



## Acorn preference by the dung beetle, *Thorectes lusitanicus*, under laboratory and field conditions

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Processes of seed predation and dispersal are crucial for tree regeneration and forest dynamics. To understand the role of the dung beetle, *Thorectes lusitanicus* (Col., Scarabaeoidea, Geotrupidae) as secondary seed disperser in Mediterranean oak forests, its food preference was investigated in the field and in the laboratory. This paper had for objectives (1) to explore the feeding preferences for acorns of the two species available in the field: *Quercus suber* and *Quercus canariensis*, and (2) to study the olfactory and palatability response of *T. lusitanicus* to different food resources (oak acorns and dung) under laboratory conditions. The number of beetles in the field was curvilinearly related to the number of acorns, being higher when the number of acorns was intermediate. The maximum values of *T. lusitanicus* density corresponded to those plots located under *Q. suber* trees with the highest total weight of acorns. Bioassays with a four-armed olfactometer showed that *T. lusitanicus* was clearly attracted to volatiles of *Q. suber* acorns more than to of dung. Palatability bioassays also showed significant preferences for acorns of *Q. suber* in comparison with the typical food previously described for this beetle species (dung). According to our results, we suggest that a diet based on acorns (due to their high content of polyunsaturated fatty acids) probably satisfies the nutritional requirements of *T. lusitanicus* during winter, larval development, and metamorphosis.

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Polyphagy is the main feature of Scarabaeoidea beetles feeding behaviour (Hanski & Cambefort 1991). Different patterns of resource partitioning have been shown among different biogeographic regions (Halffter & Matthews 1966; Martín-Piera & Lobo 1996; Verdú & Galante 2002). While many tropical dung beetle species are attracted to carrion, fruits, and other substances in decomposition, mainly in forest biomes (Halffter & Matthews 1966), Palearctic dung beetles are predominantly attracted to herbivore and omnivorous dung, and occasionally to carnivore dung (Martín-Piera & Lobo 1996). Even though most Palearctic dung beetle are trophic generalists, a few species seem to prefer a specific type of dung (Verdú & Galante 2002, 2004), although this specialized habit can vary among geographical areas (Barbero et al. 1999). In some cases, dung

beetles assume a role as secondary seed dispersers; they bury faeces in the soil with seeds inside, favouring the establishment of seedlings (Sheperd & Chapman 1998; Vulinec 2000; Andresen 2002; Vander Wall & Longland 2004). In a recent work, an astonishing case of interaction between *Thorectes lusitanicus* Jeckel and the seeds of two *Quercus* species in the forests of South Spain has been documented. From the point of view of the oak tree, *T. lusitanicus* beetles not only act as postdispersal seed predators but also as authentic secondary dispersers, collecting and burying a great number of viable acorns. As only a small proportion of these buried acorns are completely consumed, this behaviour enables a higher survivorship of the seeds as well as their protection from more efficient seed predators (Pérez-Ramos et al. 2007). Because of the probable relevance of this behaviour on the ecology of Mediterranean forests, more research is needed about the evolutionary changes in anatomy, physiology, forage behaviour, and life cycle of this intriguing dung beetle. Olfaction is the primary

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sense used by insects to detect and locate food resources (Whittaker & Feeny 1971; Tumlinson et al. 1993; Pernal & Currie 2002). Food partitioning in dung beetle assemblages also depends on the insect's abilities to detect and select different odours, however, the influence of odour effluents emitted by different resources has received little attention (e.g. Dormont et al. 2004; Schmitt et al. 2004).

The purpose of this study was to examine feeding preferences of *T. lusitanicus* in field and laboratory conditions. For field experiments, we analyse feeding preferences between the two acorn species available in the field: *Quercus suber* and *Quercus canariensis*. For laboratory tests, we describe a new four-arm olfactometer adapted to flightless beetles in which three odours can be offered to test a great number of beetles at the same time.

