

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

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Abstract

Carabid beetles are important predators in agricultural landscapes feeding on a range of prey items. However, their role as predators of the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), one of the most serious pests of olives, is unknown. In this context, the feeding preferences and the functional responses of two carabid beetle species, *Calathus granatensis* (Vuillefroy) and *Pterostichus globosus* (Fabricius), were studied under laboratory conditions. Feeding preference assays involved exposing carabid beetles to different ratios of *B. oleae* pupae and an alternative prey, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Both species fed on *B. oleae* pupae however, *C. granatensis* always showed a significant preference for that prey whereas *P. globosus* switched to *C. capitata* pupae when the offered ratio was below 0.5. The total prey biomass consumed was significantly higher for *P. globosus* than for *C. granatensis*. Functional response curves were estimated based on different densities of *B. oleae* pupae and both carabid beetle species exhibited a type II functional response using Rogers' random-predator equation. *P. globosus* showed shorter handling time (1.223 ± 0.118 h) on *B. oleae* pupae than *C. granatensis* (3.230 ± 0.627 h). Our results suggest that both species can be important in reducing the densities of *B. oleae* in olive groves, although *P. globosus* was more efficient than *C. granatensis*.

Keywords: *Calathus granatensis*, *Pterostichus globosus*, consumption, biomass, pest, predator

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Introduction

Carabid beetles are important polyphagous predators in agroecosystems (Lövei & Sunderland, 1996; Lövei, 2008). Most of them consume other insects, molluscs or millipedes, and a range of plant material such as seeds, or are scavengers (Kromp, 1999; Symondson *et al.*, 1999; Honek *et al.*, 2003; Foltan, 2004; Wallace, 2004; Wallinger *et al.*, 2015). Due to their predatory behavior, carabid beetles can be important natural control agents of crop pests (Kromp, 1999).

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is considered the major pest of olives in most

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commercial olive growing regions worldwide (Nardi *et al.*, 2005; Daane & Johnson, 2010). Damages caused by this insect include the premature fall of infested fruits, pulp consumption by developing larvae and a general reduction in olive oil quality (Pereira *et al.*, 2004). Although control options for this pest are still based on insecticides, recent efforts intend to promote biological control. So far, the use of natural enemies, mainly parasitoids, is still unsuccessful (Daane & Johnson, 2010). Moreover, the action of predators on larvae is difficult once this stage develops inside the olive fruit. However, pupation occurs on 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 & 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). In this context, carabid beetles are considered voracious feeders and studies of their feeding preferences and consumption rates are essential to understand basic trophic relationships and their potential efficacy as natural control agents. However, their efficiency may be affected by the simultaneous occurrence of alternative prey resulting in a decreased predation on the target pest species. 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). Predatory functional responses are typically described by three types of curves depending on prey density. Thus, for types I, II and III functional responses, the number of prey consumed increases linearly, asymptotically to a plateau and sigmoidally with increasing prey density, respectively (Holling, 1966).

In the olive grove, carabid beetles can have an important role as natural enemies of the olive fruit fly population. Previous studies showed that they are abundant insects among the edaphic arthropod community of the olive grove (Santos *et al.*, 2007; Gonçalves & Pereira, 2012), mainly in autumn (Oliveira, 2013) coinciding with the increase of pupae on the soil. Moreover, generalist carabid beetles (i.e., common species such as *Carabus banonii* Dejean and *Pterostichus creticus* (Frivaldsky) were referred to predate pupae of the olive fruit fly in the 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 carabid beetles as natural enemies 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; Dinis *et al.*, *in press*), representing interesting species for evaluating predation on pupae of the olive fruit

fly under laboratory conditions. We tested the hypothesis that specimens belonging to the largest species, *P. globosus* have higher predation rates than the smallest species, *C. granatensis*.

Material and Methods

Test organisms

Laboratory rearing of *B. oleae* was initiated in October/November 2013 with pupae obtained from infested olive fruits collected in several olive groves in the region of Mirandela (Northeastern Portugal). Adult flies were kept in poly-methyl-methacrylate cages (40 × 30 × 30 cm³) and every 2 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. Rearing was maintained under controlled conditions at 21 ± 1°C, 70 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) at the School of Agriculture (ESA), Bragança. Pupae from the 2nd to the 5th generation were used in the experiments.

