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MECANISMOS Y PROCESOS IMPLICADOS EN LA REGENERACIÓN DEL BOSQUE MEDITERRÁNEO EN RESPUESTA A LA HETEROGENEIDAD AMBIENTAL: DESDE LA FISIOLOGÍA HASTA LA DEMOGRAFÍA

TESIS DOCTORAL

José Luis Quero Pérez
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MECANISMOS Y PROCESOS IMPLICADOS EN LA REGENERACIÓN DEL BOSQUE MEDITERRÁNEO EN RESPUESTA A LA HETEROGENEIDAD AMBIENTAL: DESDE LA FISIOLOGÍA HASTA LA DEMOGRAFÍA

Memoria que el Licenciado José Luis Quero Pérez presenta para aspirar al Grado de Doctor por la Universidad de Granada

Esta memoria ha sido realizada bajo la dirección de:
Dr. Rafael Villar Montero y Dr. Regino Zamora Rodríguez

Ldo. José Luis Quero Pérez

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Dr. Rafael Villar Montero, Profesor Titular de Ecología de la Universidad de Córdoba **y Dr. Regino Zamora Rodríguez**, Profesor Titular de Ecología de la Universidad de Granada

CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: "Mecanismos y procesos implicados en la regeneración del bosque mediterráneo en respuesta a la heterogeneidad ambiental: desde la fisiología hasta la demografía"**, son aptos para ser presentados por el Ldo. José Luis Quero Pérez ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad de Granada.**

Y para que así conste, en cumplimiento de las disposiciones vigentes, extendemos el presente certificado a 14 febrero de 2007

Dr. Rafael Villar Montero **Dr. Regino Zamora Rodríguez**

Durante el tiempo de realización de esta Tesis Doctoral he disfrutado de una Beca del Programa Nacional de Formación de Personal Investigador del Ministerio de Educación y Ciencia Ref. (BES–2003–1716).

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A Josefa, Amalia, Gonzalo y Teresa,

A los enanos

A Maria

A fuerza de horas de exposición,
una placa fotográfica situada en el foco
de un anteojo dirigido al firmamento,
llega a revelar astros tan lejanos
que el telescopio más potente es incapaz de mostrarlos;
a fuerza de tiempo y atención, el intelecto
llega a percibir un rayo de luz en las tinieblas
del más abstruso problema.
La comparación precedente no es del todo exacta.
La fotografía astronómica limítase a registrar
actos pre-existentes de tenue fulgor;
mas en la labor cerebral se da un acto de creación.

Santiago Ramón y Cajal

Voy a hacer una estatua ecuestre,
cuestre lo que cuestre.

Les Luthiers

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ÍNDICE

1. RESUMEN

1.1. RESUMEN

La presente tesis doctoral versa sobre la respuesta a la heterogeneidad ambiental de diferentes especies leñosas mediterráneas, gran parte de ellas distribuidas en el Parque Nacional de Sierra Nevada, al sureste de la Península Ibérica. En ella, se evalúan las diferentes variables ambientales que pueden afectar al crecimiento y supervivencia de las plantas desde diferentes aproximaciones. El ciclo de regeneración de una especie puede verse seriamente limitado e incluso colapsado por cualquier etapa demográfica cuya probabilidad de establecimiento exitoso esté próxima a cero. En ambientes Mediterráneos, la fase de plántula suele ser la más limitante para el establecimiento, ya que es la más sensible ante cualquier circunstancia adversa. Es por ello que en el primer bloque de esta tesis, se analizan las condiciones ambientales donde las plántulas potencialmente pueden establecerse. Estas áreas suelen ser conocidas como micrositios, definidos como el entorno que inmediatamente rodea a una plántula y los cuales están influenciados por un conjunto de variables ambientales que pueden variar en el espacio a pequeña escala. Así, en el **capítulo 1**, en la zona del Trevenque (P. Nac. de Sierra Nevada), caracterizada por diferentes unidades de paisaje (bosque autóctono, repoblaciones forestales y zonas de matorral pionero) se llevo a cabo un estudio de aproximadamente 1000 micrositos potenciales en cada una de ellas, con un diseño espacialmente explicito, es decir, conociendo la posición relativa en el espacio de cada uno de los puntos de muestreo. De esta manera, podemos conocer la variación en el espacio de las variables y comprobar si dichas se distribuyen a lo largo del mismo, de manera aleatoria, regular, o agregada. En cada punto de muestreo, se evaluaron un conjunto de variables, de las que destacan la humedad del suelo en diferentes épocas cruciales para la plántula, la disponibilidad lumínica, la compactación del suelo o la microtopografía. De esta manera, y mediante técnicas de análisis espacial como SADIE

(análisis espacial basado en índices de distancia) se evaluó la heterogeneidad ambiental en cada una de las unidades de paisaje, se comparó entre ellas, además de analizar la co-variación espacial de las distintas variables. Los resultados más relevantes de este estudio fueron que el bosque autóctono presentó la mayor heterogeneidad espacial, es decir, fue la zona donde las variables se distribuían espacialmente de una manera más agregada, seguido de la reforestación y la zona de matorral pionero. Por otro lado se encontraron diferentes variables que estaban asociadas o disociadas en el espacio, es decir, en el caso de estar asociadas espacialmente, valores altos de las variables (parches) coincidían en el espacio; por el contrario, en el caso de estar disociadas, valores altos de una variable coincidían espacialmente con valores bajos (claros) de otra. Tal era el caso de la disociación existente entre la compactación del suelo y la profundidad de hojarasca: zonas con una alta compactación del suelo, se correspondían con zonas con una baja profundidad de hojarasca. Adicionalmente pudimos observar asociaciones espaciales como la que se apreciaba en el caso de la disponibilidad lumínica con la humedad del suelo. Zonas con valores altos de disponibilidad lumínica coincidían en el espacio con zonas de alta disponibilidad hídrica. Este fenómeno podría atribuirse a la alta cobertura de herbáceas existente en estas zonas, los cuales forman una capa de vegetación que reduce la perdida de humedad en los primeros centímetros del suelo dejando estas zonas con un mayor contenido hídrico.