## METHODS

### Study Sites

The study area was located in the Aljibe Mountains, near the Strait of Gibraltar, in south Spain. The climate is sub-humid Mediterranean-type, with cool, wet winters alternating with warm, dry summers. The January mean temperature is 12°C while the mean annual rainfall is 950 mm. Vegetation is dominated by the evergreen cork oak, *Q. suber*, mixed with deciduous oaks, *Q. canariensis*, which are more abundant on north-facing slopes and near stream beds. Most of the forested area has been protected as *Los Alcornocales* Natural Park, covering about 1700 km<sup>2</sup>. In some places, domestic cows are allowed to graze freely in the forest.

Two forest sites within this study area were used for the field experiments: San Carlos del Tiradero (TI) located at 36°9'46"N, 5°35'39"W, and La Saucedá (SA) located at 36°31'54"N, 5°34'29"W, about 40 km apart. The two forest sites differed in the presence of cattle, being sporadic in TI and very abundant in SA, through all the seasons.

### Study Organism: *T. lusitanicus*

*Thorectes lusitanicus* (Scarabaeoidea, Geotrupidae) is a flightless dung beetle endemic of the southern Iberian Peninsula (Spain and Portugal; López-Colón 1981, 1995; Martín-Piera & López-Colón 2000). The biology of this beetle species is poorly known, although it can be assumed to be similar to the close relative *Thorectes laevigatus* from North Africa (Klemperer & Lumaret 1985). The life cycle begins in the autumn (between September and November) when females lay their eggs in buried masses of herbivore dung (Martín-Piera & López-Colón 2000; J. R. Verdú, unpublished data). After immature development of 6–7 months, the adults emerge in spring. During the dry and warm summer they aestivate, while during the autumn they become active again and are quite visible crawling on the forest floor (Pérez-Ramos et al. 2007).

### Field Measurement of Feeding Preference

Field measurements were carried out during the autumn (November 2005), coinciding with the natural availability

of acorns of both oak species in the soil and the maximum peak of activity of *T. lusitanicus*. In each forest site (TI and SA), 10, 1-m<sup>2</sup> plots for each *Quercus* species were randomly selected beneath the canopy of adult trees to estimate the number of individuals of *T. lusitanicus* buried in the first 5 cm with or without acorns. The average distance between plots was 100 m (range 40–200 m). All acorns present at the surface of the soil of each plot were collected. Once in the laboratory, the number of these acorns and their total dry weight were calculated to obtain a precise measurement of food availability in the field for each plot. Moreover, in each plot, the ground was excavated and sifted meticulously to find all the beetles buried up to 10–15 cm of depth as well as the buried acorns partially or almost completely consumed by beetles. Consumed acorns were easily identified by a characteristic circular hole and the frequent presence of the beetle inside the acorn. When an individual (adult or larva) was found, its sex (if adult) and the type of buried food were recorded.

### Laboratory Experiments of Feeding Preference

Behavioural experiments were carried out in November–December 2005 on beetles collected in autumn in the field to test the response of *T. lusitanicus* to different food sources. A four-arm olfactometer was developed to test the effects of different volatiles emitted by two types of dung (cow and rabbit) and acorns. The apparatus consisted of a central arena with four exits (Fig. 1). The central arena consisted of a plastic truncate cone (60 cm superior radius and 40 cm inferior radius) with sterile dry leaves as substrate. The role of this substrate was to eliminate stress for beetles prior to each experiment. There were four 5-cm-diameter holes to attach the tubes (arms) containing the plastic containers with the test samples at the ends. Each tube consisted of a methacrylate cylinder (50 cm length, 5 cm external radius and 4.75 cm internal radius) placed horizontally. The plastic containers were made to capture the beetles that responded positively to the tested resources. Air, which had been passed through an activated carbon filter, was drawn into the plastic containers of the olfactometer. In the centre of the arena, there was a 12-cm hole to attach a tube with an air out ventilator. Complete sealing of the system was ensured with the use of teflon to join all connections. The temperature in the bioassay room was maintained at 25–27°C. Odour sources were randomly placed in the olfactometer in each trial.

Firstly, a total of 60 males and 60 females were used to test the effectiveness of the apparatus in six separate experiments (20 beetles per session). For this test, two containers with odour sources (acorns) as well as two empty containers were used. After placing the beetles in the arena, there was a 10 min wait before starting the experiment to allow the beetles to adapt to the new conditions. All insects were given 24 h to select a container, after which they all were removed and a new group was released.

