



## Impact of vertebrate acorn- and seedling-predators on a Mediterranean *Quercus pyrenaica* forest

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### Abstract

We have experimentally investigated the impact of biotic factors, acting at the seed and seedling stages, on a *Quercus pyrenaica* forest in the Sierra Nevada mountains (SE Spain). We monitored the natural establishment of the oak for 3 years in two forest plots and two shrubland plots, by counting seedlings and juveniles. In addition, we established several experiments in these plots to examine acorn and seedling survival, while also considering the microhabitat effect on survival probability. Dispersed acorns were quickly consumed by several species of predators, particularly wild boar (*Sus scrofa*) and woodmouse (*Apodemus sylvaticus*). Less than 4% of the experimental acorns survived to produce seedlings, even when they were buried 4 cm in soil, simulating caches. No effect of microhabitat was found on predation, and thus no safe site appears to exist for *Q. pyrenaica* acorns in the study area. Some 98% of the 1000 experimental seedlings were killed by herbivores, notably woodmice, wild boar, and domestic and wild ungulates. Seedling survival varied spatially, being significantly higher under shrubs (4%) than in any other microhabitat (less than 0.5%). Both acorn and seedling survival were much lower in the shrublands than in the forests. In shrubland plots, the main agent of seedling mortality was trampling by domestic ungulates foraging in herds. This study suggests that the regeneration of *Q. pyrenaica* forests in Mediterranean mountains can be limited by herbivores acting at several life-history stages.

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### 1. Introduction

Recruitment is a long and complex process in woody plant species, involving several sequentially connected life-history stages (Herrera et al., 1994). The collapse of any stage results in the limitation of

the overall recruitment process (Herrera et al., 1994; Houle, 1995; Jordano and Herrera, 1995; Schupp, 1995; Schupp and Fuentes, 1995). In plants, regeneration can be limited by the amount of seeds produced, by the effectiveness of dispersers, by the availability of microsites for seed germination and seedling establishment and by the activity of herbivores feeding on seeds, seedlings and juveniles (Schupp, 1995; Schupp and Fuentes, 1995; Hulme, 1996). In addition, the effect of all these factors is in many cases non-exclusive, and

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evidence is accumulating that recruitment of many long-lived, woody perennials is limited by the synergistic action of several species of herbivores inducing propagule mortality at consecutive life stages (Crawley and Long, 1995; Hulme, 1996). Furthermore, herbivores can also hinder recruitment of plant species indirectly by specifically affecting the few seeds dispersed to the high-quality microsites (Callaway, 1992; Crawley and Long, 1995; Rousset and Lepart, 2000). When the activity of herbivores varies between microhabitats, the spatial distribution of seeds and seedlings can alter their own probability of survival and passage to the next demographic stage (Rey and Alcántara, 2000). Therefore, it is necessary to consider the spatial pattern of recruitment in order to gather accurate information about the biotic and abiotic factors limiting plant regeneration (Jordano and Herrera, 1995; Schupp and Fuentes, 1995; Rey and Alcántara, 2000).

In this paper, we experimentally investigate the incidence of acorn and seedling predators of the oak *Quercus pyrenaica* Willd. (Fagaceae) in the Sierra Nevada mountains (SE Spain). This resprouter oak is deciduous, monoecious and wind pollinated. Like most oaks, it does not form a persistent seed bank in the soil. Acorns ripen during autumn (late October–November in the study area) and are dispersed both abiotically by gravity and biotically by European jay (*Garrulus glandarius*) and woodmouse (*Apodemus sylvaticus*). Abiotic dispersal usually occurs during strong winds and results in most acorns being dispersed directly under the oak canopies. *Q. pyrenaica* is distributed from SW France to N Morocco, and many woodlands are threatened in the Iberian Peninsula due to the combined effects of several management practices. In the Sierra Nevada high mountains, this oak species is found from 1600 to 1800 m a.s.l., invariably inhabiting siliceous, schist soils. In these mountains, the oak forests form the treeline, bounded above by a homogeneous subalpine shrubland spreading up to 2500 m a.s.l. Our specific goals are to: (1) describe the patterns of natural regeneration of *Q. pyrenaica*; (2) experimentally estimate the survival probability of acorns and seedlings; (3) identify the mortality factors acting on the two life-cycle stages; and (4) experimentally test whether there are between microhabitat differences in the effect of the mortality agents.

## 2. Material and methods

### 2.1. Study area

The present study was conducted from 1997 to 2000 in a *Q. pyrenaica* forest (37°05'N, 3°26'W; Parque Nacional de Sierra Nevada, Granada Province, Spain). This forest is formed by dense clumps of oak trees and scattered man-made gaps which are recolonized by four species of shrubs: *Genista versicolor* (Fabaceae), *Cytisus reverchonii* (Fabaceae), *Adenocarpus decorticans* (Fabaceae) and *Compositae campestris* (Asteraceae). Above the timberline, the subalpine shrubland is composed mainly of *G. versicolor*, with a few isolated oaks and maples. The forest is used by domestic livestock (goats and sheep) and Spanish ibex (*Capra pyrenaica* L.) during summer, and by free-ranging cattle and wild boar (*Sus scrofa* L.) all year around. The subalpine shrubland is used only during summer by goats, sheep and ibex.

