

Incorporating insect infestation into rodent seed dispersal: better if the larva is still inside

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Abstract Many nutritious seeds are commonly attacked by insects which feed on the seed reserves. However, studies have not fully explored the ecological implications of insect infestation in animal seed dispersal and subsequent plant regeneration. Our question is whether the fact that an infested seed still contains the larva or not might increase/decrease the probability of being successfully dispersed by animals. This study examines the effects of weevil-infested seeds on the natural regeneration of a rodent-dispersed oak species. Rodents showed a high ability to discriminate between sound and infested seeds, even when the larva was still inside. As a result, rodents caused differential seed dispersal for sound and infested seeds by modifying multiple aspects of the dispersal process. We found that, for the same seed weight, infested acorns with a larva still inside can contribute to natural regeneration (0.7 % of seedlings in next summer), although in comparison to sound acorns they suffered higher predation rates by rodents (both partial and complete), were removed later from the ground (less preferred), cached less frequently, and dispersed to shorter distances, which reduced their potential to colonize new environments. However, infested seeds with exit holes are notably less preferred by rodents and, when dispersed, they are mostly deposited on the litter (uncached) with shorter dispersal distances and lower emergence success. Thus, the probability that larval-holed acorns will produce viable seedlings

is extremely low (null in this study). Whether infested seeds still contain a larva or not clearly determines the probability of being successfully dispersed. Premature seed drop prolongs the presence of the larva inside the acorn after seed drop, and could be a possible mechanism to allow dispersal of infested seeds.

Keywords Acorn dispersal · *Apodemus sylvaticus* · *Curculio* · Seed deposition · Seedling establishment

Introduction

Seeds are the products of sexual reproduction in most vascular plants, and are the principal means by which plants move across landscapes (Vander Wall et al. 2005). Fruits and seeds have evolved to facilitate dispersal, using biotic or abiotic agents. Thus, one common mode of dispersal is by animals, which move the seeds inadvertently or purposely. However, many other animals attack the seeds before dispersal and thus reduce plant reproduction efficiency (Vander Wall 2001). Larvae of numerous insect groups (e.g. weevils, moths, wasps, bruchid beetles) feed on the seeds and are considered the most prominent guild of pre-dispersal seed predators (Hulme and Benkman 2002). Insect infestation of seeds has been considered an important limitation for plant reproduction (Harper 1977; Andersen 1988; Crawley and Long 1995). However, it has been demonstrated that infested seeds can still germinate and even establish if the embryo remains undamaged (Steele et al. 1993; Branco et al. 2002; Xiao et al. 2007; Perea et al. 2011a).

Although some guilds of seed foragers only consume seeds (predators), some others also contribute to effective seed dispersal (Herrera 2002). Scatter-hoarding rodents are

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known to be one of the main guilds of seed dispersers (Xiao et al. 2005; Den Ouden et al. 2005; Steele et al. 2007; Briggs et al. 2009). Thus, hoarding behaviour increases the probability of seedling recruitment by storing seeds in favourable sites (Soné and Kohno 1996; Vander Wall 2001; Gómez et al. 2008; Perea et al. 2011b). However, rodents do not select all seeds equally; they show preferences for certain seeds, mainly due to nutritional properties, seed size and palatability (Steele et al. 1996; Pons and Pausas 2007; Wang and Chen 2008, 2009). It is not clear that insect infestation reduces the nutritional value of the seeds (Gálvez and Jansen 2007), because larvae synthesize proteins, fat or vitamins which may compensate for the nutritional loss (Semel and Andersen 1988; Valburg 1992). Moreover, infested seeds may either increase (Borowicz 1988) or decrease seed palatability (e.g. through the frass produced by the larvae; Muñoz and Bonal 2008). Consequently, insects may affect seed dispersal by making seeds less/more attractive to seed dispersers (Crawley and Long 1995; Steele et al. 1996; Gálvez and Jansen 2007; Muñoz and Bonal 2008). As a result, seed dispersers may exhibit different responses to infested seeds. Such responses commonly involve seed discrimination (see Gálvez and Jansen 2007 for a review) and/or changes in seed-hoarding behaviour. Those studies conclude that rodents tend to consume low-value seeds in situ and hoard proportionally more high-value seeds (Steele et al. 1996; Smallwood et al. 2001; Xiao et al. 2003; Cheng and Zhang 2011). However, multiple aspects of the dispersal process of infested seeds and their ecological implications for natural regeneration remain unknown. Unexplored aspects include microsite of seed deposition, partial consumption, recovery rates and re-caching behaviour, among others. These aspects will determine dispersal distances, eventual seed survival and germination, and subsequent seedling establishment, which are crucial for understanding the real contribution of infested seeds to plant reproduction.

Acorns are highly nutritious seeds that are commonly attacked by weevil larvae, which feed on the cotyledons (usually a single larva per acorn; Desouhant et al. 2000). Those infested seeds fall onto the ground with the larvae still developing inside until they exit a few days after the seed drop (average of 20 days for *Curculio elephas*; Bonal 2005). Weevil infestation can affect a high proportion of the acorn crop (Leiva and Fernández-Alés 2005; Xiao et al. 2007; Bonal et al. 2007) and thus plenty of infested seeds (with and without a larva inside) are available for scatter-hoarding rodents, which are considered one of the main guilds of acorn dispersers (Pulido and Díaz 2005; Steele et al. 2007; Perea et al. 2012). Experienced rodents can accurately discriminate infested seeds when the larva has emerged (Muñoz and Bonal 2008; Perea et al. 2012), but it is not yet clear if they can do so when the larva is still

inside (Semel and Andersen 1988; Steele et al. 1996; Dixon et al. 1997; Xiao et al. 2003; Cheng and Zhang 2011). Furthermore, the relationship among acorns, insects and rodents is probably not exclusively reduced to seed discrimination, and a more complex interaction might exist. Although it is suggested that pre-dispersal seed predation may influence the population dynamics of many plant species (Crawley 2000), studies have neglected to fully explore the ecological implications of insect infestation in the natural regeneration of rodent-dispersed plants.