En el **capítulo 2**, evaluamos la influencia de la heterogeneidad ambiental sobre la supervivencia de diferentes especies leñosas. Para ello, en una submuestra de puntos del capítulo 1, se realizaron siembras con encina (*Quercus ilex*) y serbal (*Sorbus aria*), a las que se les realizó un seguimiento para determinar el patrón espacial de la supervievencia. Se realizaron dos siembras, una en 2004, un año relativamente húmedo y 2005, relativamente seco. El patrón espacial de supervivencia se analizó utilizando regresión múltiple asociada a un método de partición de la variación que discrimina la parte de variación explicada por las variables ambientales y la explicada por el patrón espacial de los datos. La supervivencia de las especies evaluadas siguió una distribución agregada pero esto sólo ocurrió en el año seco, donde las condiciones extremas del verano se intensificaron, con lo que sólo sobrevivieron las plántulas en ciertas manchas que se correspondían fundamentalmente con zonas donde la humedad del suelo fue mayor. Las variables que seleccionó el modelo estadístico como mas importantes, fueron la disponibilidad lumínica y la humedad del suelo en verano. Por tanto, podemos concluir que la heterogeneidad espacial de variables ambientales, puede llegar a determinar el patrón espacial de la supervivencia de las especies estudiadas.

En el capítulo 2 del primer bloque se determinó que la luz y el agua fueron los factores mas importantes que influyeron en la supervivencia. Es por ello que en el siguiente bloque de la tesis se estudiaron diferentes combinaciones de luz y agua en condiciones controladas, para evaluar los mecanismos de respuesta de las plántulas a estas dos variables. Se llevó a cabo un macro experimento del que se han extraído los tres siguientes capítulos de la presente tesis doctoral. **El capítulo 3** versa sobre un estudio de la influencia del peso de semilla sobre la plántula sometida a tres condiciones lumínicas diferentes tras 50 días de crecimiento. Se eligieron cuatro especies del género *Quercus* con las que se evaluaron tres hipótesis sobre la influencia del peso de la semilla: 1) las semillas más grandes retienen una mayor proporción de sus reservas para afrontar riesgos potenciales, 2) plántulas provenientes de semillas más grandes tienen una menor tasa de crecimiento relativo para así consumir menos reservas y poder utilizarlas ante futuros riesgos y 3) semillas más grandes generan plántulas más grandes. Las diferentes hipótesis se evaluaron a lo largo de los distintos ambientes lumínicos mediante “Standard major axis regression”, una técnica estadística que permite comparar las pendientes de las regresiones lineales sin efecto de dependencia de una variable sobre otra. La hipótesis más ampliamente aceptada en este experimento es que las semillas más grandes, generaron plántulas de mayor tamaño y, que la dependencia del efecto de la semilla fue más fuerte en bajas intensidades lumínicas. Adicionalmente, se construyó un modelo causal conectando las tres hipótesis. El resultado principal del modelo propuesto es que existe alta probabilidad de encontrar un efecto de la semilla en generar plántulas más grandes con independencia de que las hipótesis 1 y 2 se cumplan.

Dentro de este bloque, el **capítulo 4** trata sobre la influencia de la luz y el agua en las respuestas a nivel foliar. Las cuatro especies objeto de estudio anteriormente descritas fueron sometidas a tres niveles de luz y dos de agua. En este capítulo nos preguntamos 1) si la combinación de condiciones limitantes de luz y agua (sombra y sequía) es positivo, negativo o independiente sobre el funcionamiento de la planta, 2) si las distintas especies o grupos funcionales (caducifolias vs. perennifolias) responden de diferente manera, 3) qué variables fueron mas afectadas por el estrés combinado de sombra y sequía y 4) qué relaciones funcionales hay entre cuatro variables importantes relacionadas con el funcionamiento de las planta: concentración de N foliar, área específica foliar, conductancia estomática y tasa de fotosíntesis. Después de 7 meses de crecimiento, dentro de cada tratamiento, se realizaron medidas de intercambio gaseoso en respuesta a diferentes niveles de luz. Los datos resultantes se ajustaron a un modelo de fotosíntesis en respuesta a la luz y del que se desprendieron diferentes parámetros fotosintéticos como la fotosíntesis máxima, la respiración o el punto de compensación a la luz. Todas ellas formaban el conjunto de las variables fisiológicas. Por otro lado las hojas analizadas fueron cosechadas para medir diferentes variables morfológicas como el área específica foliar (SLA), la concentración de carbono y nitrógeno. En total se analizaron 7 variables morfológicas y 13 variables fisiológicas a nivel foliar en cada especie y por combinación de luz y agua (6 tratamientos). A partir de los resultados obtenidos se concluyó que 1) la combinación de la sombra y sequía no tenía efectos negativos a nivel foliar, sino más bien lo contrario, es decir, el efecto de la sombra mitigaba las condiciones de sequía, 2) las especies caducifolias difirieron en las respuestas morfológicas y fisiológicas con respecto a las perennifolias, 3) tanto las variables fisiológicas como morfológicas tuvieron una alta respuesta a la variación de la luz sin embrago las variables morfológicas tuvieron una respuesta relativamente baja a la variación en la disponibilidad de agua y 4) en cuanto a la relación entre variables, el área específica foliar explicó la concentración de nitrógeno la cual afectaba a la conductancia estomática que regulaba la tasa de fotosíntesis. Además, existió una relación directa entre la tasa de fotosíntesis y el área específica foliar no mediada por la concentración de nitrógeno.