Two 0.5 ha plots were randomly established both in the forest at 1700 m a.s.l. and in the subalpine shrubland at 1850 m a.s.l. (habitats, hereafter). Plots were similar in soil and physiognomy. The plots located in the same habitat were separated by about 0.2 km, whereas the distance between plots in different habitats was approximately 0.7 km. Three major microhabitats can be found in the forest plots: (1) *under oak*, under the canopy of reproducing *Q. pyrenaica* individuals; (2) *under shrub*, under the canopy of any of the four species of shrubs inhabiting the forest; and (3) *open*, the open interspaces between the trees still not colonized by shrubs. In the subalpine shrubland only two major microhabitats can be found: (1) *under shrub*, under the canopy of *G. versicolor* individuals; and (2) *open*, the open interspaces between the shrubs.

### 2.2. Natural pattern of acorn dispersal, seedling establishment and juvenile abundance

The seed shadow generated by abiotic acorn dispersal was studied from 1998 to 2000, by quantifying the number of acorns in each microhabitat of the four plots during the peak of the dispersal period (10–20 400 cm<sup>2</sup> quadrats per microhabitat, plot and year,  $N = 180$  quadrats in total). This estimate of abiotic dispersal is conservative, since it does not consider the number of acorns quickly removed by predators or

secondary dispersers. Just after the snowmelt (early April), we quantified the number of acorns still lying on the plot surface. Every acorn appearing in this last period was collected, to determine whether they were sound, preyed upon by insects or undeveloped.

In winter 1997–1998 we located at random 20 permanent 5 m × 2 m transects per microhabitat in all four plots ( $n = 200$  transects). In June of 1998, after natural seedling emergence, we counted the number of current-year seedlings and the number of established juveniles (lower than 150 cm tall) per transect. Each juvenile was permanently tagged in order to quantify height (in cm) and damage intensity (in percentage of current-year shoots damaged by ungulates). We also noted the number of adventitious shoots in each microhabitat of the forest plots. No adventitious shoots were observed in the scrubland. We revisited the transects in June 1999 and 2000, noting the number of seedlings.

### 2.3. Experimental studies of acorn predation

Several experiments were carried out during the period 1997–1999 to investigate acorn survivorship and the factors limiting it. Acorns were collected during the peak of natural availability (October–November) close to the experimental plots and stored for several weeks in a cold room at 2–4 °C. Non-viable acorns (empty or preyed upon by insects) were excluded by floating in water (see Bonfil, 1998). In each plot the experimental acorns were placed separated from each other by 50 cm in grids labelled in their outer part. Marks were located 1 m from the grid border to avoid attraction effect. All experiments were checked after 7 and 15 days, noting the number of acorns predated in situ, the number removed, and, when possible, the identity of the predators. To differentiate between predator species was easy based on several traits. Only wild boars and woodmice feed on buried acorns in the study zone. Wild boar encountered the acorns during rooting activity, mauling and breaking them longitudinally. By contrast, woodmice extract from the soil the acorn without any sign of rooting activity, and gnaw a hole in the base of the acorns and feed on the cotyledons. By contrast, when acorns are lying on the ground, there are consumed not only by wild boars and woodmice but also by livestock and Spanish ibex, and it is not possible to differentiate

between these mortality agents. In early spring we checked whether the acorns left by the predators germinated and produced seedlings.

#### 2.3.1. Effect of habitat and microhabitat

A preliminary experiment was set up to investigate the intensity of predation suffered by acorns, and the effect of habitat and microhabitat. In winter 1997 we planted 50 acorns per microhabitat and plot in the forest and 75 acorns per microhabitat and plot in the scrubland ( $N = 600$  acorns). We used *G. versicolor* in this experiment, because it was the only shrub inhabiting all the plots. Acorns were buried horizontally into the soil at 3–4 cm, simulating dispersal by European jays (Bossema, 1979; Gómez et al., pers. obs.).

#### 2.3.2. Effect of burial

To test the effect of burial on acorn survival, we planted in winter 1998 200 acorns per microhabitat in each forest plot and 100 acorns per microhabitat in each scrubland plot ( $N = 1600$  acorns). Half the acorns were buried 3–4 cm, and the remaining were laid on the ground, simulating abiotic (by gravity) seed dispersal. The shrub species was again *G. versicolor*.

#### 2.3.3. Effect of nurse shrub species

This experiment tested the effect of the identity of the nurse shrub species on acorn survival. In one forest plot in winter 1999 we planted 100 acorns 3–4 cm deep in each of the six microhabitats: open, under oak, under *G. versicolor*, under *C. reverchonii*, under *A. campestris*, and under *A. decorticans* ( $N = 600$  acorns).