Our question is whether the fact that a seed still contains the larva after the seed drop (premature seed drop) might increase the probability of being successfully dispersed as a possible mechanism to better tolerate insect damage. Our hypothesis is that infested acorns will have a similar dispersal process (dispersal quality and quantity) and ability to produce seedlings regardless of whether the larva has exited the seed or is still inside. We first wonder whether sound and infested seeds (both with and without larvae) show differences in germination ability and seedling performance in the laboratory to assess the potential contribution of infested seeds to oak regeneration. Then, we test whether sound and infested seeds (both with and without larvae) are treated differentially by acorn-dispersing rodents throughout the dispersal process, which will determine the eventual seed fate and the real probability of successful establishment. By considering the interaction between pre-dispersal acorn predators (weevil larvae) and the main guild of acorn dispersers (rodents) from seed production to seedling establishment, we expect to gain a better understanding of the real ecological implications of insect infestation for the natural regeneration of oaks.

Materials and methods

Study area

This study was conducted in a mixed oak stand of *Quercus pyrenaica* Willd. (271 stems ha⁻¹; basal area 18.4 m² ha⁻¹) and *Quercus petraea* (Matt.) Liebl. (109 stems ha⁻¹; basal area 3.8 m² ha⁻¹) along with scattered European beech *Fagus sylvatica* L. (83 stems ha⁻¹; basal area 0.8 m² ha⁻¹). The understorey was consisted mainly of a few shrub species (*Erica arborea* L., *Ilex aquifolium* L., *Genista florida* L., *Crataegus monogyna* Jacq. and *Rosa* sp.). The study area was located in the Ayllon mountain range in central Spain (3°30'W, 41°07'N, Madrid province), at 1,400 m a.s.l., under a submediterranean climate with 958 mm annual rainfall and a two-month dry summer. Weevil larvae (*Curculio* sp.) are the main predispersal seed predators of European oaks (Siscart et al. 1999; Leiva and Fernández-Alés 2005; Bonal et al. 2007). Female weevils

oviposit into the acorns and the larva feeds on the cotyledons to complete its development. In European oak forests, wood mice (*Apodemus sylvaticus* L.) are known to be one of the main acorn removers, and are also effective dispersers of acorns, playing an important role in the natural regeneration of oaks (Den Ouden et al. 2005; Pulido and Díaz 2005; Gómez et al. 2008; Perea et al. 2012).

Acorn crop

To estimate the crop size, we used 1 m² seed traps in two sampling plots. Plot 1 had 48 traps (6 × 8 rectangular grid) and plot 2 had 61 traps (12 × 5 rectangular grid plus an extra trap). Seed traps were located 7 m from each other inside the grid. Traps were covered with a metallic mesh (hexagonal openings with a 2 cm radius) to avoid seed predation by small mammals and birds. Seeds were collected from the traps and counted every month from September to December for four consecutive years (2007–2010). Two acorn categories were distinguished: sound and infested.

Acorn collection and classification

All experiments were carried out with *Quercus pyrenaica* acorns. Seeds were collected in the study area or in nearby locations during September and October of 2009 and 2010. We established six types of acorns as a result of the combination of two acorn sizes (large: 5.5–7.5 g and small: 2.0–4.0 g) and three categories [sound acorns, infested acorns with an emergence hole of weevil-larvae (*Curculio* sp., Col.: Curculionidae), and infested acorns with a larva still developing inside]. Infested seeds with a larva still inside were distinguished by the puncture of the seed coat, as made by the female in order to oviposit, and the lack of emergence holes (see Bonal et al. 2007). In addition, we used 100 acorns classified as infested with a larva inside and opened them to ensure that they were indeed infested (92 % reliability).

Potential ability of infested seeds to produce seedlings

We assigned a total number of 180 *Quercus pyrenaica* acorns to individual containers of length 300 mm and diameter 50 mm. Acorns were divided into the six abovementioned categories (two acorn sizes and three infestation types), with 30 acorns per category. Acorns were sown in October 2009 into a substrate consisting of 3:1 peat:perlite. Acorns were partially covered in substrate (1–2 cm) and kept under the same environmental conditions in a chamber: 60 % humidity, 14 h of light per day at 24 °C, and 10 h in darkness at 19 °C. Containers were watered and checked three times a week for 18 weeks for

the appearance of photosynthetic tissue above the substrate level, and taken out from the chamber once the stem was longer than 1 cm. Thus, successful emergence was considered when seedlings germinated and produced 1 cm of green stem above the substrate level. At this stage, seedlings are able to produce adequate photosynthate to survive by producing their own supply. Thus, we considered that seeds were potentially able to produce seedlings when seedling emergence occurred. Since root development is critical for the successful establishment of the seedling (Grossnickle 2005), we measured the development of the roots at the seedling emergence stage. Thus, we removed the root from each seedling and measured the root length and root diameter at the root collar. We then washed all roots, dried them in an oven at 80 °C for 48 h, and weighed them. After 18 weeks, we emptied all the remaining containers and looked for acorns that germinated but did not show photosynthetic tissue.