Ceratitis capitata (Wiedemann) pupae were originally collected from the stock colony in the Unidad de Protección de Cultivos of Technical University of Madrid, and rearing has been maintained at the ESA, Bragança since September 2012. Adult flies were kept in poly-methyl-methacrylate cages (40 × 30 × 30 cm³) under controlled conditions at 24 ± 2°C; 60 ± 5% RH and a photoperiod of 16:8 (L:D). 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 yeast hydrolysate at a ratio 4:1 (based on dry weight). *C. capitata* pupa was used as model alternative prey in preference experiments and was selected due to its similarity with *B. oleae* trying to mimic less mobile prey items present in the olive grove.

Adult specimens of *C. granatensis* and *P. globosus* were hand collected in an organic olive grove in the region of Mirandela (Northeastern Portugal) between September 2013 and May 2014. Specimens found on the ground, leaf litter or under the stones were placed in plastic boxes (7.5 cm in diameter × 4.5 cm height) and carried out to the laboratory where the identification of the species was confirmed and each species was transferred to different rearing plastic cages (15 × 37 × 53 cm³) containing dry natural soil (a layer of about 8 cm height) and several stones scattered on the surface to provide shelter. The soil used in the rearing cages was collected in the olive grove, sieved to <2.0 mm and air dried. Beetles were fed every 5 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. Specimens were acclimatized, at least, for 2 months before the beginning of the experiments.

Feeding preferences, predation efficiency and functional responses

Specimens of *C. granatensis* and *P. globosus* were transferred from the rearing cages and placed individually in plastic containers (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. A hole of 6.0 cm in diameter was made on the lid of the containers and substituted by a permeable piece of cloth (1.0 mm mesh) to ensure ventilation. Experiments were performed under controlled conditions at

$21 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D). Carabid beetles were starved for seven days prior to the start of each experiment.

Feeding preferences

Eight treatments, corresponding to eight different prey ratios, were offered to each carabid beetle species. The following prey ratios were tested: (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. For each prey ratio, pupae were randomly allocated in a Petri dish (6.0 cm diameter and 1.0 cm height), and then placed inside each testing container. A total of 25 individuals of each species were tested in each treatment for 24 h. Pupae consumed by each beetle were calculated by counting the non-consumed pupae of each prey and subtracting them to the initial number. Each specimen was used once in each combination.

Predation efficiency

The weight of 30 randomly selected 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 were 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 ten adult specimens of each carabid beetle species as replicates in each density. Different densities of the prey (pupae of *B. oleae*) were offered to each species. *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 h, the number of pupae consumed was recorded.

Data analysis

Statistical analyses were performed using R (R Core Team, 2015). Firstly, feeding preferences of *C. granatensis* and *P. globosus* were assessed by performing a multivariate two-sample Hotelling's T^2 test (Lockwood, 1998) using the *hotelling.test* function from the *Hotelling* package. The percentage of biomass of prey consumed was used as explanatory variables in order to reduce the noise introduced by the different total percentage of prey consumption within each arena and 10,000 permutations were carried out to lead with the lack of independence on data. The consumed ratios of *B. oleae* pupae were calculated by dividing the number of *B. oleae* pupae by the total number of pupae consumed. Then, Manly's preference index (Manly *et al.*, 1972) was calculated; this is a method to evaluate preference that takes into account the prey

densities depletion by predation during experiments (Cock, 1978) as following:

$$a = \frac{r_1/n_1}{r_1/n_1 + r_2/n_2} \quad (1)$$

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 consumed pupae, the total biomass of consumed pupae (calculated as the weight of the pupae \times number of consumed pupae) and the percentage of biomass of consumed pupae (calculated as the total biomass consumed/total biomass offered, in percentage).

The consumed ratios of *B. oleae* pupae, the Manly's preference index values, the total number of pupae consumed, the total biomass and the percentage of biomass of consumed pupae were compared between species of carabid beetles using two-sided *t*-tests and subsequently a pairwise procedure was followed correcting the alpha threshold ($0.05/21 = 0.0024$) in order to uncover differences between the offered ratios of *B. oleae* pupae within each beetle species and the same alpha threshold was used all along the analyses. All statistical outputs were summarized in the Appendix.

