).

On the other hand, the presence of alternative prey items and switching behavior can be seen as positive factors contributing to biological control by increasing predator's abundance when the levels of the pest in the agroecosystem are low (Settle *et al*., 1996). Thus, the ability for consuming other prey items further than *B. oleae* pupae can be advantageous for *P. globosus* that will have conditions to reach high populations.

Moreover, in olive groves, both carabid species have peaks of activity in autumn, coinciding with the peak of abundance of *B. oleae* pupae on soil. Thus, in this period, both *P. globosus* and *C. granatensis* can significantly contribute to reduce pest levels, the former because the prey is present in high proportion and the latter because it gives preference to this prey.

Acknowledgments

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2.5. References

Ayre, K. 2001. Effect of predator size and temperature on the predation of Deroceras reticulatum (Muller) (Mollusca) by carabid beetles. Journal of Applied Entomology. 125:389–395.

Barney, R.J., Pass, B.C. 1986. Foraging behavior and feeding preference of ground beetles (Coleoptera: Carabidae) in Kentucky alfalfa. Journal of Economic Entomology. 79:1334-1337.

Begon, M., Townsend, C.R., Hayer, J.L. 2006. Ecology: From individuals to ecosystems. 4th Edition. Wiley-Blackwell, Oxford, UK.

Cock, M.J.W. 1978. The assessment of preference. Journal of Animal Ecology. 47:805-816.

Cárdenas, A.M., Bach, C. 1988. Contribución al conocimiento de los carábidos (Col. Carabidae) de Sierra Morena Central. 1ª Parte. Boletín Asociación Española de Entomología. 12: 9-25.

Cárdenas, A.M., Bach, C. 1993. Descripción de los estadios larvarios *Calathus granatensis* Vuillefroy, 1866 (Col., Carabidae). Boletín Asociación Española de Entomología. 17:109-121.

Cárdenas, A. M., Bach, C. 1985. Fenología de las especies de carábidos (Col. Carabidae) más abundantes en la cuenca del Bembezar (NW. de la provincia de Córdoba). Mediterránea-Serie de Estudios Biológicos. 8:147-163.

Civantos, M. 1999. Olive pest and disease management, Conseil Oleicole International, Madrid, Spain.

Crowson, R.A. 1981. The Biology of the Coleoptera. Academic Press, London.

Daane, K.M. and Johnson, M.W. 2010. Olive Fruit Fly: Managing an Ancient Pest in Modern Times. Annual Review of Entomology. 55:151–169.

DeBach, P., Rosen, D. 1991. Biological control by natural enemies. (2nd ed.). Cambridge University Press. New York, USA.

Ernsting, G., Vanderwerf, D.C. 1988. Hunger, partial consumption of prey and prey size preference in a carabid beetle. *Ecological Entomology.* 13:155–164.

Gonçalves, M.F., Pereira, J.A. 2012. Abundance and diversity of soil arthropods in the olive grove ecosystem. Journal of Insect Science. 12:20 available online: insectscience.org/12.20

Foltan, P. 2004. Influence of slug defense mechanisms on the prey preferences of the carabid predator *Pterostichus melanarius* (Coleoptera: Carabidae). European Journal of Entomology. 101:359–364.

González-Núñez, M. 1998. Uso conjunto de plaguicidas y enemigos naturales en el olivar: Optimización del manejo de *Opius concolor* Szépligeti, parasitoide de la mosca del olivo, *B. oleae* (Gmelin). Tesis Doctoral. Universidad Politécnica de Madrid. ETSI Agrónomos. Madrid. 175 pp.

Hassell, M.P., Lawton, J.H., Beddington, J.R. 1977. Sigmoid functional response by invertebrate predators and parasitoids. Journal of Animal Ecology. 46:249–262.

Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. Canadian Entomologist. 91:385-398.

Holling, C.S. 1966. The functional response of invertebrate predators to prey density. Memoirs of the Entomological Society of Canada. 98:5-86.

Juliano, S.A. 2001. Nonlinear curve fitting: predation and functional response curve. In Scheiner S.M. and Gurevitch J. (eds): Design and analysis of ecological experiments. Oxford University Press, New York, pp. 178–196.

Hengeveld, R. 1980. Polyphagy, oligophagy and food specialization in ground beetles (Colepotera; Carabidae). Netherlands Journal of Zoology. 30:585-594.

Kakani, E.G., Mathiopoulos, K.D. 2008. Organophosphosphate resistance-related mutations in the acetylcholinesterase gene of Tephritidae. Journal of Applied Entomology. 132:762–771.

Kendra, P.E., Montgomery, W.S., Epsky, N.E., Heath, R.R. 2006. Assessment of female reproductive status in *Anastrepha suspensa* (Diptera: Tephritidae). Florida Entomologist. 89:144-151.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture, Ecosystems & Environment. 74:187-228.

Lester, P.J., Harmsen, R. 2002. Functional and numerical responses do not always indicate the most effective predator for biological control: an analysis of two predators in a two-prey system. Journal of Applied Ecology. 39:455-468.

Livdahl, T.P., Stiven, A.E. 1983. Statistical difficulties in the analysis of predator functional response data. Canadian Entomologist. 115:1365-1370.

Lövei, G.L. 2008. Ecology and conservation biology of ground beetles (Coleoptera: Carabidae) in an age of increasing human dominance. Doctoral dissertation, Aarhus University, Aarhus, Denmark.

Lövei, G., Sunderland, K.D. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology. 41:231-256.

Manly, B.F.J., Miller, P., Cook, L.M. 1972. Analysis of a selective predation experiment. The American Naturalist. 106:719-736.

Mckemey, A.R., Symondson, W.O.C., Glen, D.M., Brain, P. 2001. Effects of slug size on predation by *Pterostichus melanarius* (Coleoptera: Carabidae). Biocontrol Science and Technology. 11:81–91.

Murdoch, W.W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecological Monographs. 39:335–354.

Murdoch, W.W., Oaten, A. 1975. Predation and population stability. Advances in Ecological Research. 9:1-131.

Nardi, F., Carapelli, A., Dallai, R., Roderick, G.K., Frati, F. 2005. Population structure and colonization history of the olive fruit fly, *Bactrocera oleae* (Diptera, Tephritidae). Molecular Ecology. 14: 2729–2738.

Neuenschwander, P., Bigler, F., Delucchi, V., Michelakis, S. 1983. Natural enemies of preimaginal stages of Dacus oleae Gmel. (Dipt., Tephritidae) in Western Crete. I. Bionomics and phonologies. Bollettino del Laboratorio di Entomologia Agraria. “Filippo Silvestri”. 40: 3-32.

Oberholzer, F., Escher, N., Frank, T. 2003. The potential of carabid beetles (Coleoptera) to reduce slug damage in oilseed rape in the laboratory. European Journal of Entomology. 100:81-85.

Odoguardi, R., Bonnacci, T., Bruno, L., BrandMayr, P., Zetto, T. 2008. Carabid beetles as potential natural predators of olive fly pupae. Young Ideias in Insect Science. 1st meeting of PhD students and Post-Doctoral Fellows. Florence, Italy.

Oliveira, J.M.S. 2013. Abundância e diversidade de carabídeos ao longo de um gradiente crescente de práticas agrícolas no olival transmontano. MSc. Thesis, Polytechnic Institute of Bragança, Bragança, Portugal.

Orsini, M.M., Daane, K.M., Sime, K.R., Nelson, E.H. 2007. Mortality of olive fruit fly pupae in California. Biocontrol Science and Technology. 17:797-807.

Ortuño, V.M. 1990. Estudio sistemático del género *Steropus* (*sensu* Jeannel, 1942) de la fauna Ibero-mauritánica (2^a parte). El género *Corax* (Coleoptera, Caraboidea, Pterostichidae). Nova Acta Científica Compostelana (Bioloxía). 1:31–46.

