Title: Seedling establishment in a deciduous and an evergreen oak under simulated climate change

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Abstract

Climate change constitutes a major threat to global biodiversity and to the success of natural and assisted tree regeneration. Oaks are among the most emblematic tree species in the Northern Hemisphere, so it is crucial to understand the impact of changing climate on seedling recruitment and early development. In this study, we investigated the effect of air warming on the early development of one deciduous species – *Quercus faginea* – and one evergreen species – *Quercus ilex* subsp. *ballota*. Acorns of both species were seeded in an alluvial valley in southern Spain and subjected to an air warming treatment with *Open-Top Chambers* (OTC), which increased air temperature by 2ºC. We monitored seedling emergence, growth, chlorophyll concentration, and mortality in the first growing season. The simulated climate change treatment accelerated plant emergence in early spring, reduced spring shoot growth, and increased mortality from ~23% in control plots to ~40% inside OTCs. Although *Q. ilex* and *Q. faginea* are sympatric species, *Q. faginea* showed lower performance under simulated climate change in terms of growth. In addition, acorn fresh weight was positively related with the probability and speed of emergence (only for *Q. faginea*), seedling size, and relative chlorophyll content, and plants that emerged earlier had a greater likelihood of surviving. In short, larger acorns partly counter-balanced the negative impact of temperature increase on plants. This study highlights the importance of understanding plant response to climate change both to forecast potential changes in species composition and to choose adequate species and traits such as acorn size in restoration projects.

Keywords: Direct seeding, management, natural regeneration, holm oak, Lusitanian oak, sowing

1. Introduction

Climate change is degrading forests and altering ecological processes, ranging from the physiology and phenology of species to the distributions of communities at a global scale (Piao et al., 2019; Scheffers et al., 2016; Trisos et al., 2020). For plants, key problems arise at the phase of regeneration, as early life stages are particularly sensitive to extreme climatic conditions (Bykova et al., 2019). As a result, understanding the factors that modulate the effects of climate change on the performance of plants in early life stages may help predict vegetation responses and improve restoration programmes.

In the Mediterranean Basin, oak forests and oak-dominated savannah-like *dehesas* are widely extended vegetation types. Many of such stands are facing regeneration challenges driven by biotic and abiotic factors (Arosa et al., 2015; Gómez-Aparicio et al., 2008; Ibáñez et al., 2017). Additionally, ongoing climate change is increasing aridification, which threatens the long-term persistence of Mediterranean oak forest (Matías et al., 2019). While changes in the persistence of mature stands –e.g., through the mortality of adult trees– have been much explored (e.g. Hartmann et al., 2022), the potential shift in the regeneration capacity has received less attention. Ongoing land abandonment and the subsequent availability of open spaces could provide new opportunities for natural regeneration (Kouba et al., 2012), yet tree recruitment rates in Mediterranean areas are generally low (Ramírez and Díaz, 2008) and it is important to further assess how regeneration responds to changes in climate.

Ecological constraints to natural regeneration can include predation (Arosa et al., 2015; Leal et al., 2022; Leverkus et al., 2015b) and emerging factors due to climate change, such as lower quality and quantity of acorn crops (Garcia-Fayos et al., 2020; Sánchez-Humanes and Espelta, 2011). Another relevant climate change effect is warmer air temperature, which can increase soil aridity and seedling transpiration, leading to higher

water demand by plants (Hatfield and Dold, 2019). In areas with low water availability, such as those under Mediterranean climate, the effects of warmer air temperatures may add to chronic water limitation. Moreover, higher temperatures could also accelerate seed desiccation and reduce germination capacity. Climate change may also accelerate seedling phenology (e.g. earlier emergence and leaf unfolding), leading to an extended growing season, increased water use throughout the year, and potentially elevated plant mortality rates (Morin et al., 2010). Such effects could have long-term negative consequences for regeneration capacity and seedling recruitment, thereby undermining the persistence of populations (Sánchez-Humanes and Espelta, 2011).

Tolerance to high temperatures and water limitations may differ considerably between coexisting species as a result of various physiological traits (Alonso-Forn et al., 2021a; Camarero et al., 2016; Forner et al., 2018; Montserrat-Martí et al., 2009; Nardini et al., 1999; Peguero-Pina et al., 2020). Compared to deciduous trees, evergreen trees have a slow relative growth rate, an effective photoprotective mechanism (Alonso-Forn et al., 2021a), and a more conservative water use due to tighter control of stomatal conductance (Lopez-Iglesias et al., 2014). Meanwhile, deciduous trees have an earlier phenological development and higher conductance, resulting in a lower water-use efficiency (Camarero et al., 2016; Maltez-Mouro et al., 2009; Montserrat-Martí et al., 2009; Valladares and Sánchez-Gómez, 2006). In addition, morphological traits such as acorn mass can affect seedling survival, and they should therefore be considered when evaluating the success of regeneration (Gómez, 2004; González-Rodríguez et al., 2011; Marañón et al., 2004; Ramírez-Valiente et al., 2009). Understanding seedling establishment from seeds under climate change is fundamental to evaluate the effect of species and their traits on regeneration (Pérez-Ramos et al., 2013; Rodríguez-Calcerrada et al., 2011), both to forecast future species distribution and migration but also for direct seeding as an assisted regeneration strategy.

In this study, we aim to understand the impact of warmer air temperature, in a simulated climate change scenario, on the early performance of seeded oaks. We applied an experimental climate-change treatment with *Open-Top Chambers* (OTC) to acorns seeded in a field in southern Spain, and we compared the response of emerged seedlings during the first growing season – a major bottleneck for oak regeneration (Castro et al., 2006; García de Jalón et al., 2020; López-Sánchez et al., 2019; Pérez-Ramos et al., 2012; Ramírez-Valiente et al., 2018). We used two sympatric Mediterranean oak species with different summer-drought and heat tolerance: the evergreen holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) and the deciduous Lusitanian oak (*Quercus faginea* Lam.) (Alonso-Forn et al., 2021b; Mediavilla and Escudero, 2003; Montserrat-Martí et al., 2009; Peguero-Pina et al., 2020). Our hypotheses are that a) air warming increases the speed of emergence and b) physiological stress, which would reduce the early growth and survival of the seedlings. Finally, we hypothesise that c) the evergreen holm oak would be less affected by additional stress conditions than the deciduous Lusitanian oak. This study will provide input to future restoration projects and contribute to understanding and predicting the response of oak species facing climate change.