Functional response – A logistic regression analysis was used to determine the shape of the functional response with the proportion of predated pupae versus initial density of pupae (Trexler *et al.*, 1988). In the regression, it was fitted a polynomial function (Juliano, 2001) as the following:

$$\frac{N_e}{N_0} = \frac{\exp(\beta_0 + \beta_1 N_0 + \beta_2 N_0^2 + \beta_3 N_0^3)}{1 + \exp(\beta_0 + \beta_1 N_0 + \beta_2 N_0^2 + \beta_3 N_0^3)} \quad (2)$$

where N_e represents the number of *B. oleae* pupae consumed, N_0 is the initial density of *B. oleae* pupae, 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 likelihood (Juliano, 2001). A negative linear coefficient means a better adjustment to type II, whereas a positive linear coefficient and a negative quadratic coefficient imply that the data fit a type III functional response. Significance level was established at $P = 0.001$.

Discrimination between types I and II responses has previously been carried out by comparing proportional mortality at different prey densities (Rogers, 1972; Juliano, 2001). The data indicated type II functional responses for both carabid species and because there was no prey replacement, the random predator equation was fitted (Rogers, 1972), excluding those densities in which all prey were consumed in 24 h, following equation (3).

$$N_e = N_0[1 - \exp\{a(T_h N_e - T)\}] \quad (3)$$

where a represents the attack rate (searching efficiency per time), T_h the handling time (time to attack, kill and eat each prey) and T the time of the experiment (24 h).

Rogers' random predator equation was primarily fitted using the *nlsLM* and *lambertW* functions of the *minpack.lm* and *emdbook* packages respectively in R, but an overestimated result for the parameter a was obtained. Therefore, a second

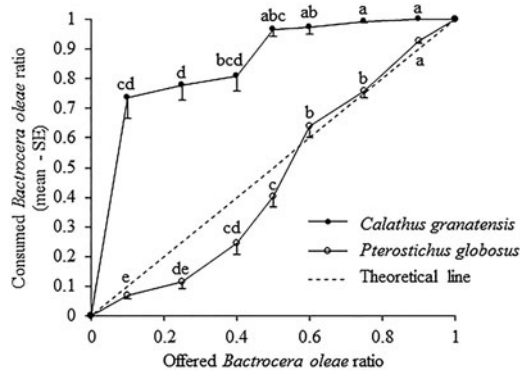


Fig. 1. Consumed ratio of *Bactrocera oleae* pupae (mean – standard error of the mean, SE) for *Calathus granatensis* and *Pterostichus globosus*. Means with different letters for each carabid species were significantly different ($P < 0.0024$). In all cases, the different consumed ratios were significantly different between the two carabid beetle species ($P < 0.0024$).

fitting was performed establishing an upper limit for this parameter. The value used was the asymptote of the model, i.e., the inverse of the handling time ($1/T_h$) estimated after the first model fitting (Bolke, 2007).

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. Data are presented as mean values ± 1 standard error (SE).

Results

Feeding preferences

The Hotelling's T^2 test showed statistically significant differences for the proportional consumption of the two prey species ($T^2 = 441.93$, $P < 0.001$). For *C. granatensis*, the consumed ratio decreased when the offered ratio of *B. oleae* pupae decreased but was always superior to the offered ratio of *B. oleae* (fig. 1). 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 *B. oleae* pupae decreased with the decrease of the offered ratio of *B. oleae* pupae (fig. 1).

Manly's preference index was significantly different over the offered ratio of *B. oleae* pupae for each carabid beetle species (Appendix 1). Comparing both species, *C. granatensis* showed significantly higher Manly's preference indexes than *P. globosus* for all offered ratios of *B. oleae* pupae (table 1). For *C. granatensis*, the Manly's preference index increased as the offered ratio of *B. oleae* pupae 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 higher than 0.5, this species showed a preference above 50%, however, when the offered ratio reached 0.5 the index decreased rapidly reaching 24%.

Predation efficiency

The average (\pm SE) weight of *C. granatensis* was 47.5 ± 2.2 mg and the average weight of *P. globosus* was 248.8 ± 7.4 mg. For the prey, the average weight of *B. oleae* pupae was 5.0 ± 0.2 mg,

Table 1. Manly's preference indexes (mean \pm SE) for different ratios of offered *Bactrocera oleae* pupae for adult *Calathus granatensis* and *Pterostichus globosus*.