En la última parte de este bloque, el **capítulo 5**, se estudiaron las respuestas a la luz y a el agua pero en este caso a nivel de planta completa. Los objetivos que contemplamos en este capítulo fueron 1) evaluar el efecto del tamaño de semilla tras 7 meses de crecimiento bajo los distintos niveles de luz y agua, 2) analizar las respuestas del crecimiento relativo (RGR), biomasa total y distribución de biomasa de las cuatro especies así como de los dos grupos funcionales a los que pertenecen (caducifolias y perennifolias), 3) determinar qué componentes del crecimiento influyen en la variación de RGR y 4) comprobar la capacidad de escalar las respuestas observadas a nivel foliar (capítulo 4) con las respuestas observadas a nivel de toda la planta. La tasas de crecimiento relativo se calcularon a partir de una cosecha inicial a los 4 meses de crecimiento y una cosecha final a los 7 meses, una vez finalizado el experimento. Una vez extraídas las plantas, los distintos órganos (raíz, tallo y hojas) se pesaron en seco para determinar la biomasa total y la distribución de biomasa. Para cumplir el objetivo

4) se realizó una estima de la ganancia total de carbono por parte de la hoja teniendo en cuenta la radiación incidente durante los meses de crecimiento y se relacionó con la tasa de crecimiento relativo (RGR). Los resultados indicaron que después de 7 meses de crecimiento, aun había un efecto de la semilla sobre la biomas total, aunque el uso de las reservas varió a lo largo de las especies. Al igual que a nivel foliar, se encontraron efectos interactivos de la luz y el agua en la planta completa, aunque fueron de menor intensidad debido principalmente a la progresiva sequía aplicada en el experimento. En cuanto a los componentes del crecimiento, RGR estuvo muy relacionada con parámetros fisiológicos del crecimiento mas que con morfológicos, lo que sugiere que la plantas respondieron a la luz y a el agua ajustando sus actividades fisiológicas y no las morfológicas. Por último, la tasa de crecimiento relativo estuvo relacionada con la estima de ganancia de carbono calculada a partir de las medidas a nivel foliar, lo que indica el potencial para predecir las respuestas del crecimiento en distintas combinaciones de luz y agua a partir de medidas de intercambio gaseoso a nivel foliar.

Finalmente, en el tercer y último bloque de esta tesis doctoral (**capítulo 6**) se realizó una aproximación observacional sobre el nicho de regeneración y el cambio ontogenético del mismo. El estudio se realizó cuantificando el nicho de regeneración de individuos de la especie *Acer opalus* subsp. *granatense* en diferentes estadios demográficos, para poder contestar a las siguientes preguntas 1) ¿experimenta *A. opalus* cambios ontogenéticos durante los estadios tempranos del ciclo de vida (brinzales de un año, juveniles de dos a cinco años y juveniles mayores de cinco años?; 2) si así ocurriese, ¿estos cambios reflejarían expansión o contracción del nicho de regeneración?; y 3) ¿qué variables están rigiendo estos cambios? De los resultados obtenidos, se concluyó que *A. opalus* experimenta cambios en el nicho de regeneración con la edad, reflejando una contracción del nicho, localizándose principalmente en matorrales no palatables. Los factores que condujeron este proceso fueron la profundidad de hojarasca, las coberturas arbórea y de matorral así como la herbivoría por parte de ungulados.

1.2. ABSTRACT

This PhD thesis deals about response to environmental heterogeneity of different Mediterranean woody species, most of them distributed in Sierra Nevada National Park at Iberian Peninsula. From different approaches, different environmental variables that potentially can affect to the plant growth and survival are evaluated. The regeneration cycle of species can be seriously limited and even collapsed by any demographic stage whose probability of successful establishment is next to zero. In Mediterranean areas, seedling stage is usually limiting for establishment, since it is more sensible to any adverse circumstance. For that reason, in the first part of this thesis, the environmental conditions where seedlings potentially can establish have been analysed. These areas usually are known as microsites, the areas that immediately surround to seedling being influenced by a set of environmental variables that can vary in the space at small scale. Thus, in **chapter 1**, in Trevenque area (Sierra Nevada National Park), characterized by different landscape units (native forest, reforestation stand and shrubland) a study was carried out of approximately 1000 potentials microsites in each landscape unit, with a spatial explicit design for knowing the relative position in the space of each sampling points. In this way, we can know the variation in the space of variables and verify whether the spatial pattern of them is regular, random or aggregates. In each sampling point, a set of variables have been evaluated (i. e., soil moisture at different crucial times for seedlings, light availability, soil compaction or microtopography). Using This techniques of spatial analysis as SADIE (space analysis based on distance indices) the environmental heterogeneity in each one of the landscape units was evaluated and compared among them. Additionally, spatial co-variation of different variables was also evaluiated. The most excellent results of this study were that the native forest presented the highest spatial heterogeneity, that is mean, it was the zone where the spatial

