EFFECTS OF COTYLEDON EXTIRPATION AND SEEDLING AGE ON THE SURVIVAL AND PERFORMANCE OF OAK SEEDLINGS: APPLICATION TO RESTORATION PRACTICES

Máster Universitario en Restauración de Ecosistemas

Presentado por: D. Guillermo Carranza Jiménez

Directores: Dra. Dª Marta Peláez Beato y Dr. D. Ramón Perea García-Calvo

Tutor académico: Dr. D. Juan Antonio Oliet Pala

En Madrid, a 23 de junio de 2022

Table of contents

Abstract

In Mediterranean oak ecosystems, drought and ungulate populations can be considered one of the main abiotic and biotic stresses threatening natural regeneration. Furthermore, many restorations fail due to the challenges of overcoming these pressures. In fact, how different sources of stress act and vary across time in oak seedlings plantations remains still largely unknown. Thus, in Cabañeros and Doñana National Parks, with high wild ungulate densities, we planted different oak seedling species (200 *Quercus ilex* seedlings and 100 *Q. suber,* respectively). We examined how temporal variation of abiotic and biotic stress agents affected seedling survival across two different seedling ages (1 vs 2-year-old) and between seedlings with the acorn attached or not (cotyledon removal). All oak seedlings (100%) suffered some type of stress. Drought was the main cause of mortality followed by wild boar. Deer, insect and fungi damage was not strong enough to cause the death of any seedling. We revealed a strong temporal variation in the occurrence of stressors as the risk of being damage by wild boar was highest between February and April, and by drought, after the onset of the dry season. In Cabañeros no differences were found for all combinations of plant age and acorn treatment. However, in Doñana we observed that wild boar uprooted 1-year-old seedlings significantly more often than 2-years-old, and within the same age group, we could perceive a weak but consistent preference towards those with the acorn still attached. In addition, we found that cotyledon removal from the seedling does not affect their growth or physiology. Thus, in areas with high densities of wild boar, removing the cotyledon reserves from 1-year-old seedlings, and, at least on sandy soils, planting 2-year-old seedlings instead of 1-year-old seedlings could be a good strategy to avoid or reduce wild boar damage.

Keywords

Sus scrofa, assisted regeneration, temporal variation, stress agents, leaf physiological content

Resumen

En los ecosistemas mediterráneos dominados por *Quercus* spp., la sequía y las poblaciones de ungulados pueden considerarse los principales estreses abióticos y bióticos que amenazan la regeneración natural. Además, muchas restauraciones fracasan debido a los retos que supone superar ambos estreses. De hecho, todavía se desconoce en gran medida cómo actúan las diferentes fuentes de estrés y cómo varían a lo largo del tiempo en las plantaciones de *Quercus*. Así, en los Parques Nacionales de Cabañeros y Doñana, con altas densidades de ungulados silvestres, plantamos diferentes especies de *Quercus* (200 plántulas de *Quercus ilex* y 100 de *Q. suber*, respectivamente). Examinamos cómo la variación temporal de los agentes de estrés abiótico y biótico afectaba a la supervivencia de las plántulas en dos edades diferentes (1 savia vs 2 savias) y entre plántulas con la bellota adherida o sin ella (eliminación de cotiledones). Todas las plántulas de *Quercus* (100%) sufrieron algún tipo de estrés. La sequía fue la principal causa de mortalidad, seguida del jabalí. Los daños causados por el ciervo, los insectos y los hongos no fueron lo suficientemente fuertes como para causar la muerte de ninguna plántula. Se reveló una fuerte variación temporal en la ocurrencia de los estreses, ya que el riesgo de daño por jabalí fue mayor entre febrero y abril, y por sequía, tras el inicio de la estación seca. En Cabañeros no se encontraron diferencias para todas las combinaciones de edad de la planta y tratamiento de la bellota. Sin embargo, en Doñana observamos que los jabalíes arrancaron plántulas de 1 año con una frecuencia significativamente mayor que las de 2 años, y dentro del mismo grupo de edad, pudimos percibir una preferencia débil pero consistente hacia las que tenían la bellota aún adherida. Además, descubrimos que retirar los cotiledones de la plántula no afecta a su crecimiento ni a su fisiología. Por lo tanto, en zonas con altas densidades de jabalíes, eliminar los cotiledones de las plántulas de 1 año y, al menos en suelos arenosos, plantar plántulas de 2 años en lugar de las de 1 año, podría ser una buena estrategia para evitar o reducir los daños por jabalí.

Palabras clave

Sus scrofa, regeneración asistida, variación temporal, agentes de estrés, contenido fisiológico de las hojas

1. INTRODUCTION

One of the major challenges facing ecologists today is to understand how global change is altering the structure, diversity and dynamics of plant communities (Peñuelas et al., 2004; Gómez-Aparicio et al., 2008). Anthropogenic climate change and land use changes are considered important drivers of these processes which, among others, have led to the reduction of tree recruitment rates in many areas of the world (Kerr, 2007; Wright, 2010; Wu, 2019). In the Mediterranean basin, climate change has produced an increase in the frequency and intensity of droughts and heat waves (IPCC, 2019) as well as a variation in precipitation patterns (Peñuelas et al., 2004; IPCC, 2014; Fernández-Manjarrés et al., 2018). Indeed, drought is already the main abiotic stress threatening Mediterranean ecosystems and compromising forest regeneration, with climate change models predicting an increase in temperatures and droughts (Peñuelas et al., 2004; Peñuelas et al., 2017; Fernández-Manjarrés et al., 2018, IPCC, 2021). Land use changes have created the perfect conditions for a rapid increase of ungulate populations (Klein, 1968; Chapin et al., 1996), driven by socio-economic factors such as the abandonment of agricultural fields or extensive livestock farming, rural depopulation, the extinction or reduction of large predators and the intensification and spread of commercial hunting (Coté et al., 2004; Perea et al., 2014a; Carpio et al., 2017; Valente et al., 2020). Although this increase can be observed across all the northern hemisphere (Coté et al., 2004; Carpio et al., 2020), wild ungulate populations in Mediterranean environments have rocketed in recent decades, especially in high-diversity Mediterranean oak (genus *Quercus*) ecosystems where they are producing a huge biotic stress, hindering natural regeneration of key native oak species (Gill, 1992; San Miguel et al., 1999; Coté et al., 2004; Acevedo et al., 2008, Muñoz et al., 2009, San Miguel et al., 2010, Perea et al., 2014b).

The Mediterranean basin is a highly anthropized landscape with a long history of human occupation, and thus, contain extensive degraded areas due to the excessive exploitation of the land (i.e. intensive agriculture, overgrazing, repeated burning, deforestation, etc). In this context, restoration is a necessary tool to recover ecological processes and functioning but, unfortunately, many restoration works fail due to the challenges of overcoming both, abiotic factors (drought) and biotic conditions (ungulate pressure; Castro et al., 2004; Perea & Gil, 2014a; Rey-Benayas et al., 2015; López-Sánchez et al., 2019). Therefore, new tools for restoration practices are needed based on the understanding of natural regeneration patterns (Castro et al., 2004), and seedling planting plays a key role in forest restoration (Andivia et al., 2021). Plant recruitment is a multi-stage process that can be affected by, both, abiotic and biotic stressors during the demographic process (seed deposition, dispersal, germination and seedling or sapling survival) and thus, a bottleneck on any stage could limit the entire recruitment process. In Mediterranean systems, a critical stage for the regeneration of *Quercus* is the seedling stage (Espelta et al., 1995; Rey-Benayas, 1998; Retana et al., 1999; Lookingbill & Zavala, 2000), as the combined effects of increasing droughts and high ungulate densities is threatening their long-term persistence (Tyler et al., 2006; Perea & Gil, 2014a; López-Sánchez et al., 2016).

Among abiotic stresses, water deficit during the summer drought is responsible for the majority of seedling mortality (Rey-Benayas, 1998; Gómez-Aparicio et al., 2004; Marañón et al., 2005; Pulido & Díaz, 2005; Gómez-Aparicio et al., 2008; Gómez & Hódar, 2008), and drought is expected to increase in the Mediterranean basin (IPCC, 2021). Young seedlings have a poorly developed root systems to reach deep water in the dry period (Matzner et al., 2003; Tyler et al., 2008; López-Sánchez et al., 2019). Many biotic stresses affect oak seedlings including pathogens, herbivores and competition with other plants (Cuesta et al., 2010). Pathogens and insects can affect the above-ground part, such as leaves (Humphrey & Swaine ,1997; Dunning et al., 2002), and ungulates can have severe effects on the fate of seedlings by trampling, browsing or rooting them (Gómez et al., 2003; Perea & Gil, 2014a). Browsing is one of the major factors constraining oak forest regeneration in Mediterranean environments (Pulido & Díaz, 2005; López-Sánchez et al., 2016; Leal et al., 2022). During the periods of food shortage (end of winter and summer), the lack of grass production forces wild ungulates to browse on woody species. In areas of high ungulate densities, an unsustainable level of browsing can be reached. As a consequence, oak seedling growth and development is constrained, suppressing future plant size and reproduction (for re-sprouting oak species; Rooney & Waller, 2003; Gómez & Hódar, 2008; Perea et al., 2015; López-Sánchez et al., 2019) or even causing seedling mortality (for non-sprouting species). Ultimately, by browsing preferentially on palatable species and rejecting other species, large herbivores can alter the floristic composition (Augustine & Naughton, 1998; Perea et al., 2014b), causing biotic homogenization of plant communities, forcing, ultimately, vegetation dynamics towards early successional stages (Hobbs, 1996; Rooney, 2008; Perea et al., 2015a). Other ungulates, such as the wild boar, cause primarily below-ground damage through foraging or rooting, causing great mortality of seedlings (Focardi et al., 2000; Gómez et al., 2003; Pulido & Díaz, 2005; Gómez & Hódar, 2008; Perea & Gil, 2014a). Unlike deer, which consume acorns mostly under the canopy of oak trees, wild boars also consume acorns in areas away from trees (open and shrub areas) and search and forage the buried acorns with a high efficiency (Herrera, 1995; Gómez et al., 2003). When saplings or seedlings are growing, boars can uproot them to consume the acorns and chew the swollen roots, or accidentally, when looking for fungi and other buried food items, leaving the stems, leaves and roots on the soil surface (Herrera, 1995; Focardi et al., 2000; Gómez et al., 2003; Gómez & Hódar, 2008) which eventually cause them to die from dehydration. Presumably, wild boars cause major damages to oak plantations as they uproot seedlings planted in the search for acorns or food (Perea & Gil, 2014a). Active restoration by planting is a particularly suitable tool to increase seedling survival rates (Rey-Benayas, 2005; Andivia et al., 2021), however, in ecosystems with a high density of wild ungulates there is a high failure rate, because the synergistic effect of ungulate browsing and wild boar rooting activity is deadly to the survival of oak seedlings (Perea et al., 2014b).

Some studies have shown that taking into account spatio-temporal variability of different stresses may increase seedling survival (Gómez et al., 2003; Gómez-Aparicio et al., 2005), because the occurrence and intensity of each type of stress may vary with space and time (López-Sánchez et al., 2019). For example, numerous studies have already demonstrated the importance of microsites, such as nurse plants which provide protection against biotic and abiotic factors (Castro et al., 2002; Rey-Benayas et al., 2002; Gómez-Aparicio et al., 2005; Cuesta et al., 2010; Pugnaire et al., 2015; Pelaéz et al., 2019), for instance, by hiding seedlings that go unnoticed (Callaway et al., 2000; Zamora et al., 2004). However, some ungulates such as wild boar, are able to detect the acorn of the seedling very efficiently (Groot Bruinderink et al., 1994; Gómez et al., 2003). When seedlings are older, and the cotyledon reserve is almost depleted, acorn predators became less important (Gómez et al., 2003), so removing the acorn from the seedling may reduce the effect of wild boar stress. However, the effect of removing the acorn attached to the seedling on wild boar damage remains largely unknown (but see Yi et al., 2015). Other artificial methods such as protectors have been shown to be an effective method to reduce the stressors (Reque & Martin, 2015; Oliet et al., 2015) but the costs could increase considerably the plantation budget (Perea & Gil, 2014a). Sometimes, protectors are not removed from the field and may end up as trash in the environment and, thus, the use of recycled materials in restoration applications is encouraged (Urreaga et al., 2020)

Another relevant aspect to take into account in assisted oak regeneration, is the intraspecific differences in seedling survival according to their attributes (Andivia et al., 2021), such as plant age Navarro et al. (2006). For oak species, there are studies that show a higher survival of 1-year-old seedlings for *Quercus ilex* L. and *Quercus suber* L. (González-Rodríguez et al., 2011) or for *Quercus faginea* Lam. (Nicolás-Peragon et al., 2004) when compared to 2-year or seedlings or older. In addition, some authors such as Navarro et al. (2006) and Villar-Salvador et al. (2021) recommend using 1 over 2-year-old seedlings if it is possible to obtain the adequate quality. In contrast, other authors have shown higher survival rates in 2-year-old oak seedlings (Bonfil et al., 2000). In 2-year-old seedlings, the acorn is presumably almost or totally depleted, making the seedlings less attractive to predators (Gómez et al., 2003). Therefore, the damage caused by wild boars can be expected to be less detrimental for 2-year-old compared to 1-year-old seedlings with attached and non-depleted acorns.