Secondly, odour source preference was tested using the following four odour sources: (1) 50 g of fresh cow dung,

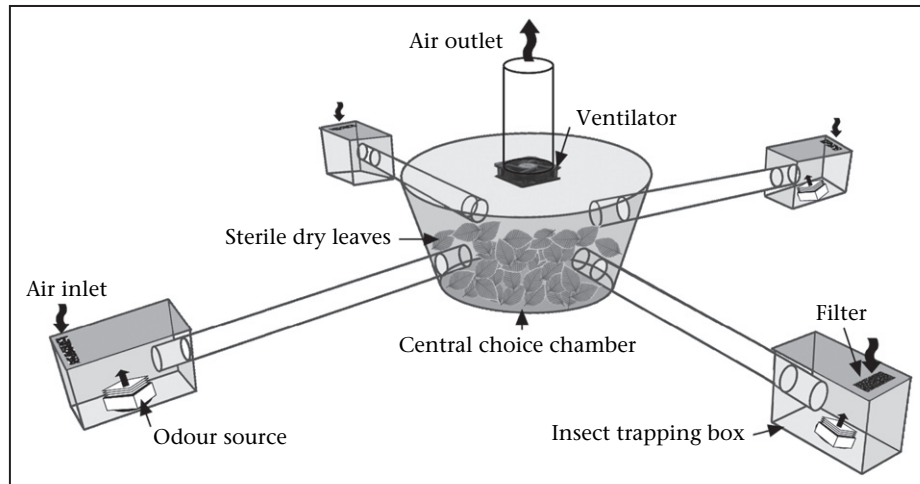


Figure 1. A schematic overview of the four-arm olfactometer used in laboratory bioassays. See text for a complete explanation.

(2) 50 g of fresh rabbit dung, (3) 50 g of mature *Q. suber* acorns, and (4) a control consisting of an empty container. For this bioassay, a total of 100 males and 100 females were used in 10 separate experiments (20 beetles per session). To corroborate food attraction preference, a palatability test was conducted to measure the beetles' preference for different food types. This test was carried on consisting of the same three food resources (50 g of cow dung, 50 g of rabbit dung, and 50 g of *Q. suber* acorns) separated by 15 cm in an arena consisting of a plastic truncate cone (80 cm superior radius and 60 cm inferior radius). A total of 100 males and 100 females were used in 10 different experiments (20 beetles per session). All insects were given 20 min to make a choice (based on food permanence after this time), after which they all were removed and a new group was released. The rate of consumption was not measured, considering a positive choice when the species begin to eat on the selected food source. If no choice was made after this time, the beetles were considered a null response. Rabbit dung was chosen because many of the species of this genus are associated with this type of dung (Verdú & Galante 2004).

Since the availability of *Quercus* species in natural conditions can vary, a last test was made to determine the preference of *T. lusitanicus* towards the different types of acorns. A complementary palatability test was made, consisting of three new food resources (*Quercus suber*, *Quercus canariensis*, and *Quercus rotundifolia* acorns). Acorns of this last *Quercus* species were included in this palatability test to examine the attractiveness of the acorns from more xeric species. A total of 100 males and 100 females were used in 10 different experiments (20 beetles per session). All insects were given 20 min to make a choice, after which they were removed and a new group was released. In all cases, each beetle was tested only once and treatments were randomly interchanged.

### Data Analysis

For field experiments, the nonparametric Spearman rank correlation and the nonparametric test of Mann–Whitney

were used. As the distribution of the number of *T. lusitanicus* in the plots fits a Poisson distribution, Generalized Linear Models were used with a logarithmic link between this dependent variable and the explanatory ones (*Quercus* species, acorn weight, acorn abundance and forest site). The best model was selected based on the Akaike Information Criterion (AIC; Akaike 1973).

In each laboratory experiment, the mean number of individuals obtained from the olfactometer and the scores of the palatability test were compared using the non-parametric test of Mann–Whitney and the Kruskal–Wallis test with the Dwass–Steel–Chritchlow–Fligner post hoc test for pairwise comparisons (Critchlow & Fligner 1991). Possible differences between sexes were investigated using the Wilcoxon signed-rank test. The binomial test was used to analyse the deviation of the sex ratio from 1:1. We used Statistica 6.0 software (StatSoft, Inc. 2001) and StatsDirect 2.5.7 (StatsDirect, Ltd. 2005) for analyses.