R Core Team 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org

Santos, S.A.P., Cabanas, J.E., Pereira, J.A. 2007. Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. *European Journal of Soil Biology*. 43:77-83.

Sengonca, C., Al-Zyoud, F., Blaeser, P. 2005. Prey consumption by larval and adult stages of the entomophagous ladybird *Serangium parcesetosum* Sicard (Col., Coccinellidae) on the cotton whitefly, *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae), at two different temperatures. Journal of Pest Science. 78:179-186.

Settle, W.H., Ariawan, H., Astuti, E.T., Cahyana, W., Hakim, A.L., Hindayana, D., Lestari, A.S. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. Ecology. 77:1975-1988.

Solomon, M.E. 1949. The natural control of animal populations. Journal of Animal Ecology 18:1-35.

SPSS Inc., IBM Company 2010. IBM, SPSS Statistic for Windows, version 19.0.0. New York.

Suenaga, H., Hamamura, T. 1998. Laboratory evaluation of carabid beetles (Coleoptera: Carabidae) as predators of diamondback moth (Lepidoptera: Plutellidae) larvae. Environmental Entomology. 27:767-772.

Sueldo, M.R., Bruzzone, O.A., Virla, E.G. 2010. Characterization of the earwig, *Doru lineare*, as a predator of larvae of the fall armyworm, *Spodoptera frugiperda*: A functional response study. Journal of Insect Science. 10:38. available online: insectsicence.org/10.38

Symondson, W.O.C., Erickson, M.L. Liddell, J.E. 1999. Development of a monoclonal antibody for the detection and quantification of predation on slugs within the *Arion hortensis* agg. (Mollusca: Pulmonata). Biological Control. 16:274–282.

Trexler, J.C., McCulloch, C.E., Travis, J. 1988. How can the functional response best be determined? Oecologia. 76:206-214.

Thiele, H.U. 1977. *Carabid beetles in their environments. A study on habitat selection by adaptation in physiology and behavior.* Springer, Berlin, 369 pp.

van Leeuwen, E., Jansen, V.A.A., Bright, P.W. 2007. How population dynamics shape the functional response in a one-predator-two-prey system. Ecology. 88: 1571–1581.

Wallace, S.K. 2004. Molecular gut analysis of carabids (Coleoptera: Carabidae) using aphid primers. MSc Thesis, Montana State University, Bozeman.

Wheater, C.P. 1988. Predator-prey size relationships in some Pterostichini (Coleoptera, Carabidae). *Coleoptera Bulletin*. 42:237–240.

Zbyšek, Š. (2012) Changes in carabid communities (Insecta: Coleoptera) along an urbanization gradient in Madrid (Spain). Studii şi comunicări. Ştiinţele Naturii. Muzeul Olteniei Craiova. Oltenia. 28. 20 pp.

Chapter 3

Predation by edaphic arthropods on pupae of *Bactrocera oleae* (Diptera: Tephritidae) under field conditions

Predation by edaphic arthropods on pupae of *Bactrocera oleae* (Diptera: Tephritidae) under field conditions

Abstract

Edaphic arthropods can provide valuable services within agroecosystems, such as the biological control of crop pests that spend part of their life cycle on the ground. This is a characteristic of the olive fruit fly, *Bactrocera oleae* (Rossi), one of the most important pests of olives. In this work, the impact of edaphic arthropods on the abundance of *B. oleae* pupae of *B. oleae* was evaluated by using a paired exposed-exclusion box method and their contribution for biological control services index (BSI) was quantified. Exclusion and exposed boxes were installed in two olive groves and BSI was calculated based on the relative suppression of *B. oleae* pupae throughout five sampling periods, from January to May 2014. Pitfall traps were installed close to exposed-exclusion boxes for sampling active edaphic arthropods and correlate their abundance with BSI. The community of arthropods was dominated by Formicidae, Araneae and Forficulidae. Considering the trophic guild of arthropods captured, omnivorous were the most abundant, followed by granivorous and predators. Forficulidae dominated the community during the winter period while Formicidae dominated in spring. BSI values increased with sampling time reaching the maximum value (1) in the third sampling period, coinciding with the beginning of spring. From all the groups of arthropods captured, the abundance of Forficulidae was highly correlated with the increase of BSI. These results indicate that edaphic arthropods may have strong impact in the abundance of *B. oleae* pupae in olive groves. Moreover, the biological control of *B. oleae* can be more effective by promoting the complementarity of edaphic species.

Key-words: *Bactrocera oleae*, edaphic arthropods, predators, omnivorous, Forficulidae, Biological Control Services Index.

3.1. Introduction

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is the key pest of commercial olive crops worldwide, surviving and developing in any area where olive trees grow (Daane and Jonhson, 2010; Matallanas *et al*., 2013). Losses caused by this insect include the premature fall of infested fruits, pulp consumption due to the larvae development and more importantly, 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), spinosad 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; Torres *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 *Psyttalia concolor* (Szepligeti) (Hymenoptera: Braconidae) that revealed low effectiveness and low rate of establishment and persistence (Kapatos *et al*., 1977; Del Rio *et al*., 2005; Wang *et al*., 2012). 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 constrains, other approaches are needed, such as exploring the potential of edaphic arthropods as predators of *B. oleae*. This group of arthropods represents an important part of the biodiversity in olive groves (Morris and Campos, 1999; Santos *et al*., 2007b; Gonçalves *et al*., 2012), where they can provide multiple ecosystem services, such as biological control of crop pests (Odoguardi *et al*., 2008). This service helps maintaining agricultural productivity and reduces the need of pesticide inputs (Isaacs *et al*., 2009).

Edaphic predators can have an important action against the olive fruit fly, especially in its pupal stage (Neuenschwander *et al*., 1983; Odoguardi *et al*., 2008). This pest spends almost the entire larval phase inside the olive fruit, as many tephritid species, and pupates on the soil becoming exposed and vulnerable to the attack of different predaceous species (Dimou *et al*., 2003).

Olive groves comprise complex predaceous 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) indicated that some carabids, staphylinids, centipedes and ants can be potential predators of pupae (Neuenschwander *et al*., 1983; Orsini *et al*., 2007; Odoguardi *et al*., 2008), although the impact of these edaphic predators have on olive fruit fly populations is poorly known.

Under field conditions, there are some methods that can be used to demonstrate this impact and also to measure rates of consumption and determine biocontrol services indices (BSIs) (Gardiner *et al*., 2009). Among these, exclusion methods can offer valuable clues to examine linkages between predaceous communities and biological control services 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 action of edaphic arthropods as potential natural control agents of olive fruit fly pupae using a paired exposed-exclusion method and quantify their contribution for BSI.

3.2. Material and Methods

3.2.1. Rearing of *B. oleae*

Bactrocera oleae pupae were obtained from field-collected infested olive fruits in several olive groves in the region of Mirandela (northeastern Portugal) in October/November 2013 and kept under controlled conditions at 21 ± 1 ºC, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. Pupae were collected and transferred to poly-methyl-methacrylate cages (40 × 30 × 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). Around 100 healthy olive fruits per box were provided every two days as oviposition places. After the fourth generation, 900 pupae were separated and used in field assays.

3.2.2. Exposed and exclusion boxes

Potential predation exerted by natural control agents on pupae of the olive fruit fly was tested by using exposed and exclusion boxes. A hundred and eighty plastic Petri dishes (6.0 cm diameter and 1.0 cm height) were prepared so that the bottom was removed and replaced by a permeable piece of cloth (1.0 mm mesh) in order 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. Half of the Petri dishes (90) were 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 boxes remained uncovered – exposed boxes.

3.2.3. Study Areas

The study areas were located in two olive groves near Mirandela, respectively in Valbom-dos-Figos (41˚ 33’ 00.58’’ N, 7˚ 08’ 39.92’’ W) and Cedães (41˚ 29’ 16.86’’ N, 7˚ 07’ 34.02’’ W) (Figure 3.1A).