The physiological responses of oak seedlings depend on the identity of the plant, therefore differences in growth and physiological parameters are found across species and ages (Chaney & Byrnes, 1993; López-Sánchez et al., 2020). Physiological parameters such as the content of chlorophyll, flavonols, anthocyanins and nitrogen are easy to measure in the field (e.g., using leaf-clip optical devices) and provide useful information about plant status. Chlorophyll is involved in photosynthetic activity (Percival, 2005), flavonols and anthocyanins provide a defence against biotic and abiotic stressors (Chalker-Scott, 1999; Hernández et al., 2004; López-Sánchez et al., 2020) and nitrogen, a major plant food, is an essential constituent of proteins and chlorophyll and plays a very important role in the process of plant metabolism (Leghari et al., 2016).

In summary, this study aims to further enhance our knowledge on oak seedling restoration practices providing direct applications for forest management that would help increase the survival rates on plantations. We used two sites located in Mediterranean National Parks, where wild ungulates (red deer -*Cervus elaphus* L.- and wild boar -*Sus scrofa* L.-) are present in high densities, to investigate: 1) the effect of cotyledon removal (manual acorn extirpation) on the temporal variation of stress agents (biotic and abiotic) for two plant ages (1 and 2-year-old seedlings) and two oak species (*Quercus ilex* in Cabañeros National Park, and *Quercus suber*, in Doñana National Park) and; 2) the effect of cotyledon removal on the physiological parameters of seedlings and their growth during the growing season.

We specifically hypothesised that:

a) seedlings without the acorn attached would display higher survival rates as they will not be so frequently affected by wild boars;
b) higher damage to 1-year-old than 2-year-old seedlings because of the higher acorn nutrient content in 1-year-old seedlings;
c) removing the attached acorn in 2-year-old seedlings will have less effect on seedling survival;
d) removing the acorn attached to the seedling will not affect growth and physiology, being this particularly true for 2-year-old seedlings.

2. MATERIALS AND METHODS

2.1. Study area

The experiment was conducted in two Spanish protected areas with high density of wild ungulates (mainly red deer and wild boar) and characterised for presenting a pronounced summer drought period: 1) Cabañeros National Park (northwest of Ciudad Real) and 2) Doñana National Park (southeast of Huelva). The climate in both parks is Mediterranean with dry and hot summers (Csa, according to Köppen climate classification), but Doñana is considered sub-humid with Atlantic influence that softens temperatures in winter and summer (600 mm average annual rainfall and 17ºC mean temperature; Miteco, 2011) because is located at an altitude of 10 m a.s.l and Cabañeros has cold winters (550 mm of ppt., 12.4ºC of annual mean temperature; García-Herrera et al., 2011) and is located at 700 m a.s.l. The topography of Cabañeros is characterized by a relief of Appalachian type formed by the so-called "Sierras", mountain ranges of quartzite and siliceous slate, and the "Rañas" plains formed by alluvial hillside deposits of clay and quartzite pebbles (García-Herrera et al., 2011). The plantation was carried out in the soils of the "Raña", which have an argillic horizon (Bt, according to USDA) that facilitates the formation of water layers that saturate the soil, causing an extensive hydromorphy that acidifies the soil (Pardo, 1995). The topography of Doñana is the result of coastal-littoral sedimentation processes that have formed a large aeolian landform (Clemente et al., 1998). The soils are poor in organic matter, slightly acidic and consist mainly of sands with some silts and clays, that allow rapid water circulation due to the low retention capacity that hinders the establishment of vegetation (Ordoñez et al., 1998; Moral et al., 2002).

The vegetation in both parks is sclerophyllous. In Cabañeros is dominated by semidecidious oak forests and woodlands composed by *Quercus ilex, Quercus suber, Quercus faginea* and *Quercus pyrenaica* Willd. (Perea & Gil, 2014a), with a typical Mediterranean understory dominated mainly by evergreen shrubs such as *Cistus ladanifer* L., *Erica australis* L., *Erica scoparia* L., *Rosmarinus officinalis* L., *Lavandula pedunculata* (Mill.) Cav., *Thymus mastichina* (L.) L., *Phillyrea angustifolia* L. and *Rubus ulmifolius* Schott. Doñana is dominated by monospecific and mixed forests of *Pinus pinea* L. and *Quercus suber* and large areas of shrubland which are divided into the so-called: "Monte Blanco" an open shrub composed by *Halimium halimifolium* (L.) Willk., *Rosmarinus officinalis*, *Ulex australis* Clemente, *Cistus libanotis* L., *Lavandula stoechas* L. and *Thymus mastichina* and; "Monte Negro" a dense and impenetrable thicket in areas where soil moisture is much higher, and composed mostly of *Erica scoparia*, *Erica umbellata* Loefl. ex L., *Erica ciliaris* Loefl. ex L, *Calluna vulgaris* (L.) Hull, *Rubus ulmifolius*, *Phillyrea angustifolia*, *Pistacea lentiscus* L., *Cytisus grandiflorus* (Brot.) DC., *Daphne gnidium* L., *Osyris alba* L. and *Ulex minor* Roth (Valdés et al., 2007)

The main wild ungulates in both national parks are the wild boar (*Sus scrofa*) and the red deer (*Cervus elaphus*; Perea & Gil, 2014a; Gortázar et al., 2008). In Cabañeros, current densities of red deer are 31 individuals per km^2 and for wild boar 2.5 per km^2 (Linares & Urivelarrea, 2020), and in Doñana for red deer are 6 per km^2 and for wild board 5 per km^2 (Laguna, 2015), although the ungulate pressure may strongly vary in space and time. In both parks, the regeneration processes of the main oak species are in decline due to high pressure from wild ungulates (Perea & Gil, 2014a; Fedriani et al., 2016). Red deer browse more intensely in winter (December–March) and summer (July–September) when no alternative high-quality food is available (green grass or acorns; Perea et al., 2014b). Therefore, evergreen shrubs constitute an important and permanent source of medium-low quality food for herbivores (San Miguel et al., 1999; Perea & Gil, 2014a). Wild boars search for acorns more intensively from November onwards, when acorns on the soil are depleted, they forage the buried acorns killing the seedlings by uprooting them (Herrera, 1995; Gómez & Hódar, 2008; Focardi et al., 2000). Currently, in both national parks, red deer and wild boar are controlled mainly by capturing the animals alive to reduce populations as they lack natural predators (Perea & Gil, 2014a; Linares & Urivelarrea, 2020). Abundances of lagomorphs (rabbit - *Oryctolagus cuniculus* L.- and hare – *Lepus granatensis* Rosenhauer-) for Doñana are 2.5 individuals per km (Beltran et al., 2022) and in Cabañeros density is unknown but presumably low throughout the park (Delibes-Mateos et al., 2008; Ferreras et al., 2021)

2.2. Experimental design

In both parks, the experiments were located in areas of shrubland with low density of trees. More specifically, in Cabañeros, the plantation was carried out in “Las Canalejas” (39°21'14.5"N, 4°26'05.4"W), a transition zone between the dehesa and the high mountain zones, and in Doñana on “Monte Blanco” in the area of “La Rocina” (37°07'36.3"N, 6°30'33.6"W). In Cabañeros, an old rectangular wild ungulate exclusion fence created to enhance the rabbit's habitat (year 2016), was selected to perform the plantation outside. We used photo-trapping (Browning motion detection cameras, Model BTC-5PXD) to ensure the presence-absence of herbivores outside and inside the exclosures (3 cameras inside and 3 outside each national park). Rabbit population is low or extinct in the plots located at Cabañeros (photo-trapping) and thus, we used the exclosure (with no herbivores) as control (see Figure 1). In Doñana, there was no control treatment as there were rabbits in the fenced area.

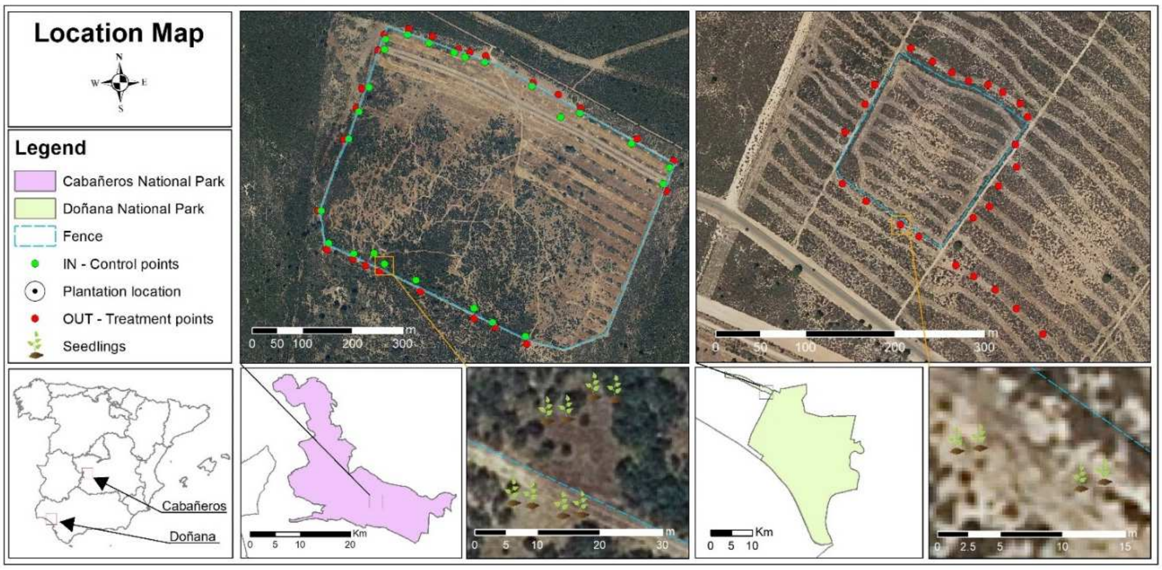

Figure 1: Experiment design with the location of the sites (left Cabañeros National Park, right Doñana National Park) and the points where the seedlings were planted. The wild ungulate exclusion fence used to carry out the treatments is shown. A total of 200 *Quercus ilex* seedlings were planted in Cabañeros (25 points x 2 acorn treatments x 2 ages x 2 fenced sites) and 100 *Quercus suber* seedlings in Doñana (25 points x 2 ages x 2 acorn treatments).

In each park, 25 plantation locations (points) around the fenced area (non-fenced treatment) were selected at a minimum distance of 20 m from each other. Two different treatments were identified at each point: *i)* seedlings with acorn attached; *ii)* seedlings without acorn (cotyledon removal). For each treatment (with and without acorn), a pair of 1 and 2-year-old seedlings was planted at a distance of 50 cm from each other. In Cabañeros, it was repeated inside the fence as a control plot against ungulate herbivory (fenced treatment) but not in Doñana (no control treatment). In February 2021, we planted 200 seedlings in Cabañeros (25 points x 2 acorn treatments x 2 ages x 2 fenced sites) of *Quercus ilex* from the National Park nursery, and in Doñana 100 seedlings of *Quercus suber* (25 points x 2 acorn treatments x 2 ages) from the Seville Council plant nursery (Junta de Andalucía). We selected pairs of seedlings of similar height, diameter and number of leaves.

At plantation, seedlings were labelled and their GPS coordinates recorded. Monthly revisions were carried out from February to September (end of the experiment). Each visit consisted of recording: the seedling survival (alive vs. dead), the damage caused by the different biotic and abiotic stress agents (wild boar, deer, rabbit, fungi, insect or drought) and the main stress agent. Wild boar damage was assigned when we found over-turned soil from rooting and plants were dug out or uprooted (Herrera, 1995; Perea & Gil, 2014a; see Figure 2a, 2b), by deer when the twigs were torn by browsing (Pierson & Decalesta, 2015), per rabbit when the terminal shoots were clip in angled from the seedlings (Dolbeer et al., 1994; Taylor II, 2011; see Figure 2c), by fungi when visual symptoms (stems lesions, foliar chlorosis and wilting) were observed (Linaldeddu et al., 2009; see Figure 2d), by insects when leaves were defoliated or nests were present (Humphrey & Swaine, 1997; Dunning et al., 2002) and for drought when the leaves showed signs of dehydration. Seedling damage can be quantified, as an indicator of potential stress, using a damage score from 0 to 5: 0 = No evidence of damage; 1 = Light (<10% of leaves damaged); 2 = Low (10%–30% of leaves damaged); 3 = Intense (30%–60% of leaves damaged); 4 = Heavy (60%–90% of the leaves); and 5 = Maximum damage (>90% of leaves damaged; Fernandez-Olalla et al., 2006; Perea et al., 2015). To assign the main stress agent we chose the stress that caused the death of the plant or, in the case of seedlings that survived, the stress with the highest intensity (range 0 to 5) of the last visit (September).

Figure 2: A) wild boar damage (rooting activity) in Cabañeros National Park and, B) in Doñana National Park. C) Rabbit damage (stemp clip in angled). D) Fungi damage (genus Mycosphaerella).