distribution of variables were more aggregated, followed by reforestation stand and shrubland. On the other hand, different variables were spatially associated or dissociated, such as spatial dissociation between soil compaction and litter depth: zones with high soil compaction spatially coincided with lower litter depth. Additionally, spatial associations were observed such a light availability and soil moisture: zones with high values of light availability coincided in the space with zones of high soil moisture. This phenomenon was attributed to the higher herbaceous cover in these zones, which reduced soil water lost at the first centimetres of the ground. In **chapter 2**, the influence of the environmental heterogeneity on the survival of different woody species was evaluated. For this proposal, in a subsample of points from chapter 1, two species was sowed, *Quercus ilex* and *Sorbus aria*, which were monitorized to determine survival spatial pattern. Two sowings were done, in 2004, a year relatively humid and 2005, relatively dry one. Survival spatial pattern was analysed using multiple regression analysis associate to a variation partition method, which discriminates the variation explained by the environmental variables and the explained one by the space pattern of the data. Survival of the evaluated species followed a aggregated distribution but this only happened in the dry year, where the extreme conditions of the summer intensified. Therefore, Patches of seedling survival was found in areas corresponding with zones where soil moisture was higher. Another variable selected by statistical model was light availability. As conclusion, spatial heterogeniety of environmental variables, can determine the survival spatial pattern the studied species.

In chapter 2 of the first part, light and the water availability were ones of the most important factors influencing survival. For that reason, the following part of this thesis studied different combinations from light and water in controlled conditions, to evaluate the seedling responses mechanisms to these variables. Three chapters was dedicated to evaluated these responses. **Chapter 3** was a study of seed mass effect on seedlings under three different light conditions after 50 days of growth. Four *Quercus* species were chosen to evaluated three hypotheses: 1) the larger seeds retain a larger proportion of their reserves on the face of potential hazards, 2) seedlings from larger seed have a lower relative growth rate (RGR) to consume less reserves being used in front of future risks and 3) larger seeds generate larger seedlings. The different hypotheses was evaluated under different light conditions using "Standard major axis regression", a statistical technique that allows to compare slopes of the linear regressions without dependency effect of a variable on another one. The hypothesis widely accepted in this experiment is that the larger seeds generated larger seedlings of and dependency of seed mass effect was higher under low irradiance level. Additionally a causal model was constructed to connect the three hypotheses. The main result of the proposed model is that there is high probability for accepting hypothesis 3 independently of hypotheses 1 and 2 are fulfilled. The second chapter of this part, **chapter 4**, deals with the influence of light and the water leaf-level responses. The target species previously described, were subjected under three levels of light and two levels of water. In this chapter the following question were addressed: 1) does the combination of extreme conditions of light and water (shade and drought) is positive, negative or independent?; 2) do the different species or functional groups (evergreens vs. deciduous) respond in different way?; 3) which physiological and structural leaf traits are most affected by the combined stress?; and 4) what are the functional relationships among those variables? After 7 months of growth, within each treatment, photosynthetic light response curve were made at different light levels. The resulting data was adjusted to a photosynthetic model to derive different photosynthetic parameters such a maximum photosynthetic rate, stomatal conductance or light compensation point (LCP). All of them were the set of the physiological variables. On the other hand sampled leaves were harvested to measure different morphological variables such a specific leaf area (SLA), carbon and nitrogen contents. Altogether, 7 morphological variables and 13 physiological ones at leaf level were analysed in each species and light per water combination (6 treatments). From the obtained results was concluded that 1) the combination of the shade and drought did not have negative effects at leaf level, that is mean, the effect of the shade mitigated the drought conditions, 2) deciduous species differed in the morphological and physiological responses with respect to evergreen, 3) physiological and morphological variables had a high response to light variation whereas morphological variables had a relatively low response water variation, and 4) with respect to relation among variables, specific leaf area explained the nitrogen concentration which affected stomatal conductance regulating photosynthetic rate. In addition, a direct relation between photosynthetic rate specific leaf area existed to foliar non-mediated by nitrogen concentration.

In the last chapter of this part, **chapter 5**, responses to light and water at whole-plant level were studied. The objectives of this chapter were 1) to evaluate the effect seed after 7 months of growth under the different light and water levels, 2) to analyse the relative growth rate (RGR), total growth and distribution of biomass on the four species and the two functional groups (deciduous and evergreen), 3) to determine which growth components influence in RGR variation and 4) to verify the capacity to scale the observed responses at leaf-level (chapter 4) with the observed ones at whole-plant level. RGR was calculated from an initial harvest after 4 months of growth and a final harvest at the end of the experiment (7 month). Once extracted the plants, the different components (root, stem and leaves) were weighed to determine the biomass distribution and the total growth. In order to fulfil objective 4), an estimate of the total leaf carbon gain was calculated having in account the incident radiation during the growth months and it was related to the rate of relative growth (RGR). As results, after 7 months of growth, even there was an effect of the seed in the total growth although the use of the reserves varied throughout the species. Such as leaf-level (chapter 4), there were interactive effects of light and water, although they were not too strong, mainly due to the progressive drought applied in the experiment. Concerning to growth components, RGR was related to physiological growth parameters more than morphological ones, suggesting that plants responded to light and water fitting its physiological activities. Finally, RGR was related to the estimate of leaf carbon gain, indicating the potential to predict the growth responses under different combinations of light and water from gas exchange measurements at leaf-level.