Acorn dispersal by rodents and final fate

Four sites were chosen within the study area. Distances between sites were at least 200 m to avoid overlapping sites for rodents and ensure statistical independence (home range of *A. sylvaticus* is around 0.10 ha in nonbreeding seasons and deciduous woodlands; Corp et al. 1997). Since shrub cover plays an important role in rodent activity (Manson and Stiles 1998; Perea et al. 2011b), we considered two microhabitats for offering acorns: under oak trees with no shrub cover within a 5 m radius (only litter), and under shrub cover beneath oak trees. Each site contained two acorn supply stations, one for each microhabitat. Stations within each site were located 7–10 m from each other and built with a wire mesh (0.5 m × 0.5 m) that was placed approximately 3 cm above the ground surface to exclude removers other than small mammals. Five groups (one every 15 days from the first of October to the first of December 2010) of 42 tagged acorns were placed on the ground in each supply station. Each group of 42 acorns contained 7 acorns of the six established types (2 sizes and 3 acorn categories). In total, 1,680 acorns were offered to the rodents (4 sites × 2 microhabitats × 5 groups × 42 acorns). For acorn tagging, a wire (3 cm long and 0.6 mm wide) was attached to the acorns by drilling a hole with a needle in the approximate centre of the side of the acorn and as close as possible to the pericarp to use as little wire as possible and to minimise animal disturbance. Then a 14.5 × 1.2 cm yellow plastic tag was attached to the wire and numbered with waterproof permanent ink. We used this wire tagging method because Xiao et al. (2006) found that it does not significantly alter acorn dispersal patterns and yields a high recovery rate. Wire plus tag constituted 7.1–12.3 % of the acorn weight. We monitored seed

removal (noted the acorns that were still present in the supply stations) and searched for dispersed acorns daily during the first three days after seed offer, and then on days 7 and 15 of each trial. Tag number, distance from the supply station, burial depth, microhabitat (shrub vs. open), distance to the closest shrub, and survival category (intact, completely consumed or partially eaten) were recorded for each relocated acorn. For the partially eaten acorns we recorded the region damaged (pericarp, basal, lateral or apical portion), the approximate percentage of cotyledon lost (visual estimate, using the wire position in the centre of the acorn as a reference for 50 % consumption) and the embryo status (damaged or not). Dispersed acorns were marked with wooden sticks (50 cm × 0.5 cm) to allow easy relocation. Marked sticks have no significant effect on seed removal (Xiao et al. 2005). We also covered the yellow plastic tags with litter to avoid attracting foragers. On every day of fieldwork, we monitored previously found caches (including all previous trials), noted whether acorns were still present, and searched for seeds that were dispersed again. Thus, we assessed the repeated movement of seeds throughout the experiment (October–December), since rodents often re-disperse the acorns (Perea et al. 2011b). During the following spring, in June 2011, we revisited all marked dispersed seeds to check whether they had turned into seedlings. The aerial part of the encountered seedlings was harvested and oven dried at 80 °C for 48 h to obtain the shoot dried biomass.

Data analysis

To analyse whether successful seedling emergence in the laboratory is affected by infestation type and acorn size, we used a generalized linear model with a binary response variable (seedling emerged or not). Predictors were acorn size (large and small) and infestation type (sound, infested with the larva still inside, and infested with exit hole). We also used two-way ANOVA to analyze seedling emergence characteristics. Response variables were root length, dry root biomass and time to produce green tissue, and factors were acorn category and acorn size. Tukey HSD post hoc tests were performed for multiple comparisons. Finally, to analyze whether root biomass, root length and time to establish were explained by acorn weight, we used linear regressions.

We used a generalized linear mixed model (GLMM) to analyse acorn selection. We took a binary response variable (seed selected or not) for the first day that rodents encountered the supply stations (when all acorns were available). Seeds were considered not to have been selected when they remained intact and to have been selected when they were dispersed or eaten in situ. We included acorn category (sound, infested with an exit hole, and infested

with a larva inside), acorn weight and microhabitat of origin as fixed effects. Group of seeds (acorns placed together on the same day and in the same supply station) was considered a random effect. Each group was assigned to a specific microhabitat within a site and within a trial (date). We used another GLMM to analyse acorn survival (embryo damage). We took a binary response variable (whether the embryo was damaged or not). Fixed effects were acorn weight and acorn category, and we used the same structure of random effects as above. Acorn weight was always the sum of acorn, wire and tag weights.

Linear mixed models were used to analyse the effects on dispersal distance. We first checked whether dispersal distance was correlated with number of dispersal movements. We fitted the response variable (distance to supply station) to a normal distribution by a log transformation. We used the same structure of random effects as in the model above, but we also included number of dispersal movements to avoid re-dispersal as a confounding factor. Fixed effects were acorn category and acorn weight. Deposition conditions (buried vs. not buried and within the litter vs. on the litter) were analysed with GLMM in relation to acorn category and weight. To analyse the effects on burial depth, we removed the values equal to zero in the burial depth response variable. We built the same model as the distance model (log-transformed). All models included the interactions between the main effects and were built using the R 2.11.1 software package.

Results

Annual crop size throughout the years varied from 0.14 to 11.8 acorns m⁻² year⁻¹. Prevalence of weevil infestation varied greatly across years (18–82 % of the crop size).