2. Materials and methods

2.1. Study site

The experiment was conducted at the “Huerta de La Paloma” farm (37° 10′ 03.2″ N, 3° 36′ 58.1″ W), Granada, Southern Spain), a flat (slope ca. 2%) agricultural terrain located at 654 m a.s.l. in Vega de Granada (WorldClim database, Fick and Hijmans 2017). The site is situated in the Depression of Granada, an enclosed valley filled with sedimentary materials deposited by watercourses exiting from the nearby Sierra Nevada and with deep, fertile soils (Castillo, 2005). The area is characterised by small, flat, and irrigated farm plots mainly used

for production of crop such as vegetables, maize, tree plantations and pasture (Castro et al., 2017). Climatic conditions are typical Mediterranean, characterised by dry and hot summers and mild wet winters, with a mean annual temperature of 15.4ºC and mean annual precipitation of 356.5 mm (period 2006-2022; IFAPA 2022). The maximum temperature recorded in this period was 43.8ºC (August 2021) with a mean potential evapotranspiration of 3.31 mm (IFAPA, 2022). Besides, no precipitation was recorded from July to mid-September, which could affect the seedling size at the end of summer measures (IFAPA, 2022).

2.2. Acorn collection and preparation

The experiment was conducted from November 2020 to September 2021. In November 2020 we collected mature acorns from 11 maternal trees of each of the two target species – *Quercus ilex* subsp. *ballota* and *Q. faginea*. The maternal trees were located in the surroundings of the study area, all within the Sierra Nevada provenance region, and we collected a minimum of 130 seeds per tree. Acorns were stored in plastic bags separated by parent tree, and they were kept refrigerated for 1–2 weeks. Thereafter, acorns were visually inspected and subjected to a flotation test to remove damaged and non-viable seeds (Gribko and Jones, 1995). Two of the 11 maternal trees per species showed poor acorn quality, so we removed these acorn batches. All viable acorns were subsequently weighed and labelled with a unique code reflecting the species, maternal tree and seed number. Before seeding, acorns were submerged in water for 4 h to assure the full hydration of acorns.

2.3. Experimental design

The experiment was established in an agricultural field of 640 m^2 (10 × 64 m). Site preparation consisted in mechanically ploughing the ground with a rotovator to remove weeds and roots and to soften the soil. After that, we established 160 hexagonal plots, 1 m wide, distributed in 5 columns by 32 rows, whose centres were separated by 2 m. In each plot, we installed six seeding points at the vertices of a hexagon of 32 cm per side (which was contained in the middle of the larger hexagon). The seeding points included three individuals per species that were placed at random seeding points within each plot, totalling 960 points. Seeds were randomly hand-picked, placed individually ca. 3 cm under the soil, and covered with a seed protector for physical protection from rodents. This device, named *Seed Shelter*, consist of a truncated polypropylene pyramid, which allows the shoot to exit directly from the upper opening and the root to exit at the bottom (Castro et al., 2015); for this study, we used only the upper part.

Finally, we installed a hexagonal *Open-Top Chamber* (OTC) over every second plot (Figure S1). The OTCs consisted of six transparent methacrylate plates (32cm upper width x 41cm height x 50cm lower width), connected by aluminium joints and held by steel bars. OTCs were lifted 5–10 cm above the ground by metal bars to allow air circulation. OTCs produce a passive air Warming Treatment (hereinafter WT), and they are widely used in ecosystem warming experiments (Hollister and Webber, 2000).

To measure the variations in air temperature and relative humidity produced by OTCs, we installed 32 data loggers in 16 pairs of OTC and control plots (EasyLog EL-USB-2 LASCAR, Wiltshire, United Kingdom). The devices were attached to wooden sticks at ca. 30 cm from the ground and protected from direct sunlight with single-use plastic cups cut in half and open to the North. Measurements were made every 6 h (Fig. 1). Air temperature during this experiment was significantly higher inside OTCs (mean ± SE = 22.1 ± 0.12ºC) than in control plots (20.1 ± 0.11ºC; LMM, $\chi^2 = 225.78$, df = 1, $P < 0.001$), while humidity was

slightly lower (58.2 ± 0.23%) than in the controls (59.1 ± 0.22%; LMM, $\chi^2 = 4.67$, df = 1, $P = 0.031$). Mean vapour pressure deficit, calculated from air temperature and humidity, was higher inside OTCs (0.0264 ± 0.0003 mol mol^{-1}) than in control plots (0.0192 ± 0.0002 mol mol^{-1}).

2.4. Site maintenance

To avoid confounding the effect of the higher air temperature induced by the WT with that of increased competition by herbs (as early herb emergence and growth were greatest inside OTCs), we periodically removed weeds with a garden tiller and applied herbicide (Roundup®, glyphosate at the dosage recommended by the manufacturer) twice between the plots, and within plots we weeded manually by cutting the aboveground plant mass. We also frequently cleaned OTCs with wet clothes to guarantee maximum sunlight permeability. Finally, to reduce the impact of ants, we applied an insecticide composed of 1% alpha-cypermethrin (Paparrin®, Comercial Química Massó S.A., Barcelona, Spain), around each ant nest entrance located inside the plots.

2.5. *Sampling*

We monitored seedling emergence and survival weekly from early March to mid-July 2021, totalling 20 revisions throughout the growing season. Two more revisions were carried out during the summer (mid-August) and at its end (mid-September).