Finally, in the last part of this thesis, **chapter 6** was included. An observational approach about ontogenetic niche shift was done. The study was made quantifying the regeneration niche of *Acer opalus* subsp. *granatense* individuals at different demographic stages. The following questions were addressed: 1) do ontogenetic niche shifts occur in *A. opalus*?, 2) if so, do they reflect niche expansion or contraction among stages?, and 3) what variables drive such shifts? From results, it was concluded that Acer suffered ontogenetic niche shifts reflecting a niche contraction of the niche towards non-palatable shrub. Drivin factors of this process were litter depth, tree and shrub cover, and ungulate herbibory.

2. INTRODUCCIÓN

2.1. INTRODUCCIÓN GENERAL

CONCEPTO DE HETEROGENEIDAD

Ya sea "mezcla de partes de diversa naturaleza en un todo", "consistente de distintos constituyentes" o "cualidad que se aplica a los conjuntos formados por cosas diferentes entre sí y a las cosas que los forman", cualesquiera de las definiciones que aparecen en los diccionarios convencionales (RAE, Maria Moliner, 1998) atisban el sentido de heterogeneidad en ecología. Una de las definiciones más completa viene dada por Milne (1991) donde propone que la heterogeneidad es la complejidad resultante de las interacciones entre la distribución de los factores ambientales y la respuesta diferencial de los organismos a esos factores.

Por tanto, y según esta definición, los organismos viven en hábitats que son altamente heterogéneos tanto en el espacio como en el tiempo (Stewart *et al.*, 2000). Aunque esto hoy día esta ampliamente aceptado, la tendencia tradicional ha sido la de asumir que los sistemas en la naturaleza son homogéneos con el objetivo de simplificar y comprender los procesos e interacciones que en ella se desarrollan. Ello ha contribuido al desarrollo de la teoría ecológica, pese a la pérdida de realidad manifiesta a la hora de trasladar la base teórica y experimental a la investigación en campo (Wiens, 2000).

La heterogeneidad ambiental es un concepto acuñado en los primeros pasos de la historia de la ecología (McIntosh, 1991). A mitad del siglo XX ya se había demostrado mediante experimentos de laboratorio que la heterogeneidad ambiental podría alterar la dinámica de las poblaciones y comunidades (Gause 1935; Huffaker 1958). Con esto se demuestra que la ecología, a pesar de la tendencia tradicional, no ha ignorado a la heterogeneidad. En cualquier caso, ha sido en las últimas décadas cuando se ha forjado un renovado interés por incorporar la heterogeneidad en diversas aproximaciones teóricas a problemas ecológicos relevantes (Terradas, 2001). Wiens (2000) define este hecho como un cambio de paradigma en la ecología. Así por ejemplo, si recordamos el modelo de Lotka-Volterra que describe la interacción entre depredador y presa, asume que el ambiente es homogéneo, ya que la disponibilidad de presas en el espacio no varía. La introducción de la heterogeneidad dio lugar a la teoría de la inversión óptima de esfuerzo en la búsqueda de alimento (*optimal foranging theory*) en la que se reconoce que el individuo debe maximizar su eficacia a la hora de encontrar alimento ya que se encuentra en un ambiente donde las presas se distribuyen de manera irregular. Hoy por hoy, son muchos los ejemplos (la teoría biogeográfica de islas, la ecología de metapoblaciones, las invasiones, la dispersión de organismos sésiles, etc.) en los que la teoría ecológica se construye teniendo en cuenta este concepto.

LA HETEROGENEIDAD EN LA REGENERACIÓN

La regeneración de los ecosistemas es otro tema en el que la heterogeneidad puede desempeñar un importante papel. En ambientes mediterráneos, a escala temporal, se están llevando a cabo estudios predictivos del futuro del bosque mediterráneo (Sabaté *et al*., 2002). A escala espacial, la heterogeneidad puede condicionar en gran medida los procesos de regeneración de las plantas y la estructura de las poblaciones. Como consecuencia, ha recibido merecida atención en diversas escalas de observación, desde escalas que engloban el área de distribución geográfica de una especie (Gómez-Aparicio *et al*., 2005a), hasta nivel de rodal (Gómez-Aparicio *et al*., 2005b). A pesar de ello, en pocas ocasiones se ha explorado la heterogeneidad espacial a pequeña escala y sus consecuencias en los procesos de regeneración (Maestre *et al*., 2003).

El ciclo natural de regeneración de cualquier especie leñosa mediterránea puede estar seriamente limitado, incluso colapsado, por cualquier etapa demográfica cuya probabilidad de establecimiento exitoso esté próxima a cero. En ambientes Mediterráneos la fase de plántula suele ser más limitante para el establecimiento, ya que ésta es más sensible ante cualquier circunstancia adversa. En tal caso, el éxito de establecimiento dependerá de la disponibilidad de micrositios adecuados, entendiendo por micrositio la zona que inmediatamente rodea a una plántula. La calidad de un micrositio, generada por el conjunto de factores abióticos y bióticos (p. ej., humedad y compactación del suelo, disponibilidad de luz, profundidad de hojarasca, etc.) puede variar metro a metro, de ahí la importancia que puede tener la heterogeneidad espacial a pequeña escala.