To analyze and compare seedling growth and physiological responses to different treatments, and plant ages, several non-destructive measures of chlorophyll, anthocyanin and flavonoid concentrations and the nitrogen balance index (NBI) were obtained from five leaves of each seedling. We used a fluorescence sensor Dualex ® TMScientific+, Force One CNRS-Lure, France, with a leaf clamp sensor (Agati et al., 2016; López-Sánchez et al., 2020). The nitrogen balance index (NBI) is the ratio between the chlorophyll and flavonols (related to nitrogen/carbon allocation) that can be used as a proxy of nitrogen status in plants (Cartelat et al., 2005; Agati et al., 2016; Padilla et al. 2016; López-Sánchez et al., 2020). The Dualex measures the chlorophyll content of the leaf using a transmittance ratio at two different wavelengths, and the flavonoid and anthocyanin content of the leaf epidermis using a differential chlorophyll fluorescence ratio (ForceA-Dualex). Measurements were taken on old leaves (post-planting growth) previously marked, from top to bottom and taking 5 leaves. In addition, to analyse the physical growth, the number of shoots and the length of each shoot (mm) were measured to obtain the growth (total sum of the length of each shoot for each seedling). We only measured alive seedlings.

2.3. Data analysis

To analyze differences in seedling survival according to the different treatments: acorn (with acorn *vs* no acorn), age (1 *vs* 2 years), fenced (inside *vs* outside), we used Cox proportional hazard models (Cox, 1972). Response variable was time at death (in months). Seedling survival was also included in the model codified as 0 if the seedling was alive at the end of the study (censored data) and 1 if it was dead (event). Predictors were Acorn, Age, Fenced and their two-way interactions (note that Doñana analysis did not include the predictor Fenced as all seedlings were located outside the fence). We also included the location point of each seedling (n=25) as cluster in the model to group correlated observations. We used the function "Coxph" from the R survival package to calculate the estimated time at death for each treatment and to disentangle differences in predicted time at death among treatments. We also used the function "survfit" and "ggsurvplot" from the library "survminer" to generate the figures. We used full model results to compare differences among predictors and obtain significance levels.

Similarly, we used multinomial models to assess probability of stress agent occurrence. The response variable was main stress (a multinomial factor) while predictors were Time (month of main stress occurrence), Acorn, Age, Fenced and their two-way interactions. The location point of each seedling (n=25) was also included as cluster in the model. We used the function multinom from the package "nnet". We also used the function "mnl_pred_ova" and "mnl_fd_ova" from the package "MNLpred" to graphically represent model predictions. We performed model selection based on AIC and selected all models with DeltaAIC<2 and averaged the model coefficients.

In addition, to evaluate and compare differences in physiological variables (chlorophyll, flavonol, anthocyanins and the NBI) and the physical growth (total sum of shoots in cm) depending on treatments, seedling age and acorn attached, we obtained the mean of each parameter for all seedlings and for each month. All analysis were performed using the R 4.1.2 software (www.r-project.org).

3. RESULTS

3.1. Climatic conditions in the study period compared to the average of the period 2000-2020

During the study period (Feb-Sep), the accumulated precipitation in Cabañeros was 210 mm, 4% less than the average of the last 20 years (2000-2020), and in Doñana 174 mm, 34% less than the average. From plantation (Feb) to the onset of summer drought (beginning of May), the accumulated precipitation in Cabañeros was 129 mm, 2.4% more than the average (rainfall accumulated mainly in April), and in Doñana 110 mm, approximately 40% less than the average. During the month of May, precipitation in Cabañeros was 11 mm, 70% less than average, and in Doñana 10 mm, 63% less than average (see Figure S1).

3.2. Oak seedling survival analysis

In Cabañeros National Park, results of the proportional hazard model analysis revealed no significant differences in seedling time at death between any of the treatments once the experiment finished. Indeed, no significant differences were found between the fenced treatment and the non-fenced as seedling survival by September was low (9 seedlings; 4.5% of the total). Furthermore, seedling survival depending on plant age revealed no significant differences both outside and inside the exclusion fence at the end of the experiment ($p>0.05$, respectively). Similarly, seedling survival depending on the plant with and without the acorn attached was non-significant at the end of the experiment ($p>0.05$, respectively).

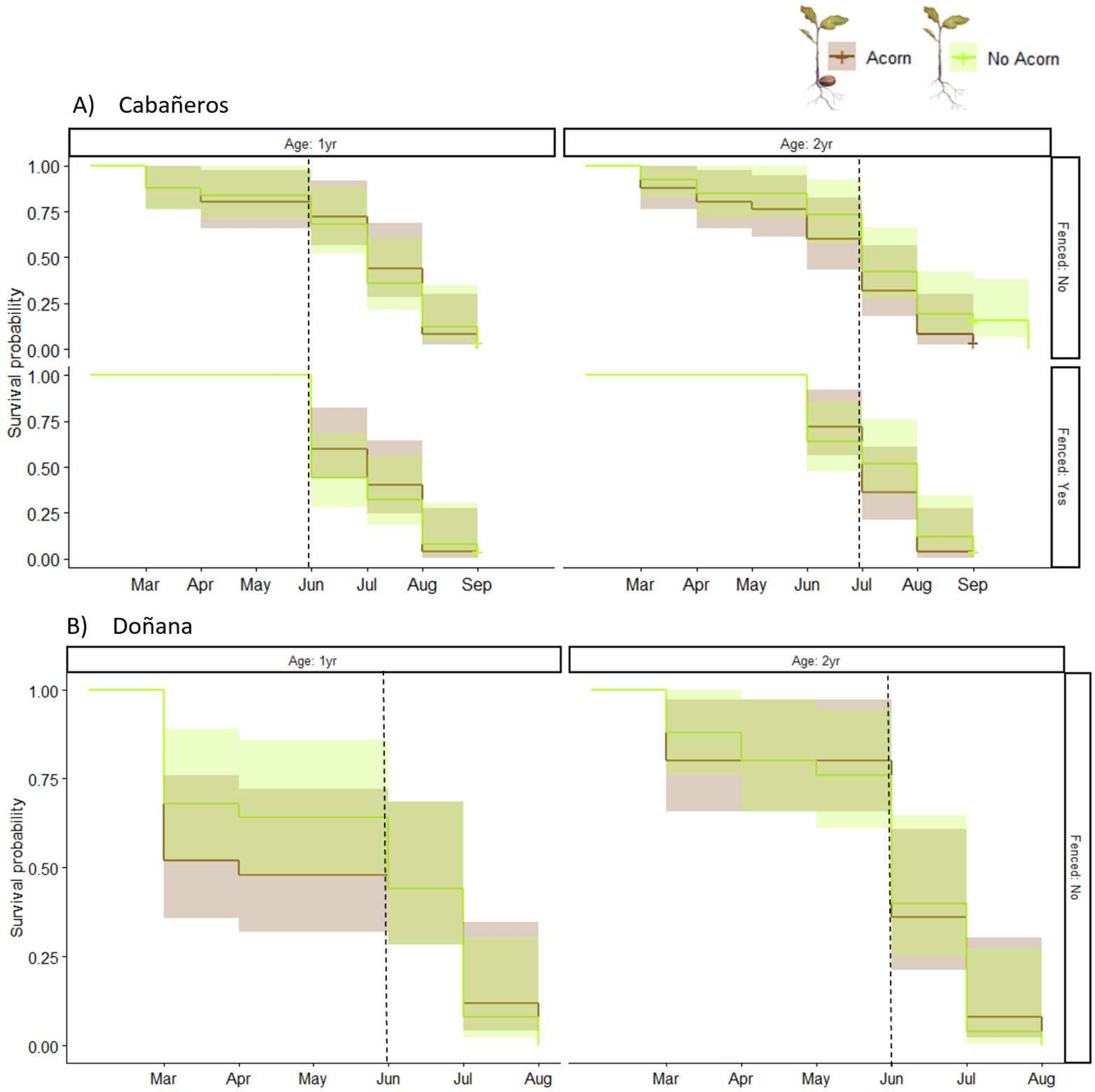

Figure 3: Survival probability of seedlings depending on Time (in months) with and without acorns according to plant Age (1-year-old vs 2-years-old) and Fenced (Fenced vs non fenced). A) Results observed in Cabañeros National Park and B) in Doñana National Park. Dashed horizontal line divides the period before and after the onset of the summer drought.

However, as Figure 3a shows, some temporal variation on seedling survival can be observed within the first months of the study (February to May, see also Table S1). For this reason, we repeated the analysis using a subset of data from February to May.

As expected, Cabañeros NP results suggested a significant increase in seedling survival inside the fence for all combinations of plant age and acorn treatment (Figure S2). Furthermore, no seedling died inside the fence during that period while there was a decrease in survival outside. In addition, no significant differences between plant Age or Acorn treatment were found outside or inside the fence. However, as indicated by the model coefficient sign, a non-significant decreased in survival probability was found on seedlings with acorns when compared to their same-age pair without acorn (P=0.578 and P=0.435, respectively for 1-year-old seedlings and 2-years-old; See Table S2).

In Doñana NP (non-fenced treatment), results were in accordance with those obtained in Cabañeros. Cox proportional hazard models showed no significant differences at the end of the study for any of the treatments (Age or Acorn; Figure 3b). However, when only using data before the onset of the dry season (i.e. from February till May), seedling survival according to plant age revealed that 1-year-old seedlings survival was significantly reduced compared to 2-years-old ($p<0.05$; Figure S3). Furthermore, within the 1-year-old group, a non-significant decrease in survival probability for the plants with acorns was observed (P= 0.25; Table S3) but not for the 2-years-old group (P=0.81; See Table S3).

3.3. Stress agents and their effect on oak seedling survival

In Cabañeros National Park, all seedlings (N=200; 100%) experienced some or all stress agents. Six per cent of seedlings experienced one, 33% two, 49% three and 12% four or more stress agents. For the non-fenced treatment (N=100), 73% were affected mainly by drought, 21% by wild boar damage, 2% by unknown causes, 2% due the post-planting causes, 1% by fungal pathogens and 1% by lagomorphs (hare/rabbit) herbivory. When wild boar was the main stress, no seedling survived and when drought was the main stress, only 4% of seedlings survived. Damage caused by fungi pathogens was not strong enough to cause the death of any seedling. Instead, when rabbit was the mains stress, no seedling survived. Insect damage was not severe enough to be the main stress agent for any seedling, even though the damage caused in some seedlings was severe with 50% of the leaves showing damage. At the end of the experiment, 95 % of all seedlings died. Drought was the main cause of mortality (69%), followed by wild board damage (21%), unknown causes (2%), post-plantation causes (2%) and rabbit (1%). In the case of the fenced treatment, 99% were affected mainly by drought, which killed all affected seedlings, and 1% by fungi but the affected seedling survived. In Doñana NP (non-fenced), all seedlings (N=100, 100%) experienced one (29%), two (10%), three (26%), four (30%) or five stress agents (5%). Around 68% of seedlings were affected mainly by drought, 31% by wild boar damage and 1% by accidental car damage. The damage caused by the stress agents was so strong that no seedlings survived at the end of the experiment.

3.4. Spatio-temporal variation in the appearance of stress agents and the role of the plant age

As expected, we found a strong temporal variation in the occurrence of the main stress in Cabañeros National Park. Results from the multinomial model suggested that main variables affecting the seedling was Time, Fenced and Age (model averaged results based on DeltaAIC<2). Thus, at spatial level, we found that drought was the only cause of mortality inside the fence, while many other factors influenced mortality outside, being wild boar and drought the main causes of mortality (Figure 4). In addition, these factors also had a marked temporal pattern, being the risk of mortality by wild boar higher at the beginning of the study (February and April) while drought was the main factor after the onset of the dry season (from June till September).

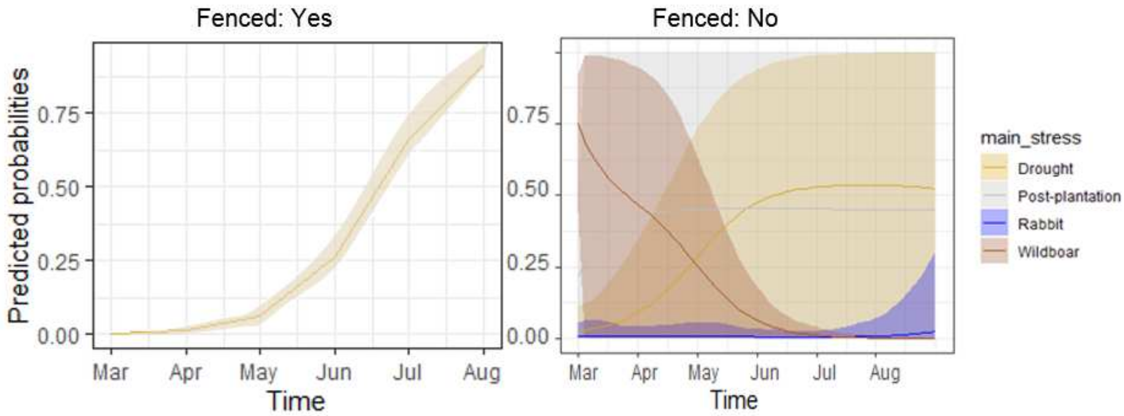

Figure 4: Results from the multinomial model to analyze the temporal variation of main stress occurrence inside and outside the fence in Cabañeros National Park

Finally, although seedling age was an important factor in the model, the differences in stress probability between 1 and 2-year-old seedlings were non-significant.