Offered ratio of <i>B. oleae</i> pupae	Manly's preference indexes	
	<i>C. granatensis</i>	<i>P. globosus</i>
0.9 (18/20)	1.000 ± 0.000 (a)	0.646 ± 0.046 (a)*
0.75 (15/20)	0.988 ± 0.012 (ab)	0.535 ± 0.034 (ab)*
0.6 (12/20)	0.972 ± 0.027 (abc)	0.556 ± 0.040 (ab)*
0.5 (10/20)	0.966 ± 0.022 (abc)	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 (c)	0.240 ± 0.044 (c)*
0.1 (2/20)	0.924 ± 0.021 (bc)	0.333 ± 0.050 (c)*

SE, standard error of the mean.

Means within a column with different letters were significantly different at $P < 0.0024$.

The asterisks (*) mean that, within the row, carabid species were significantly different for the same ratio of *B. oleae* pupae at $P < 0.0024$.

whereas that of *C. capitata* was 8.0 ± 0.1 mg. The ratio between the weight of the predator and that of *B. oleae* pupae was 9.5 for *C. granatensis* and 49.8 for *P. globosus*.

The total number of consumed pupae was significantly different for *C. granatensis* over the offered ratios of *B. oleae* pupae (table 2) and also between species for each offered ratio of *B. oleae* pupae (table 2). *C. granatensis* consumed a significantly higher number of pupae in the 0.9 ratio of *B. oleae* pupae when compared with the 0.1 ratio of *B. oleae* pupae while no differences were detected for *P. globosus* (table 2).

The percentage of biomass consumed over the offered ratios of *B. oleae* pupae differed significantly for *C. granatensis* but it was not different for *P. globosus* (table 2). There were significant differences between the percentage of biomass consumed by both species; 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).

The total biomass consumed over the offered ratio of *B. oleae* pupae differed significantly for *P. globosus* (increasing the total biomass of prey consumed as the offered ratio of *B. oleae* pupae decreased (table 2)). However, for *C. granatensis* the total biomass consumed did not differ significantly with the decrease of the offered ratio of *B. oleae* pupae. The total biomass consumed was significantly higher for *P. globosus* than for *C. granatensis* (table 2).

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 a type II functional response for both species based on the respective linear coefficient obtained, $\beta_1 = -3.425 \pm 0.736$, $P < 0.001$ and $\beta_1 = -0.312 \pm 0.090$, $P < 0.001$.

Both carabid beetle species showed an increase in predation with the increase of the density of *B. oleae* pupae, although *C. granatensis* reached a plateau at lower prey densities (fig. 2). For *P. globosus*, the estimated handling time (T_h) was 1.223 ± 0.118 h and the coefficient of attack rate (a) was 0.281 ± 0.165 h⁻¹, resulting in a maximum attack rate (T/T_h) of 19.6 pupae. For *C. granatensis*, the estimated handling time was 3.230 ± 0.627 h and the coefficient of attack rate

Table 2. Total number of pupae, percentage of total biomass and total biomass consumed (mean \pm SE) of *Bactrocera oleae* pupae plus *Ceratitis capitata* pupae (Bo + Cc) in 24 h by *Calathus granatensis* and *Pterostichus globosus* for different ratios of offered *B. oleae* pupae.

Carabid species	Offered ratio of <i>B. oleae</i> pupae	Number of pupae (Bo + Cc) consumed	Total biomass (Bo + Cc) consumed (%)	Total biomass (Bo + Cc) consumed (mg)
<i>C. granatensis</i>	0.9 (18/20)	5.88 \pm 0.659 (a)*	27.22 \pm 3.051 (a)*	29.9 \pm 3.3 (a)*
	0.75 (15/20)	4.88 \pm 0.681 (ab)*	20.43 \pm 2.860 (ab)*	24.52 \pm 3.4 (a)*
	0.6 (12/20)	4.36 \pm 0.635 (ab)*	16.79 \pm 2.431 (ab)*	22.16 \pm 3.2 (a)*
	0.5 (10/20)	4.44 \pm 0.507 (ab)*	16.29 \pm 1.907 (ab)*	22.8 \pm 2.7 (a)*
	0.4 (8/20)	5.04 \pm 0.599 (ab)*	19.46 \pm 2.515 (ab)*	28.8 \pm 3.7 (a)*
	0.25 (5/20)	4.68 \pm 0.515 (ab)*	17.18 \pm 2.181 (ab)*	27.48 \pm 3.5 (a)*
<i>P. globosus</i>	0.1 (2/20)	3.12 \pm 0.463 (b)*	11.65 \pm 2.083 (b)*	20.4 \pm 3.6 (a)*
	0.9 (18/20)	15.00 \pm 1.450 (a)	73.22 \pm 7.197 (a)	79.08 \pm 7.8 (a)
	0.75 (15/20)	15.16 \pm 1.053 (a)	72.47 \pm 5.097 (a)	86.96 \pm 6.1 (ab)
	0.6 (12/20)	14.08 \pm 1.150 (a)	65.24 \pm 5.558 (a)	86.12 \pm 7.3 (ab)
	0.5 (10/20)	13.80 \pm 0.926 (a)	66.34 \pm 4.132 (a)	92.88 \pm 5.8 (ab)
	0.4 (8/20)	14.80 \pm 0.938 (a)	71.32 \pm 3.828 (a)	105.56 \pm 5.7 (ab)
	0.25 (5/20)	13.36 \pm 0.914 (a)	63.20 \pm 3.903 (a)	101.12 \pm 6.3 (ab)
0.1 (2/20)	15.04 \pm 0.908 (a)	67.93 \pm 4.013 (a)	116.84 \pm 6.9 (b)	