Potential ability of infested seeds to produce seedlings

Just over half of the acorns (55 %; $N = 99$) produced photosynthetic tissue. Larger acorns showed higher percentages of successful seedling emergence (Table 1), which produced significant differences between sizes ($Z = 4.948$; $P < 0.001$). Sound acorns had significantly higher emergence percentages than infested holed acorns ($Z = -3.995$; $P < 0.001$; Table 1) but no significant differences were found between sound and infested holed acorns with a larva inside ($Z = -1.842$; $P = 0.066$). We also obtained significant differences in successful seedling emergence between both types of infested acorns ($Z = -2.301$; $P = 0.021$). Time to produce photosynthetic tissue (time to establish) was not correlated with acorn weight ($t = 0.83$; $P = 0.413$; adjusted $R^2 = 0.008$). Multiple comparisons revealed that larval-holed acorns required

Table 1 Seedling emergence (%) and time to produce photosynthetic tissue (mean \pm CI days) for the three acorn categories

	Small acorns (2.00–4.00 g)			Large acorns (5.50–7.50 g)		
	%	<i>N</i>	Time to emerge (days)	%	<i>N</i>	Time to emerge (days)
Sound	50	15	61 \pm 13	93	28	53 \pm 4
Infested with a larva inside	43	13	57 \pm 11	70	21	48 \pm 8
Infested with an exit hole	17	5	34 \pm 7	57	17	37 \pm 5

significantly less time to establish than both sound ($P = 0.041$) acorns and those infested with a larva inside ($P = 0.047$; Table 1).

All seedlings produced similar root lengths, and no differences were observed in infestation category or acorn size (Fig. 1a). Production of root dry biomass was not affected by infestation category either, but larger acorns produced significantly more root biomass (Fig. 1b; $F_{1,95} = 10.10$; $P = 0.003$). Root biomass was positively related to acorn weight in linear regressions [root biomass (g) = $-0.00579 + 0.01362 \times$ (acorn weight in g); $t = 3.49$; $P = 0.001$; $R^2 = 0.25$]. However, root length was not correlated with acorn weight ($t = 0.75$; $P = 0.460$; $R^2 = 0.013$).

Initial acorn fate

Only 7.6 % ($N = 128$) of the acorns offered remained intact in the supply stations. Most of the intact acorns were infested with exit holes, and no sound acorns remained intact (Fig. 2). The proportion of partially consumed acorns in situ was higher for infested seeds with exit holes than for sound and infested acorns with a larva inside (Fig. 2). The initial fates of sound and infested seeds with a larva inside were similar (Fig. 2), though the infested seeds with a larva inside remained longer in the supply stations because sound acorns were preferred and thus removed first. At the end of autumn 2010 (December 15th), there were still 166 dispersed and surviving acorns (23.1 % of the dispersed and relocated acorns), and 51.9 % of them ($N = 86$) were sound acorns (Fig. 2). We found significant differences in acorn survival rates among the three acorn categories at the end of autumn ($\chi^2_2 = 10.86$; $P = 0.004$).

Acorn selection

Rodents selected 556 acorns (33.1 % of the original amount) on the first day that supply stations were encountered. Sound acorns were preferred ($N = 302$; 54.3 % of the selected seeds) over those infested with exit holes ($N = 64$; 11.5 %) and those infested with a larva inside ($N = 190$; 34.2 %), leading to significant differences among the three categories ($P < 0.001$ in all cases).

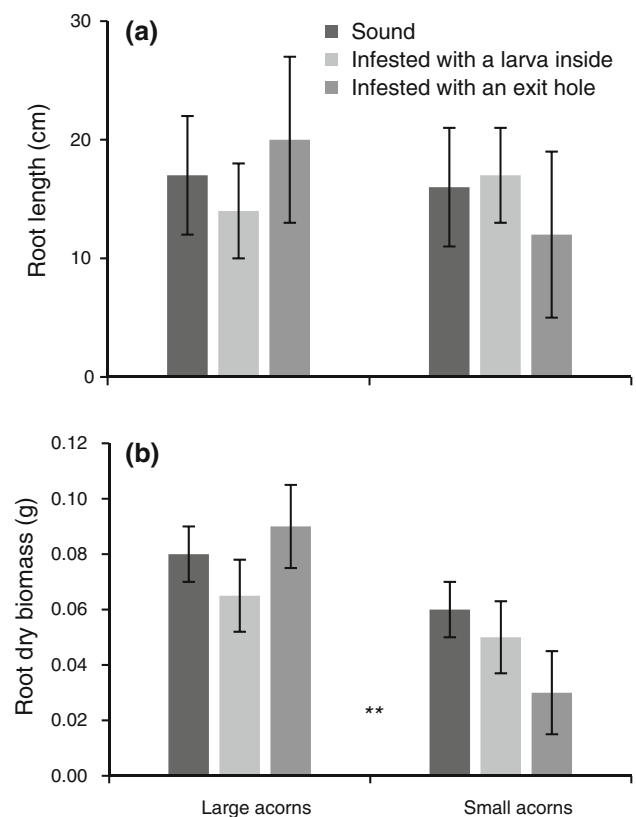


Fig. 1 Variations (mean \pm SE) in **a** root length and **b** root dry biomass for large and small acorns in relation to category of infestation. Symbols located between acorn sizes indicate difference between small and large acorns, $**P < 0.01$

Microhabitat of origin did not have a significant effect on the seed selection ($Z = -0.410$; $P = 0.682$). Larger acorns were significantly preferred only for infested acorns with an exit hole ($Z = 4.951$; $P < 0.001$). No significant seed size effect was found for sound ($Z = 1.634$; $P = 0.102$) or for infested acorns with a larva inside ($Z = 1.581$; $P = 0.114$).