### 2.4. Experimental study of seedling survival

In autumn 1997, several thousand ripe acorns were collected from at least 20 trees located in the forest but outside the marked plots. Acorns were pooled to avoid problems related to inter-individual differences in acorn traits, and stored in a cold room (2–4 °C) for several weeks. In December, acorns were planted individually in cylindrical pots (7 cm wide × 20 cm deep) located in a fenced plot situated approximately 4 km far from the forest and at the same altitude (1650 m a.s.l.). Acorns germinated in February–March and seedlings emerged in late May and June,

as under natural conditions. The seedlings were kept all the summer in the nursery and when they were 6 months old, 1000 seedlings were randomly taken, root pruned to a root length of 20–25 cm, and transported to the forest. In November 1998 in each plot and microhabitat, we planted 100 seedlings at random, but located at least 1 m apart. All seedlings still had the acorns attached to the plant, and mean shoot length was  $9.6 \pm 0.2$  cm (range: 5.5–14.5).

Seedling survival was recorded during early June 1999, November 1999, early June 2000 and November 2000. When possible, we noted the mortality causes of the dead seedlings. *Q. pyrenaica*, like many species of oaks, readily resprouts after shoot damage due to browsing or drought. For this reason, we collected the seedlings still left in the study area at the end of the experiment, in order to check whether live tissue persisted in the roots. We assumed that seedlings were dead if the main root, after transversally cutting, was completely dry.

## 2.5. Data analysis

The natural spatial pattern of acorn dispersal and juvenile abundance were not normally distributed, and thereby were analysed by using Generalized Linear Models (Proc GENMOD; SAS, 1997). To compare juvenile abundance (number per census) between habitats and microhabitats, we fitted data to a Poisson probability distribution using the log as a link function (SAS, 1997). Acorn abundance (number per quadrat) between habitats and microhabitats was compared by using a negative binomial as error distribution, since data were highly clumped and departed from a Poisson distribution. These latter analyses were computed using the SAS Macro-program GLM-LOG NEGATIVE BINOMIAL REGRESSION Version 1.0 for Proc GENMOD (Hilbe, 1994). Pairwise comparison between microhabitats was made by Wald chi-square, using the CONTRAST statement in Proc GENMOD (SAS, 1997).

Acorn predation experiments were analysed by Generalized Logits Models (Proc CATMOD, SAS, 1997) using maximum likelihood estimations (Stokes et al., 1995). Microhabitat was nested into plot and plot nested into habitat, and the dependent variables were binomial, whether cumulative acorn survival when analysing effect on acorn predation, or predator

identity when analysing effect on the kind of acorn predators. In this latter analysis, we considered only those acorns for which we could clearly identify the predator.

Differences in seedling survivorship were explored by the Cox's Proportional Hazards semiparametric model (Proc PHREG), using the maximum partial likelihood as estimation method (Allison, 1995). To handle the ties in survival times occurring in a data set, we used the EXACT method that assumes a true but unknown ordering for the tied event times (Allison, 1995). The temporal and spatial variability in the cumulative effect of the several mortality agents of the seedling was analysed by a multinomial Generalized Logits Model (Proc CATMOD, SAS, 1997; Stokes et al., 1995), introducing as dependent variable the mortality agent (multinomial variable), and as independent the date at which each mortality agent acted, the habitat, the microhabitat nested into plot, the plot nested into habitat and the interactions between these variables and the date. This latter variable was considered ordinal in the model (Stokes et al., 1995). Throughout this paper, means are shown  $\pm 1$  S.E. unless otherwise indicated.

## 3. Results

### 3.1. Natural pattern of acorn dispersal, seedling establishment and juvenile abundance

Acorn production varied markedly between years. In 1998 no acorns found at all, whereas in 1999 there were  $28.3 \pm 6.2$  acorns/m<sup>2</sup> in the two forest plots ( $N = 120$  quadrats). In 2000 there was again a low acorn production ( $0.01 \pm 0.10$  acorns/m<sup>2</sup>). In the sub-alpine shrubland we did not find any acorn during the study period. Between microhabitat difference in acorn abundance was found in 1999 ( $\chi^2 = 805.8$ ,  $P < 0.0001$ , Proc GENMOD), since more than 99.5% of the acorns were under oaks (Table 1). Almost all acorns had disappeared after 1 month, most of them having being eaten by wild boar and goats, although there was still between microhabitat difference in acorn abundance ( $\chi^2 = 291.7$ ,  $P < 0.0001$ , Proc GENMOD). However, more than 85% of the acorns found on the forest floor after winter were empty and damaged by weevils. After omitting

Table 1

Acorn abundance (number/m<sup>2</sup>) on the forest ground (both forest plots pooled) due to abiotic (by gravity) dispersal in each of the six microhabitat considered for the 1999 dispersal period<sup>a</sup>

Microhabitats	November 1999	April 2000
Oak	160.00 ± 17.99 (20)a	1.00 ± 0.60 (100)a
Open	3.75 ± 2.05 (20)b	0.1 ± 0.10 (100)b
<i>G. versicolor</i>	1.25 ± 1.25 (20)b	0.1 ± 0.10 (100)b
<i>A. decorticans</i>	1.25 ± 1.25 (20)b	0.1 ± 0.10 (100)b
<i>C. reverchonii</i>	2.50 ± 1.72 (20)b	0.0 ± 0.00 (100)b
<i>A. campestris</i>	1.25 ± 1.25 (20)b	0.25 ± 0.25 (100)b

<sup>a</sup> Number of quadrats is shown in brackets. Column with different superscript letters are different at  $\alpha < 0.05$ .

these non-viable acorns, no between microhabitat differences in acorn abundance were found after winter ( $P = 0.41$ ).