Valbom-dos-Figos has been conducted according 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 × 10 m apart. The predominant cultivars are Cobrançosa and Verdeal Transmontana. No sprays were done against olive pests. Every year, an application of cupper has been applied in February. Considering soil coverage, a mixture of leguminous plants (*Trifolium repens, Trifolium fragiferum, Trifolium incarnatum* and lupines) was sown for the first time in 2008 and regularly grazed by sheep.

Cedães has been conducted 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 were spaced 7 × 7 m apart and the dominant cultivar is Cobrançosa. No sprays were done against olive pests or diseases. Soil was covered by spontaneous plants.

Both groves were rain-fed and no vegetation cuts occurred during the field assay.

3.2.4. 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 leveled with the soil surface (Figure 3.1B-D). Sets were placed in an arrangement of 3 × 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 (anti-freeze liquid) and a lid supported by iron wires was placed to exclude rain, debris and small vertebrates. Pitfalls were used to assess edaphic arthropod activity and density near exposed and exclusion boxes. Every three 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), the period between the end of winter period and beginning of spring (day 63) and the spring period (day 84 till day 105).

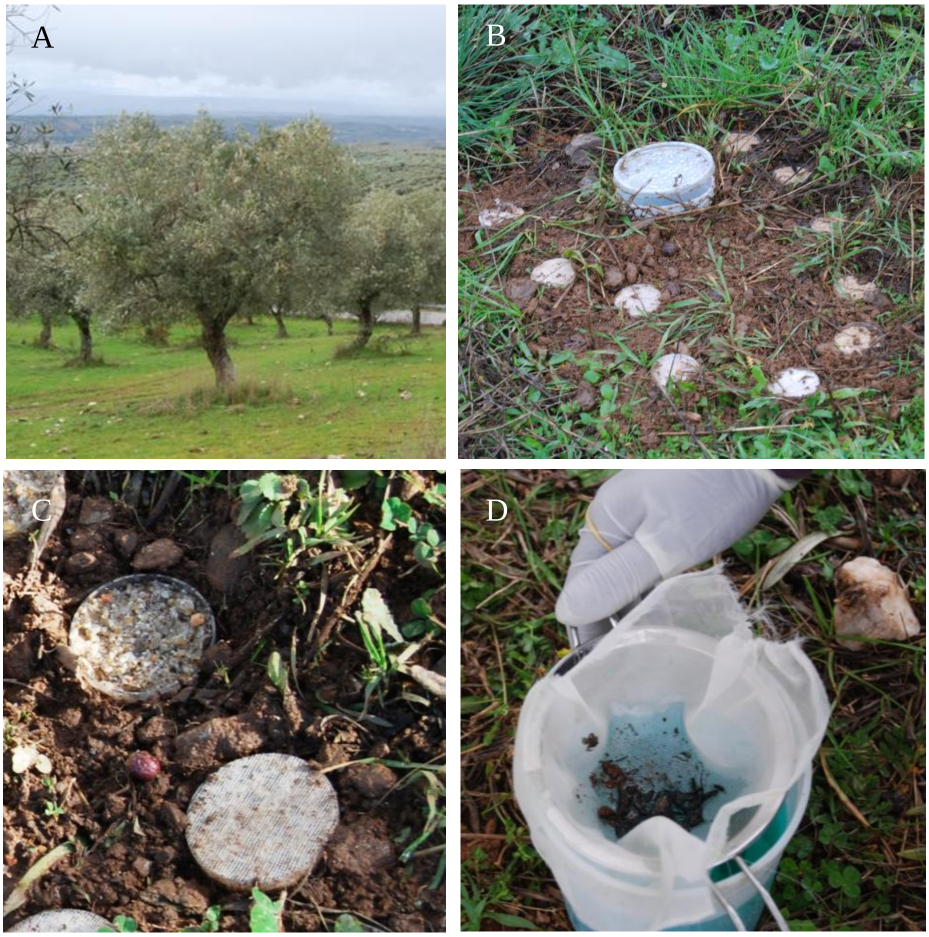

Figure 3.1. A – Aspect of an olive tree; B – Detail of a set showing the disposition of exclusion and exposed boxes and the pitfall trap; C – Detail of a paired open/exclusion boxes; D – Collection of pitfall trap content.

3.2.5. Predation of the olive fruit fly

In the laboratory, sand was removed from each box, spread on the bottom of a container (15 × 7 × 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 symptoms of predation. Apparently intact pupae were placed under controlled conditions at 21 ± 1 ºC, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h for evaluating emergence rates.

3.2.6. Arthropod identification

All captured individuals 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 Prince (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), mainly predators (MP) that complement their diets with other materials or have scavenger or opportunistic habits, omnivorous that have different food sources (OM), granivorous that eat seeds or are seed harvesters (G), saprophagous/fungivorous (SFA) that feed on organic matter or microorganisms and non-identified that included taxa with phytophagous, predators or parasitoid species (NI). NI individuals were excluded from the analyses.

3.2.7. Data analysis

Statistical analyses were performed with IBM-SPSS statistics, version 19.0.0 (SPSS Inc. IBM Company, 2010). 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.

A one-way analysis of variance was used to compare the mean abundance of arthropods, trophic guilds, richness and diversity of taxa between the five sampling dates. Data values were transformed as log(x + 1) prior to analysis.

Richness of families and Simpson Diversity index were calculated for each sampling date. Simpson's diversity index was calculated as $1/D$ using the formula (Eq. 1):

$$1/D = 1/\sum_{i=1}^{s} p_i^2 \qquad \text{(Eq. 1)}$$

where p_i^2 is the proportion of individuals of the i^{th} species and S is the total number of families. The minimum value of $1/D$ is 1, which is reached when the community has only a single family and the maximum is S, which is reached when a community has all families with equal abundance (Magurran, 2004).

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.

Data from exposed and exclusion boxes were used to calculate a biological control services index (BSI) (Gardiner et al., 2009) that expresses the change in the number of pupae of the olive fruit fly in the presence of edaphic arthropods (Eq. 2).

$$\mathrm{BSI} = \frac{\sum_{p=1}^{18} \frac{(Bex,s - Be,s)}{Bex,s}}{n} \qquad \text{(Eq. 2)}$$

To calculate BSI at each sampling period, counts of pupae of *B. oleae* on exclusion boxes (*Bex*) were compared with pupae of *B. oleae* exposed to arthropods (*Be*), these differences were measured for each set (*p*), summed, and divided by the number of replicates for each grove (*n*). The resulting BSI, varied from 0 to 1, with values increasing as the number of pupae found in the exposed boxes decreases.

Curve estimation regression analyses were performed in order to determine the best model fitting the relationship between the abundance of captured arthropods (considering taxa and trophic guilds) and BSI values. Accumulated abundance of arthropods through time was used.

3.3. Results

3.3.1. Composition of the community of edaphic arthropods

A full list of the abundance, mean ± standard error (SE) and trophic guilds of captured taxa in total pitfall traps in the two olive groves is provided in Appendix 1 and resumed in Table 3.1. A total of 6967 arthropods were captured in both olive groves (Table 3.1). 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. Within Insecta, the family Formicidae was the most numerous, representing 43.3% of the total captures, followed by the family 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%. Considering the trophic guild of arthropods captured in pitfall traps, omnivorous were the most abundant representing 32.6% of the total captures, followed by granivorous representing 24.5% and predators, representing 22.1%.

Table 3.1. Abundance (N) and mean ± standard error (SE) of taxa and trophic guilds captured in total pitfall traps in two olive groves, January-May 2014.