## RESULTS

### Acorns Availability across Species and Sites

The average number of acorns per 1 m<sup>2</sup> was 98.8 (SE = 19.4,  $N = 40$ ), varying between 0 for a plot of *Q. suber* in TI and 500 acorns/m<sup>2</sup> in the case of a plot of *Q. canariensis* in SA. More than half of the plots had less than 50 acorns. Neither the abundance nor the average weight of the acorns in the plots differed significantly between forest sites (Mann–Whitney  $U$  tests: acorns' abundance,  $U = 196.5$ ,  $N_1 = N_2 = 20$ ,  $P = 0.92$  and average weight of the acorns,  $U = 182.0$ ,  $N_1 = N_2 = 20$ ,  $P = 0.63$ ). Nevertheless, the availability of acorn resources under *Q. canariensis* was greater than under *Q. suber*. The average abundance of acorns under the cork oak trees, *Q. suber*, was significantly smaller (mean  $\pm$  SE;  $41.8 \pm 13.6$ ,  $N = 20$ ) than under *Q. canariensis* trees ( $155.9 \pm 31.9$ ,  $N = 20$ ; Mann–Whitney  $U$  test:  $U = 3.27$ ,  $N_1 = N_2 = 20$ ,  $P = 0.001$ ). The average weight of the acorns was also lower under cork oaks ( $25.3 \pm 9.1$  mg) than in *Q. canariensis* trees ( $55.7 \pm 11.1$  mg; Mann–Whitney  $U$  test:  $U = 115.0$ ,  $N_1 = N_2 = 20$ ,  $P = 0.02$ ). This

fact explains the positive and significant correlation between the abundance of acorns in each plot and the average weight of individual acorns (Spearman rank correlation:  $r_s = 0.93$ ,  $N = 40$ ,  $P < 0.0001$ ). The smaller availability of resources under the cork oaks was observed even when considering only whole acorns without evidence of damage (Mann–Whitney  $U$  test:  $U = 92.0$ ,  $N_1 = N_2 = 20$ ,  $P = 0.003$  for the abundance and  $U = 116.0$ ,  $N_1 = N_2 = 20$ ,  $P = 0.02$  for the weight). The adjustment of the probability values by a simultaneous-inference significance test for multiple testing of the data set also generates statistically significant scores.

### Sex Ratio of Beetles

Of the individuals captured while buried, 58 of 101 (57.4 %) were female and the sex ratio did not differ significantly from 1:1 (binomial test:  $P = 0.111$ ). Similarly, in the 13 of 40 plots in which acorns were partially consumed by the beetles, the sex ratio was also 1:1 (binomial test:  $P = 0.868$ ).

### *Thorectes* Density and Acorn Availability

The average weight of acorns per 1 m<sup>2</sup> was 40.5 g (SE = 7.5,  $N = 40$ ). The average number of *T. lusitanicus* per 1 m<sup>2</sup> was 2.52 (SE = 0.80), varying between 0 and 21 (total abundance = 101). Only 15 larvae were found, and all were at the SA site. In 21 plots, no beetles were found, whereas the abundance of beetles in 10 plots was higher than three individuals per 1 m<sup>2</sup>. The beetles collected in these plots ( $N = 89$ ) represented 88% of the total, and they were collected at SA site (four plots under *Q. canariensis* and six under *Q. suber*). The correlation between the number of acorns and the number of beetles was not statistically significant (Spearman rank correlation:  $r_s = 0.17$ ,  $N = 40$ ,  $P = 0.30$ ), and neither was the correlation between the weight of acorns and the abundance of beetles ( $r_s = 0.15$ ,  $N = 40$ ,  $P = 0.36$ ).