In Doñana NP, we also found a clear temporal pattern on the main stress occurrence outside the fence. Model selection indicated that the variables Time and Age were key to predict the main stress probability. Therefore, wild boar was, by far, the main stress agent at the beginning of the study. Wild boars were particularly active from February to April, reducing their rooting activity from May onwards (Figure 5). Indeed, wild boar killed up to 38% of all 1-year-old seedlings and 18% of 2-years-old during the first two months of study. On the contrary, drought dramatically increased from June onwards, being the main cause of mortality. Human driven seedling mortality (cars) did not have a temporal pattern, being an occasional cause of mortality (just two plants).

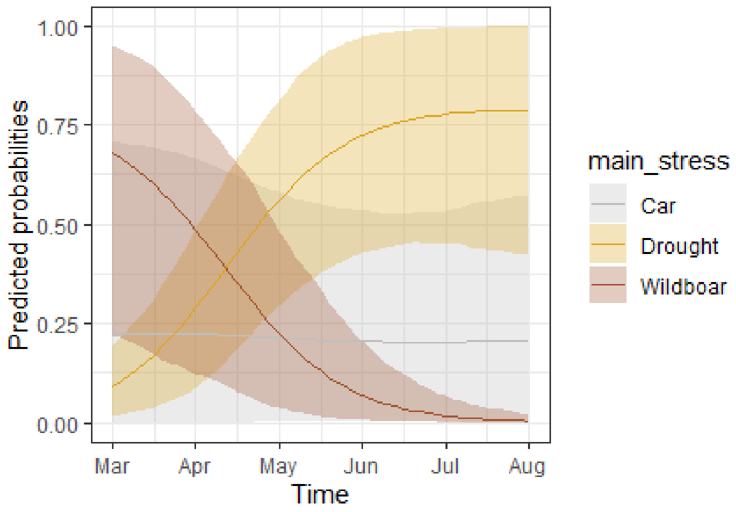

Figure 5: Predicted variation of main stress probability through time in Doñana National Park.

In addition, we found that seedlings age also played an important role in determining the probability or risk of being killed. One year-old seedlings faced an increased risk of being uprooted by wild boar when compared to 2-year-old seedlings. On the other hand, 2-year-old seedlings suffered more from summer drought (Figure 6).

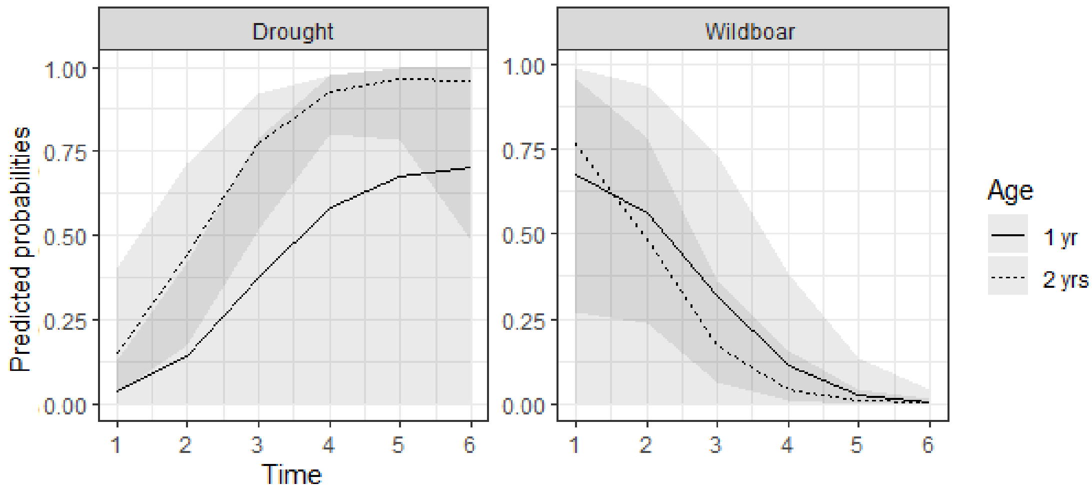

Figure 6: Time dependent main stress probabilities for each seedling age class in Doñana National Park. The time represents each month where 1 is February and 6 is July.

3.5. Seedling growth

In Cabañeros NP, we observed that seedling inside the fenced (Figure 7a) grew slightly more than outside the fence (growth is defined as total shoot length; Figure 7b). Regarding the age of the seedlings, it was observed that 2-year-old seedlings grew considerably more than the 1-year-old. In addition, we observed that the effect of removing the acorn attached to the seedling did not affect seedling growth. In Doñana, the seedlings suffered a first loss of leaves due to post-sowing stress, therefore, during the months of March and April seedling grew in a wide range, so the values do not show any difference between treatments, nor plant age, or in the effect of acorn removal (Figure 7c).

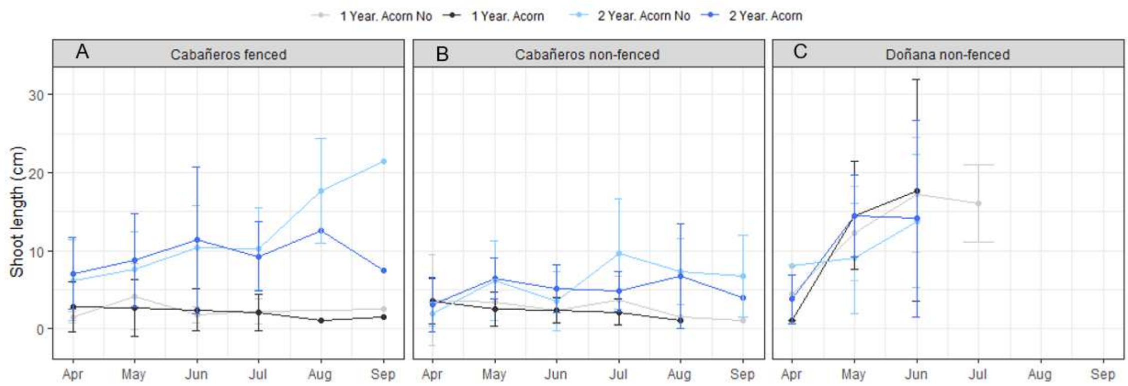

Figure 7: Line graph with mean total shoot length (in cm) representing the longitudinal growth of seedlings. Seedlings of 1 and 2 years (Age) with and without the acorns attached (Acorn treatment) are shown. Above is the length of the shoots and below the months. In addition, the standard deviation of the data set is shown.

3.6. Physiological parameters depending on plant age, acorn treatment and oak species.

In both National Parks, we found differences in the physiological parameters across the plant age. Instead, no differences were found for the acorn removal treatment. In Cabañeros, for *Quercus ilex*, 2-year-old seedlings showed higher content of chlorophyll, NBI and flavonols (Figure 8a,b,d,e,g,h) that 1-year-old, contrary to anthocyanins (Figure 8j,k) that showed a lower content in 2-year-old seedlings. Conversely, in Doñana, 1-year-old seedlings showed higher content of chlorophyll, NBI and flavonol (Figure 8c,f,i) and lower content of anthocyanins (Figure 8l) than 2-year-old seedlings. We also observed, slightly in Doñana and strongly in Cabañeros, a clear decrease in the content of chlorophyll and NBI over time and while seedlings were dying.

In contrast, anthocyanins showed an increase over time. Flavonol content shows both increasing and decreasing values over time and therefore did not show a clear pattern.

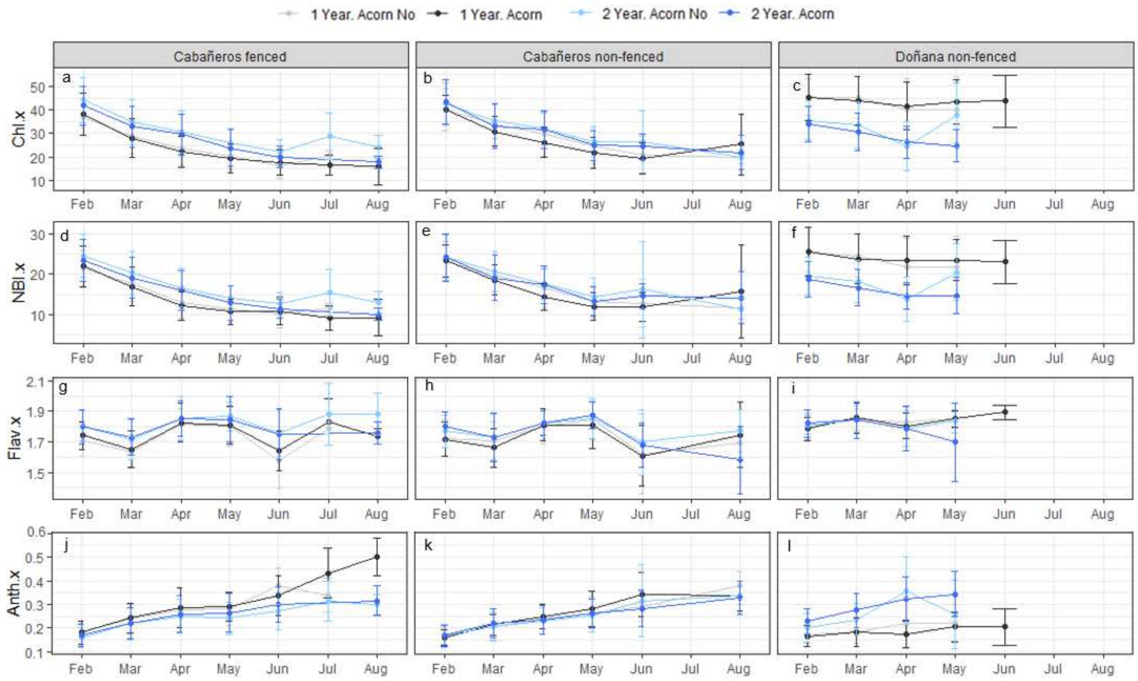

Figure 8: Average values of physiological parameters of chlorophyll content, flavonoid content, anthocyanin content and nitrogen balance index (NBI) for 1 and 2-year-old seedlings (age) and if they have the acorn attached or not (treatment). The standard deviation for the data set is shown.

4. Discussion

Our results show a strong temporal variation in the occurrence of the different stress agents that influences the establishment of *Quercus* seedlings. Thus, we found that different biotic and abiotic stress agents occur at different time of the year and may affect seedling differently depending on their individual characteristics (i.e. age) or treatment (i.e. with or without the acorns). Furthermore, this temporal dimension also determined the dominance, intensity and lethality of each stress agent, as also reported by López-Sánchez et al. (2019). Therefore, when performing forest restoration, such as seedling plantations to assist natural regeneration, we should evaluate first the main abiotic and biotic factors present in our site but also the time-dependent effect of each factor in order to increase the success of the plantation (Gómez-Aparicio et al., 2012). For example, in the context of global change, drought is clearly the main cause of seedling mortality in Mediterranean plantations (Rey-Benayas, 1998; Gómez-Aparicio et al., 2008; Perea & Gil, 2014a; López-Sánchez et al., 2019). However, many of the oak seedlings will have no chance to survive the dry period if they are previously damaged by other biotic stresses (i.e. insect, fungi or wild boars). Therefore, a first target just after plantation is to reduce the damage caused by these biotic factors (mainly wild boars) and thus, increase the number of seedlings that reach early summer.

4.1. Effect of acorn removal on the survival of oak seedling according to their age

The study period (February to September) was very dry compared to the average of the last twenty years, causing the death of most seedlings due to drought (summer period). Therefore, at the end of the experiment, in both National Parks, no significant differences were observed between the treatments (fenced and non-fenced, 1 vs 2-years-old or with and without acorn). Nevertheless, if the hydrological year had been wetter, significant differences between some treatments could have been found as seedling survival within the first months of the study differed between treatments.

During early spring, as water deficit is usually not a limiting factor, we found that biotic factors, mainly wild boar activity, strongly affected seedling survival. This biotic stress was highly lethal as no seedlings survived just after being damaged. Therefore, in Cabañeros, we found differences between fenced and non-fenced treatments, which show higher probability of seedling survival inside the areas protected from ungulates. However, as wild boar uprooted only <20% of the seedlings outside the fence, no differences were found for all combinations of plant age (1 and 2-year-old) and acorn treatment (acorn attached or not). This may be due to the fact that the soils in Cabañeros are rocky and highly clayey and when the water level is low they become hard and difficult for wild boar to root. Nevertheless, we still could detect a slight pattern of lower survival probability for same age seedlings with acorn attached compared to those without acorn, although these differences were not significant. On the contrary, wild boar rooting activity in Doñana was much higher, damaging almost 38% of seedlings during the first months after plantation. We believe this increase damage is due to the higher wild boar densities in Doñana but also due to the decreased soil resistance of sands, which allows wild boar to overturn extensive areas with minimum effort (Fern et al., 2020). Hence, outside the fence in Doñana, we found significant differences in mortality according to plant age (i.e. 1-year-old oak seedlings survival was lower than 2-year-old). Furthermore, we could also observe a non-significant increase in the survival of 1-year-old seedlings without the acorn attached compared to those with acorn, suggesting a positive effect of acorn removal on the survival of seedlings against wild boar activity. However, further studies, with greater sample size, are probably needed to confirm these preliminary results.