Group	**N (n=90)**	**Mean ± SE**	**$F_{4, 85}$**	***P***
Insecta				
Formicidae	3014	33.49 ± 9.28	7.95	<0.001
Forficulidae	987	10.97 ± 1.68	7.56	<0.001
Staphylinidae	586	6.51 ± 2.31	2.07	0.09
Carabidae	79	0.88 ± 0.18	2.06	0.09
Other Coleoptera	621	6.90 ± 1.47	8.87	<0.001
Arachnida				
Araneae	1361	15.12 ± 1.26	4.73	0.002
Acari	307	3.41 ± 1.48	6.32	<0.001
Chilopoda				
Scolopendromorpha	12	0.13 ± 0.08	-	-
Total arthropods	6967	43.68 ± 6.06	2.13	0.08
Trophic Guilds				
Predators	1541	17.10 ± 1.29	4.50	0.002
Mainly Predators	120	1.33 ± 0.20	3.66	0.008
Omnivorous	2272	25.24 ± 5.68	1.38	0.25
Granivorous	1704	18.93 ± 7.20	7.21	<0.001
Saprophagous/Fungivorous	404	4.49 ± 7.20	3.33	0.014
Non-Identified	928	10.31 ± 2.28	8.01	<0.001

n = total number of samples. F and *P* are statistical results for comparisons of abundance between sampling dates.

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). Within 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 *Ocypus* (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%, Zodariiae with 9.7% and Thomisidae with 9.4% (Appendix 1).

The abundance of the different groups of edaphic arthropods varied through the sampling period (Figure 3.2), and one-way ANOVA showed significant differences for Araneae, Formicidae and Forficulidae ($P < 0.05$). During the winter period, the edaphic community was dominated by Forficulidae, followed by Araneae and Formicidae. From the end of the winter period until the spring period the community was dominated by Formicidae, Araneae and Staphylinidae.

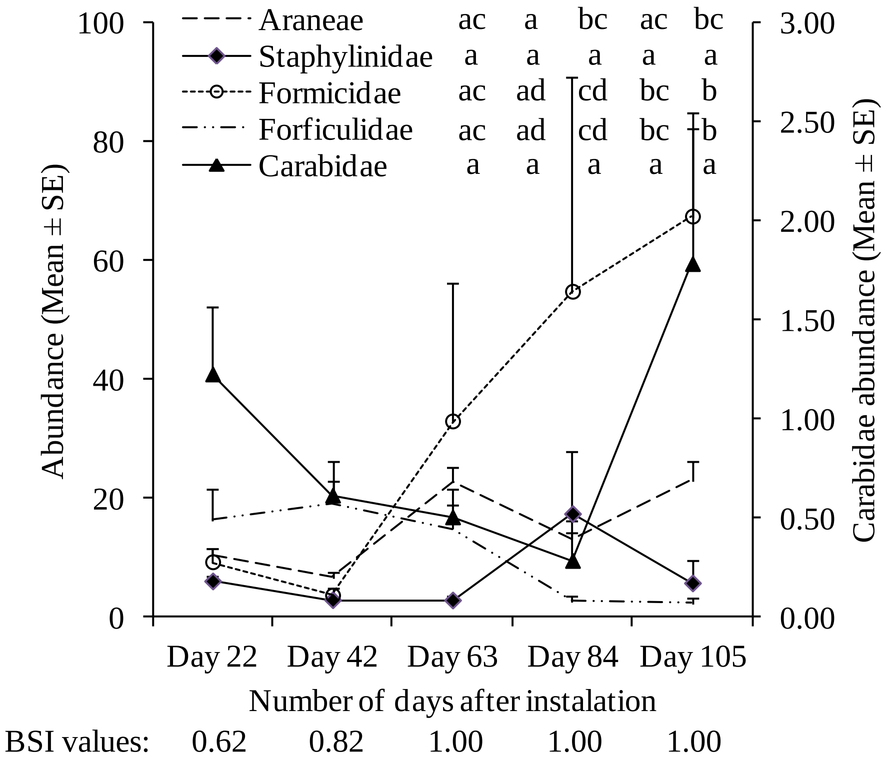

Figure 3.2. Dynamics of the abundance (mean ± standard error - SE) of edaphic arthropods captured in pitfall traps in two olive groves. The x-axis represents the number of days after the installation of pitfall traps on the 21^{st} of January 2014 and the respective Biological Control Services Indexes (BSI). Note different scales of right and left y-axes. n = 18 for each sampling period. The letters on the right side of the legend represent the results of the post-hoc Tukey test for comparisons of the abundance of taxa between sampling dates. Dates sharing the same letter are not significantly different at P > 0.05.

The dynamics of abundance of different functional groups through sampling time is shown in Figure 3.3. In the winter period and in the first sampling date of spring, the community was dominated by omnivorous, predators and granivorous. In the two last sampling dates, granivorous, omnivorous and predators dominated the community (Figure 3.3).

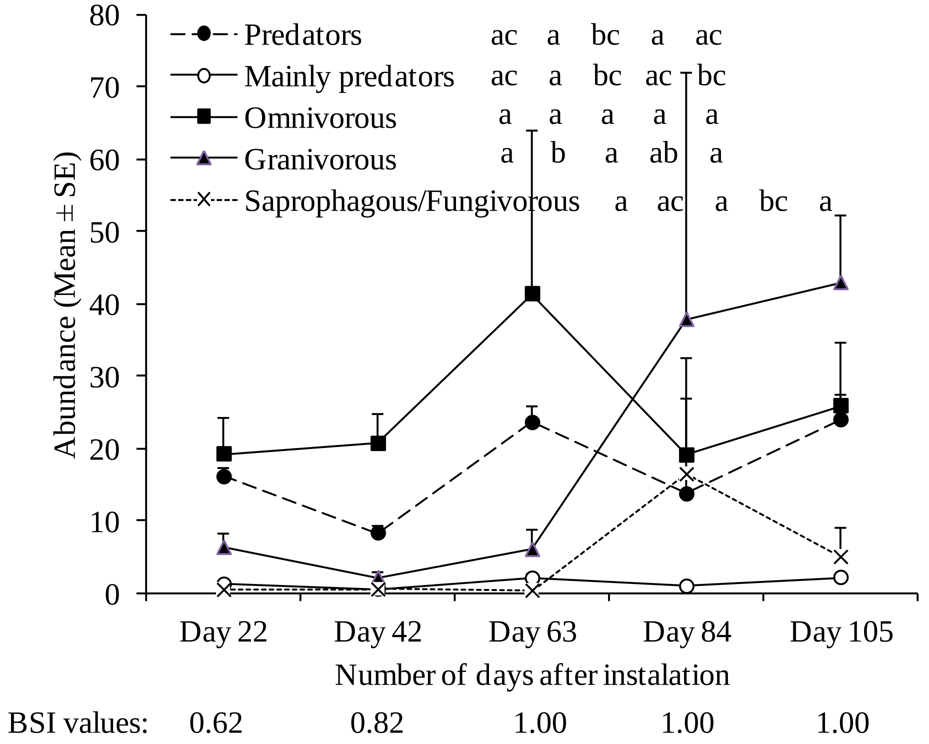

Figure 3.3. Abundance (mean ± standard error - SE) of trophic groups captured in pitfall traps in two olive groves. The x-axis represents the number of days after the installation of pitfall traps on the 21st of January 2014 and the respective Biological Control Services Indexes (BSI). n = 18 for each sampling period. The letters on the right side of the legend represent the results of the post-hoc Tukey test for comparisons of the abundance of each trophic group between sampling dates. Dates sharing the same letter are not significantly different at $P > 0.05$.

Richness of families and Simpson's diversity index was higher in the period around the 63th day after the installation of the experiment, which corresponds to the end of winter and beginning of spring with a peak of seven families. The minimum value was reached in the second sampling date of winter period for richness of families (Figure 3.4a) and in the last sampling date for Simpson's diversity index (Figure 3.4b). Both richness and Simpson's diversity index were statistically significant different between sampling dates ($F_{4, 85} = 3.16$, $P = 0.018$ and $F_{4, 85} = 5.72$, $P < 0.001$, respectively).