We measured the growth of each independent stem of each seedling twice: in mid-July, as a proxy of spring growth, and in mid-September to estimate summer growth. We used a flexometer to measure stem height, and a digital calliper to measure two perpendicular diameters at the base of each stem. Relative chlorophyll content was measured three times (July, August, and September) in three randomly selected leaves of

the upper third of the shoot in each seedling using a portable chlorophyll meter (SPAD-502 Plus by Konika Minolta Inc, Osaka, Japan). This device determines the relative chlorophyll content of leaves by measuring the absorbance in two wavelength regions without causing damage to leaves (Konica Minolta, 2009). The measurements were made on shoots of live plants. Differences between species in SPAD units cannot be directly compared due to each species has a different signal as a baseline, however, these measures allow us to compare differences between treatments.

2.6. *Statistical analyses*

First, to analyse seedling emergence, we used a generalized linear mixed-effects model (GLMM) assuming a binomial distribution. Emergence was the binomial response variable, and the model tested the effect of species (*Q. ilex* or *Q. faginea*), WT (control or OTC), acorn fresh weight, and the interactions among these variables as fixed effects. Additionally, we fitted row and column as covariates; they account for the spatial location of each plot in the experiment and for possible gradients in environmental conditions in the field, yet we do not present the results for these variables. Mother tree identity was included in the model as a random factor. Time of emergence as response variable (days from seeding to recorded seedling emergence) was analysed with a GLMM assuming a Poisson distribution and following the above-described model structure. Similarly, seedling survival was analysed with a GLMM assuming binomial distribution and adding the time of emergence as a covariate, as well as the interactions between time of emergence and the other fixed effects. Only the fourth-level interaction was excluded due to model overparameterization.

Second, we analysed plant spring and summer growth with linear mixed-effects models (LMM). The models had the same structure of fixed and random effects as for the seedling emergence models. As response variables, we used a) total plant height in July, b)

relative growth rate (RGR) of plant height in summer (from July to September), c) total stem section area (SSA) in July, and d) RGR of stem section area in summer. Total height was calculated by adding the height of all stems of each individual and total stem section area by calculating the area of each stem ($\pi \cdot r^2$) and then adding the areas of all stems for a given plant. RGR was calculated for two months as the natural logarithm of the quotient of final height (or SSA) divided by the initial value. Finally, for the analyses of relative chlorophyll content (RCC) we used the average of the three measurements made per plant. Previous to the LMMs, the data were square root-, inverse-, or log-transformed, or outliers removed (using Interquartile Range method), when necessary, to fulfil the model assumptions. The model for height RGR could not estimate the random effect of parent tree, so a linear model (LM) was fitted instead.

All models were simplified applying a stepwise backward selection procedure to test for the significance of all main terms and interactions (Crawley, 2012). The significance of the terms was assessed through likelihood ratio tests during simplification; at each step, the least significant term was removed, starting with the highest-order interactions. All analyses were conducted in R 4.1.3 (R Core Team, 2022). The GLMMs and LMMs were run with the *glmer* and *lmer* functions in the *lme4* package (Bates et al., 2015).

3. Results

In most of the models described below, there were spatial trends within the field that were accounted for by the models and that we do not describe further (Table 1 & 2, results for Row and Column).

3.1. Seedling emergence and survival

Of the 960 seeded acorns, 608 (63%) emerged. Acorn fresh weight, which varied between 1.86 g and 10.89 g (mean ± SE = 4.71 ± 0.05 g), was positively related to the probability of seedling emergence, yet there were no effects of WT or species (Table 1A). However, WT and species did have an effect on the timing of emergence, which occurred between 115 and 273 days after seeding. The peak of seedling emergence occurred 14 days earlier inside OTCs than outside (Table 1B, Fig. 2A). *Quercus faginea* seedlings emerged, on average, 12.1 days before *Q. ilex*, and acorn fresh weight accelerated the emergence of *Q. faginea* seedlings while *Q. ilex* showed no such association (species × acorn fresh weight interaction, Table 1B, Fig. 2B). Overall, the models for emergence and timing of emergence showed a moderately high variance explained by maternal effects (24.6% and 16.4%, respectively, Appendix B).

Out of the 608 emerged plants, 420 (69.08%) still survived by the end of the summer, with greater seedling survival in control plots (77.02%) than in OTCs (60.87%) and no differences in survival across species. Seedling survival was positively associated with an early emergence for both species, but especially for *Q. faginea*, and most strongly in control plots (Table 1C, Fig. 3).

3.2. Seedling growth

The warming treatment reduced the average spring height growth of *Q. faginea* seedlings, while this effect was small for *Q. ilex* (WT × species interaction; Table 2; Fig. 4A). Spring height growth was also positively related to acorn mass (Fig. 4B). In turn, height growth in summer only differed between species, with positive values for *Q. faginea* and negative ones for *Q. ilex* (Fig. 4C).

In terms of section area, in spring *Q. faginea* seedlings grew a greater stem section area than *Q. ilex* (Fig. 5A), and section area was reduced by OTCs for both species (Fig. 5B).

Acorn fresh weight was also positively associated with stem area for both species in spring (Fig. 5C). The RGR in stem section area during summer averaged -0.10±0.02 and was not affected by any of the tested variables (Table 2).

Finally, mother trees explain more variance in the spring plant height model (12.5%) than in stem section area ones (0.7% for spring and 1.1% for summer, Appendix B).

3.3. Relative chlorophyll content

The WT produced differences in SPAD values only in July, with slightly greater RCC in control plots than inside OTCs (Table 2C, Fig. 6A). RCC showed a positive relationship with acorn fresh weight throughout all measurements, but this was only significant in August (Table 2C, Fig. 6B). There were no significant interactions for this variable.

4. Discussion

Our experimental emulation of climate change by rising air temperatures with OTC's increased the speed of emergence of both, *Q. ilex* and *Q. faginea* seedlings, but also the mortality rates of seedlings during the Mediterranean summer. Despite known differences in the stress tolerance among these species, we found little differences in performance among them. We did, however, find that seedling performance was conditioned by the individual-level trait of seed fresh weight and by advance in emergence. These results contribute to our understanding of the response of oak natural regeneration to climate change and can improve the success of ecosystem restoration through direct seeding.