SE, standard error of the mean.

For each carabid species, means within a column with different letters were significantly different at $P < 0.0024$.

The asterisks (*) mean that, within a column, carabid species were significantly different for the same ratio of *B. oleae* pupae at $P < 0.0024$.

was $0.300 \pm 0.939 \text{ h}^{-1}$, resulting in a maximum attack rate of 7.4 pupae.

Discussion

This study shows that both *C. granatensis* and *P. globosus* were able to feed on *B. oleae* pupae, although they had significantly different feeding preferences and abilities to respond to increasing prey densities. Thus, *C. granatensis* had a 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. Diverse prey items, such as slugs (Oberholzer *et al.*, 2003), lepidopteran pests (Suenaga & Hamamura, 1998) and dipteran pupae including *B. oleae* pupae (Neuenschwander *et al.*, 1983; Odoguardi *et al.*, 2008) are commonly present in the diet of these carabid beetles.

P. globosus consumed a significantly higher amount of total biomass than *C. granatensis* probably because the former is larger (14–22 mm in length) than the latter with 9–12 mm in length (Aguiar & Serrano, 2012) and larger carabid beetles have larger guts and consequently are able to consume more biomass (Wallace, 2004). Such differences can also justify the results obtained in the food preference experiments as *P. globosus* seemed to select prey items that were more valuable in terms of energy intake per unit of handling time. In previous studies conducted to evaluate feeding preferences of carabid beetles on different slug species, the weight of the slug was considered the main factor influencing the choice of the predator (Thiele, 1977; Ernsting & Vanderwerf, 1988; Wheeler, 1988; Ayre, 2001; McKemey *et al.*, 2001; Hatteland *et al.*, 2013) 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 gained energy. 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 carabid beetles (Hengeveld, 1980; Barney & Pass, 1986). On the other hand, 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 choice of the predator.

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

Both carabid beetle species exhibited a type II functional response in which the consumption rate of *B. oleae* pupae rose with prey density, but gradually decelerated until a plateau was reached and the consumption rate remained constant with the increase of *B. oleae* pupae density. The plateau was reached at lower numbers of consumed prey by *C. granatensis* than by *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