Partial consumption

Among the relocated sound acorns, 12.3 % ($N = 51$) had been partially consumed. A significantly higher proportion

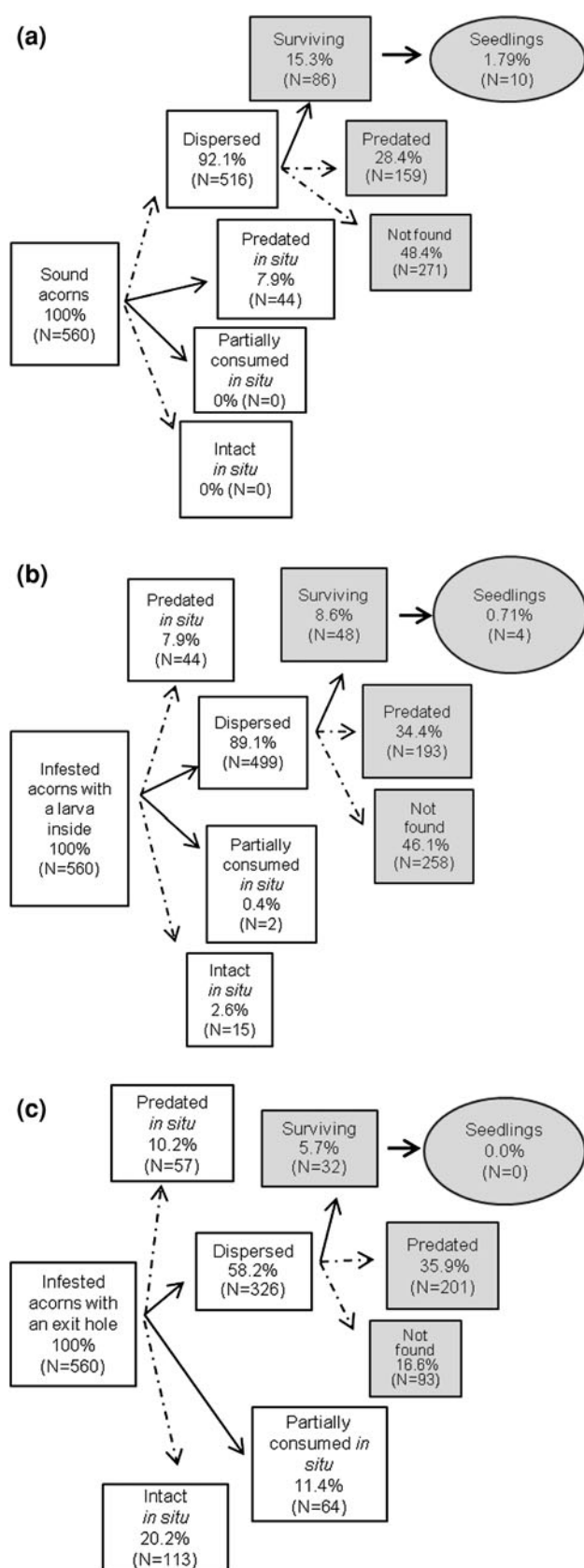


Fig. 2 Diagrams representing initial seed fate (white squares), medium-term fate (grey squares), and final fate (grey circles) for the three acorn categories: **a** sound, **b** infested with a larva inside, and **c** infested acorns with an exit hole. *Intact in situ* refers to acorns that were never moved from the supply stations. *Initial fate* refers to the first change from the original acorn status (under the mesh), and *medium-term fate* refers to the last visit (end of autumn). *Final fate* represents the acorns that turned into seedlings the following summer

of partially consumed acorns was found for both types of infested acorns in comparison to the sound acorns ($P < 0.001$ in both cases). No significant differences were found in the proportion of partial consumed acorns between both types of infested acorns ($Z = -1.461$; $P = 0.144$). Cotyledon loss caused by rodents was lower for infested acorns with an exit hole (mean \pm SD = 29 ± 26 %) than for sound acorns (42 ± 20 %) and infested acorns with the larva inside (38 ± 24 %), but only marginally significant differences were found between sound and infested acorns with an exit hole ($t = -1.633$; $P = 0.097$). Sound and infested acorns with a larva inside were mostly consumed in the basal and lateral parts, whereas acorns that were infested with exit holes were more frequently consumed in the apical part, causing significant differences in the proportion of embryo damage ($P < 0.013$ in both cases). Acorn weight also affected the proportion of acorns with embryo damage ($\chi^2 = 11.166$; $P < 0.001$). Thus, small acorns accounted for 72.8 % ($N = 59$) of all acorns with embryo damage in comparison to large acorns (27.2 %; $N = 22$). We found a significant interaction between acorn weight and category ($\chi^2 = 12.108$; $P = 0.007$), due to the higher proportion of embryo damage in large larval-holed acorns compared with large acorns in the two other categories.

Dispersal movements and final dispersal distances

Infested acorns with exit holes showed lower numbers of repeated dispersal movements (re-dispersal) in comparison to both sound acorns ($t = -8.319$; $P < 0.001$) and those infested with a larva inside ($t = 4.345$; $P < 0.001$; Fig. 3). Sound acorns also showed higher numbers of dispersal movements than infested acorns with a larva inside ($t = -2.517$; $P = 0.012$). Number of dispersal movements was positively correlated with dispersal distance from the supply station ($t = 19.321$; $P < 0.001$; $R^2 = 0.59$). For each single dispersal movement (correcting by the number of dispersal movements), sound acorns were dispersed significantly farther than those infested with a larva inside ($t = -2.796$; $P = 0.005$) and with an exit hole ($t = -5.263$; $P < 0.001$; Fig. 3). In addition, infested acorns with a larva inside were found significantly farther from the supply station than infested acorns with exit holes ($t = -3.175$; $P = 0.002$; Fig. 3). Finally, larger acorns were dispersed significantly farther from the supply station than

both infested seeds with an exit hole ($t = 2.675$; $P = 0.008$) and acorns with a larva inside ($t = 8.597$; $P < 0.001$), but no significant difference were found among the sizes of the sound acorns ($t = -1.617$; $P = 0.107$).