4.1. Advance in emergence due to increased temperatures

Although induced air warming did not reduce the proportion of seedlings emerged for either species, it influenced the temporal pattern of emergence, thereby supporting our first

hypothesis. Our results build on existing evidence that seedling emergence may advance with increasing temperatures (Gordo and Sanz, 2010; Morin et al., 2010), as the warming treatment induced a two-week advance in the peak of emergence in both oak species albeit the temperature differences having been smallest around the time of germination (Fig. 1A). In line with this, our results also show that early emergence increased the probability of seedling survival irrespective of species, but most intensively for *Q. faginea*. This could result from the benefit of an extended growing period, which gives more time to seedlings to develop shoots before the summer drought stress starts, then receive light and achieve a positive carbon gain (Augspurger, 2008), and deeper roots to guarantee water access (Ramírez-Valiente et al., 2018; Villar-Salvador et al., 2012). In our study, this positive effect may have conferred seedlings greater tolerance to summer drought and a competitive advantage that outbalanced potential negative effects of advanced emergence such as a greater susceptibility to freezing events or longer summer drought periods (Arosa et al., 2015; Castro, 2006; Verdú and Traveset, 2005; Vizcaíno-Palomar et al., 2014). This resulted in higher survival, as has also been found in other studies with these and other species (Bianchi et al., 2019; Castro, 2006). Besides, for *Q. faginea,* which emerged earlier than *Q. ilex,* seedlings tended to emerge earlier from heavier acorns, and this early emergence increased seedling survival especially for this species. This suggests that the effects of warming could be partly mitigated by traits such as greater acorn fresh weight.

4.2. Mortality and performance

By the end of the summer, there was low to moderate overall seedling mortality, yet the warming treatment doubled mortality irrespective of species, thereby supporting our second hypothesis. Seedlings subjected to the OTC's were under warmer and drier conditions than in control plots, likely exacerbating and prolonging the effects of summer drought.

The effect of the warming treatment on seedling performance may have been mediated by changes in photosynthetic capacity. The optimum temperature for net photosynthesis (A_{net}) is about 20ºC in several Mediterranean species including *Q. ilex* (Gratani et al., 2000), and higher temperatures than those will reduce A_{net}, as inside OTC. Besides, our relative chlorophyll content data –which are sensible to physiological stress (Fang and Xiong, 2015; Munné-Bosch and Alegre, 2004)– together with low growth and high temperatures inside OTCs, suggest that photosynthetic capacity in July was undermined by the warming treatment. Besides, adverse Mediterranean summer conditions, exacerbated by the higher vapour pressure deficit inside OTCs, may have indirectly caused severe water shortage by increasing evaporative demand from soil and plant, coupled with greater water need for leaf cooling. In accordance to our second hypothesis, both species showed a growth reduction under emulated climate change conditions, probably caused by this water stress.

The two species, *Q. faginea* and *Q. ilex*, have different strategies to cope with abiotic stress. *Quercus faginea* maximizes its carbon uptake when the conditions are favourable, with a shorter but more intense growing period than *Q. ilex*, which has a more conservative carbon and water use strategy, with a longer vegetative period (Alonso-Forn et al., 2021a; Corcuera et al., 2004; Montserrat-Martí et al., 2009)□ In fact, *Q. faginea* showed a noticeable growth during spring outside OTCs, compared with *Q. ilex*. Summer height growth was minimal or even slightly negative in both species, although this “decrease” might have resulted from the dieback of some upper part or entire stems of multi-stemmed individuals (Wang et al., 2023). In line with this, the reduction of stem section area in summer could be an effect of low water potential of plants caused by drought and high temperatures which affect equally at all seedlings. However, in accordance with our third hypothesis, the climate change treatment produced the greatest effects in *Q. faginea,* by reducing the spring

growth of seedlings (2.06 cm less height in OTC plots on average) more than that of *Q. ilex* seedlings (where OTCs only reduced 0.33 cm). The effect was probably due to the higher stomatal sensitivity to soil water deficiency and higher transpiration rate of *Q. faginea* (Alonso-Forn et al., 2021a; Mediavilla et al., 2003). Under conditions of prolonged and intense drought, the conservative strategy can result in greater losses in terms of growth, as found in our study in spring, or ultimately to death by carbon starvation (McDowell et al., 2008). However, we only found different responses of the two species to warming in terms of growth, but not survival, and only in the growth that occurred in spring. This suggests that the deciduous *Q. faginea* was affected since spring, while the evergreen *Q. ilex* was initially more resistant in terms of growth to the induced heat (as also found elsewhere (Alonso-Forn et al., 2021a; Forner et al., 2018; Montserrat-Martí et al., 2009). In terms of survival, the prolonged summer also took its toll on the evergreen *Q. ilex*, whose strategy of remaining photosynthetically active during summer can result in xylem embolism and death by cavitation (Martín-Sánchez et al., 2022).

4.3. Effects of acorn size

Acorn fresh weight was positively related to the probability of emergence, growth and relative chlorophyll content, and these relationships were consistent across both species. The relationship of acorn size with overall seedling performance is largely known (Gómez, 2004; Löf et al., 2019). In accordance with other studies in oak species (Carroll et al., 2019; Mechergui et al., 2021; Sánchez-Montes de Oca et al., 2018), our results show that larger acorns have a higher probability of emergence and a positive relationship with seedling size. Given that most of the resources for initial seedling growth depend on acorn reserves rather than soil nutrients (Shi et al., 2019, 2018; Villar-Salvador et al., 2009), larger acorns can retain a greater proportion of their reserves after germination and produce larger seedlings

(Gómez, 2004; Quero et al., 2007). Therefore, these larger seedlings can have higher performance during summer drought than small seedlings derived from small acorns (Cuesta et al., 2010a, 2010b).

The ultimate effect of heavier acorns on oak regeneration, however, should be evaluated in the light of multiple ontogenetic stages. These include, for instance, the preference for pre- and post-dispersal seed predation by insects and granivorous species (Gómez, 2004; Villalobos et al., 2020), the relationship with seedling responses to freezing events in early spring (Aizen and Woodcock, 1996; Donohue et al., 2010), and the effect of acorn mass on the sensitivity to extreme summer drought, as found here and elsewhere (Gómez, 2004; Ramírez-Valiente et al., 2009). Although the relationship between acorn size and seedling performance diminishes with seedling age, some effects can remain in the populations for years (Ramírez-Valiente et al., 2009; Shi et al., 2018).