The species of *Quercus* did not affect the average number of beetles buried (Mann–Whitney  $U$  test:  $U = 177.0$ ,  $N_1 = N_2 = 20$ ,  $P = 0.53$ ), while the number of beetles was significantly higher at SA than TI (Mann–Whitney  $U$  test:  $U = 68.5$ ,  $N_1 = N_2 = 20$ ,  $P = 0.0004$ ). Nevertheless, the variables that better explained the abundance of beetles were the interaction between the species of *Quercus* and the weight of the acorns (43% of total deviance) and the

**Table 1.** Variables, interactions between them, deviance (D) and percentage of deviance that can be explained by the abundance of *Thorectes lusitanicus* considering all plots (A) and plots only with data from SA (B)

	A		B	
	D	%D	D	%D
<i>Quercus</i> species*acorn weight	146.56	43.1	76.52	45.6
Site*acorn weight	148.25	42.4	—	—
Site	157.38	38.9	—	—
Acorn abundance ( $f^2$ )	177.80	31.0	67.33	52.2
<i>Quercus</i> species*acorn abundance	189.72	26.3	101.27	28.0
Site*acorn abundance	205.18	20.3	—	—
Acorn weight	224.30	12.9	106.04	24.7
Site* <i>Quercus</i> species	243.71	5.4	—	—
<i>Quercus</i> species	247.92	3.8	128.43	8.7

%D = percentage of deviance;  $f^2$  = quadratic function.

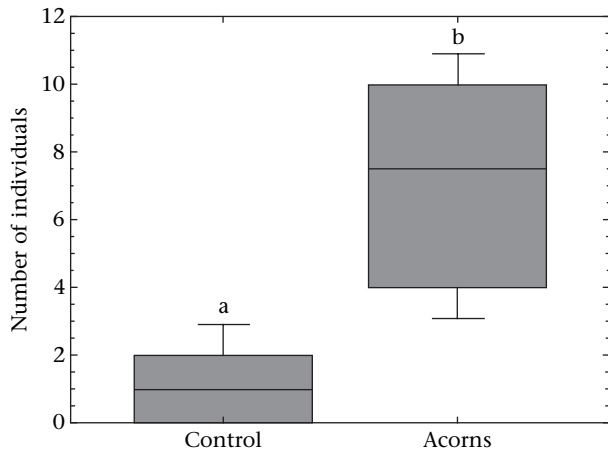
interaction between the site and the weight of acorns (42%; see Table 1). They were followed in order of importance by the site (39%) and the quadratic function of the abundance of acorns (31%). The two first interactions themselves explained 53% of the total variability in the number of beetles, whereas the four previously mentioned variables explained 77% of this variability. The resulting final model of the joint inclusion of all the significant variables, selected by means of the Akaike criterion (AIC = 114.3), allowed for the explanation of 84.6% of the variability in the number of beetles (deviance = 39.78).

Because of the scarcity of collected beetles in the TI forest site (only five of the 101 beetles) and the low number of acorns partially consumed in TI (only one of the 66 buried and consumed acorns), these analyses were repeated including only the data from SA. In this case, the number of acorns was the most influential variable, followed by the interaction between the weight of the acorns and the species of *Quercus* (Table 1). Both variables explained 65.4% of the variability in the number of beetles. The final model included five variables (AIC = 142.5) and explained 82.9% of the variation in the number of collected beetles (deviance = 24.10; Table 2). The number of beetles was curvilinearly related with the number of acorns, being greater when the number of acorns was intermediate. These maximum values of beetle density corresponded to those plots located under *Q. suber* trees with the higher total weight of acorns.

**Table 2.** Parameters of the final model to explain *Thorectes lusitanicus* abundance in SA

	Parameter	SE	Wald	P
Intercept	0.2896	0.3343	0.74	0.39
Acorn abundance	0.0291	0.0077	14.22	0.0002
Acorn abundance <sup>2</sup>	-0.0001	0.0001	6.94	0.008
<i>Quercus</i> species*acorn weight	0.0379	0.009	17.49	0.00003
<i>Quercus</i> species*acorn abundance	-0.0174	0.0045	15.15	0.0001
Acorn weight	-0.0547	0.0159	11.83	0.0006
<i>Quercus</i> species	0.6787	0.3012	5.08	0.02