Deposition conditions

Infested acorns with exit holes were found under shrub cover more frequently than both sound acorns ($Z = 3.965$; $P < 0.001$) and infested acorns with a larva inside ($Z = -3.124$; $P = 0.002$; Fig. 4a). No differences in microhabitat of destination were found between sound and infested acorns with a larva inside ($Z = 1.679$; $P = 0.093$). Acorn weight also had a significant effect on the microhabitat of destination ($Z = -2.767$; $P = 0.006$), with larger acorns being more frequently dispersed to open areas, except for infested acorns with an exit hole, which were mainly dispersed to sites with shrub cover destination (Fig. 4a). Sound acorns were found buried more frequently than both types of infested acorns (Fig. 4b), but significant differences in burial were only observed between sound and infested acorns with exit holes ($Z = -3.584$; $P < 0.001$), not between sound and infested acorns with a larva inside ($Z = -1.272$; $P = 0.203$). Acorn weight also had a significant effect on the proportion of caches ($Z = 0.062$; $P < 0.001$); larger acorns of all three categories were more frequently buried (Fig. 4b). Finally, no significant differences in burial depth were found between the three acorn categories ($P > 0.264$). Instead of being buried, infested acorns with exit holes were mostly found on the litter rather than within the litter; in this respect, they showed significant differences from both sound ($Z = -7.157$; $P < 0.001$) and infested acorns with larvae inside ($Z = 2.827$; $P = 0.004$). Moreover, a higher proportion of sound acorns were found within the litter than infested acorns with a larva inside were ($Z = -5.093$; $P < 0.001$; Fig. 4b). Small acorns were deposited significantly more frequently on the litter than large acorns ($Z = 4.032$; $P < 0.001$) for all acorn categories (Fig. 4b).

Acorn final fate

In June 2011, we found 14 seedlings from the marked acorns (8.4 % of the acorns surviving from last December). The rest of the marked acorns were either moved again and not found ($N = 119$; 71.7 %) or were predated ($N = 33$; 19.9 %). No seedlings came from acorns with exit holes. Four seedlings emerged from infested acorns that contained a larva inside (8.3 % of the surviving acorns in December) and ten from sound acorns (12.1 % of the surviving acorns in December). We found significant

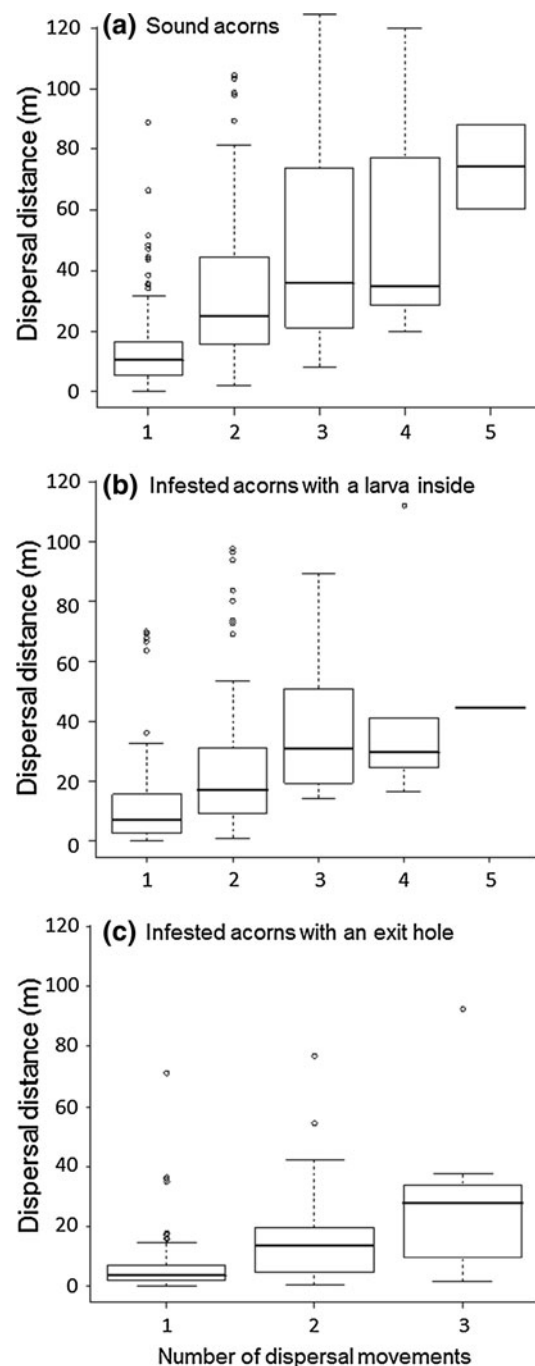


Fig. 3 Dispersal distances in relation to the number of dispersal movements (number of times that seeds were re-dispersed) for **a** sound acorns, **b** infested acorns with a larva inside, and **c** infested acorns with an exit hole. Each box shows the median (band in the middle of the box) and the first and third quartiles (edges). Whiskers represent the lowest and highest values within the 1.5 interquartile range of the lower and upper quartiles

differences among the three acorn categories in the number of acorns that turned into seedlings ($\chi^2_2 = 10.86$; $P = 0.004$), but no differences were found between sound acorns and those infested with a larva inside ($\chi^2_1 = 2.57$;

$P = 0.108$). Seedling dry biomass (aerial part) was higher for sound (mean \pm SE = 0.39 ± 0.06 g) than for infested acorns containing a larva (mean \pm SE = 0.26 ± 0.04 g), with marginally significant differences ($t = 1.97$; $P = 0.074$).