LA IMPORTANCIA DE LA LUZ, EL AGUA Y OTROS FACTORES

Por otro lado, de entre los factores abióticos que afectan al establecimiento, la luz y el agua se identifican como los mas limitantes en ambientes mediterráneos (Marañón et al. 2004). La combinación de sendos factores genera diferentes ambientes a los que las plántulas tienen que responder. Así, en el bosque mediterráneo, a lo largo del gradiente de luz y agua, las plántulas pueden encontrarse bajo el sotobosque donde los niveles de radiación fotosintéticamente activa pueden reducirse hasta un 3 %, con el agravante de una fuerte reducción de la disponibilidad hídrica en el estío (situación de sombra seca). En el otro extremo, las plántulas medran en zonas desprovistas de vegetación donde el déficit hídrico estival, puede combinarse con radiaciones intensas de hasta 2000 µmoles m^{-2} s^{-1}. Las plantas responden a las diferentes combinaciones de luz y agua a través de mecanismos a nivel foliar y a nivel integrado de toda la plántula, modulando tanto rasgos morfológicos como fisiológicos. Por ejemplo, a nivel foliar, las plantas bajo sombra profunda pueden aumentar su área específica foliar (SLA[1]) para maximizar la

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[1] Del ingles Specific Leaf Area

recepción de fotones (respuesta morfológica) y reducir sus tasas de respiración para maximizar la ganancia de carbono (respuesta fisiológica). A nivel integral, una solución para afrontar la sequía estival, puede ser una mayor inversión en raíz en detrimento de la parte aérea, con lo que al mismo tiempo se consigue acceder a niveles edáficos más profundos y se reducen las pérdidas de agua por transpiración. Sin embrago, situaciones como la sombra seca podrían provocar en las plantas situaciones de conflicto, es decir, basándonos en el mecanismo de compensación propuesto por Smith & Huston (1989) la sombra profunda agravaría el estrés impuesto por la sequía, ya que las plantas al inicio de su crecimiento en primavera, podrían responder a la sombra invirtiendo más parte aérea (hojas, para captar mas fotones y tallo, como sostén de aquéllas) lo que comprometería la captura de agua (por una baja inversión en raíces) a la llegada del verano. Se han planteado diferentes hipótesis en la literatura científica para abordar las respuestas de las plantas a la interacción de la luz y el agua. Parece que los diferentes resultados encontrados dependen de los rangos de la irradiancia y humedad del suelo evaluados (Aranda et al 2005), de las variables respuesta medidas (Quero et al., 2006), incluso de las especies (Prider & Facelli, 2004) o fenotipos estudiados (Valladares et al., 2005) dentro de especie.

En las primeras fases de crecimiento, las plántulas dependen de las reservas de la semilla y ello puede permitir resistir situaciones limitantes, y ser mas independientes de la heterogeneidad ambiental. Se han propuesto diferentes mecanismos por los que el tamaño de la semilla puede contribuir al éxito de las plántulas en el medio (Westoby et al. 1996). El efecto reserva[2], predice que las semillas más grandes retienen una mayor proporción de sus reservas para poder afrontar riesgos potenciales, tales como la destrucción de la parte aérea, ya sea por herbivoría, sequía etc. El efecto metabólico[3]

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[2] traducción libre del ingles “reserve effect”

[3] traducción libre del ingles “metabolic effect”

propone que las semillas mas grandes, disminuyen su tasa de crecimiento (RGR[4]) de manera que los recursos son consumidos más lentamente permitiendo soportar a la planta periodos mas largos de estrés (Green & Juniper 2004). Por último, el efecto tamaño de plántula[5] establece que las semillas mas grandes darán como resultado mayores plántulas con lo que éstas últimas disminuirán las probabilidades de mortalidad asociadas al tamaño de plántula, es decir, plántulas mas grandes pueden captar más luz, más agua o incluso emerger de capas de suelo y hojarasca mas profundas (Foster 1986, Metcalfe & Grubb 1997, Bond et al 1999).

En definitiva, luz, agua y la cualidad inherente del tamaño de semilla son tres importantes fuentes de variación a las que las plantas responderán de diversas maneras, ya sea a nivel de hoja, de plántula, morfológica o fisiológicamente. Investigar cómo estos tres factores interactúan es de esencial valor científico, ya que, por un lado no existen trabajos que combine estos tres factores a un mismo tiempo y, por otro, dada la variedad y complejidad de resultados encontrados en la literatura (Holmgren 2000, Sack & Grubb 2002, Valladares & Pearcy 2002) este problema está aún sin resolver.