The number of seedlings was extremely low in our study site. In 1998 and 1999 no seedling established in the permanently marked transects. In 2000 we found only one seedling established under *Adenocarpus* ( $0.0002 \pm 0.0003$  seedlings/m<sup>2</sup>) in our forest plots, and none in any of the other microhabitats and none in the shrubland.

Juvenile abundance differed significantly between habitats ( $\chi^2 = 52.6, P < 0.0001$ , Proc GENMOD) and microhabitats ( $\chi^2 = 61.9, P < 0.0001$ , Proc GENMOD). Juveniles were extremely scarce in the shrubland ( $0.001 \pm 0.010$  juveniles/m<sup>2</sup>), and invariably located under shrubs. Nevertheless, juvenile abundance was also low in the forest ( $0.02 \pm 0.03$  juveniles/m<sup>2</sup>) and located non-randomly with respect to the microhabitat ( $\chi^2 = 9.46, P = 0.009$ , Proc CATMOD), since 66.7% were under shrubs, 29.6% in the open but only 3.7% under oaks ( $N = 27$  juveniles), despite this latter microhabitat occupied 80% of the forest plot surface.

By contrast, sprouts were abundant in the forest ( $0.15 \pm 0.02$  sprouts/m<sup>2</sup>). There were between microhabitat differences in sprout abundance ( $\chi^2 = 1500.2, P < 0.0001$ , Proc GENMOD), with most (99%) located under oaks.

Some  $41.8 \pm 6.6\%$  of the current-year shoots of the juveniles were damaged by ungulates. Although juveniles in open interspaces and under oaks tended to suffer more damage, no statistical difference was found ( $\chi^2 = 4.56, d.f. = 2, P = 0.10$ , Kruskal–Wallis test, Proc NONPAR).

### 3.2. Effect of habitat and microhabitat on acorn predation

Only nine acorns survived to spring (1.6%), all of these germinating and producing seedlings. For this reason, no effect of habitat (Wald  $\chi^2 = 0.02, d.f. = 1, P = 0.89$ ), plot (Wald  $\chi^2 = 0.3, d.f. = 3, P = 0.95$ ) or microhabitat (Wald  $\chi^2 = 0.365, d.f. = 2, P = 0.83$ ) was found in survival probability. Most acorns (99.2%) were preyed upon by woodmice, the remaining being consumed by wild boar. Sixty percentage of the acorns encountered by woodmice were consumed in situ. No effect of habitat (Wald  $\chi^2 = 0.01, d.f. = 1, P = 0.90$ ), plot (Wald  $\chi^2 = 0.001, d.f. = 3, P = 0.99$ ) or microhabitat (Wald  $\chi^2 = 1.03, d.f. = 2, P = 0.60$ ) was found for the kind of predator.

### 3.3. Effect of burial on acorn predation

Only 58 (3.6%) acorns survived to germinate and produce seedlings. Although more acorns survived when buried than when placed on the ground surface (49 versus 9 acorns), no single factor affected acorn predation ( $P > 0.90$  in all cases, Proc CATMOD). About 44.7% of the buried acorns were preyed upon by wild boar (it was not possible to differentiate between mortality agents when analysing the mortality of acorns lying on the ground, see methods), whereas the remaining were removed by woodmice. Fifty percentage of the acorns encountered by rodents were removed without being consumed in situ. There was between microhabitat difference in the kind of predator ( $\chi^2 = 314.8, d.f. = 2, P < 0.0001$ , Proc CATMOD). Whereas only 11.6% of the acorns located under oaks and 41.8% of those under shrubs were consumed by wild boar, 66.7% of the acorns in the open microhabitat were consumed by these mammals.

### 3.4. Effect of nurse shrub species on acorn predation

No acorn survived to germinate in this experiment ( $n = 600$  acorns), and thus, no between microhabitat difference was found in survival. About 72.5% of the acorns were consumed by wild boar and 27.5% by woodmice. Nevertheless, the type of predator differed between microhabitats (Wald  $\chi^2 = 220.66, d.f. = 5, P = 0.0001$ ), since 66% of the

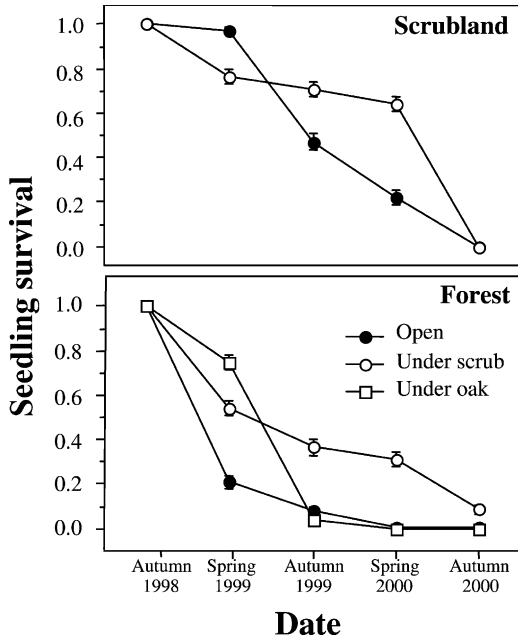


Fig. 1. Survival functions for *Q. pyrenaica* seedlings planted in several microhabitats in the forest and the subalpine scrubland. Shown are survival values (means  $\pm$  1 S.E.) resulting from life-table estimations using LIFETEST procedure (SAS, 1997).

acorns located under *A. decorticans*, 96% under *A. campestris*, 87% under *C. reverchonii*, 99% in the open and 65% under oak were consumed by wild boars, against 22% under *G. versicolor*.