Therefore, acorn fresh weight should be considered as a factor that could counter-balance the negative effects of predicted climate change scenarios. However, the possible effects of a strong selection of acorn size should be further evaluated in terms of potential reductions in intrapopulation genetic and phenotypic variability, which could result in other side-effects on restored populations such as modifications in the capacity of response to environmental conditions (Morcillo et al., 2020), acorn dispersal (Chen et al., 2023) and population expansion (Woziwoda et al., 2023).

4.4. Applied implications

Given rapid climate change, ecological restoration considering future climate is warranted to aid in the maintenance of local populations and support ecosystem adaptation (Hoegh-Guldberg et al., 2008; Leverkus et al., 2015a). According to our findings, an advance in emergence could partially balance the negative effects of climate change, but rising

temperature may cause an overall increase in seedling mortality and a reduction in growth, suggesting that natural regeneration would be limited. Although our study was conducted under the expectation that one species would be less affected than the other by temperature increase, both species had similar losses in terms of survival, growth and physiological status. Then, although selecting the less sensitive species for ecosystem restoration projects could improve the overall establishment of individuals from revegetation –and thereby help adapt the future forest to hotter conditions through assisted compositional change (Lunt et al., 2013)–, species considered to be more sensitive can sometimes yield surprisingly positive results, as in our case. Further, selecting different species to create mixed forests increases diversity and may drive resistance to natural disturbances such as mammal herbivory, insect pests, fungal diseases, fires, drought events and windstorms (Jactel et al., 2017; Jactel and Brockerhoff, 2007; Kelty, 2006; Pardos et al., 2021; Stemmelen et al., 2022).

Larger acorns increased the probability of emergence, produced larger seedlings and advanced seedling emergence, especially in *Q. faginea*, which improved seedling survival and may thereby help regenerate oak stands. Thus, acorn size can represent a key trait to consider for assisted oak regeneration in a context of climate change, which may be complemented by known restoration techniques such as facilitation by nurse plants (Gómez-Aparicio, 2009; Plieninger et al., 2010) to increase the success of oak recruitment. Therefore, we conclude that, for the most susceptible species and under more stressful scenarios, it is not necessary to completely replace one species with other more resistant ones, but selecting traits –such as larger acorns– could help favor adaptation to future conditions.

5. Conclusions

Climate change, simulated with *Open-Top Chambers*, sped up the emergence of *Q. ilex* and *Q. faginea* seedlings and increased physiological stress and death rates during their first summer. Seedling performance was especially reduced for *Q. faginea,* yet only in terms of growth and only early in the summer. Early emergence partially balanced the negative effects of climate change by showing a positive relationship with seedling survival. Heavier acorns improved many of the measured parameters –including the probability of emergence, seedling growth, and relative chlorophyll content– regardless of species and climatic scenario. Besides, heavier acorns of *Q. faginea* emerged earlier and are therefore likely to increase their survival rates. Future restoration projects should consider appropriate selection of traits such as seed size to improve early revegetation success, and they should not overlook local species considered to be more sensitive to changing conditions.

Data availability

The dataset underlying this study is available in the Zenodo repository at https://doi.org/XXXXXXX

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FIGURE LEGENDS

Figure 1. Effect of warming treatment (control vs. OTC) on mean daily a) temperature and b) relative humidity during the experiment. Measurements were made with data loggers in 15 OTC and 14 control plots. The grey shaded area indicates 95% confidence intervals.