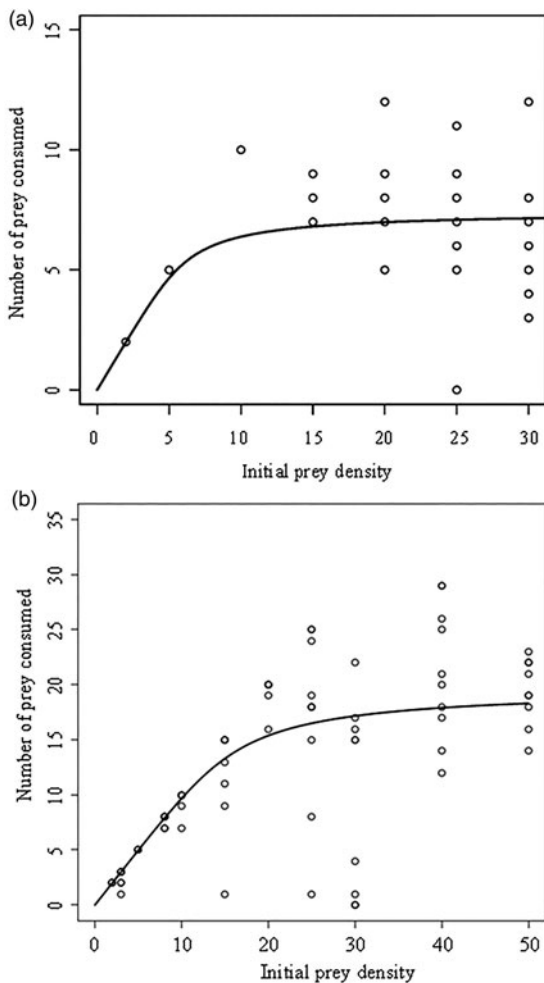


Fig. 2. Functional responses of adult *Calathus granatensis* (a) and *Pterostichus globosus* (b) fed for 24 h on increasing densities of pupae of *Bactrocera oleae*. Circles represent the number of prey eaten in each density of offered pupae and lines represent the sketched fitted values.

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 of *B. oleae* pupae for *C. granatensis* was, in average, 2.6 times longer than for *P. globosus*. Thus, although both carabid beetles exhibited the same kind of functional response, the time required for handling *B. oleae* pupae may indicate different abilities to deal with increasing *B. oleae* densities and different levels of satiation, voracity or digestive rates between them. *P. globosus* can consume more pupae before satiation and can be more efficient in handling pupae than *C. granatensis*.

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 & Harmsen,

2002). *C. granatensis* showed higher preference for *B. oleae* pupae in detriment of the alternative prey, thus, for this species, the presence of alternative prey items in olive groves might affect less its consumption on *B. oleae* pupae. However, other studies need to be done using prey items smaller than *B. oleae* pupae in order to clarify the feeding habits of this species. Although *P. globosus* consumed more *B. oleae* pupae than *C. granatensis*, the presence of alternative prey items in olive groves can originate a decrease of the consumption of *B. oleae* pupae due to switching to more energetic prey items and, consequently, to higher levels of satiation given by that prey (Murdoch, 1969; Murdoch & 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 the abundance of the predator when pest levels in the agroecosystem are low (Settle *et al.*, 1996; Symondson *et al.*, 2002). Thus, the ability for consuming other prey items can be more advantageous for *P. globosus* that will have conditions to reach high populations. Moreover, in olive groves, both carabid beetle 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 prefers this prey. Further studies (e.g., semi-field assays or PCR-based gut content analysis for tracking *B. oleae* predation) will need to be done in order to confirm the contribution of both species as biocontrol agents of *B. oleae*. Moreover, the development of *B. oleae* biocontrol strategies should take into account the conservation or enhancement of these species of carabid beetles in olive groves. Therefore, management practices such as tillage or herbicide application should be avoided whereas non-crop habitats (plants and stones) may be established in the agroecosystem since they provide shelter and alternative food resources for carabid beetles.

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APPENDIX

Statistics of comparisons between carabid beetle species for consumed ratios, Manly's preference index, number of pupae consumed, percentage of biomass consumed and total biomass consumed for each offered ratio of *Bactrocera oleae* pupae.

Offered ratio of <i>B. oleae</i> pupae	Consumed ratios			Manly's preference index			Number of pupae consumed			% of total biomass and total biomass consumed		
	<i>t</i>	d.f.	<i>P</i>	<i>t</i>	d.f.	<i>P</i>	<i>t</i>	d.f.	<i>P</i>	<i>t</i>	d.f.	<i>P</i>
0.9 (18/20)	7.54	24.00	<0.001	7.70	24.00	<0.001	-5.71	33.47	<0.001	-5.88	32.36	<0.001
0.75 (15/20)	10.00	26.05	<0.001	12.45	29.77	<0.001	-8.19	41.10	<0.001	-8.90	37.75	<0.001
0.6 (12/20)	7.76	41.77	<0.001	8.38	41.90	<0.001	-7.40	37.37	<0.001	-7.99	32.86	<0.001
0.5 (10/20)	13.96	40.83	<0.001	13.96	40.83	<0.001	-8.87	37.21	<0.001	-11.00	33.78	<0.001
0.4 (8/20)	9.41	45.96	<0.001	8.88	46.52	<0.001	-8.77	40.76	<0.001	-11.33	41.47	<0.001
0.25 (5/20)	12.11	32.82	<0.001	12.09	41.06	<0.001	-8.27	37.85	<0.001	-10.29	37.65	<0.001
0.1 (2/20)	9.75	25.28	<0.001	11.09	32.32	<0.001	-11.69	35.69	<0.001	-12.44	36.06	<0.001