3.5. Seedling survival

Only  $1.7 \pm 0.7\%$  (mean survivorship  $\pm$  binomial S.E., Proc LIFETEST) of the 1000 seedlings were still alive at the end of the experiment. There was between habitat difference in survivorship ( $\chi^2 = 13.88$ , d.f. = 1,  $P < 0.0002$ , Cox proportional hazards regression), since mortality began earlier in the forest than in the scrubland (Fig. 1), but all surviving seedlings were at the forest plots. In addition, there was also between microhabitat difference ( $\chi^2 = 13.18$ , d.f. = 1,  $P < 0.0003$ ), since no seedling survived under oaks, 16 seedlings survived under shrubs and one at open.

We identified five causes of seedling mortality ( $N = 817$  seedlings): trampling by livestock, presumably feral cattle and domestic goats (36.9% of the dead seedlings), acorn predation by wild boar and hares *Lepus granatensis* (39.5%), woodmice (11.7%), abiotic factors, mainly desiccation (11.3%), and browsing by livestock (1.0%). Thus, herbivores killed most of the seedlings, although the way in which they severed the seedlings was very diverse. Woodmice killed the seedlings by consuming the acorns attached to the root, usually cutting the stems before removing the acorns. Wild boar and hares killed the seedlings by uprooting them before consuming the acorns and chewing the swollen roots. Bovids killed the seedlings by consuming the above-ground biomass or, more frequently, by trampling.

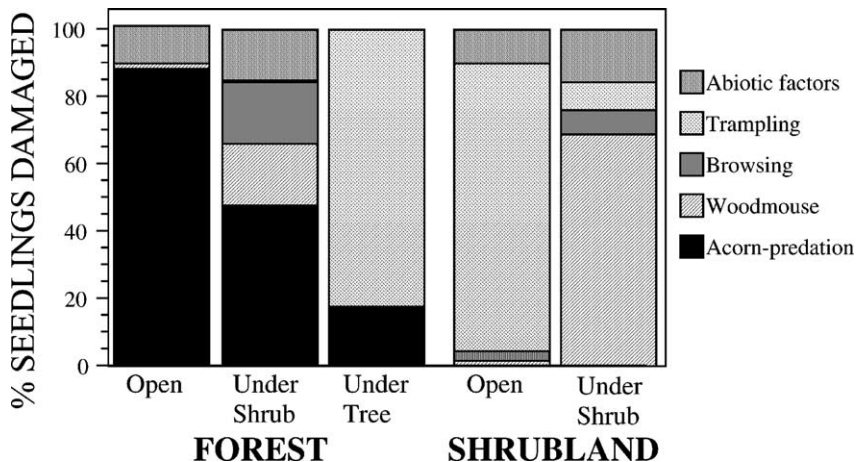


Fig. 2. Between habitat and microhabitat differences in the cumulative predation by each of the seven agents of seedling mortality considered in this study.

There was spatial variability in the effect of each mortality agent, both between habitats ( $\chi^2 = 47.81$ ,  $P < 0.0001$ ) and microhabitat ( $\chi^2 = 184.15$ ,  $P < 0.0001$ , Proc CATMOD). The probabilities of seedling death by wild boar and hares were much higher in the forest than in the shrubland, and in open sites than in any other microhabitat (Fig. 2), whereas predation by woodmice was highest under shrub in both habitats (Fig. 2). Trampling was especially severe under oak in the forest and in open interspaces in the shrubland plots (Fig. 2).

There was also significant temporal heterogeneity in the activity of mortality agents ( $\chi^2 = 111.1$ ,  $P < 0.0001$ , Proc CATMOD). The earliest mortality factors, acting during winter just after planting, were acorn predators and woodmice. During the first summer of the experiment (1999), the main mortality agents were drought and especially trampling. Trampling persisted as an important mortality factor during the next winter and summer. During the second summer of the experiment, drought was no longer a major mortality factor.

#### 4. Discussion

Our observations and experiments have shown that dispersed acorns are quickly consumed, and that, although a high number of acorns were abiotically dispersed to the forest floor during masting years, no single acorn remains after a period of 3 months. In addition, contrary to observations made in other locations, as for example in temperate oak forests of Japan (Kikuzawa, 1988), England (Crawley and Long, 1995), Canada (Fuchs et al., 2000) or Central Europe (Kollman and Schill, 1996) and in Mediterranean woodlands of California (Borcher et al., 1989) and SW Spain (Herrera, 1995), burial did not increase acorn survival in our study. This overall dramatic predation occurs surely because acorns are consumed by several generalist species, such as rodents and wild boar, able to detect buried acorns with very high efficiency (Groot Bruinderink et al., 1994). Although *Apodemus* spp. can act also as dispersers of some oaks (Jensen and Nielsen, 1986; Kollman and Schill, 1996; Pulido, 1999), in our case they were mainly predators, since they consumed many of the acorns in situ, and no single seedling was found in the plots the years after the experiment. Other studies have also found that mice are mainly predators, because they usually hoard