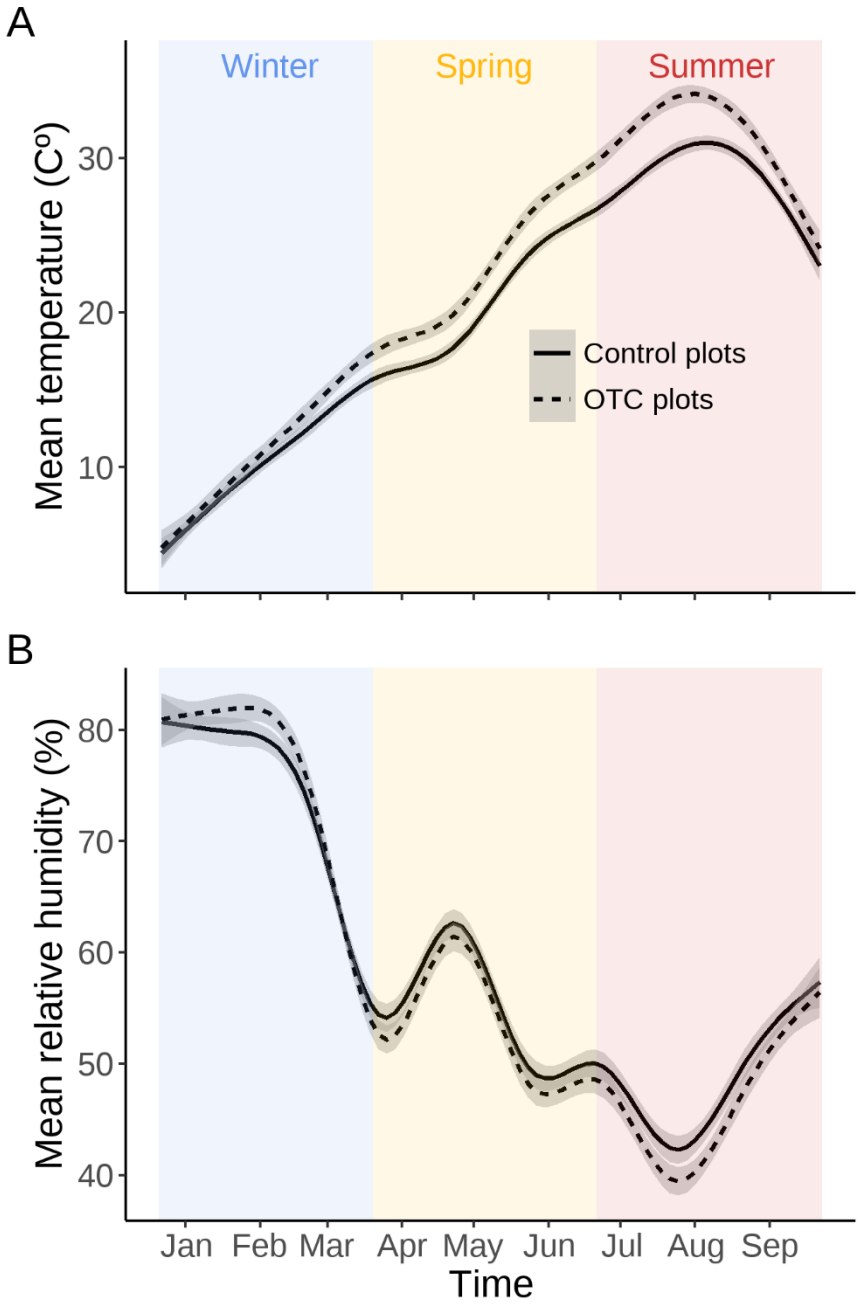


Figure 2. Response of emergence time to A) warming treatment and B) the species × acorn mass interaction. The numbers indicate time from seeding to peak emergence, and the dotted vertical lines in panel A show the average emergence time. The lines in panel B come from the GLM shown in Table 1B.

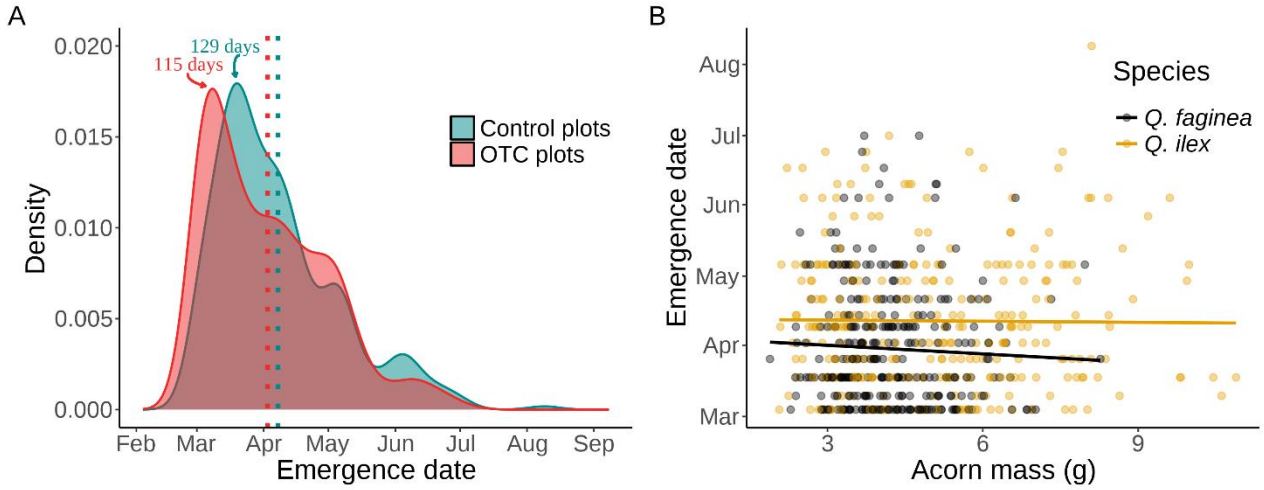


Figure 3. Probability of survival in September as a function of the timing of emergence, separating by A) the warming treatment and B) oak species. The lines show model predictions, and the shaded grey lines the confidence interval at 95%.

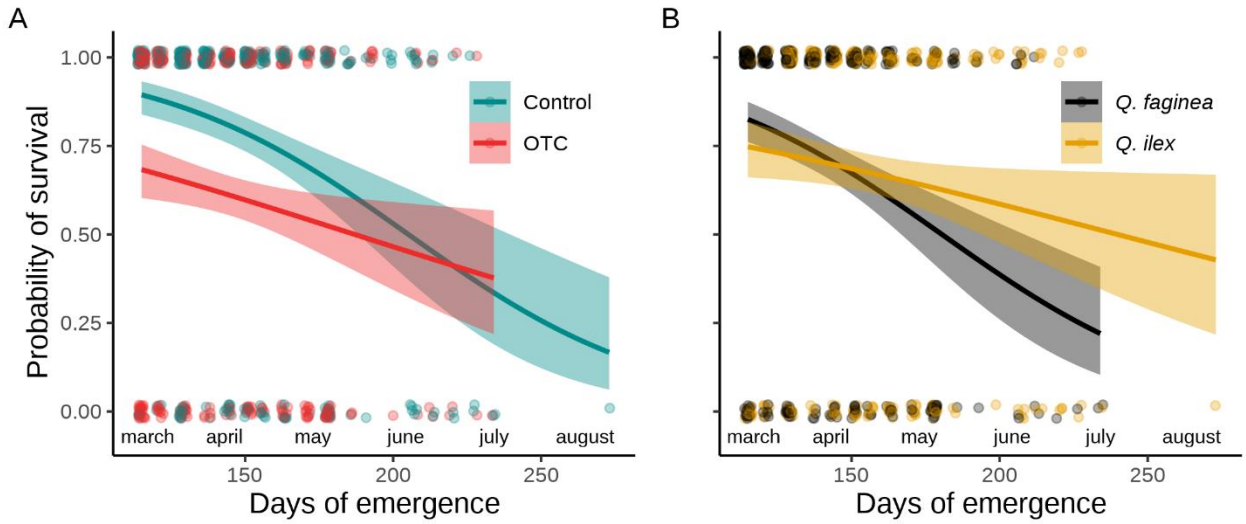


Figure 4. Response of spring growth (mean (±SE) seedling height in July) A) to a species × warming treatment interaction and B) to acorn mass, and C) of summer growth (Relative Growth Rate from July to September; RGR) to species. The values in A and C and the line in B show model predictions, and the shaded grey lines in B the confidence interval at 95%.