Thus, results agree with our main hypothesis suggesting that wild boar are actively searching for acorns during early spring, focusing mostly on those with a nutrient-filled acorn attached. As seedlings age (from 1-yr to 2-yrs), the acorn would become rotten and thus, less nutritious (Gómez et al., 2003) and therefore, less attractive to wild boar. Indeed, Doñana NP results confirmed this preference of wild boar, uprooting 1-year-old seedlings more often than 2-years-old. However, while this pattern was clear and significant in Doñana NP, it was not found for Cabañeros. Other authors have suggested that the odour of freshly overturned or disturbed soil around the seedlings may represent an attractant for the boars to investigate each plantation spot (Mayer et al. 2000). We believe this could be true in some cases but, in this study, we observed a more selective pattern. In fact, Mayer et al. (2000) found that wild boar did not randomly uproot all nine species used in the plantation. On the contrary, only four of the nine species used were affected by wild boar, being two of them oak species. Thus, we strongly believe that in their search, wild boar are showing their preferences for some species that they feed on, although sometimes they may also investigate disturbed soils. According to Fern et al. (2020), a factor that could explain this variation is that wild boars attack plantations more often when they know that the planted seedlings are a good source of food but, if they do not recognize the species, the damage became less severe. Furthermore, we observed a hierarchical pattern of wild boar preferring 1-year-old seedlings vs. 2-years-old but, within the same age group, we could perceive a weak but consistent preference towards those with the acorn still attached. It is known that acorns decompose and give off an odour that can attract animals (Colville et al., 2012; Löf et al., 2019) and they may be able to identify those seedlings with acorns. However, this seems to be less relevant than age. Thus, we agree with Fern et al. (2020) who suggests that a combination of ecological factors may interplay in the decision of wild boar to uproot specific seedling individuals inside plantations. Therefore, it is necessary to understand better the behaviour of wild boars in forest plantations due to the huge damage they can inflict. Unfortunately, sample size (25 plants per treatment; age x acorn) did not allow us to draw stronger conclusions or obtain significant results on some of the interactions between variables (age and acorn). We believe further experiments should increase sample size to allow drawing much stronger conclusions.

Nevertheless, we found that removing the cotyledons reserve (acorn) did not affect the physiology of the seedlings (i.e. content of chlorophyll, NBI, flavonols and anthocyanin) neither their growth (i.e. shoot length) as differences in mean value for each treatment were not significant. Indeed, these results were in line with those of Yi et al. (2015), who also demonstrated that the removal of the cotyledon reserves from the seedling does not affect their growth or survival. Thus, although the evidence of a positive effect of removing the acorn in ungulate dominated environments is still weak, it seems that, at least, it might not be detrimental.

4.2. Temporal variation on stress agent occurrence and management implications

In both National Parks, we found a large temporal variation in the occurrence of stress agents. In fact, seedling survival was highly dependent on temporal variation, as other results confirm (López-Sánchez et al., 2019; Perea & Gil, 2014a). We revealed a common temporal pattern in Cabañeros and Doñana, in which the risk of being damaged by wild board is highest between February and April. From May onwards, wild boars reduce their rooting activity and drought become the main cause of mortality after the onset of the dry season (May till September).

As we observed in this study, wild boar produces below-ground damage while foraging or rooting. When uprooting the seedling and exposing the roots to the air and sunlight, the plant desiccates in just a couple of days. Indeed, previous research studies have also shown wild boar to be a very important cause of oak seedling mortality in plantations (Fern et al., 2020; Focardi et al., 2000; Mayer et al., 2000; Gómez et al., 2003; Pulido & Díaz, 2005; Gómez & Hódar, 2008; Perea & Gil, 2014a). In most cases, wild boar damage was detected because of the exposure of overturned soil at the location of the seedling. In addition, we typically found the uprooted seedling in the vicinity, with the root masticated or the acorn removed, as has been also found by Fern et al. (2020).

Similar results were obtained by Perea & Gil (2014a) who showed that rooting activity was higher in spring than in summer and also by Fern et al. (2020) who found that 74% of the documented wild boar damage to seedlings occurred during spring. Perea & Gil (2014a) explained this variation by the fact that in late winter and early spring soils contained more water and are softer and easier to dig up by boars than in summer, and also buried seeds are easier to detect olfactorily when they have higher water content while, in the summer of Mediterranean environments, acorns are desiccated. However, Fern et al. (2020) found that even though the year was dry, wild boars continued to root in sand and silt areas, as they are easy to overturn. However, in Doñana, we did see a decrease in rooting activity even though sandy soils offer littler resistant to rooting during the summer so, maybe, the hypothesis that acorns are desiccated in summer might have more relevance in this context.

Our results are in accordance with previous research (Herrera, 1995; Focardi et al., 2000; Gómez et al., 2003; Pulido & Díaz, 2005; Gómez & Hódar, 2008; Perea & Gil 2014a; Fern et al., 2020), which have found that wild boar starts searching for surface acorn from late autumn (acorn fall), mainly due to the availability of food on the surface. When the reserves of acorns on the ground are depleted (normally late winter or early spring) and the soil is still moist, wild boars search for buried acorns, roots and other food resources (rooting activity), causing the death of established seedlings.

We observed that fencing was the best method to increase survival of the seedlings during the first months after the plantation (Feberuary to May). In Cabañeros, we found significant differences between fenced and non-fenced treatments, showing much higher probability of seedling survival inside the areas protected from ungulates. However, this may be a high impact infrastructure that some National Parks would rather avoid. To reduce wild boar predation or damage without fencing, previous studies have found that rooting activity is higher in the forest and open sites than in shrublands where the recruitment rate increases (Gómez et al., 2003; Gómez & Hódar, 2008) and that acorns buried alone under dense heath scrub are more likely to survive predation (Herrera, 1995). Other authors have demonstrated the importance of microsites, such as nurse plants which provide protection against biotic and abiotic factors (Castro et al., 2002; Rey-Benayas et al., 2002; Gómez-Aparicio et al., 2005; Zamora et al., 2004; Cuesta et al., 2010; Pugnaire et al., 2015; Pelaéz et al., 2019). Therefore, it would be useful to study more in depth which shrubs might facilitate the survival of seedlings in order to reduce wild boar damage in the plantations while also improving the deleterious effect of abiotic factors such as drought. For instance, Perea and Gil (2014a) demonstrated that spiny and intricated shrubs such as *Rubus* spp. work better than other shrubs against wild boar rooting activity for *Quercus pyrenaica* seedlings.

In both National Parks, water stress (drought period in summer) was the most frequent stress agent that caused the highest mortality of seedlings. The intensity of this stress increased progressively from May until September producing a peak of seedling mortality during the month of June and July. In Mediterranean environments, water deficit has been described as one of the most important limiting factors for the establishment of oak trees (López-Sánchez et al., 2019), being the summer drought one of the main causes of unsuccessful reforestations (Rey et al., 2009; González-Rodríguez et al., 2011). Furthermore, in both National parks, during the study period, rainfall has been lower than the average of the last 20 years; especially in the months prior to the drought season (i.e. average rainfall during May was reduced by 60-70%). Also, in both parks the availability of water in summer is very low and the seedlings end up dying due to drought, since in Doñana the sandy soils hardly retain water, and in Cabañeros the clay soils dry out and become impenetrable for the roots of the seedlings. Thus, water deficit during this experiment has been above average due to the exceptionally dry hydrological year, being a key limiting environmental factor for the survival of the seedlings.

Currently, water stress is a very difficult abiotic factor to overcome, and droughts are expected to increase in the Mediterranean basin due to climate change (IPCC, 2021). In addition, Gómez-Aparicio et al. (2008), found that climate change can have a double negative impact on *Quercus* regeneration, as extremely dry years reduce the probability of seedling survival and cancel out the positive effects of the (increasingly more infrequent) wet years. To enable seedlings to resist water stress during the summer, some authors recommend applying irrigation during the peak of the dry season to increase survival rates (Rey-Benayas, 1998; Siles et al., 2010). Other authors recommended applying drought hardening in the nursery to gain drought tolerance and transplanting performance in some oak species (for holm oak see Villar-Salvador et al., 2004). An experimental study of the benefits of irrigation during summer on seedlings survival (at least twice during the whole summer) should be also performed to quantify the benefits of this treatment in ungulate-dominated areas. However, further studies should investigate if irrigation the seedlings may have a trade-off in high ungulate abundance sites, as irrigation during the summer could re-hydrate the acorn, making it easier for wild boar to detect it olfactorily (Perea & Gil, 2014a).

Surprisingly, the incidence and damage caused by deer browsing was quite low and not strong enough to be the main stress agent for any seedlings, despite the high density in both National Parks. As Perea & Gil (2014b) and López-Sánchez et al. (2019) pointed out, deer browsing should be higher and occur earlier in drier year (as in the study period) compared to wet years, because forage quality and quantity is lower in a drier year. However, our study year was exceptionally dry and the seedlings were already dry at the beginning of the summer and thus, not available for browsing due to their low palatability when dead. Finally, other studies suggest that deer may also browse more on saplings and shrubs than seedlings (López-Sánchez et al., 2014; Perea et al., 2016), although we could not assess the role of seedling age on browsing damage as deer were not the main stress of any plant.

Finally, fungi and insect damage was not severe enough to cause the death of any seedling, although insect damage was severe for some seedlings (>50% of the leaves damaged). Furthermore, other authors have pointed out that insect damage may affect the growth of oak seedlings and consequently their competitive ability (Wright et al., 1989; López-Sánchez et al., 2019). In addition, pathogens and insects affecting an oak plant, along with other stress agents such as herbivory and water stress, may accelerate and promote plant death (Ghanbary et al., 2021). In environments where drought is the main stress agent, insect and fungal damage becomes less notable, but may still affect oak seedlings and cause their death together with other stress agents.

Finally, we observed that when the seedlings are under stress, their physiology changes, as chlorophyll and nitrogen decrease over time, while anthocyanins increase. This is because when seedlings feel any stress they increase the concentration of anthocyanins to defend and enhance their resistance capacity, as has been the case in other studies (Chalker-Scott, 1999; Ghanbary et al., 2021).

4.3. Conclusions

This study highlights the role of wild boar as a limiting agent for oak recruitment and shows the impact that it might inflict on the management of forest plantations. As a conclusion, we have seen that a combination of ecological factors influences the impact of wild boar damage in plantations, so it is necessary to better understand the behaviour of wild boars in forest plantations.

Therefore, we proved that 2-year-old seedlings have a lower probability of being damaged by wild boar than 1-year-old seedlings in an area of sandy soils such as Doñana NP. To the best of our knowledge, no previous research has obtained these results. In addition, we suggest a possible benefit from removing the acorn attached, particularly from 1-year-old seedlings. Thus, removing the cotyledon reserves from 1-year-old seedling could be a good strategy in assisted oak plantations in areas with high densities of wild boar, as they will be less damaged. Similarly, planting 2-years-old seedlings instead of one-year-old seedlings could be a good strategy to avoid damage by wild boar, at least, in sandy soils.

Unfortunately, sample size was one of the main limitations of our study and further experimental plantations should put more effort into planting higher number of seedlings per treatment (> 50 plants), as wild boar seems to be attracted not only by the acorn odour but also by the plant age and, potentially, by the soil being removed.

ACKNOWLEDGEMENTS

I am enormously grateful to Marta Peláez for her hard work, support, perseverance and trust in me, as well as for guiding me and walking by my side during the year and a half that this project has taken. Thanks to all her time and affection, I have been able to have the opportunity to carry out this work that means a lot to me. Thank you for being an exemplary and exceptional academic tutor.

I would also like to thank Ramón Perea for all the knowledge he has passed on to me, inspiring me, advising me, and giving me the opportunity to work with him and his team on his research projects in National Parks.

To my family and especially to my father, for his love and constant support throughout my university years.

Finally, I thank my field colleagues Daniel, Carlota, Pedro and Carmen, for all the hours working and having good times in the national parks, and J.M. Fedriani (CSIC) for plant transportation and advice.

Both my directors and I would like to thank the personnel of Cabañeros and Doñana National Park for all the support provided during these months of study. We also acknowledge the logistic and technical support provided by ICTS-RBD in Doñana National Park and from the Director and rangers of Cabañeros National Park. We also thank the supply of oak seedlings to the Seville Council plant nursery (Junta de Andalucia) and Cabañeros plant nursery. This work was funded by the Spanish Ministry of Ecological Transition through the HEREGE project (2020/8). MP acknowledges the support of "Margarita Salas postdoctoral Fellowship" from the Ministerio de Universidades - UPM funded by the European Union NextGenerationEU.