Comparison between each offered ratio combination of *Bactrocera oleae* for consumed ratios, Manly's preference index, number of pupae consumed, percentage of biomass consumed and total biomass consumed. Within each parameter (first column), *P*-values for *t*-tests are provided on the upper side of diagonals for *Calathus granatensis* and the lower side corresponds to *Pterostichus globosus*.

	Offered ratio	0.9 (18/20)	0.75 (15/20)	0.6 (12/20)	0.5 (10/20)	0.4 (8/20)	0.25 (5/20)	0.1 (2/20)
	of <i>B. oleae</i> pupae							
Consumed ratios	0.9 (18/20)	–	0.327	0.327	0.136	<0.001	<0.001	<0.001
	0.75 (15/20)	<0.001	–	0.439	0.207	<0.001	<0.001	<0.001
	0.6 (12/20)	<0.001	0.008	–	0.768	0.003	0.001	0.002
	0.5 (10/20)	<0.001	<0.001	<0.001	–	0.004	0.002	0.003
	0.4 (8/20)	<0.001	<0.001	<0.001	0.003	–	0.647	0.370
	0.25 (5/20)	<0.001	<0.001	<0.001	<0.001	0.006	–	0.613
	0.1 (2/20)	<0.001	<0.001	<0.001	<0.001	<0.001	0.074	–
	0.9 (18/20)	–	0.327	0.327	0.136	<0.001	<0.001	<0.001
Manly's index	0.75 (15/20)	0.059	–	0.608	0.391	0.002	0.003	0.011
	0.6 (12/20)	0.154	0.689	–	0.865	0.013	0.036	0.168
	0.5 (10/20)	<0.001	0.009	0.006	–	0.012	0.032	0.165
	0.4 (8/20)	<0.001	<0.001	<0.001	0.103	–	0.439	0.095
	0.25 (5/20)	<0.001	<0.001	<0.001	<0.001	0.314	–	0.292
	0.1 (2/20)	<0.001	0.002	0.001	0.250	0.694	0.169	–
	0.9 (18/20)	–	0.297	0.103	0.090	0.350	0.158	0.001
	0.75 (15/20)	0.929	–	0.579	0.607	0.861	0.816	0.039
Number of pupae consumed	0.6 (12/20)	0.622	0.492	–	0.922	0.440	0.697	0.122
	0.5 (10/20)	0.490	0.337	0.850	–	0.448	0.741	0.061
	0.4 (8/20)	0.909	0.799	0.630	0.452	–	0.651	0.015
	0.25 (5/20)	0.345	0.203	0.627	0.737	0.277	–	0.029
	0.1 (2/20)	0.982	0.932	0.516	0.344	0.855	0.199	–
	0.9 (18/20)	–	0.111	0.010	0.004	0.056	0.010	<0.001
	0.75 (15/20)	0.932	–	0.337	0.234	0.799	0.370	0.017
	0.6 (12/20)	0.385	0.343	–	0.872	0.449	0.906	0.115
% Biomass consumed	0.5 (10/20)	0.412	0.356	0.875	–	0.320	0.760	0.1075
	0.4 (8/20)	0.817	0.859	0.372	0.381	–	0.496	0.021
	0.25 (5/20)	0.229	0.156	0.765	0.583	0.144	–	0.073
	0.1 (2/20)	0.525	0.488	0.697	0.784	0.544	0.402	–
	0.9 (18/20)	–	0.430	0.513	0.161	0.008	0.032	<0.001
	0.75 (15/20)	0.310	–	0.930	0.485	0.030	0.112	0.002
	0.6 (12/20)	0.122	0.618	–	0.473	0.042	0.126	0.004
	0.5 (10/20)	0.127	0.694	0.879	–	0.124	0.338	0.011
Total biomass consumed	0.4 (8/20)	0.905	0.402	0.183	0.197	–	0.601	0.213
	0.25 (5/20)	0.691	0.548	0.267	0.292	0.797	–	0.098
	0.1 (2/20)	0.061	0.371	0.661	0.540	0.096	0.143	–