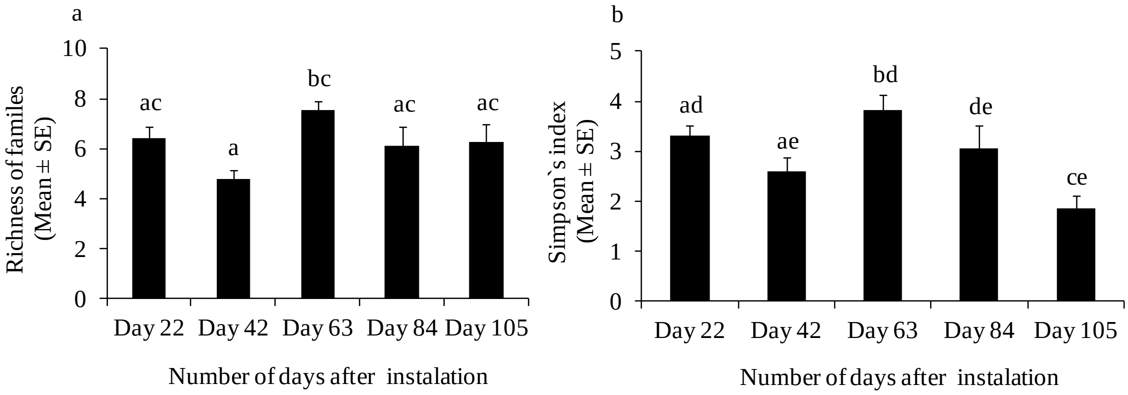

Figure 3.4. Richness of families (a) and Simpson's diversity index for families (b) (mean ± standard error) captured in pitfall traps in two olive groves. The xx axis represents the number of days after the installation of pitfall traps on the 21st of January 2014. n = 18 for each sampling period. The letters on the columns represent the results of the post-hoc Tukey test for comparisons between sampling dates. Dates sharing the same letter are not significantly different at $P > 0.05$.

3.3.2. Exposed versus exclusion boxes and biological control services index

The Wilcoxon test showed that the abundance of pupae of *B. oleae* in exposed boxes was significantly different from exclusion boxes ($P < 0.05$). BSI values were calculated for each sampling time and varied between 0.62 in the first date and 1.00 in the last date (Figures 3.2 and 3.3).

During the field assay, from a total of 450 pupae placed in 90 exposed boxes, only 41 pupae were recovered; in the first sampling time, 31 pupae (34.4%) were recovered from exposed boxes, from which 13 pupae (41.9%) had signs of predation (Figure 3.5) and three pupae (23.0%) emerged in laboratory conditions. In the second sampling date, 10 pupae (11.1%) were recovered, from which eight pupae (72.1%) had signs of predation and none emerged in laboratory conditions. In the three last sampling dates, no pupae were recovered from exposed boxes.

Figure 3.5. Remains of pupae of *Bactrocera oleae* (Rossi) recovered from the exposed boxes with signs of predation.

Regression analyses showed that relationships between the accumulated abundance of Forficulidae, Araneae, total arthropods, predators, mainly predators and omnivorous and BSI was described by logarithmic models and between Carabidae, Staphylinidae and Formicidae and BSI was described by inverse models (Table 3.2).

Table 3.2. Values for the coefficient of determination (R^2) and significance (*P*) of the curve estimation regression analyses between the mean accumulated abundance of groups and the biological control services index.

Group	**R^2**	***P***	**Best curve fitting**
Taxa			
Formicidae	0.881	0.018	Inverse model
Forficulidae	0.996	<0.001	Logarithmic model
Staphylinidae	0.810	0.037	Inverse model
Carabidae	0.880	0.018	Inverse model
Araneae	0.818	0.035	Logarithmic model
Total arthopods	0.799	0.041	Logarithmic model
Trophic Guilds			
Predators	0.810	0.037	Logarithmic model
Mainly Predators	0.787	0.045	Logarithmic model
Omnivorous	0.911	0.012	Logarithmic model
Total	0.875	0.020	Logarithmic model

Figure 3.6 shows the regression curves obtained for predators, omnivorous, Forficulidae and Formicidae. For the first three groups, their higher abundance observed in the winter period than in spring is related with the increase of BSI. Forficulidae and omnivorous arthropods (where Forficulidae was included) registered the highest values for the coefficient of determination. Considering Formicidae, inverse model indicates that their abundance mainly increases in spring, when BSI values reached the maximum value of 1.

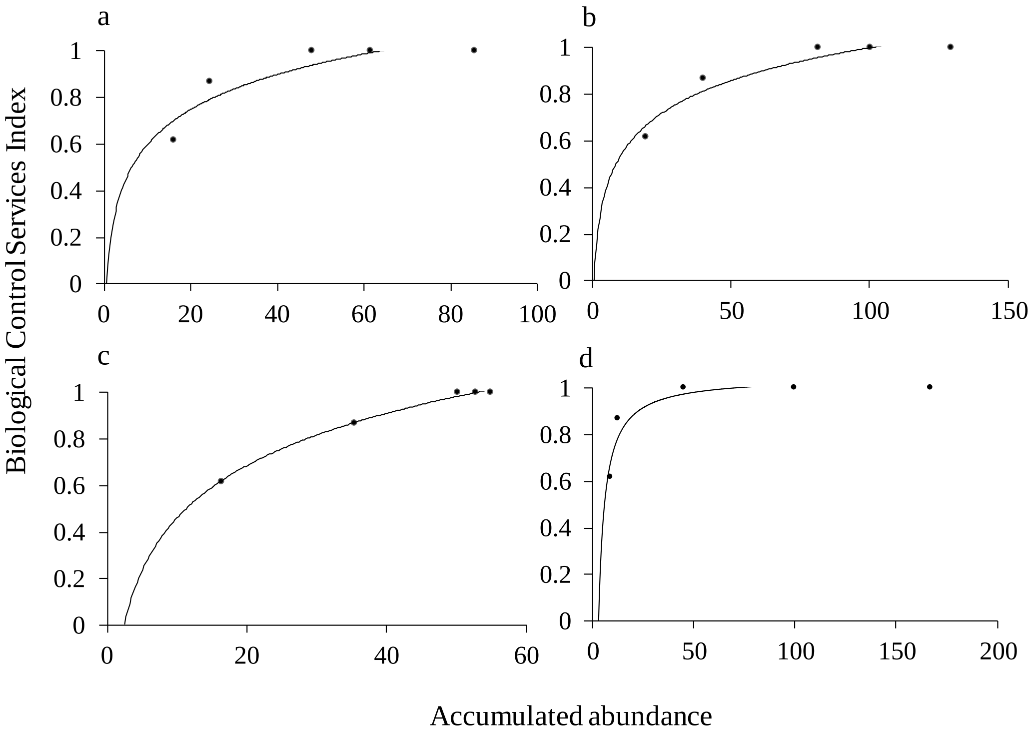

Figure 3.6. Regression curves between the accumulated abundance of predators (a), omnivorous (b), Forficulidae (c) and Formicidae (d) and biological control services indexes observed in each sampling period.

3.4. Discussion

During this study, there were several evidences that edaphic arthropods could have impact on the abundance of pupae of the olive fruit fly. These evidences were mainly supported by the functional composition of edaphic arthropod community, by the decrease in the number of pupae in the exposed boxes when compared to the exclusion boxes and by the remains of pupae recovered from exposed boxes with signs of predation. BSI values ranged from 0.68 to 1.00 indicating that edaphic arthropods can actively reduce *B. oleae* in its pupal stage, with high levels of predation occurring in the first three sampling dates.