5. REFERENCES

Acevedo, P., Ruiz-Fons, F., Vicente, J., Reyes-García, A. R., Alzaga, V., & Gortázar, C. (2008). Estimating red deer abundance in a wide range of management situations in Mediterranean habitats. Journal of Zoology, 276(1), 37-47. https://doi.org/10.1111/j.1469-7998.2008.00464.x

Agati, G., Tuccio, L., Kusznierewicz, B., Chmiel, T., Bartoszek, A., Kowalski, A., … Kaniszewski, S. (2016). Nondestructive Optical Sensing of Flavonols and Chlorophyll in White Head Cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*) Grown under Different Nitrogen Regimens. *Journal of Agricultural and Food Chemistry, 64*(1), 85–94. https://doi.org/10.1021/acs.jafc.5b04962

Andivia, E., Villar-Salvador, P., Oliet, J. A., Puértolas, J., Dumroese, R. K., Ivetić, V., Molinas-Venegas, R., Arellano, E. C., Guolei, L.i, Ovalle, J. F. (2021). Climate and species stress resistance modulate the higher survival of large seedlings in forest restorations worldwide. *Ecological Applications, 31*(6). https://doi.org/10.1002/eap.2394

Augustine, D. J., & McNaughton, S. J. (1998). Ungulate Effects on the Functional Species Composition of Plant Communities: Herbivore Selectivity and Plant Tolerance. *Journal of Wildlife Management, 62*(4), 1165-1183. https://doi:10.2307/3801981

Beltrán, J. F., Rau, J. R., Soriguer, R. C., Kufner, M. B., Delibes, M., & Carro, F. (2022). Effects of Population Declines on Habitat Segregation and Activity Patterns of Rabbits and Hares in Doñana National Park, Spain. *Land, 11*(4), 461. https://doi.org/10.3390/land11040461

Bonfil, S. C., Rodríguez Vega, H., & Peña Ramírez, V. (2020). Evaluación del efecto de las plantas nodrizas en el establecimiento de una plantación de *Quercus* L. *Revista Mexicana De Ciencias Forestales, 25*(88), 59–73. Retrieved from http://177.242.149.221/index.php/forestales/article/view/923

Callaway, R. M., Kikvidze, Z., & Kikodze, D. (2000). Facilitation by unpalatable weeds may conserve plant diversity in overgrazed meadows in the Caucasus Mountains. *Oikos, 89*(2), 275-282. https://doi.org/10.1034/j.1600-0706.2000.890208.x

Carpio, A. J., Apollonio, M., & Acevedo, P. (2020). Wild ungulate overabundance in Europe: contexts, causes, monitoring and management recommendations. *Mammal Review, 51*(1), 95–108. https://doi:10.1111/mam.12221

Carpio, A. J., Guerrero-Casado, J., Barasona, J. A., & Tortosa, F. S. (2017). Ecological impacts of wild ungulate overabundance on Mediterranean basin ecosystems. *Ungulates Evolution, Diversity and Ecology*. Hauppauge, New York: Nova Science Publishers, 111-149.

Cartelat, A., Cerovic, Z., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.-L., Barbottin, A., Jeuffroy, M. H., Gate, P., Agati., Moya, I. (2005) Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). *Field Crops Research, 91*(1), 35–49. https://doi.org/10.1016/j.fcr.2004.05.002

Castro, J., Zamora, R., Hodar, J. A., & Gómez, J. M. (2002). Use of Shrubs as Nurse Plants: A New Technique for Reforestation in Mediterranean Mountains. *Restoration Ecology, 10*(2), 297–305. https://doi:10.1046/j.1526-100x.2002.01022.x

Castro, J., Zamora, R., Hodar, J. A., Gómez, J. M., & Gómez-Aparicio, L. (2004). Benefits of Using Shrubs as Nurse Plants for Reforestation in Mediterranean Mountains: A 4-Year Study. *Restoration Ecology, 12*(3), 352–358. https://doi:10.1111/j.1061-2971.2004.0316.x

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology, 70* (1), 1–9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x

Chaney, W. R., & Byrnes, W. R. (1993). Effect of seedling age and taproot length on performance of oak. *Northern Journal of Applied Forestry, 10(*4), 175-178. https://doi.org/10.1093/njaf/10.4.175

Chapin, F. S., Torn, M. S., & Tateno, M. (1996). Principles of Ecosystem Sustainability. *The American Naturalist, 148*(6), 1016–1037. https://doi:10.1086/285969

Clemente, L. S., García-Fernández, L.V. & Siljestrom, P. (1998). *Los suelos del Parque Nacional de Doñana*. Organismo Autónomo de Parques Nacionales (España).

Colville, L., Bradley, E. L., Lloyd, A. S., Pritchard, H. W., Castle, L., & Kranner, I. (2012). Volatile fingerprints of seeds of four species indicate the involvement of alcoholic fermentation, lipid peroxidation, and Maillard reactions in seed deterioration during ageing and desiccation stress*. Journal of Experimental Botany, 63*(18), 6519–6530. https://doi.org/10.1093/jxb/ers307

Côté, S. D., Rooney, T. P., Tremblay, J.-P., Dussault, C., & Waller, D. M. (2004). Ecological Impacts of Deer Overabundance. *Annual Review of Ecology, Evolution, and Systematics, 35*(1), 113–147. https://doi:10.1146/annurev.ecolsys.35.021103.105725

Cox, D. R. (1972). Regression models and life-tables. *Journal of the Royal Statistical Society: Series B (Methodological), 34(*2), 187-202. https://doi.org/10.1111/j.2517-6161.1972.tb00899.x

Cuesta, B., Villar-Salvador, P., Puértolas, J., Rey-Benayas, J. M., & Michalet, R. (2010). Facilitation of *Quercus ilex* in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. *Journal of Ecology, 98*(3), 687–696. https://doi:10.1111/j.1365-2745.2010.01655.x

Delibes-Mateos M, Ferreras P, Villafuerte R (2008) Rabbit populations and game management: the situation after 15 years of rabbit haemorrhagic disease in central-southern Spain. Biodiv Conserv 17:559–574.

Delibes-Mateos, M., Ferreras, P., & Villafuerte, R. (2008). Rabbit populations and game management: the situation after 15 years of rabbit haemorrhagic disease in central-southern Spain. *Biodiversity and Conservation, 17*(3), 559–574. https://doi.org/10.1007/s10531-007-9272-5

Dolbeer, R. A., Holler, N. R., & Hawthorne, D. W. (1994). Identification and assessment of wildlife damage: an overview. In S. Hygnstrom, R. Timmand G. Larson (Eds.), *The Handbook: Prevention and Control of Wildlife Damage* (pp. A1-A18). Internet Center for Wildlife Damage Management, University of Lincoln.

Dunning, C. E., Paine, T. D., & Redak, R. A. (2002). Insect-oak interactions with coast live oak (*Quercus agrifolia*) and Engelmann oak (*Q. engelmannii*) at the acorn and seedling stage. In R. B. Standiford, D. McCreary, & K. L. Purcell (Tech. Eds.). *Proceedings of the Fifth Symposium on Oak Woodlands: Oaks in California's Challenging Landscape* (pp. 205-218). Gen. Tech. Rep. PSW-GTR-184, Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Espelta, J. M., Riba, M., & Javier, R. (1995). Patterns of seedling recruitment in West-Mediterranean *Quercus ilex* forest influenced by canopy development. *Journal of Vegetation Science, 6*(4), 465–472. https://doi:10.2307/3236344

Fedriani, J. M., García, L. V., Sánchez, M. E., Calderón, J., & Ramo, C. (2016). Long-term impact of protected colonial birds on a jeopardized cork oak population: conservation bias leads to restoration failure. *Journal of Applied Ecology, 54*(2), 450–458. https://doi:10.1111/1365-2664.12672

Fern, M. P., Armstrong, J. B., Barlow, R. J., & Kush, J. S. (2020). Ecological factors influencing wild pig damage to planted pine and hardwood seedlings. *Human–Wildlife Interactions, 14*(2), 228-238. https://digitalcommons.usu.edu/hwi/

Fernández-Manjarrés, J., Ruiz-Benito, P., Zavala, M., Camarero, J., Pulido, F., Proença, V., Navarro, L., et al.. (2018). Forest Adaptation to Climate Change along Steep Ecological Gradients: The Case of the Mediterranean-Temperate Transition in South-Western Europe. S*ustainability, 10*(9), 3065. https://doi.org/10.3390/su10093065

Fernández-Olalla, M., Muñoz-Igualada, J., Martínez-Jauregui, M., Rodríguez-Vigal, C., & San Miguel-Ayanz, A. (2006). Species selection and effect of red deer (Cervus elaphus L.) on shrublands at the Montes de Toledo range, central Spain. Forest Systems, 15(3), 329-338. https://doi.org/10.5424/srf/2006153-00975

Ferreras, P., Jiménez, J., Díaz-Ruiz, F., Tobajas, J., Alves, P. C., & Monterroso, P. (2021). Integrating multiple datasets into spatially-explicit capture-recapture models to estimate the abundance of a locally scarce felid. *Biodiversity and Conservation, 30*(14), 4317-4335. https://doi.org/10.1007/s10531-021-02309-1

Focardi, S., Capizzi, D., & Monetti, D. (2000). Competition for acorns among wild boar (Sus scrofa) and small mammals in a Mediterranean woodland. *Journal of Zoology, 250*(3), 329–334. https://doi:10.1111/j.1469-7998.2000.tb00777.x

ForceA-Dualex. (n.d.) Measuring the chlorophyll and polyphenols contents in plants. https://www.force-a.com/wp-content/uploads/2019/09/BROCHURE-DUALEX-1.pdf

García-Herrera, J.J., Redondo, M.C., Manzaneque, A.G., López, J.B., Valero, E.F. (2011). *Guía de visita del Parque Nacional de Cabañeros* (pp.39-43). Organismo Autónomo Parques Nacionales.

Ghanbary, E., Fathizadeh, O., Pazhouhan, I., Zarafshar, M., Tabari, M., Jafarnia, S., Parad, G. A., Karl-Friedrich, M. B. (2021). Drought and Pathogen Effects on Survival, Leaf Physiology, Oxidative Damage, and Defense in Two Middle Eastern Oak Species. *Forests, 12*(2), 247-270. https://doi.org/10.3390/f12020247

Gill, R.M., (1992). A review of damage by mammals in north temperate forests: 1. Deer. *Forestry An International Journal of Forest Research, 65(2)*, 45-169. https://doi.org/10.1093/forestry/65.2.145

Gómez, J. M., Garcıa, D., & Zamora, R.(2003). Impact of vertebrate acorn- and seedling-predators on a Mediterranean Quercus pyrenaica forest. *Forest Ecology and Management, 180*(1-3), 125–134. https://doi:10.1016/s0378-1127(02)00608-4

Gómez, J. M., & Hódar, J. A. (2008). Wild boars (Sus scrofa) affect the recruitment rate and spatial distribution of holm oak (*Quercus ilex*). *Forest Ecology and Management, 256*(6), 1384–1389. https://doi:10.1016/j.foreco.2008.06.045

Gómez-Aparicio, L., Ibáñez, B., Serrano, M. S., De Vita, P., Ávila, J. M., Pérez-Ramos, I. M., García, L. V., Sanchez, E., Marañón, T. (2012). Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. *New Phytologist, 194*(4), 1014–1024. https://doi.org/10.1111/j.1469-8137.2012.04108.x

Gómez-Aparicio, L., Pérez-Ramos, I. M., Mendoza, I., Matías, L., Quero, J. L., Castro, J., & Marañón, T. (2008). Oak seedling survival and growth along resource gradients in Mediterranean forests: implications for regeneration in current and future environmental scenarios. *Oikos*, *117*(11), 1683–1699. https://doi:10.1111/j.1600-0706.2008.16814.x

Gómez-Aparicio, L., Valladares, F., Zamora, R., & Luis Quero, J. (2005). Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. *Ecography, 28*(6), 757–768. https://doi:10.1111/j.2005.0906-7590.04337.x

Gómez-Aparicio, L., Zamora, R., Gómez, J. M., Hódar, J. A., Castro, J., & Baraza, E. (2004). Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications, 14*(4), 1128–1138. https://doi:10.1890/03-5084

González-Rodríguez, V., Navarro-Cerrillo, R. M., & Villar, R. (2011). Artificial regeneration with *Quercus ilex* L. and *Quercus suber* L. by direct seeding and planting in southern Spain. *Annals of forest science, 68*(3), 637-646. https://doi.org/10.1007/s13595-011-0057-3

Gortázar, C., Torres, M. J., Vicente, J., Acevedo, P., Reglero, M., de la Fuente, J., Negro J. J., Aznar-Martín, J. (2008). Bovine Tuberculosis in Doñana Biosphere Reserve: The Role of Wild Ungulates as Disease Reservoirs in the Last Iberian Lynx Strongholds. *PLoS ONE, 3*(7), e2776. https://doi.org/10.1371/journal.pone.0002776

Groot Bruinderink, G. W. T. A., Hazebroek, E., & van der Voot, H. (1994). Diet and condition of wild boar,Sus scrofu scrofu, without supplementary feeding. *Journal of Zoology, 233*(4), 631–648. https://doi.org/10.1111/j.1469-7998.1994.tb05370.x

Hernández, I., Alegre, L., & Munné-Bosch, S. (2004). Drought-induced changes in flavonoids and other low molecular weight antioxidants in Cistus clusii grown under Mediterranean field conditions. *Tree Physiology, 24*(11), 1303–1311. https://doi.org/10.1093/treephys/24.11.1303

Herrera, J. (1995). Acorn predation and seedling production in a low-density population of cork oak (*Quercus suber* L.). *Forest Ecology and Management, 76*(1-3), 197-201. https://doi.org/10.1016/0378-1127(95)03566-S

Hobbs, N. T. (1996). Modification of Ecosystems by Ungulates. *The Journal of Wildlife Management, 60*(4), 695–713. https://doi.org/10.2307/3802368

Humphrey, J. W., & Swaine, M. D. (1997). Factors Affecting the Natural Regeneration of *Quercus* in Scottish Oakwoods. II. Insect Defoliation of Trees and Seedlings. *Journal of Applied Ecology, 34*(3), 585–593. https://doi.org/10.2307/2404909

IPCC. (2014). *Climate Change 2013 – The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press. https://doi:10.1017/CBO9781107415324

IPCC. (2019). Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. Retrieved from: OECC 2019. Climate Change and Land Use. Spanish Office for Climate Change. Ministerio para la Transición Ecológica, Madrid.

IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, In press, https://doi:10.1017/9781009157896.

Kerr, R. A. (2007). Climate Change: Global Warming Is Changing the World. *Science, 316*(5822), 188–190. https://doi:10.1126/science.316.5822.188

Klein, D. R. (1968). The Introduction, Increase, and Crash of Reindeer on St. Matthew Island. *The Journal of Wildlife Management, 32*(2), 350–367. https://doi.org/10.2307/3798981

Laguna, E. (2015). *Desarrollo de un índice de agregación de individuos espacialmente explícito: los ungulados de Doñana como caso de estudio* [Master 's thesis, Universidad de Castilla-La Mancha]. DIGITAL.CSIC http://hdl.handle.net/10261/145797

Leal, A. I., Bugalho, M. N., & Palmeirim, J. M. (2022). Effects of ungulates on oak regeneration in Mediterranean woodlands: A meta-analysis. *Forest Ecology and Management, 509*, 120077. https://doi.org/10.1016/j.foreco.2022.120077

Leghari, S. J., Wahocho, N. A., Laghari, G. M., HafeezLaghari, A., MustafaBhabhan, G., HussainTalpur, K., Bhutto, T. A., Wahocho, S. A., & Lashari, A. A. (2016). Role of nitrogen for plant growth and development: a review. *Advances in Environmental Biology, 10*(9), 209.

Linaldeddu, B. T., Sirca, C., Spano, D., & Franceschini, A. (2009). Physiological responses of cork oak and holm oak to infection by fungal pathogens involved in oak decline. *Forest Pathology, 39*(4), 232–238. https://doi.org/10.1111/j.1439-0329.2008.00579.x

Linares, L. & Urivelarrea, P. (2020). *Estudio para el conocimiento de las poblaciones de ungulados silvestres en el Parque Nacional de Cabañeros. Bases ecológicas para su gestión.* Organismo Autónomo de Parques Nacionales, España.

Löf, M., Castro, J., Engman, M., Leverkus, A. B., Madsen, P., Reque, J. A., Villalobos, A., Gardiner, E. S. (2019). Tamm Review: Direct seeding to restore oak (Quercus spp.) forests and woodlands. *Forest Ecology and Management, 448*, 474–489. https://doi.org/10.1016/j.foreco.2019.06.032

Lookingbill, T. R., & Zavala, M. A. (2000). Spatial pattern of *Quercus ilex* and *Quercus pubescens* recruitment in Pinus halepensis dominated woodlands. *Journal of Vegetation Science, 11*(4), 607–612. https://doi:10.2307/3246590

López-Sánchez, A., Johnson, I., Dirzo, R., & Perea, R. (2020). Matching species traits and microsites improves regeneration in mixed oak woodlands. *Applied Vegetation Science, 24*(1), e12536. https://doi.org/10.1111/avsc.12536

López-Sánchez, A., Peláez, M., Dirzo, R., Fernandes, G. W., Seminatore, M., & Perea, R. (2019). Spatio-temporal variation of biotic and abiotic stress agents determines seedling survival in assisted oak regeneration. *Journal of Applied Ecology, 56*(12). https://doi.org/10.1111/1365-2664.13500

López-Sánchez, A., Perea, R., Dirzo, R., & Roig, S. (2016). Livestock vs. wild ungulate management in the conservation of Mediterranean dehesas: Implications for oak regeneration. *Forest Ecology and Management, 362*, 99–106. https://doi:10.1016/j.foreco.2015.12.002

López-Sánchez, A., Schroeder, J., Roig, S., Sobral, M., & Dirzo, R. (2014). Effects of cattle management on oak regeneration in northern Californian Mediterranean oak woodlands. *PLoS ONE, 9*, e105472. https://doi.org/10.1371/journal.pone.0105472

Marañón, T., Pérez-Ramos, I. M., Rodríguez Urbieta, I., Quero Pérez, J. L., Villar Montero, R., & Zavala, M. A. (2005). Ecología de la regeneración de bosques de *Quercus* en el sur de España. *Teoría y práctica de la conservación de plantas en el sur de la Península Ibérica y en Marruecos*, 51-52. http://hdl.handle.net/10261/48323

Mariscal, I., (2008). *Recuperación de la calidad de Ultisoles mediterráneos degradados, mediante la aplicación de enmiendas y formas alternativas de uso* [Doctoral dissertation, Universidad Politécnica de Madrid]. Archivo Digital UPM. https://oa.upm.es/1597/

Matzner, S. L., Rice, K. J., & Richards, J. H. (2003). Patterns of stomatal conductance among blue oak *(Quercus douglasii*) size classes and populations: Implications for seedling establishment. *Tree Physiology, 23*(11), 777–784. https://doi.org/10.1093/treephys/23.11.777

Mayer, J. J., Nelson, E. A., & Wike, L. D. (2000). Selective depredation of planted hardwood seedlings by wild pigs in a wetland restoration area. *Ecological engineering, 15*, S79-S85. https://doi.org/10.1016/S0925-8574(99)00074-9

Moral, F. J., Giráldez Cervera, J. V., & Laguna Luna, A. M. (2002). La hidrofobia en los suelos arenosos del parque natural de Doñana: caracterización y distribución. *Ingeniería Del Agua*, *9*(1), 37–50. https://doi.org/10.4995/ia.2002.2606

Muñoz, A., Bonal, R., & Díaz, M. (2009). Ungulates, rodents, shrubs: interactions in a diverse Mediterranean ecosystem. *Basic and Applied Ecology, 10*(2), 151–160. https://doi:10.1016/j.baae.2008.01.003

Navarro, R. M., Villar-Salvador, P., del Campo, A. (2006). Morfología y establecimiento de los plantones. In Cortina J, Peñuelas JL, Puértolas J, Savé R, & Vilagrosa A (Eds). *Calidad de planta forestal para la restauración en ambientes mediterráneos degradados. Estado actual de conocimientos* (pp 67–88). Organismo Autónomo Parques Nacionales.

Nicolás-Peragón J.L., Villar-Salvador, P., & Peñuelas Rubira, J.L. (2004). Efecto de la edad de la planta y el tipo de preparación del suelo en la supervivencia y crecimiento de *Quercus faginea* Lam. cultivado en contenedor. Cuadernos de la Sociedad Española de Ciencias Forestales, 17, 205-209.

Oliet, J. A., Vázquez de Castro, A., & Puértolas, J. (2015). Establishing Quercus ilex under Mediterranean dry conditions: sowing recalcitrant acorns versus planting seedlings at different depths and tube shelter light transmissions. *New Forests, 46*(5), 869-883. https://doi.org/10.1007/s11056-015-9495-3

Ordoñez, R., Haro, J. M, & Giráldez, J. V. (1998). Estudio de la recarga de los suelos arenosos del entorno de El Abalario, Parque Natural de Doñana. In *Progresos en la investigación en zona no saturada* (p. 117.127). Universidad de Huelva.

Padilla, F. M., Peña-Fleitas, M. T., Gallardo, M., & Thompson, R. B. (2016). Proximal optical sensing of cucumber crop N status using chlorophyll fluorescence indices. *European Journal of Agronomy, 73*, 83–97. https://doi.org/10.1016/j.eja.2015.11.001

Pardo García, E. (1995). *Morfología, mineralogía y génesis de los suelos desarrollados sobre distintas superficies morfológicas relacionadas con la vertiente norte de los Montes de Toledo* [Doctoral dissertation, Universidad Complutense de Madrid]. E-Prints Complutense.

Peláez, M., Dirzo, R., Fernandes, G. W., & Perea, R. (2019). Nurse plant size and biotic stress determine quantity and quality of plant facilitation in oak savannas. *Forest Ecology and Management*, *437*, 435–442. https://doi:10.1016/j.foreco.2019.02.010.

Peñuelas, J., Sabaté, S., Filella, I., & Gracia, C. (2004). Efectos del cambio climático sobre los ecosistemas terrestres: observación, experimentación y simulación. In Valladares F (ed) Ecología del bosque mediterráneo en un mundo cambiante. Ministerio de Medio Ambiente, Madrid, pp. 425-460.

Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., et al.. (2017). Impacts of Global Change on Mediterranean Forests and Their Services. *Forests, 8*(12), 463. https://doi:10.3390/f8120463

Percival, G.C. (2005). The Use Of Chlorophyll Fluorescence To Identify Chemical And Environmental Stress In Leaf Tissue Of Three Oak *(Quercus*) Species. *Formerly the Journal of Arboriculture, 31*(5), 215-227. https://doi.org/10.48044/jauf.2005.028

Perea, R., & Gil, L. (2014a). Tree regeneration under high levels of wild ungulates: The use of chemically vs. physically-defended shrubs. *Forest Ecology and Management, 312*, 47–54. https://doi:10.1016/j.foreco.2013.10.022

Perea, R., & Gil, L. (2014b). Shrubs facilitating seedling performance in ungulate-dominated systems: Biotic versus abiotic mechanisms of plant facilitation. *European Journal of Forest Research, 133*, 525–534. https://doi.org/10.1007/s10342-014-0782-x

Perea, R., Girardello, M., & San Miguel, A. (2014a). Big game or big loss? High deer densities are threatening woody plant diversity and vegetation dynamics. *Biodiversity and Conservation, 23*(5), 1303–1318. https://doi:10.1007/s10531-014-0666-x

Perea, R., Valbuena-Carabaña, M., San Miguel, A., Gil, L. (2014b). El efecto de los ungulados silvestres sobre la supervivencia de las plántulas de *Quercus pyrenaica*: Importancia del matorral no apetecido. *Cuadernos de la Sociedad Española de Ciencias Forestales, 40,* 167-174.

Perea, R., Perea-García-Calvo, R., Díaz-Ambrona, C. G., & San Miguel, A. (2015). The reintroduction of a flagship ungulate Capra pyrenaica: Assessing sustainability by surveying woody vegetation. *Biological Conservation, 181*, 9–17. https://doi:10.1016/j.biocon.2014.10.018

Perea, R., López-Sánchez, A., & Roig, S. (2016). The use of shrub cover to preserve Mediterranean oak dehesas: A comparison between sheep, cattle and wild ungulate management. *Applied Vegetation Science, 19*, 244–253. https://doi.org/10.1111/avsc.12208

Pierson, T.G., & Decalesta, D. (2015). Methodology for Estimating Deer Browsing Impact. *Human–Wildlife Interactions, 9*(1), e7.

Pugnaire, F.I., Schöb, C., Pistón, N., Armas, C. (2015). Facilitación de las especies almohadilladas y cambio global en las comunidades alpinas del Parque Nacional de Sierra Nevada. In *Proyectos de investigación en parques nacionales: 2010–2013* (pp. 91-104). Organismo Autónomo de Parques Nacionales.

Pulido, F. J., & Díaz, M. (2005). Regeneration of a Mediterranean oak: a whole-cycle approach. *Ecoscience, 12*(1), 92-102. https://doi.org/10.2980/i1195-6860-12-1-92.1

Reque, J. A., & Martin E. (2015). Designing acorn protection for direct seeding of quercus species in high predation areas. *Forest Systems, 24*(1), e018. https://doi.org/10.5424/fs/2015241-05632

Retana, J., Espelta, J. M., Gracia, M., & Riba, M. (1999). Seedling Recruitment. *Ecology of Mediterranean Evergreen Oak Forests. Ecological Studies, 137*, 89–103. https://doi:10.1007/978-3-642-58618-7_7

Rey-Benayas, J. M. (1998). Growth and survival in *Quercus ilex* L. seedlings after irrigation and artificial shading on Mediterranean set-aside agricultural land. *Annales Des Sciences Forestières, 55*(7), 801–807. https://doi:10.1051/forest:19980704

Rey-Benayas, J. M. (2005). Restoring Forests After Land Abandonment. In: *Forest Restoration in Landscapes*. pp. 356-360. Springer, New York, NY. https://doi.org/10.1007/0-387-29112-1_51

Rey-Benayas, J. M., López-Pintor, A., García, C., de la Cámara, N., Strasser, R., & Sal, A. G. (2002). Early establishment of planted Retama sphaerocarpa seedlings under different levels of light, water and weed competition. *Plant Ecology, 159*(2), 201-209. https://doi.org/10.1023/A:1015562623751

Rey-Benayas, J. M., Martínez-Baroja, L., Pérez-Camacho, L., Villar-Salvador, P., & Holl, K. D (2015). Predation and aridity slow down the spread of 21-year-old planted woodland islets in restored Mediterranean farmland. *New Forests 46*, 841–853 (2015). https://doi.org/10.1007/s11056-015-9490-8

Rey, P. J., Siles, G., & Alcántara, J. M. (2009). Community-level restoration profiles in Mediterranean vegetation: nurse-based vs. traditional reforestation. *Journal of Applied Ecology, 46*(4), 937–945. https://doi.org/10.1111/j.1365-2664.2009.01680.x

Rooney, T. P. (2008). High white-tailed deer densities benefit graminoids and contribute to biotic homogenization of forest ground-layer vegetation. *Plant Ecology, 202*(1), 103–111. https://doi:10.1007/s11258-008-9489-8

Rooney, T. P., & Waller, D. M. (2003). Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management, 181*(1-2), 165–176. https://doi:10.1016/s0378-1127(03)00130-0

San Miguel, A., Pérez-Carral, C., & Roig, S. (1999). Deer and traditional agrosilvopastoral systems of Mediterranean Spain. A new problem of sustainability for a new concept of land use. *Cahier Options Méditerranéennes 39*, 261-264. http://om.ciheam.org/om/pdf/c39/99600084.pdf

San Miguel-Ayanz, A., Perea, R., & Fernández-Olalla, M. (2010). Wild Ungulates vs. Extensive Livestock. Looking Back to Face the Future. *Cahier Options Meditérranneenes, 92*, 27-34.