Discussion

Our findings highlight that infestation of acorns by insects influences their dispersal in complex ways, revealing important ecological implications for oak regeneration. This study also underlines the importance of considering insect phenology (larval emergence from seeds) in disperser behaviour, which has been found to modify the whole seed dispersal process. Essentially, this study reveals that although infested seeds that still had a larva inside were less preferred by rodents than sound seeds, there were no great differences between them in many aspects of the dispersal process (from initial seed fate to seedling establishment), so they were able to generate seedlings. However, the proportion of infested acorns with a larva inside that finally turned into seedlings (0.7 %) was less than half of the corresponding proportion emerging from sound acorns (1.8 %). Further studies with a higher number of naturally established seedlings are needed to check whether this apparently lower contribution of infested acorns (with larva) to oak regeneration is actually significant. Nevertheless, the shorter dispersal distances found for infested acorns in comparison to sound acorns reduce their potential to colonize new environments (Bullock et al. 2002), and increase density-dependent mortality (Vander Wall 2001). Despite this, infested acorns with a larva still inside offer a higher probability of being successfully dispersed than infested acorns in which the larva has already exited, due to differential acorn treatment by rodents. Thus, larval-holed acorns showed a lower quantity and quality of dispersal (lower removal rates, higher proportion of embryos damaged, shorter dispersal distances and lower emergence success). Consequently, the probability that infested acorns with exit holes will produce viable seedlings away from the mother tree (effective dispersal) is very low (null in the studied year). Hence, the longer that the larva develops inside the seed (once the acorn has fallen onto the ground), the higher the probability of being successfully dispersed by rodents. This, together with the fact that rodents prey upon the larvae when encountering them (Steele et al. 1996; R. Perea, personal observation) leads us to support that premature seed drop could be a mechanism that both saves plant resources, reduces larval reproduction efficiency and allows a certain degree of successful dispersal away from the mother tree.

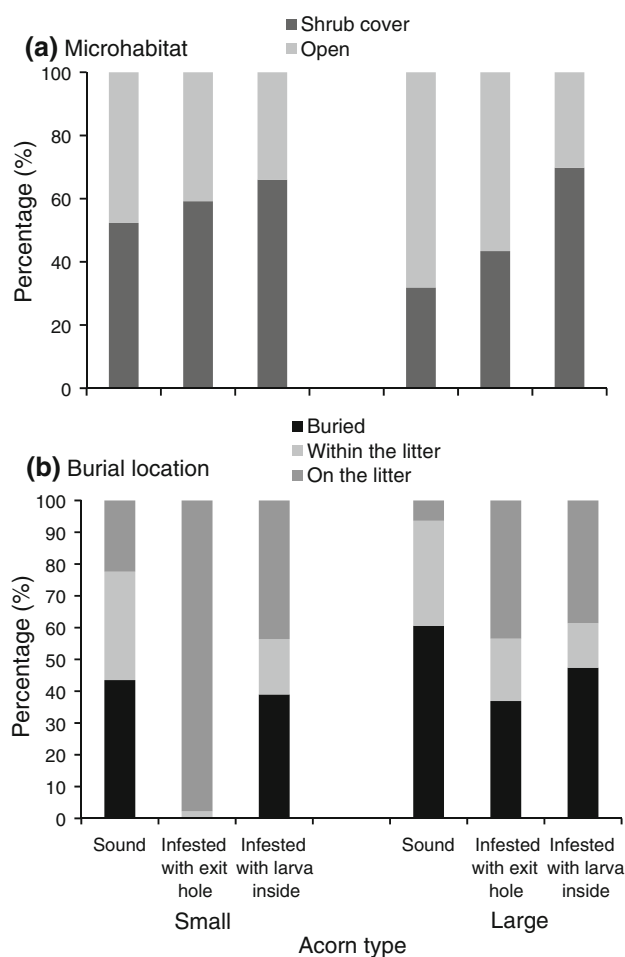


Fig. 4 Deposition conditions for the six categories of acorns: **a** microhabitat (shrub cover or open area) and **b** burial location (buried, within the litter or on the litter)

The importance of insect infestation

We confirmed that the proportion of insect infestation was not negligible and was in fact rather variable, in line with other studies (Crawley and Long 1995; Branco et al. 2002; Leiva and Fernández-Alés 2005). The low acorn crop size in the study area throughout the years studied (range $0.14\text{--}11.8$ seeds m^{-2} year $^{-1}$) could explain the high level of infestation, since lower acorn crops suffer higher infestation rates (Crawley and Long 1995).