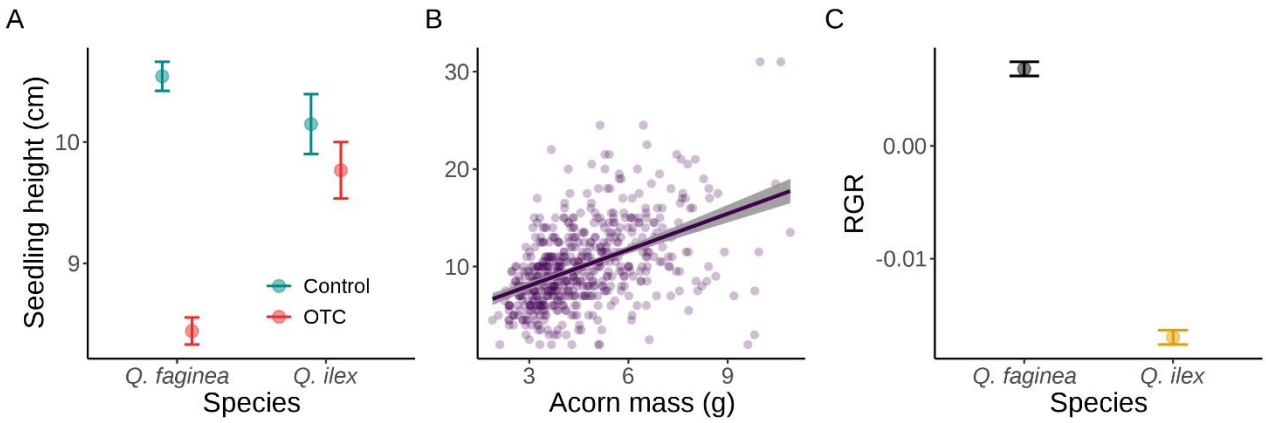


Figure 5. Response of spring growth (mean (±SE) stem section area in July) to A) species, B) warming treatment, and C) acorn mass. The values in A and B, and the lines in C, show model predictions and the shaded grey lines the confidence interval at 95%. Points in C are measured values.

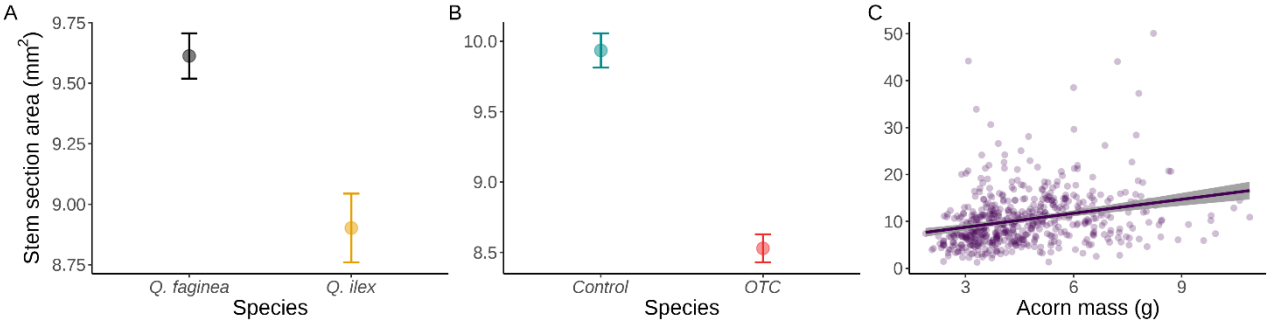


Figure 6. Response of mean (±SE) leaf relative chlorophyll content (RCR) to A) warming treatment, and B) correlation with acorn mass. The values in A and lines in B show model predictions and the shaded grey lines the confidence interval at 95%. Points in B are measured values.

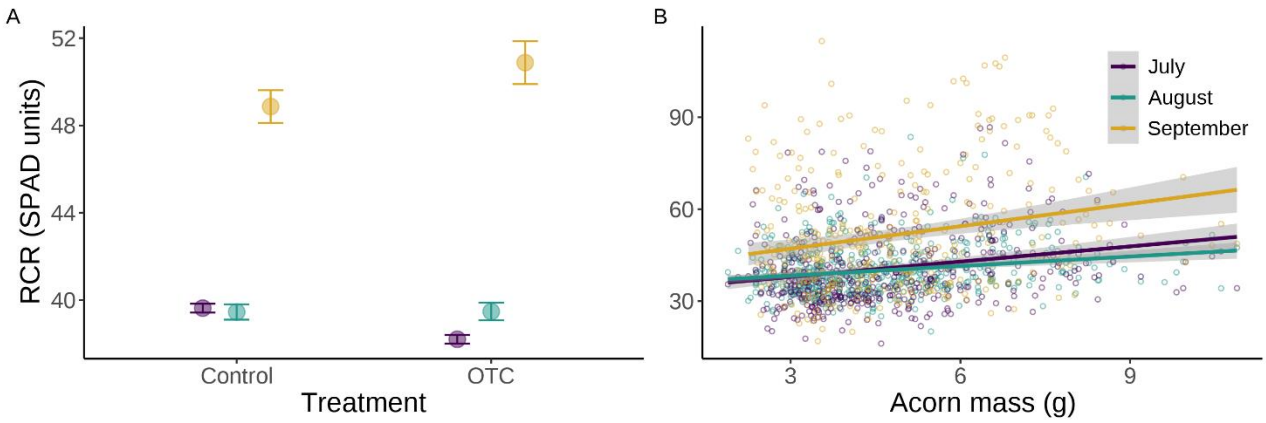


Table 1. Effects of species (*Q. faginea* vs. *Q. ilex*), warming treatment (WT: control vs. OTC) and acorn mass on A) the probability of seedling emergence, B) time to emergence, and C) seedling survival at the end of the first growing season, based on Likelihood Ratio Tests performed on generalized mixed-effects models. The effect of time to emergence was also tested for C) seedling survival.