Siles, G., Rey, P. J., Alcántara, J. M., Bastida, J. M., & Herreros, J. L. (2010). Effects of soil enrichment, watering and seedling age on establishment of Mediterranean woody species. *Acta Oecologica, 36*(4), 357–364. https://doi.org/10.1016/j.actao.2010.03.002

Taylor II, J. D. (2011). Identifying and managing for wildlife damage during stand initiation. *Northwest Woodlands Winter 2011, 29*, 16-17.

Tyler, C. M., Davis, F. W., & Mahall, B. E. (2008). The relative importance of factors affecting age-specific seedling survival of two co-occurring oak species in southern California. *Forest Ecology and Management*, *255*(7), 3063–3074. https://doi.org/10.1016/j.foreco.2008.01.073

Tyler, C. M., Kuhn, B., & Davis, F. W. (2006). Demography and Recruitment Limitations of Three Oak Species in California. *The Quarterly Review of Biology, 81*(2), 127–152. https://doi:10.1086/506025

Urreaga, J. M., Beltrán, F. R., Acosta, J., Aguinaco, T., Fonseca, C., Ochoa, A., Oliet, J. A., Sánchez, C. G., & de la Orden, M. U. (2020). Tube shelters from agricultural plastic waste: an example of circular economy. *Journal of Cleaner Production, 268*, 122401. http://dx.doi.org/10.1016/j.jclepro.2020.122401

Valdés, B., Girón, V., Sánchez Gullón, E., & Carmona, I. (2007). Catálogo florístico del espacio natural de Doñana (SO de España). Plantas vasculares. Lagascalia, 27(1), 73-362. http://hdl.handle.net/11441/54114

Valente, A. M., Acevedo, P., Figueiredo, A. M., Fonseca, C., & Torres, R. T. (2020). Overabundant wild ungulate populations in Europe: management with consideration of socio-ecological consequences. *Mammal Review, 50*, 1-14. https://doi:10.1111/mam.12202

Villalobos, A., Olsson, G., Birkedal, M., & Löf, M. (2019). The effects of four repellents on bank vole consumption and germination of beech nuts and acorns. *New Forests, 50*(2), 241-254. https://doi.org/10.1007/s11056-018-9660-6

Villar-Salvador, P., Peñuelas-Rubira, J.L., Nicolás-Peragón, J.L. (2021). La calidad de los materiales de reproducción. *Bases técnicas y ecológicas del proyecto de repoblación forestal* (pp.781-822). Ministerio para la Transición y el Reto Demográfico, España.

Villar-Salvador, P., Planelles, R., Oliet, J., Penuelas-Rubira, J. L., Jacobs, D. F., & Gonzalez, M. (2004). Drought tolerance and transplanting performance of holm oak (Quercus ilex) seedlings after drought hardening in the nursery. *Tree Physiology, 24*(10), 1147–1155. https://doi.org/10.1093/treephys/24.10.1147

Wright, S. J. (2010). The future of tropical forests. *Annals of the New York Academy of Sciences, 1195*(1), 1–27. https://doi:10.1111/j.1749-6632.2010.05455.x

Wright, S. L., Hall, R. W., & Peacock, J. W. (1989). Effect of simulated insect damage on growth and survival of northern red oak (*Quercus rubra* L.) seedlings. *Environmental Entomology, 18*, 235–239. https://doi.org/10.1093/ee/18.2.235

Wu, Z., Dai, E., Wu, Z., & Lin, M. (2019). Future forest dynamics under climate change, land use change, and harvest in subtropical forests in Southern China. *Landscape Ecology, 34*(4), 843–863. https://doi:10.1007/s10980-019-00809-8

Yi, X., Wang, Z., Liu, C., Liu, G., & Zhang, M. (2015). Acorn cotyledons are larger than their seedlings' need: evidence from artificial cutting experiments. *Scientific reports, 5* ,8112. https://doi.org/10.1038/srep08112

Zamora, R., García-Fayos, P., Gómez-Aparicio, L. (2004). Las interacciones planta-planta y planta-animal en el contexto de la sucesión ecológica. In *Ecología del bosque mediterráneo en un mundo cambiante.* (ed. F. Valladares), pp. 309-334. Madrid: Organismo Autónomo de Parques Nacionales. http://hdl.handle.net/10261/47939

6. SUPPLEMENTARY MATERIALS

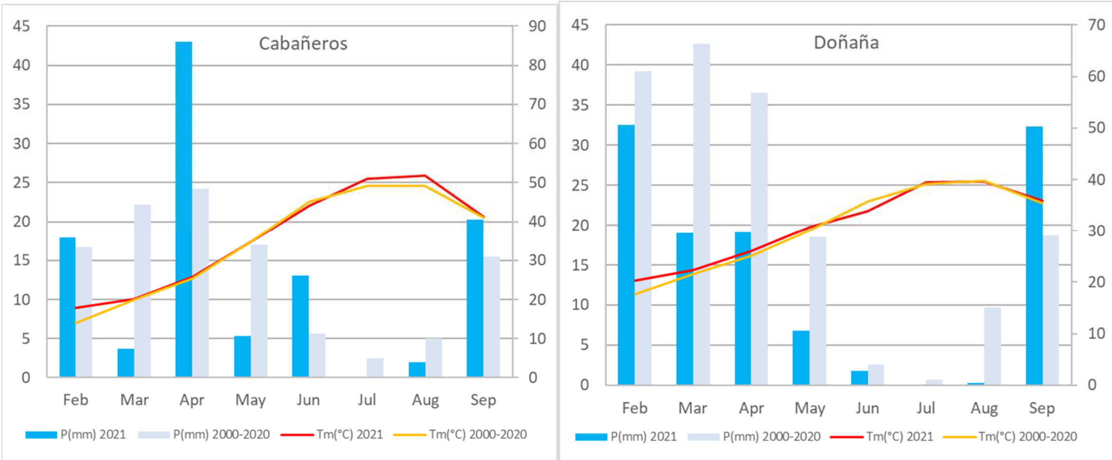

Figure S1: Meteorological data from the Porzuna weather station (14.3 km from the plantation to the SE) obtained through the SIAR (Irrigation Advisory Service of Castilla-La Mancha) of the Consejería de Agricultura, Agua y Desarrollo Rural de Castilla-La Mancha (Department of Agriculture, Water and Rural Development of Castilla-La Mancha). * Meteorological data from the Almonte station (14.1 km from the plantation to the N) obtained through the RIA (Red de Información Agroclimática de Andalucía) of the Junta de Andalucía.

Table S1. Pair-wise comparison of Cox Proportional Hazard Model significance levels among each pair of seedlings of 1-2 years-old, with or without acorns outside the fence in Cabañeros before the onset of the dry season.

Fenced- 1yr- Acorn	Fenced – 1yr- No Acorn	Fenced – 2yr - Acorn	Fenced 2yr - No Acorn	
P<0.001	P<0.001	P<0.001	P<0.001	No Fenced- Acorn -1yr
	P<0.001	P<0.001	P<0.001	No Fenced-No Acorn – 1yr
		P<0.001	P<0.001	No Fenced - Acorn – 2yr
			P<0.001	No Fenced- No Acorn - 2yr

Table S2. Pair-wise comparison of Cox Proportional Hazard Model significance levels among all combinations of seedlings of 1-2 years-old, with or without acorns outside the fence in Cabañeros before the onset of the dry season. Model coefficient and P-values are indicated. Positive coefficients indicate an increased survival of the treatment A (columns) against treatment B (rows).

1 yr –No Acorn	2 yr - Acorn	2 yr- No Acorn	
0.233 P= 0.578	0.182 P= 0.612	0.203 P=0.787	1 yr- Acorn
	0.203 P= 0.787	0.002 P= 0.975	1 yr- No Acorn
		0.436 P= 0.435	2 yr - Acorn
			2 yr – No Acorn

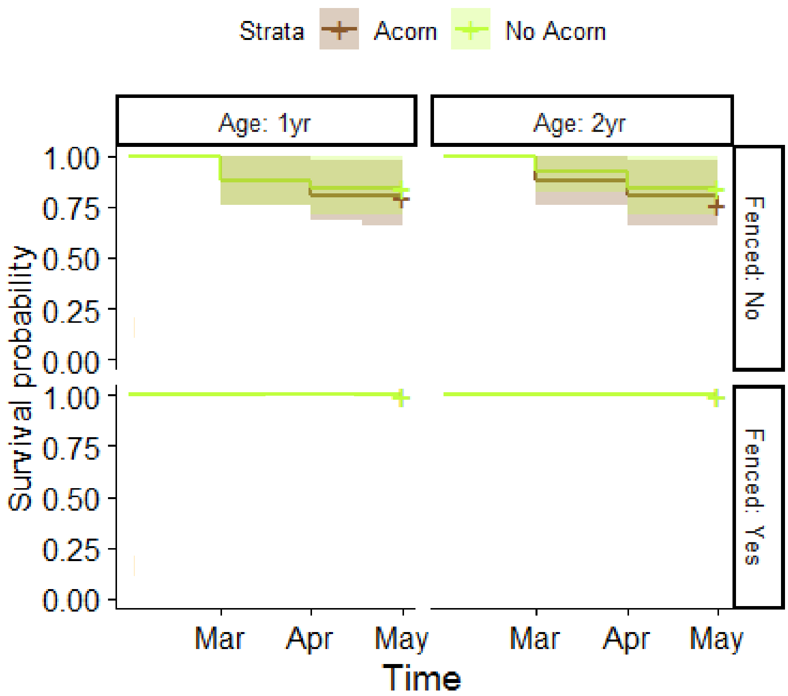

Figure S2: Survival probability of seedlings depending on Time (in months) with and without acorns according to plant Age (1-year-old vs 2-years-old) and Fence (fenced and non-fenced treatment) for Cabañeros National Park.

Table S3. Pair-wise comparison of Cox Proportional Hazard Model significance levels among all combinations of seedlings of 1-2 years-old, with or without acorns outside the fence area in Doñana before the onset of the dry season.

1 yr –No Acorn	2 yr - Acorn	2 yr- No Acorn	
0.496 P= 0.249	**1.176** **P= 0.0034**	0.633 P= 0.223	1 yr- Acorn
	0.633 P= 0.224	0.542 P= 0.101	1yr-No Acorn
		- 0.136 P= 0.81	2 yr - Acorn
			2 yr – No Acorn

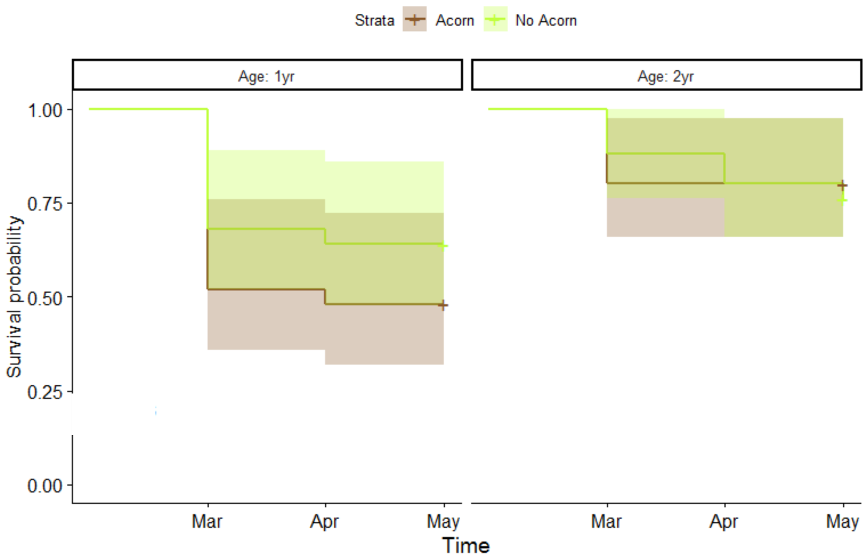

Figure S3: Survival probability of seedlings depending on Time (in months) with and without acorns according to plant Age (1-year-old vs 2-years-old) in no protected treatment (non-fenced) for Doñana National Park.