the acorns in huge and deep piles, from which successful establishment of seedlings is unlikely (Vander Wall, 1990), and relocate and consume almost all hoarded acorns (e.g. Miyaki and Kikuzawa, 1988; Wada, 1993; Kollman and Schill, 1996; Iida, 1996; Santos and Tellería, 1997; Wang et al., 1999). In addition, the high predation rate observed in this study can also be due to the high population density of wild boars occurring in the Sierra Nevada mountains nowadays. Thus, although no accurate estimates of wild boar density currently exists for the study area, indirect evidence suggests that it is higher than 10–20 individuals/100 km<sup>2</sup>, and this density has rapidly increased recently (Anonymous, 2000).

Acorn survival did not vary spatially. This was mainly due to the difference in microhabitat use shown by the acorn predators. Whereas wild boar were more active in open interspaces, rodents were more active in vegetation-covered microhabitats (under shrubs and under oaks), where they are protected against predators (see Díaz, 1992; Herrera, 1995; Kollman and Schill, 1996; Wada, 1993; Alcántara et al., 2000 for similar results). As a consequence, no safe microhabitat exists for *Q. pyrenaica* acorns in the study site.

Only 2% of the experimental seedlings survived the overall study period, most mortality was caused by several species of herbivores. It is noticeable that the main cause of mortality was ungulates trampling, whereas the summer drought, an important mortality factor for seedlings of many Mediterranean woody plants (Herrera et al., 1994; Castro et al., 1999; Rey and Alcántara, 2000; García, 2001), was not important for *Q. pyrenaica* seedlings in our study. A temporal turnover was detected in the vertebrates damaging seedlings (see also Callaway, 1992; Germaine and McPherson, 1999). During the first year, seedlings suffered damage by acorn-feeding mammals, such as wild boar, hares or woodmice, which uprooted the seedlings to consume the acorns, leaving the stems, leaves and roots on the soil surface (see also Herrera, 1995; Groot Bruinderink and Hazebroek, 1996; Germaine and McPherson, 1999). When seedlings were older, and the cotyledon reserve presumably depleted, acorn predators became less important. However, at this time goats and ibex started to damage seedlings, killing them both by browsing and also by trampling (for similar results, see McPherson, 1993; Van Hees et al., 1996; Bonfil, 1998; Germaine and McPherson,

1999; Fuchs et al., 2000; Meiner and Handel, 2000). Finally, surviving seedlings were also damaged by ungulates during their pre-reproductive stage, since the naturally established *Q. pyrenaica* juveniles have most of their shoot browsed by mammals. A major consequence of this repeated browsing is a significant delay of the oak's maturation age and size (see, e.g. Cuartas and García-González, 1992 for *Q. ilex*, Papatheodorou et al., 1993 for *Q. coccifera*).

In contrast to acorn survival, seedling survival probability varied spatially, being significantly higher under shrubs than elsewhere. Shrubs appear to facilitate the establishment of *Q. pyrenaica* during its early years. Protection by shrubs against damage by herbivory and abiotic factors has been shown for other oaks (Callaway and D'Antonio, 1991; Callaway et al., 1991; Callaway, 1992; Callaway and Davis, 1998; Rousset and Lepart, 1999, 2000). In our case, this protection also appears to apply to juvenile, since their abundance as well was significantly higher under shrubs than under oaks or open sites. In fact, we have observed that most naturally established oaks are underneath nurse shrubs in Mediterranean forests.

#### 4.1. Potential effects of predators in the regeneration of *Q. pyrenaica* forests

Our study has shown that vertebrate postdispersal predators impose many filters on *Q. pyrenaica* recruitment, by consuming most acorns and seedlings and by repeatedly browsing juveniles until the sapling reaches about 1.5 m tall. Regeneration via sexual reproduction is thus highly constrained in these Mediterranean mountains. The only way that *Q. pyrenaica* forests can regenerate at present is by resprouting. In fact, sprouts were much more abundant than juveniles in our study site. Similar cases of asexual regeneration have been reported for *Q. emoryi* in southern USA (Weltzin and McPherson, 2000) and *Q. ilex* in NE Spain (Retana et al., 1992; Espelta et al., 1995). However, due to the physiognomic structure of the *Q. pyrenaica* forests, the density of sprouts is very high under oak clumps but almost zero in the open sites or shrub clumps. This suggests that regeneration by sprouts, although capable of easily replacing adult senescent oaks, cannot easily increase the number of oaks in the forest or colonize the gaps between oak clumps. As a consequence, oak forests are currently

formed in many Mediterranean mountains by small fragments composed exclusively of adult trees.