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

Usually, Formicidae is 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 is 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 (Gonçalves and Pereira, 2012; Santos *et al.*, 2007b; Morris and Campos, 1999). The former was the dominant species in the winter period while the latter dominated in the beginning of spring, but both species were dominant in late spring and summer as shown by Morris and Campos (1999) and Santos *et al*. (2007b) reaching high abundances in soil. *Messor 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 BSI values reached 1. This corresponds to the rise of temperatures that can also promote the emergence of *B. oleae*. 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 pupae of *B. oleae* due to its granivorous habits. Although Neuenshwander et al. (1983) observed this species carrying pupae to the nest in a laboratory experiment. Thus, it is possible that this behavior could also occur in the field and contribute to the decline of pupae in the exposed cages 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, Zodariiae 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 (Allen and Hagley, 1990; Nyffeler and Benz, 1988) 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 pupae of *B. oleae* 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 pupae of *B. oleae*, although some predation can be exerted on teneral flies.

Forficulidae was composed by a single species, *F. auricularia* that dominated the community of arthropods in the winter period, decreasing its abundance in the 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. *Forficula 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 predating pupae of *B. oleae* 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 highly correlated with the increase of BSI values in that period and they were frequently found in exposed boxes.

Considering Staphylinidae, adult stages are mostly abundant in autumn (S. Santos, data not published). In this study, several larvae were collected and 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 pupae of *B. oleae* 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 pupae of *B. oleae* 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 pupae of *B. oleae* in this season.

In conclusion, an abundant and diverse edaphic arthropod community in olive groves could provide important biological control services against *B. oleae* during its pupal stage. Some groups of arthropods such as Forficulidae and Formicidae seem to be more relevant in certain periods of the year such as winter and spring while Staphylinidae and Carabidae can be important in autumn. Thus, the biological control of *B. oleae* can increase due to greater complementarity among the species in a community rather than due to a single species or group of arthropods.

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PRO/0591/2012: Olive crop protection in sustainable production under global climatic changes: linking ecological infrastructures to ecosystem functions.

3.5. References

Aguiar, C.A., Serrano, A.R.M. 2012. Coleópteros Carabídeos (Coleoptera, Carabidae) de Portugal Continental: Chaves para sua identificação. Sociedade Portuguesa de Entomologia. Lisboa. Portugal.

Allen, W.R., Hagley, E.A.C. 1990. Epigeal arthropods as predators of mature larvae and pupae of the apple maggot (Diptera: Tephritidae). Environmental Entomology. 19: 309-312.

Azcárate, F.M., Peco, B. 2003. Spatial patterns of seed predation by harvester ants (Messor Forel) in Mediterranean grassland and scrubland. Insectes Sociaux. 50: 120-126.

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) (Diptera, Tephritidae): pilot-scale feasibility studies. Journal of Applied Entomology. 126: 217-223.

Cárdenas, M., Castro, J., Campos, M. 2012. Short-term response of soil spiders to cover-crop removal in an organic olive orchard in a Mediterranean setting. Journal of Insect Science. 12:61 available online: insectscience.org/12.61

Cardoso, P., Silva, I., De Oliveira, N.G., Serrano, A.R.M. 2007. Seasonality of spiders (Araneae) in Mediterranean ecosystems and its implications in the optimum sampling period. Ecological Entomology. 32: 516–526.

Cerdá, X., Retana, J., Bosch, J., Alsina, A. 1989. Exploitation of food resources by the ant *Tapinoma nigerrimum* Hymenoptera Formicidae. Acta Oecologica Oecologia Generalis. 10: 419-430.

Chatzaki, M. 2008. A critical review of the spider family Gnaphosidae in Greece. In Makarov S.E., Dimitrijevic R.N. (eds): Advances in arachnology and developmental biology. Papers dedicated to Prof. Dr. Bozidar Curcic. Institute of Zoology, Belgrade; BAS, Sofia; Fac. Life Sci., Vienna; SASA, Belgrade & UNESCO MAB Serbia, Vienna-Belgrade-Sofia, Monographs 12, 355-374 pp.

Chisholm, P., Gardiner, M., Moon, E., Crowder, D.W. 2014. Tools and techniques for investigating impacts of habitat complexity on biological control. Biological Control. 75: 48-57.

Collingwood, C., Price, A. 1998. A Guide to ants of Continental Portugal, Supplement nº 5, Boletim da Sociedade de Entomologia. Lisboa, Portugal.

Daane, K.M., Johnson, M.W. 2010. Olive fruit fly: managing an ancient pest in modern times. Annual Review of Entomology. 55: 151‑169.

Delrio, G., Lentini, A., Satta, A. 2005. Biological control of olive fruit fly through inoculative releases of *Opius concolor* Szépl. IOBC/wprs Bulletin. 28: 53-58.

Dimou, I., Koutsikopoulos, C., Economopoulos, A.P., Lykakis, J. 2003. Depth of pupation of the wild olive fruit fly, *Bactrocera (Dacus) oleae* (Gmel.) (Diptera, Tephritidae), as affected by soil abiotic factors. Journal of Applied Entomology. 127: 12-17.

Eskafi, F.M., Kolbe, M.M. 1990. Predation on larval and pupal *Ceratitis capitata* (Diptera: Tephritidae) by the ant *Solenopsis geminata* (Hymenoptera: Formicidae) and other predators in Guatemala. Environmental Entomology. 19: 148-153.

Gardiner, M.M., Landis, D.A., Gratton, C., DiFonzo, C.D., O'Neal, M., Chacon, J.M., Wayo, M.T., Schmidt, N.P., Mueller, E.E., Heimpel, G.E. 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecological Applications. 19: 143-154.

Gobin, B., Peusens, G., Moerkens, R., Leirs, H. 2008. Understanding earwig phenology in top fruit orchards. In Ecofruit - 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 18th February to 20th February 2008 at Weinsberg/Germany. 208-212 pp.

Gonçalves, F., Santos, S.A.P., Torres, L. 2012. Efficacy of spinosad bait sprays to control *Bactrocera oleae* and impact on non-target arthropods. Phytoparasitica. 40: 17 – 28.

Gonçalves, M.F., Pereira, J.A. 2012. Abundance and diversity of soil arthropods in the olive grove ecosystem. Journal of Insect Science. 12: 1-14.

Haniotakis, G.E., Kozyrakis, E., Bonatsos, C. 1986. Control of the olive fruit fly, *Dacus oleae* Gmel. (Dipt., Tephritidae) by mass trapping: Pilot scale feasibility study. Journal of Applied Entomology. 101: 343-352.

Hawkes, N.J., Janes, R.W., Hemingway, J., Vontas, J. 2005. Detection of resistance-associated point mutations of organophosphate-insensitive acetylcholinesterase in the olive fruit fly, *Bactrocera oleae* (Gmelin). Pest Biochemistry and Physiology. 81: 154–163.

Hodgson, P.J., Sivinski, J., Quintero, G., Aluja, M. 1998. Depth of pupation and survival of fruit fly (Anastrepha spp.: Tephritidae) pupae in a range of agricultural habitats. Environmental Entomology. 27: 1310-1314.

Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., Landis, D. 2009. Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. Frontiers in Ecology and the Environment. 7: 196-203.

Kakani, E.G., Mathiopoulos, K.D. 2008. Organophosphosphate resistance-related mutations in the acetylcholinesterase gene of Tephritidae. Journal of Applied Entomology. 132: 762–771.

Kapatos, E., Fletcher, B.S., Pappas, S., Laudeho, Y. 1977. The release of *Opius concolor* and *O. concolor* var. *Siculus* [Hym.: Braconidae] against the spring generation of *Dacus oleae* [Dipt.: Trypetidae] on Corfu. Entomophaga. 22: 265-270.

Magurran, A.E. 2004. *Measuring Biological Diversity.* Blackwell Publishing, U.K.

Matallanas, B., Lantero, E., M'Saad, M., Callejas, C., and Ochando, M.D. 2013. Genetic polymorphism at the cytochrome oxidase I gene in Mediterranean populations of *Bactrocera oleae* (Diptera: Tephritidae). Journal of Applied Entomology. 137: 624-630.