EL MICROHABITAT DEL ESTABLECIMIENTO: NICHO DE REGENERACIÓN

De lo expuesto en los anteriores párrafos, se desprende que el medio donde un individuo medra es heterogéneo y que el individuo responderá de manera compleja a esa heterogeneidad. El resultado final de todo este proceso es un microambiente caracterizado por un compendio de variables que sintetizan las condiciones óptimas de establecimiento. Grubb en 1977, llamó a estas condiciones “nicho de regeneración[6]” y más adelante otros autores han considerado este concepto como crucial para entender la composición, estructura y dinámica de las comunidades (Silvertown 2004). Desde que

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[4] del ingles “relative growth rate”
[5] traducción libre del ingles “seedling size effect”

Grubb acuñara este término, son muchos los trabajos que han abordado este concepto (este artículo ha sido citado en SCI[7] 1.418 veces a fecha de 5 de febrero de 2007), sin embargo, en pocas ocasiones se le ha dado un sentido aplicado al mismo (Pywell et al. 2003). Así, la caracterización multivariante del ambiente que rodea a una plántula puede ser de vital importancia a la hora de diseñar proyectos de restauración, ya que en éstos lo que se pretende es maximizar el reclutamiento en condiciones de limitación de propágulos y de nichos de regeneración. Por tanto, para cada especie a manejar, es fundamental conocer a priori la disponibilidad de nichos potencialmente adecuados dentro de la extensa gama de microhábitats que caracterizan los ambientes mediterráneos. En un área a restaurar determinada, esto se podría conseguir realizando un estudio comparado del nicho de regeneración de todas las especies que potencialmente forman el bosque de ese área. Una aproximación observacional multivariante de los micrositios que ocupan los juveniles (ya que es el estadio demográfico en el que los individuos se consideran establecidos, Castro et al. 2006), satisface este propósito (Collins & Good 1987, Collins 1990).

Sin embargo, conviene destacar que los requerimientos de una especie pueden cambiar a lo largo del ciclo de vida (Chase & Leibold 2003, Miriti 2006), o dicho de otro modo, las plantas pueden responden a las condiciones abióticas y bióticas del medio de diferente manera a lo largo de los estadios demográficos. Estos cambios, conocidos como cambios ontogenéticos del nicho[8] (Parrish & Bazzaz 1985) han sido ampliamente estudiados en ecología animal, pero la literatura sobre este concepto en plantas es escasa. De ahí, y como complemento a la caracterización del nicho de regeneración en juveniles, cabe la necesidad de abordar este concepto ecológico, con la intención de considerarlo en las estrategias de manejo y restauración.

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[6] traducción libre del ingles “regeneration niche”
[7] base de datos del “Science citation index”

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[8] traducción libre del inglés “ontogenetic niche shifts”

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2.2. OBJETIVOS Y ESTRUCTURA DE LA TESIS

En resumen, las plantas responden a la heterogeneidad ambiental de los factores abióticos y bióticos modulando mecanismos fisiológicos y morfológicos, los cuales en última instancia determinan la regeneración. Este es el postulado general que manejaremos en la presente tesis doctoral y, del que se desprenden los siguientes objetivos generales:

1. Cuantificar la heterogeneidad ambiental a pequeña escala de las variables que pueden afectar a la regeneración de especies leñosas mediterráneas y evaluar el papel de esa heterogeneidad ambiental en la supervivencia de las especies.

2. Profundizar en los mecanismos ecofisiológicos de las plantas en respuesta a la heterogeneidad ambiental (principalmente variaciones en luz y agua).

3. Analizar los posibles cambios ontogenéticos en el nicho de regeneración.

En función de estos objetivos, la tesis se ha divido en tres grandes bloques. El primero de ellos comprende los dos primeros capítulos y versa sobre una serie de experiencias realizadas en un área mediterránea de montaña en el Parque Nacional de Sierra Nevada al sureste de la Península Ibérica. El área de estudio presenta un paisaje heterogéneo con tres tipos de rodales bien diferenciados, el bosque autóctono, las repoblaciones forestales y el matorral pionero.

En el **capítulo 1** se analiza la heterogeneidad espacial a pequeña escala de variables ambientales que pueden condicionar la regeneración. Se evalúa tanto el patrón espacial de las variables ambientales, como la covariación espacial entre las mismas, y los resultados obtenidos se comparan en los tres rodales estudiados.

En el **capítulo 2** se relaciona la heterogeneidad de las variables estudiadas con el patrón espacial de la supervivencia de plántulas en los distintos rodales. Las especies estudiadas son principales formadoras de bosque en el área de estudio, tales como el la encina [*Quercus ilex* subsp. *ballota* (Desf.) Samp.] y el serbal (*Sorbus aria* L.). Los estudios realizados en este bloque contribuirán a reconocer los micrositios óptimos para la supervivencia, mediante la detección de agregados de la supervivencia (o de variables que la determinan) en el espacio.

En el segundo bloque, que se corresponde con el segundo objetivo, se presentan los tres capítulos siguientes que tratan sobre los mecanismos implicados en las respuestas a variables ambientales tales como la luz y el agua. Todo el bloque corresponde a un experimento realizado en condiciones de invernadero en el que se sometieron 4 especies del género *Quercus* [*Quercus suber* L., *Quercus ilex* ssp. *ballota* (Desf.) Samp. (perennes), *Quercus canariensis* Willd. and *Quercus pyrenaica* Willd. (caducas)] a distintos niveles de luz y agua. Las especies estudiadas difieren en su longevidad foliar (caducifolias y perennifolias) y además presentaron un amplio rango de variación en el tamaño de semilla, tanto a nivel intraespecífico como interespecífico.

El **capítulo 3** trata sobre los mecanismos implicados en la relaciones de la plántula con el peso de semilla en los primeras fases de crecimiento y de cómo estas relaciones pueden variar con en el gradiente lumínico. Este estudio pretende evaluar la importancia funcional del tamaño de semilla y adicionalmente se propone un modelo causal que conecta los diferentes mecanismos estudiados.