In addition, our experimental data show that seed and seedling predators also can constrain the expansion of *Q. pyrenaica* into adjacent shrublands. Acorns spreading out from the current limit of the forest are preyed upon by woodmice, which are very abundant in this habitat, whereas seedlings are killed both by woodmice and by trampling. We have found that seedling mortality was even more severe in the shrubland than in the forest. Subalpine shrublands are traditionally used in these areas by large herds of ungulates, both wild and domestic, which forage on herbaceous and woody species. These results contrast with those reported by Hubbard and McPherson (1999) for *Q. emoryi* in North-American arid systems, where colonization of adjacent semiarid grasslands by oaks is possible because acorn predation is less likely in these open habitats than within woodlands.

In brief, our study strongly supports the idea that acorn and seedling predators can have a harmful effect on the regeneration of otherwise abundant *Quercus* forests in the Iberian peninsula (Herrera, 1995; Santos and Tellería, 1997). The conservation and restoration of these forests under the current ecological context of most Mediterranean mountains requires therefore the prevention of the negative impact produced by these herbivores (Zamora et al., 2001).

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#### References

- Alcántara, J., Rey, P.J., Sánchez-lafuente, A.M., Valera, F., 2000. Early effects of rodent post-dispersal seed predation on the outcome of the plant-seed disperser interaction. *Oikos* 88, 362–370.



- Allison, P.D., 1995. Survival Analysis Using the SAS<sup>®</sup> System: A Practical Guide. SAS Institute, Inc., Cary, NC, USA.
- Anonymous, 2000. Plan específico de gestión de las poblaciones de jabalí (*Sus scrofa*) en el Parque Nacional de Sierra Nevada. Comisión Mixta de Gestión de Parques Nacionales de Andalucía. Unpublished technical draft.
- Bonfil, C., 1998. The effect of seed size, cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae). *Am. J. Bot.* 85, 79–87.
- Borcher, M.I., Davis, F.W., Michaelsen, J., Oyler, L.D., 1989. Interactions of factors affecting seedling recruitment of blue oak (*Quercus douglasii*) in California. *Ecology* 70, 389–404.
- Bossema, I., 1979. Jays and oaks: an eco-ethological study of a symbiosis. *Behaviour* 70, 1–117.
- Callaway, R.M., 1992. Effect of shrub on recruitment of *Quercus douglasii* and *Quercus lobata* in California. *Ecology* 73, 2118–2128.
- Callaway, R.M., D'Antonio, C.M., 1991. Shrub facilitation of coast live oak establishment in central California. *Madroño* 38, 158–169.
- Callaway, R.M., Davis, F.W., 1998. Recruitment of *Quercus agrifolia* in central California: the importance of shrub-dominated patches. *J. Veg. Sci.* 9, 647–656.
- Callaway, R.M., Nadkarni, N.M., Mahall, B.E., 1991. Facilitation and interference of *Quercus douglasii* on understory productivity in central California. *Ecology* 72, 1484–1499.
- Castro, J., Gómez, J.M., García, D., Zamora, R., Hódar, J.A., 1999. Seed predation and dispersal in relict Scots pine forests in southern Spain. *Plant Ecol.* 145, 115–123.
- Crawley, M.J., Long, C.R., 1995. Alternate bearing, predator satiation and seedling recruitment in *Quercus robur* L. *J. Ecol.* 83, 683–696.
- Cuartas, P., García-González, R., 1992. *Quercus ilex* browse utilization by Caprini in Sierra de cazorla and Segura (Spain). *Vegetatio* 99–100, 317–330.
- Díaz, M., 1992. Rodent seed predation in cereal crop areas of central Spain: effects of physiognomy, food availability, and predation risk. *Ecography* 15, 77–85.
- Espelta, J.M., Riba, M., Retana, J., 1995. Patterns of seedling recruitment in West-Mediterranean *Quercus ilex* forests influenced by canopy development. *J. Veg. Sci.* 6, 465–472.
- Fuchs, M.A., Krannitz, P.G., Harestad, A.S., 2000. Factors affecting emergence and first-year survival of seedlings of Garry oaks (*Quercus garryana*) in British Columbia, Canada. *For. Ecol. Manage.* 137, 209–219.
- García, D., 2001. Effects of seed dispersal on *Juniperus communis* recruitment on a Mediterranean mountain. *J. Veg. Sci.* 12, 839–848.
- Germaine, H.L., McPherson, G.R., 1999. Effects of biotic factors on emergence and survival of *Quercus emoryi* at lower treeline, Arizona, USA. *Écoscience* 6, 92–99.
- Groot Bruinderink, G.W.T.A., Hazebroek, E., 1996. Wild boar (*Sus scrofa scrofa* L.) rooting and forest regeneration on podzolic soils in the Netherlands. *For. Ecol. Manage.* 88, 71–80.
- Groot Bruinderink, G.W.T.A., Hazebroek, E., van der Voot, H., 1994. Diet and condition of wild boar, *Sus scrofa scrofa*, without supplementary feeding. *J. Zool.* 233, 631–648.
- Herrera, J., 1995. Acorn predation and seedling production in a low-density population of cork oak (*Quercus suber* L.). *For. Ecol. Manage.* 76, 197–201.
- Herrera, C.M., Jordano, P., López-Soria, L., Amat, J.A., 1994. Recruitment of a mast-fruited, bird-dispersed tree: bridging frugivore activity and seedling establishment. *Ecol. Monogr.* 64, 315–344.
- Hilbe, J., 1994. Log Negative Binomial Regression using the GENMOD Procedure SAS/STAT Software. In: Proceedings of SUGI 19, SAS Institute, Cary, NC.
- Houle, G., 1995. Seed dispersal and seedling recruitment. The missing link(s). *Écoscience* 2, 238–244.
- Hubbard, J.A., McPherson, G.R., 1999. Do seed predation and dispersal limit downslope movement of a semi-desert grassland/oak woodland transition. *J. Veg. Sci.* 10, 739–744.
- Hulme, P.E., 1996. Natural regeneration of yew (*Taxus baccata* L.): microsite, seed or herbivore limitation. *J. Ecol.* 84, 853–861.
- Iida, S., 1996. Quantitative analysis of acorn transportation by rodents using magnetic locator. *Vegetation* 124, 39–42.
- Jensen, T.S., Nielsen, O.F., 1986. Rodent as seed dispersers in a heath-oak wood succession. *Oecologia* 70, 214–221.
- Jordano, P., Herrera, C.M., 1995. Shuffling the offspring: uncoupling and spatial discordance of multiple stages in vertebrate seed dispersal. *Écoscience* 2, 230–237.
- Kikuzawa, K., 1988. Dispersal of *Quercus mongolica* acorns in a broadleaved deciduous forest. 1. Disappearance. *For. Ecol. Manage.* 25, 1–8.
- Kollman, J., Schill, H.P., 1996. Spatial patterns of dispersal, seed predation and germination during colonization of abandoned grassland by *Quercus petraea* and *Corylus avellana*. *Vegetation* 125, 193–205.
- McPherson, G.R., 1993. Effects of herbivory and herb interference on oak establishment in a semi-arid temperate savanna. *J. Veg. Sci.* 4, 687–692.
- Meiner, S.J., Handel, S.N., 2000. Additive and nonadditive effects of herbivory and competition on tree seedling mortality, growth, and allocation. *Am. J. Bot.* 87, 1821–1826.
- Miyaki, M., Kikuzawa, K., 1988. Dispersal of *Quercus mongolica* acorns in a broadleaved deciduous forest. 2. Scatterhoarding by mice. *For. Ecol. Manage.* 25, 9–16.
- Papatheodorou, E., Pantis, J.D., Stamou, G.P., 1993. The effects of grazing on growth, spatial pattern and age structure of *Quercus coccifera*. *Acta Oecologica* 14, 589–602.
- Pulido, F.J., 1999. Herbivorismo y regeneración de la encina (*Quercus ilex* L.) en bosques y dehesas. Ph.D. Dissertation. Universidad de Extremadura, Cáceres, Spain.
- Retana, J., Riba, M., Castell, C., Espelta, J.M., 1992. Regeneration by sprouting of holm-oak (*Quercus ilex*) stands exploited by selection thinning. *Vegetation* 99–100, 355–364.
- Rey, P., Alcántara, J.M., 2000. Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. *J. Ecol.* 88, 622–633.
- Rousset, O., Lepart, J., 1999. Shrub facilitation of *Quercus humilis* regeneration in succession on calcareous grasslands. *J. Veg. Sci.* 10, 493–502.