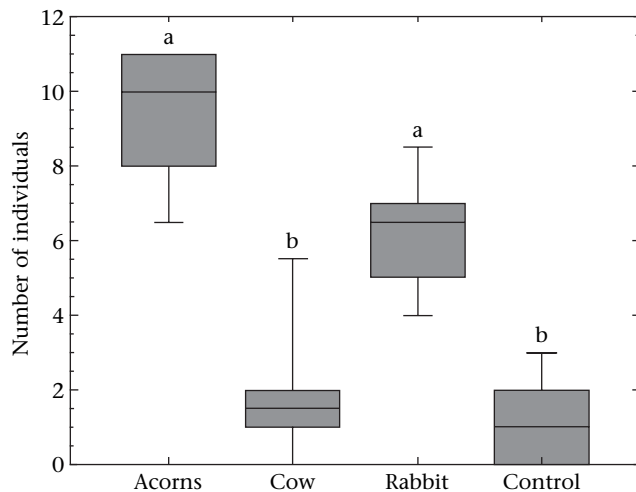
SE = standard error. The Wald statistic is a test of the significance of the regression coefficient, based on maximum likelihood estimates.



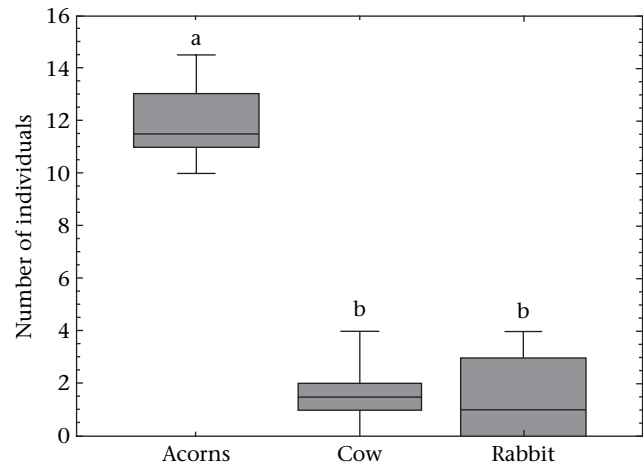
**Figure 2.** Effectiveness test of olfactometer: responses of *Thorectes lusitanicus* to acorns compared with control conditions. Horizontal lines within each box represent medians, boxes show quartiles and whiskers provide the extreme values. Treatments with the same letter did not differ from each other, for the sum of both sexes per bar ( $N = 6$ ; Kruskal–Wallis test with the Dwass–Steel–Chritchlow–Fligner post hoc test for pairwise comparisons,  $\alpha = 0.05$ ). Possible differences between sexes are explained in the text.

**Feeding Preferences under Laboratory Conditions**

In the preliminary experiment, 86% of *T. lusitanicus* were found to choose containers with acorns. Thus, the effectiveness of the olfactometer was statistically highly significant (Mann–Whitney  $U$  test:  $U = 35.50, N_1 = N_2 = 6, P = 0.005$ ; Fig. 2). There were no differences between males and females (Wilcoxon signed-rank test:  $T = -1.63, N = 10, P = 0.103$ ). Secondly, when three different odour sources

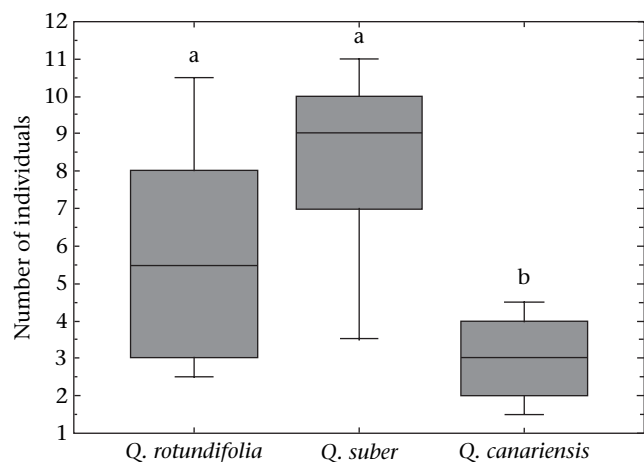


**Figure 3.** Olfactory responses of *Thorectes lusitanicus* to three different odour cues. Horizontal lines within each box represent medians, boxes show quartiles and whiskers provide the extreme values. Treatments with the same letter did not differ from each other, for the sum of both sexes per bar ( $N = 10$ ; Kruskal–Wallis test with the Dwass–Steel–Chritchlow–Fligner post hoc test for pairwise comparisons,  $\alpha = 0.05$ ). Possible differences between sexes are explained in the text.