	Emergence (N=958)	**Time to emergence (N=607)**	**Seedling survival (N=607)**
Variables [3]	**LRT (χ^2) [1]**	**LRT (χ^2) [1]**	**LRT (χ^2) [1]**
Acorn mass	4.00 *	0.78	3.37
WT	1.10	20.87 ***	20.79 ***
Species	0.48	19.91 ***	0.09
Time to emergence			21.81 ***
Row [2]	0.04	95.94 ***	5.46 *
Column [2]	8.42 **	96.17 ***	1.58
Acorn mass x WT	3.42	0.10	0.03
Acorn mass x Species	1.45	5.72 *	1.69
Acorn mass x Time to emergence			0.83
WT x Species	0.51	1.38	1.69
WT x Time to emergence			4.64 *
Species x Time to emergence			6.74 **
Acorn mass x Species x WT	0.98	0.63	1.73
Acorn mass x Species x Time to emergence			0.55
Acorn mass x WT x Time to emergence			<0.01
WT x Species x Time to emergence			0.67

[1] Significance of *P-value* is indicated as: ns>0.05; *≤0.05; **≤0.01; ***≤0.001.

[2] Spatial covariates indicative of plot coordinates within the experimental setting.

[3] Degrees of freedom = 1 for all variables and interactions tested.

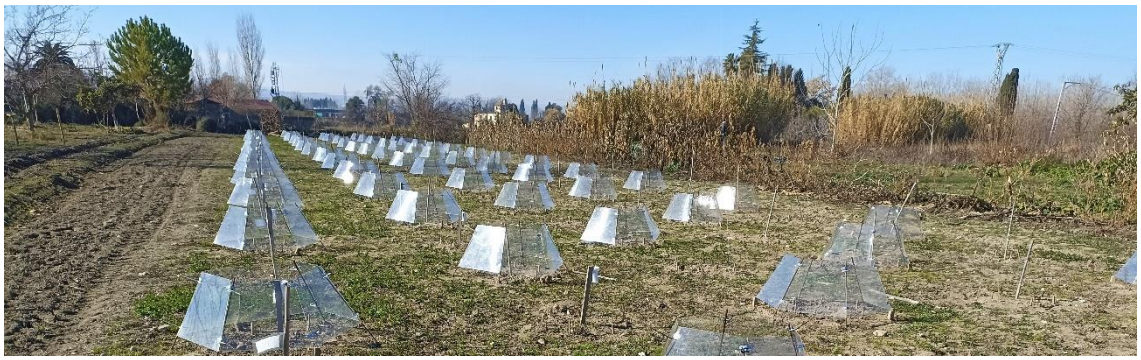
Table 2. Effects of species (*Q. faginea* vs. *Q. ilex*), warming treatment (WT: control vs. OTC) and acorn mass on A) seedling height, B) stem section area, and C) chlorophyll concentration during the first summer, based on Likelihood Ratio Tests performed on mixed-effects models. Spring growth correspond to total seedling height or stem section area in July, and summer growth to RGR from July to September.

	Seedling height		**Stem section area**		**SPAD values**		
	Spring growth (N=561)	**Summer growth (N=390)**	**Spring growth (N=561)**	**Summer growth (N=397)**	**July (N=541)**	**August (N=467)**	**September (N=340)**
Variables [3]	**LRT (χ^2) [1]**	**F-value [1]**	**LRT (χ^2) [1]**	**LRT (χ^2) [1]**	**LRT (χ^2) [1]**	**LRT (χ^2) [1]**	**LRT (χ^2) [1]**
Acorn mass	48.30 ***	0.94	55.34 ***	2.23	0.92	4.24 *	0.64
WT	23.52 ***	0.86	13.17 ***	0.83	8.47 **	0.01	0.82
Species	2.09	5.49 *	15.65 ***	0.35	29.11 ***	25.29 ***	38.64 ***
Row [2]	3.76	0.63	12.61 ***	11.39 ***	1.34	38.88 ***	3.99 *
Column [2]	0.01	2.12	<0.01	0.53	0.75	0.01	0.43
Acorn mass x WT	0.10	0.19	0.41	0.31	3.06	0.29	0.84
Species x Acorn mass	0.78	2.10	0.57	1.98	0.58	0.01	0.88
Species x WT	6.24 *	0.10	0.32	0.39	1.23	1.05	<0.01
Species x Acorn mass x WT	0.63	1.56	1.47	0.21	0.19	0.15	1.56

[1] Significance of *P-value* is indicated as: ns>0.05; *≤0.05; **≤0.01; ***≤0.001.
[2] Spatial covariates indicative of plot coordinates within the experimental setting.
[3] Degrees of freedom = 1 for all variables and interactions tested.

Appendix A

Figure S1. Experimental design of study in “Huerta la Paloma”, where an OTC was displayed every second plot. Each plot received 6 acorns (for details, see Methods).



Appendix B.

Table S1. Coefficients of determination for Generalized mixed-effect models extracted of all statistical models tested in this study. The results show the variance explained by the fixed effects (marginal) and by the entire model including fixed and random effects (conditional). The last column indicates the variance explained by maternal effects (random factor). The intensity of the green shading is positively related to the magnitude of the explained variance. Coefficients were calculated with the *r.squaredGLMM* function of the *MuMin* package in R (Barton, 2023; R Core Team, 2022).

Variable	R2 marginal	R2 conditional	Difference
Emergence	0.023	0.269	0.246
Speed of emergence	0.374	0.538	0.164
Survival	0.117	0.134	0.017
Spring growth (height)	0.188	0.313	0.125
Summer growth (height)	-	-	-
Spring growth (SSA)	0.152	0.159	0.007
Summer growth (SSA)	0.028	0.039	0.011
SPAD_july	0.167	0.223	0.056
SPAD_august	0.212	0.254	0.042
SPAD_september	0.298	0.383	0.085

Barton, K., 2023. MuMin: Multi-Model Inference. R package version 1.47.5, <https://CRAN.R-project.org/package=MuMIn>.

R Core Team, 2022. R: A Language and Environment for Statistical Computing.