- Rousset, O., Lepart, J., 2000. Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). *J. Ecol.* 88, 401–412.
- Santos, T., Tellería, J.L., 1997. Vertebrate predation on Holm Oak, *Quercus ilex*, acorns in a fragmented habitat: effects on seedling recruitment. *For. Ecol. Manage.* 98, 181–187.
- SAS Institute Inc. 1997. SAS/STAT Software: Changes and Enhancements, Release 6.12. SAS, Cary, NC.
- Schupp, E.W., 1995. Seed seedling conflicts, habitat choice and patterns of plant recruitment. *Am. J. Bot.* 82, 399–409.
- Schupp, E.W., Fuentes, M., 1995. Spatial patterns of seed dispersal and the unification of plant population ecology. *Écoscience* 2, 267–275.
- Stokes, M.E., Davis, C.S., Koch, G.G., 1995. Categorical Data Analysis Using the SAS<sup>®</sup> System. SAS Institute, Cary, NC.
- Vander Wall, S.B., 1990. Food Hoarding in Animals. University of Chicago Press, Chicago, IL.
- Van Hees, A.F.M., Kuiters, A.T., Slim, P.A., 1996. Growth and development of silver birch, pedunculate oak and beech as affected by deer browsing. *For. Ecol. Manage.* 88, 55–63.
- Wada, N., 1993. Dwarf bamboos affect the regeneration of zoochorous trees by providing habitat to acorn-feeding rodents. *Oecologia* 94, 403–407.
- Wang, W., Ma, K., Lu, C., 1999. Removal and predation of *Quercus liaoningensis* acorns by animals. *Ecol. Res.* 14, 225–232.
- Weltzin, J.F., McPherson, G.R., 2000. Implications of precipitation and redistribution for shifts in temperate savanna ecotones. *Ecology* 81, 1902–1913.
- Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J., García, D., 2001. Effect of browsing by ungulates on sapling growth of Scots pine in a Mediterranean environment: consequences for forest regeneration. *For. Ecol. Manage.* 144, 33–42.