**Figure 4.** Palatability test of *Thorectes lusitanicus* to three different food resources. Horizontal lines within each box represent medians, boxes show quartiles and whiskers provide the extreme values. Treatments with the same letter did not differ from each other, for the sum of both sexes per bar ( $N = 10$ ; Kruskal–Wallis test with the Dwass–Steel–Chritchlow–Fligner post hoc test for pairwise comparisons,  $\alpha = 0.05$ ). Possible differences between sexes are explained in the text.

(acorns, cow dung, and rabbit dung) were presented, *T. lusitanicus* showed a positive response to acorns which was significantly higher (Kruskal–Wallis test:  $H_3 = 28.83, N = 10, P = 0.001$ ) than to the other odour resources (Fig. 3). There were no differences between males and females in odour responsiveness (Wilcoxon signed-rank test:  $T = -0.25, N = 10, P = 0.801$ ). Preference for rabbit dung versus cow dung was observed (Dwass–Steel–Chritchlow–Fligner test:  $P = 0.009$ ).



**Figure 5.** Palatability test of *Thorectes lusitanicus* to three different acorn species. Horizontal lines within each box represent medians, boxes show quartiles and whiskers provide the extreme values. Treatments with the same letter did not differ from each other, for the sum of both sexes per bar ( $N = 10$ ; Kruskal–Wallis test with the Dwass–Steel–Chritchlow–Fligner post hoc test for pairwise comparisons,  $\alpha = 0.05$ ). Possible differences between sexes are explained in the text.

For the palatability test, *T. lusitanicus* were more likely to consume acorns than either type of dung (Kruskal–Wallis test:  $H_3 = 19.46$ ,  $P = 0.0001$ ; Fig. 4). There were no differences between the sexes (Wilcoxon signed-rank test:  $T = -0.093$ ,  $N = 10$ ,  $P = 0.926$ ).

When the three different species of acorns were presented, *T. lusitanicus* again showed a significant preference for *Q. suber* acorns than for *Q. canariensis* acorns (Kruskal–Wallis test:  $H_3 = 11.915$ ,  $P = 0.003$ ; Fig. 5), while there was no significant difference compared with *Q. rotundifolia* acorns (a *Quercus* species not present in the study area). As in all previous cases, there were no differences in food preference between sexes (Wilcoxon signed-rank test:  $T = -0.692$ ,  $N = 10$ ,  $P = 0.489$ ).

## DISCUSSION

Our results indicated that *T. lusitanicus* is highly attracted to acorns. In laboratory bioassays, this beetle showed significant preferences for acorns of one oak species (*Q. suber*) in comparison with the main type of food previously described for this beetle species (dung). Moreover, preference for rabbit dung versus cow dung corroborated the relation between *T. lusitanicus* and low water content dung, such as rabbit pellets; this behaviour has been observed in many *Thorectes* (*sensu lato*) species (Martín-Piera & López-Colón 2000; Verdú & Galante 2004). In the field study, the buried individuals were more abundant at the forest site characterized by higher cattle activity (SA), despite having similar acorn availability at both sites. Although *T. lusitanicus* showed a clear preference for acorns, it needs abundant dung to make nest masses and, consequently, to establish its populations. Thus, oak forests harbouring a high availability of dung for larvae and an abundance of acorns for adults will support the existence of well established *T. lusitanicus* populations and, in consequence, a higher proportion of acorns will be buried in the soil. This fact agrees with field observations: 65 out of the 66 acorns found partially consumed by *T. lusitanicus* were located in the forest with cattle (SA).