Acorn selection

Our results support that rodents are able to discriminate between sound and infested acorns with a larva inside, in agreement with other studies (Steele et al. 1996; Muñoz and Bonal 2008). However, some other studies (Semel and Andersen 1988; Cheng and Zhang 2011) have found that there is no difference in the removal rates of these two acorn categories. Besides, infested seeds with an exit hole

were the least preferred because they contain proportionally less cotyledon mass and no larva, which is a high-protein bonus for rodents (Steele et al. 1996; Silvius 2002). Surprisingly, acorn weight was only a determinant in infested seeds with exit holes; not in sound or infested acorns with larvae inside. The ratio of acorn size to acorn weight (which is much higher for infested seeds with exit holes) could be an important cue that is used to identify and then reject the less profitable acorns, as has been suggested by Muñoz and Bonal (2008). Thus, larger infested seeds with exit holes could be selected because the larger the larval-holed acorn the lower the size/weight ratio, due to the fact that the larva consumes proportionally less cotyledon mass in larger acorns (Bonal and Muñoz 2008; Yi and Yang 2010).

Infested seeds are more frequently partially consumed but present lower cotyledon loss

Both types of infested seeds showed higher probabilities of being partially consumed than sound seeds. Rodents prefer to cache and not to damage high-value seeds, and they consume those seeds that are less valuable (Steele et al. 1996; Xiao et al. 2005; Cheng and Zhang 2011). Our results agree with such previous investigations but incorporate new findings. First, larval-holed acorns are mostly partially consumed in situ, whereas infested acorns with larvae inside are first dispersed and then partially consumed. Infested seeds containing larvae offer plenty of energy for rodents (Silvius 2002 and references therein), so it is worth transporting them to safe sites in terms of energy loss/gain for rodents. In addition, we found that rodents consumed proportionally more cotyledon in sound seeds, probably due to the fact that infested seeds have a lower palatability and energy content since the cotyledons are partially occupied by the frass of the larvae.

Higher survival of acorns with larvae still inside than for acorns with emerged larvae

We found that sound seeds suffered no embryo damage because rodents consume the basal part first, whereas larval-holed acorns were damaged in the apical part where the embryo is located. This is because the larva starts to feed at the basal part (where the adult usually oviposits) towards the apical end (Yi and Yang 2010), so rodents eat the parts that are not affected by the larva. Higher tannin concentration around the embryo (Steele et al. 1993) may be the most plausible explanation for the low embryo damage in sound acorns. Consequently, sound acorns remain viable because the embryo is not damaged and contains enough resources to produce seedlings (Perea et al. 2011a), whereas larval-holed acorns become nonviable (embryo

death) due to the double damage caused by both larvae and rodents. Infested seeds with a larva inside suffered similar embryo damage to sound acorns and thus have a higher probability of successfully establishing than larval-holed acorns. Rodents could benefit infested acorns with a larva inside by stopping larval development and caching these surviving acorns (with no embryo damage) at suitable sites for establishment.

Infested seeds have shorter dispersal distances

Sound seeds showed a higher number of dispersal movements, probably because they are more valuable items for rodents that, therefore, manage these seeds more intensely (Vander Wall and Joyner 1998). This study indicates that re-dispersal occurs in not only sound seeds but also infested seeds. More dispersal movements led to longer dispersal distances, in agreement with other studies (Vander Wall and Joyner 1998; Perea et al. 2011b). Steele et al. (1996) found that infested acorns with a larva inside have shorter dispersal distances. In this study, we demonstrated that larval-holed acorns have even shorter dispersal distances and a lower number of dispersal movements. We conclude that seeds with lower nutritional value lead to fewer dispersal events and shorter dispersal distances.

Larval-holed seeds have low quality of seed deposition

Infested acorns with exit holes were mostly found under shrub cover, where predation rates are higher due to higher rodent activity (Perea et al. 2011b). Infested acorns with exit holes were also deposited on the ground and buried less frequently. This location increases the negative effects of being exposed to predators or desiccation, which may be fatal for recalcitrant seeds like acorns (Perea et al. 2012). However, infested acorns with larvae still inside showed similar deposition conditions to sound acorns. This study complements those which have concluded that rodents consume low-value seeds in situ and hoard proportionally more high-value seeds by adding the observation that higher seed quality leads to better seed deposition conditions for acorn survival and establishment (microhabitat of destination, cache frequency and litter cover).

Fewer infested seeds with exit holes establish, but they do so sooner

Sound acorns exhibited the highest percentage of successful germination, in agreement with other studies (Branco et al. 2002; Leiva and Fernández-Alés 2005). Branco et al. (2002) found that germination percentage depends on the damage intensity. Thus, infested acorns with exit holes suffered the most intense damage and

presented a significantly lower germination percentage and seedling survival rate. Interestingly, more rapid seedling emergence was found for acorns with exit holes. This faster development is probably due to the partial removal of the pericarp (exit hole), which facilitates water imbibition (Vallejo-Marín et al. 2006). Thus, no differences in the time for seedling emergence were found between sound and infested acorns with a larva inside, because the pericarp remained intact. These findings are consistent with other studies that show how partially eaten seeds with removed pericarp germinate and establish earlier (Steele et al. 1993; Perea et al. 2011a). In addition, time for seedling emergence was not correlated with acorn weight, so large and small acorns showed similar emergence times, in agreement with Perea et al. (2011a).

Root length is not affected by either insect infestation or acorn size

There were no differences in root length among both types of infested seeds and sound seeds for both large and small acorns. Only root dry biomass was affected by acorn size, so acorn weight and root biomass were strongly correlated. These results are in line with other studies which indicate that cotyledon loss leads to decreased root biomass (Perea et al. 2011a). Thus, seedlings from infested acorns would have lower root biomass and thus less aerial biomass, because some of the cotyledon had been consumed. Whether the infested acorn still contained a larva or not had no effect on its root development.

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