Mazomenos, B.E., Pantazi-Mazomenou, A., Stefanou, D. 2002. Attract and kill of the olive fruit fly *Bactrocera oleae* in Greece as a part of an integrated control system. Use of pheromones and other semiochemicals in integrated production. IOBC/wprs Bulletin. 25: 137-146.

Monzó, C., Mollá, Ó., Castañera, P., Urbaneja, A. 2009. Activity-density of *Pardosa cribata* in Spanish citrus orchards and its predatory capacity on *Ceratitis capitata* and *Myzus persicae*. BioControl. 54: 393-402.

Morris, T.I., Campos, M. 1999. Entomofauna depredadora del suelo del olivar. Zoología Baetica. 10: 149-160.

Neuenschwander, P., Bigler, F., Delucchi, V., Michelakis, S. 1983. Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in Western Crete. I. Bionomics and phonologies. *Bollettino del Laboratorio di Entomologia Agraria. "Filippo Silvestri".* 40: 3-32.

Nyffeler, M., Benz, G. 1988. Feeding ecology and predatory importance of wolf spiders (*Pardosa* spp.) (Araneae, Lycosidae) in winter wheat fields. Journal of Applied Entomology. 106: 123–134.

Odoguardi, R., Bonnacci, T., Bruno, L., BrandMayr, P., Zetto, T. 2008. Carabid beetles as potential natural predators of olive fly pupae. Young Ideias in Insect Science. 1st meeting of PhD students and Post-Doctoral Fellows. Florence. June 5-6. 3 pp.

Oliveira, J.M.S. 2013. Abundância e diversidade de carabídeos ao longo de um gradiente crescente de práticas agrícolas no olival transmontano. MSc. Thesis, Polytechnic Institute of Bragança, Bragança, Portugal.

Orsini, M.M., Daane, K.M., Sime, K.R., Nelson, E.H. 2007. Mortality of olive fruit fly pupae in California. Biocontrol Science and Technology. 17: 797-807.

Outerelo, R., Gamarra, P. 1985. *Las familias y géneros de los estafilinideos de la Península Ibérica. Claves de identificación de la fauna española, n. 10.* Ed. Universidad Complutense de Madrid. 139 pp.

Pascual, S., Cobos, G., Seris, E., González-Núñez, M. 2010. Effect of processed kaolin on pests and non-target arthropods in a Spanish olive grove. Journal of Pest Science. 83: 121-133.

Pereira, J.A., Alves, R., Casal. S., Oliveira, M.B.P.P. 2004. Effect of olive fruit fly infestation on the quality of olive oil from cultivars Cobrançosa, Madural and Verdeal Transmontana. Italian Journal of Food Science. 16: 355-365.

Persons, M.H., Uetz, G.W. 1996. The influence of sensory information on patch residence time in wolf spiders (Araneae: Lycosidae). Animal Behaviour. 51: 1285-1293.

Richman, D.B., Hemenway, R.C., Whitcomb, W.H. 1980. Field cage evaluation of predators of the soybean looper, *Pseudoplusia includens*. (Lepidoptera: Noctuidae). Environmental Entomology. 9: 315-317.

Roberts, M. 1985. The Spiders of Great Britain and Ireland. Atypidae to Theridosomatidae. Vol. 1. Harley Books, Colchester, Essex. 229 pp.

Roberts, M.J. 1987. The Spiders of Great Britain and Ireland. Linyphiidae. Vol. 2. Harley Books, Colchester, Essex. 204 pp.

Rovner, J.S. 1991. Evidence for idiothetically controlled turns and extraocular photoreception in lycosid spiders. Journal of Arachnology. 19: 169–173.

Ruano, F., Lozano, C., Garcia, P., Peña, A., Tinaut, A., Pascual, F. 2004. Use of arthropods for the evaluation of the olive-orchard management regimes. Agricultural and Forest Entomology. 6: 111–120.

Ruiz-Torres, N.I., Madueno-Magdaleno, C., Montiel-Bueno, A. 2004. Efficacy of ground application with Spinosad and Imidacloprid bait sprays to control olive fruit fly (*Bactrocera oleae*). Preliminary results. Boletin Sanidad Vegetal Plagas. 30: 415-425.

Santos, S.A.P., Cabanas, J.E., Pereira, J.A. 2007b. Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. European Journal of Soil Biology. 43: 77-83.

Santos, S.A.P., Pereira, J.A., Torres, L.M., Nogueira, A.J.A. 2007a. Evaluation of the effects, on canopy arthropods, of two agricultural management systems to control pests in olive groves from north-east of Portugal. Chemosphere. 67: 131-139.

Saour, G., Makee, H. 2004. A kaolin-based particle film for suppression of the olive fruit fly *Bactrocera oleae* Gmelin (Dip., Tephritidae) in olive groves. Journal of Applied Entomology. 128: 28-31.

Shaw, P.W., Wallis, D.R. 2010. Susceptibility of the European earwig, *Forficula auricularia* to insecticide residues on apple leaves. New Zealand Plant Protection. 63: 55-59.

SPSS Inc., IBM Company. 2010. IBM, SPSS Statistic for Windows, version 19.0.0. New York, USA.

Thaler, K., Zapparoli, M. 1993. Epigeic spider in an olive-grove in central Italy (Araneae). Redia. 56: 307-316.

Torres, L.M., Pereira, J.A., Bento, A., Torres, R. 2002. Experiments to control the olive fly, *Bactrocera oleae* (Gmel.) in north-eastern Portugal. VIIth European Congress of Entomology. Athens, Greece, October 2002: 155.

Wang, X.G., Levy, K., Son, Y., Johnson, M.W., Daane, K.M. 2012. Comparison of the thermal performance between a population of the olive fruit fly and its co-adapted parasitoids. Biological Control. 60: 247-254.

Wong, M.A., Wong, T.T. 1988. Predation of the Mediterranean fruit fly and the Oriental fruit fly (Diptera: Tephritidae) by the fire ant (Hymenoptera: Formicidae) in Hawaii. Proceedings of the Hawaiian Entomological Society. 28: 169-177.

Appendix 1. Total abundance (N), mean ± 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 ± SE	Trophic Guild
Carabidae			
Calathus granatensis Vuillefroy	26	0.29 ± 0.13	Predator
Pterostichus globosus (Quensel in Schonherr)	16	0.18 ± 0.07	Mainly Predator
Calathus mollis (Marsham)	4	0.04 ± 0.02	Predator
Calathus cinctus Motschulsky	1	0.01 ± 0.01	Predator
Calathus sp.	1	0.01 ± 0.01	Predator
Nebria salina Fairmaire & Laboulbene	3	0.03 ± 0.02	Predator
Amara aenea (De Geer)	6	0.07 ± 0.03	Omnivorous
Brachinus sp.	1	0.01 ± 0.01	Mainly Predator
Brachinus explodens Duftschmid	1	0.01 ± 0.01	Mainly Predator
Brachinus variventris Schaufuss	2	0.02 ± 0.02	Mainly Predator
Licinus punctatulus (Fabricius)	8	0.09 ± 0.04	Predator
Anchomenus dorsalis (Pontoppidan)	1	0.01 ± 0.01	Predator
Olisthopus fuscatus Dejean	1	0.01 ± 0.01	Predator
Parophonus maculicornis (Duftschmid)	1	0.01 ± 0.01	Omnivorous
Trechus obtusus Erichson	4	0.04 ± 0.02	Predator
Poecilus sp.	3	0.03 ± 0.02	Predator
Staphylinidae			
Ocypus sp.	76	0.84 ± 0.23	Predator
Quedius sp.	27	0.30 ± 0.07	Predator
Mycetoporus sp.	51	0.57 ± 0.14	Mainly Predator
Oxytelus sp.	16	0.18 ± 0.07	Saprophagous/Fungivorous
Tachyporus sp.	11	0.12 ± 0.05	Mainly Predator
Thinodromus sp.	7	0.08 ± 0.07	Saprophagous/Fungivorous
Othius sp.	5	0.06 ± 0.02	Predator
Gabrius sp.	2	0.02 ± 0.02	Predator
Anotylus sp.	377	4.19 ± 2.32	Saprophagous/Fungivorous
Coproporus sp.	4	0.04 ± 0.03	Mainly Predator
Philonthus sp.	1	0.01 ± 0.01	Predator
Xantholinus sp.	2	0.02 ± 0.02	Predator
Astenus sp.	1	0.01 ± 0.01	Predator
Tachinus sp.	2	0.02 ± 0.02	Mainly Predator
Carpelinus sp.	2	0.02 ± 0.02	Saprophagous/Fungivorous
Metopsia sp.	2	0.02 ± 0.02	Saprophagous/Fungivorous
Other Coleoptera	621	6.90 ± 1.47	Non-identified
Formicidae			
Messor barbarus (Linnaeus)	1681	18.68 ± 7.20	Granivorous
Messor bouvieri Bondroit	20	0.22 ± 0.18	Granivorous
Camponotus pilicornis (Roger)	23	0.26 ± 0.12	Omnivorous