As it occurs in some acorn-feeding vertebrate species (Cantos et al. 2003) these beetles show significantly preferences between the acorns of different *Quercus* species. Acorn availability in the field was the variable that offered a higher explanatory capacity in the models, besides the interaction with *Quercus* species and with the forest site. Without taking TI data into account, the abundance of *T. lusitanicus* showed a curvilinear relation with acorn availability, with maximum values appearing for intermediate densities of acorns. These points corresponded with the plots located under cork oaks which bore a higher total weight of acorns. Curiously, in spite of the lower abundance of acorns below the canopy of cork oak trees, the number of *T. lusitanicus* was considerably greater in comparison with plots located under *Q. canariensis* trees. So, according to our field results, more than 86% of acorns, which were partial or totally consumed by beetles, were found under the canopy of *Q. suber*.

The results of field observations were clearly corroborated under laboratory conditions. Olfactory responses of *T. lusitanicus* to volatiles compounds of acorns indicated that the selection of this type of food is preceded by an active search for the acorn, where antennae chemoreceptors are involved. These results suggest that *T. lusitanicus* is capable of choosing the acorns even when their odour is mixed with the volatile compounds of the different kinds of dung available in the field. The process of selection by *T. lusitanicus* should be thus mediated by emission of volatile compounds by acorns, although other factors related to acorn characteristics (e.g. acorn maturity, size, *Quercus* species, shell hardness, tannins level) could be involved in this activity.

Acorns of some oak species (e.g. *Q. suber* and *Q. rotundifolia*) are characterized by high contents of fatty acids (around 10%; see Vázquez Pardo et al. 2000; Shimada 2001; Cantos et al. 2003; Ferreira-Dias et al. 2003; Lopes & Bernardo-Gil 2005), substances related to lipid metabolism in insects (Van der Horst et al. 1993; Canavoso et al. 2001; Stanley-Samuels et al. 2005). Most insects have a nutritional requirement of fatty acids, and many studies have shown that either linoleic or linolenic acids, two of the main polyunsaturated fatty acids extracted from acorns (Lopes & Bernardo-Gil 2005), adequately satisfy this dietary need (Canavoso et al. 2001). Some Orthoptera reveal fatty acid deficiency by retarded nymph growth and the emergence of deformed adults (Dadd 1985); in the case of Coleoptera, some species show a requirement for essential polyunsaturated fatty acids that avoids slow larval growth and low adult fecundity (Dadd 1985). Furthermore, a higher fatty acid level could be an advantage for insects to form properly during metamorphosis since up to 30–40% of the dry weights of mature eggs are lipids (e.g. Kawooya & Law 1988). Although a direct relationship between fatty acid accumulation and egg production has not been shown, some dung beetles as *Aphodius ater* DeGeer and *Copris diversus* Waterhouse show a reduction in the number of eggs when the body weight is lower (Tyndale-Biscoe 1984; Hirschberger 1999). Preliminary unpublished results seem to corroborate this fact, showing that *T. lusitanicus* specimens which fed only acorns have a higher weight of fat body, are more active at low temperatures and generate mature eggs of a higher weight. Thus, as occur in some vertebrates (Smallwood & Peters 1986; Bouderoua & Selselet-Attou 2003) the proportion of fatty acids in these beetles would be significantly higher when the diet includes oak acorns. In the case of *T. lusitanicus* we suspect that a diet based on polyunsaturated fatty acids should favour: (1) formation of winter energy reserves since the fat body is the major organ involved in metabolism and the major storage site of glycogen, an antifreeze fluid (Beenackers et al. 1985), and (2) optimal larval development and metamorphosis.

Considering the originality of the behaviour of these beetles and its possible relevance as secondary seed dispersers (Pérez-Ramos et al. 2007) more investigation is needed in two main research lines, one focused to determine the real influence of buried and not completely consumed acorns on Mediterranean oak forest regeneration, and another one directed to investigate the anatomical

and physiological capacities of the species able to facilitate this rare adaptation for a dung beetle species. This paper is a first intent in this second line but more research is necessary to know: (1) what volatile compounds are responsible for the preference of *T. lusitanicus* for cork oak acorns? (2) What acorn compounds are mainly assimilated by the beetles? (3) What are the ecological and metabolic benefits (adults and larvae; males and females) of a diet based on acorns than an exclusive diet of dung? (4) What consequences can be observed in body size and/or fecundity? Little is known about the behavioural and physiological features of this group of insects, and we suggest that future research in this area can represent a bridge to understand the ecophysiology and functional diversity of Mediterranean ecosystems.

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