Camponotus aethiops (Latreille)	3	0.03 ± 0.03	Omnivorous
Camponotus piceus (Leach)	7	0.08 ± 0.06	Omnivorous
Camponotus cruentatus (Latreille)	1	0.01 ± 0.01	Omnivorous
Camponotus lateralis (Olivier)	1	0.01 ± 0.01	Omnivorous
Camponotus foreli Emery	2	0.02 ± 0.02	Omnivorous
Tetramorium forte Forel	63	0.70 ± 0.19	Omnivorous
Tetramorium semilaeve Andre	11	0.12 ± 0.05	Omnivorous
Tapinoma nigerrimum (Nylander)	890	9.89 ± 5.50	Omnivorous
Crematogaster auberti Emery	144	1.60 ± 0.32	Omnivorous
Cataglyphis hispanicus (Emery)	66	0.73 ± 0.27	Omnivorous
Cataglyphis sp.	54	0.60 ± 0.24	Omnivorous
Plagiolepis pygmaea (Latreille)	29	0.32 ± 0.13	Mainly Predator
Lasius sp.	1	0.01 ± 0.01	Mainly Predator
Goniomma sp.	3	0.03 ± 0.02	Granivorous
Aphaenogaster gibbosa (Latreille)	4	0.04 ± 0.03	Omnivorous
Aphaenogaster sp.	8	0.09 ± 0.09	Omnivorous
Pheidole sp.	1	0.01 ± 0.01	Omnivorous
Formica subrufa Roger	1	0.01 ± 0.01	Mainly Predator
Solenopsis sp.	1	0.01 ± 0.01	Mainly Predator
Forficulidae			
Forficula auricularia Linnaeus	987	10.97 ± 1.68	Omnivorous
Scolopendromorpha			
Scolopendromorpha	12	0.13 ± 0.08	Predator
Araneae			
Agelenidae	89	0.99 ± 0.17	Predator
Dysderidae	1	0.01 ± 0.01	Predator
Eresidae	1	0.01 ± 0.01	Predator
Gnaphosidae	641	7.12 ± 0.71	Predator
Linyphiidae	40	0.44 ± 0.11	Predator
Lycosidae	260	2.89 ± 0.64	Predator
Philodromidae	17	0.19 ± 0.05	Predator
Salticidae	33	0.37 ± 0.08	Predator
Sparassidae	1	0.01 ± 0.01	Predator
Tetragnathidae	1	0.01 ± 0.01	Predator
Theridiidae	17	0.19 ± 0.06	Predator
Thomisidae	128	1.42 ± 0.21	Predator
Zodariidae	132	1.47 ± 0.31	Predator
Acari	307	3.41 ± 1.48	Non-identified
Total arthropods	6967		

Chapter 4

General Discussion and Conclusions

General Discussion and Conclusions

This work aimed to study the role of edaphic arthropods on the biological control of *B. oleae*, specifically the potential of two carabids as predators of *B. oleae* pupae under laboratory conditions and also to measure the biological control service provided by edaphic predators on *B. oleae* pupae in the field.

In what concerns the first specific objective, both species of carabids, *C. granatensis* and *P. globosus* showed to prey *B. oleae* pupae in laboratory conditions. The species *C. granatensis* showed a strong preference for *B. oleae* pupae independently of the offered density of the alternative prey whereas *P. globosus* showed to be more polyphagous and in general didn´t demonstrate a preference for *B. oleae* pupae, switching between preys depending on the offered proportion.

Both carabid species demonstrated a type II functional response to the increase in the density of *B. oleae* pupae. However, *C. granatensis* showed higher handling times and higher attack rates and demonstrated satiation at lower number of consumed pupae when compared to *P. globosus.*

The predatory behavior showed by these carabids under laboratory conditions cannot be directly extrapolated to what may happen in field conditions. However some assumption can be made concerning their efficiency as potential biological control agents in the field.

The functional responses exhibited by both carabids indicated that an increase of *B. oleae* pupae on soil will generate similar responses in both carabid species. However, *P. globosus* will show lower attack rates and handling time, which reflects more capacity in predating *B. oleae* pupae.

Although, the preference demonstrated by *C. granatensis* reflects that it can be an efficient biological control agent, because the presence of an alternative prey may not change its functional response to increasing densities of *B. oleae* pupae. On the contrary, *P. globosus* may switch the consumption of the prey item in function of its abundance in the environment and, due to this reason, it can change its functional response by consuming less amounts of *B. oleae* pupae. This behavior can be considered negative in terms of efficacy as biological control agent against this pest but can also be considered positive, since the consumption of alternative prey and switching behavior may help to increasing the abundance of this predator in the field when levels of the pest are still low.

Thus, both species of carabids can contribute to reduce pest levels, *C. granatensis* due to the preference demonstrated and *P. globosus* due to its capacity in consuming high amounts of pupae.

The complementarity of these species in the biological control of *B. oleae* may happen especially in the peak of abundance of these carabids in the field, which happens in autumn and coincides with the presence of *B. oleae* pupae on the ground.

However, pupae may still be buried in the ground during the winter period and beginning of spring, where carabids are not so abundant and in that case, biological control services can be provided by other groups of edaphic arthropods, as the second specific objective demonstrated.

The community of arthropods was mainly dominated by individuals of the family Forficulidae during the winter season and Formicidae during the spring. The biological control service index increased throughout the sampling periods reaching the maximum value of 1 in the beginning of the spring which indicates that soil arthropods were able to predate *B. oleae* pupae in those periods. Moreover, it seemed likely that the biological control of *B. oleae* pupae in the field resulted of the complementary predation exerted by diverse groups of arthropods, and the relative importance of some groups depends highly on the season and the community present.

This work allowed knowing about the potentiality of two of the most abundant species of carabids in olive groves of Trás-os-Montes as predators of olive fruit fly pupae and their possible contribution for the biological control of this pest in the field. It also allowed knowing the edaphic community of arthropods in olive groves of Trás-os-Montes in the winter period and early spring and their importance on the biological control of olive fruit fly pupae in the field which was until now poorly known.

However, further investigation on the feeding habits of both carabids and predatory behavior is still needed, such as experimenting other alternative prey, present in olive groves, in the feeding preference experiments to understand if *C. granatensis* really prefers *B. oleae* pupae over other prey present in olive groves or if it demonstrates switching behavior as well as if *P. globosus* has some sort of prefer type of prey. Moreover, some traits related with pupae (e.g., color, hardness or nutritional composition) should also be investigated in order to understand if they are different. It should be also investigated the feeding habits of other abundant arthropods in olive groves, such as the groups which proved to be more related with the biological control provided.