En el **capítulo 4**, se evalúan, a nivel foliar, las respuestas morfológicas y fisiológicas a la luz y al agua, así como a la interacción entre estos dos factores. El estudio interacción luz y agua contribuirá al cuerpo de conocimiento generado en torno a este problema, aún por resolver, y del que la información en especies mediterráneas es todavía escasa.

Por último, en el **capítulo 5** de este bloque, se vuelve a incidir en las respuestas a la interacción luz y agua, pero en este caso a nivel de plántula, ya que se estudia tanto el crecimiento como la distribución de biomasa de las 4 especies. Además se evalúa la importancia del tamaño de semilla, sólo que en fases mas avanzadas del crecimiento. Adicionalmente, se pretende conectar los dos niveles de respuesta estudiados (a nivel de hoja y de planta completa) relacionando el cálculo integrado de medidas fotosintéticas de la ganancia de carbono y la tasa de crecimiento relativo (RGR). La conexión de medidas a nivel de hoja y de planta puede ser de especial interés para la validación de modelos predictivos de dinámica forestal (Zavala & Bravo 2005).

El último bloque, aborda el tercer objetivo, y engloba el último capítulo de esta tesis. El **capítulo 6** se ocupa de los cambios ontogenéticos del nicho de la especie *Acer opalus* subsp. *granatense* Boiss. Por diferentes razones que se discuten en el capítulo, se ha elegido esta especie para determinar si efectivamente el nicho puede cambiar a lo largo de diferentes estadios demográficos. Mediante una aproximación observacional se pretende evaluar el entorno inmediato de los individuos de cada una de las clases de edad (plántulas, juveniles de entre 2 y 5 años y juveniles de entre 5 y 9 años) y compararlos con micrositios tomados al azar, de manera que podamos determinar si los individuos se distribuyen al azar o por el contrario se encuentran en micrositios con unas características determinadas (Collins & Good 1987, Collins 1990). La cuantificación del nicho de regeneración de cada una de las estadios demográficos estudiados, es la base para la determinación de micrositios con alta probabilidad de supervivencia de plantones. Esta información puede ser de alto valor aplicado a la hora de optimizar programas de restauración, realizando siembras o plantaciones sólo en éstos microambientes. Se pretende por tanto en este estudio profundizar en los procesos que subyacen al cambio ontogenético del nicho y generar información básica para establecer programas adecuados de restauración.

3. BLOQUE I: HETEROGENEIDAD AMBIENTAL A PEQUEÑA ESCALA

Capítulo 1: (en ingles) Small-scale environmental heterogeneity across different landscape units in a Mediterranean mountain forest.

Small-scale environmental heterogeneity across different landscape units in a Mediterranean mountain forest.

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Abstract

To a large extend, it is known that seedling are influenced by the immediately surrounding area and environmental variables characterizing these areas should vary at meter scale. Here, we investigate small-scale spatial variations and spatial associations of environmental factors potentially influencing seedling establishment in a heterogeneous study area at larger scale, composed by three landscape units, native forest, reforestation and shrubland. Approximately, 1,000 potential microsites were sampled in each landscape unit with a spatial explicit design and small-scale spatial heterogeneity was quantified using spatial analysis by distance indices (SADIE). SADIE detected aggregated spatial patterns in most of environmental variables studied across landscape units being native forest and reforestation quantitatively more heterogeneous than shrubland. Complex spatial association/dissociation relationships among environmental variables were found, emphasizing “shade drought” phenomenon in native forest. Micro slope spatial relationships with other variables revealed the importance to detect spatial position of environmental variables from simple micro-topographic measurements. Small-scale heterogeneity studies should be essential for optimizing restoration programmes, since it is possible to detect “safe sites” for seedling establishment.

Keywords: Mediterranean mountain forest, microsite, microtopography, spatial heterogeneity, spatial pattern.

Introduction

Heterogeneity in Nature is the norm more than exception and spatial variation of environmental conditions have been commonly accepted as crucial for understanding forest ecology and dynamics (Tilman 1988, Canham et al 1994, Purves et al 2007). Environmental heterogeneity is especially important in Mediterranean areas, specially in mountain regions (Blondel & Aronson, 1999). In these zones, heterogeneity is presented across different scales, from regional areas to landscape units. In fact, contrasting ecological scenarios coexist in local scales owing to complex orography, high elevations and unpredictable climate (Blondel & Aronson, 1999). Recent studies have demonstrated the importance to incorporate environmental heterogeneity in studies of plant regeneration dynamics (Beckage & Clark 2003, Jurena & Archer 2003). In this type of studies, heterogeneity is frequently associated to the observation scale, that is, processes and interactions observed in different scales can be no coincident (Dale 1999). Results obtained from a particular ecological question are strongly dependent of scale which study is carried on (Turner et al 2001). Thus, in woody seedling studies, plant can be influenced for the immediate surrounding environment (Grubb 1977) which can vary meter by meter (microsite scale, Gómez-Aparicio et al 2005), having implications for woody seedling establishment. For example, Maestre et al (2003) have demonstrated that the spatial pattern of soil physic variables was related to survival spatial pattern of woody seedlings.

Among variables influencing seedling establishment, soil surface microtopography have been studied as an important factor in germination and growth (Smith & Capelle 1992) survival (Collins & Battaglia 2002) or plant species composition (Lundholm & Larson 2003). However, studies about how microtopography affect spatial pattern of others environmental variables are scarce. On the other hand, small-scale environmental heterogeneity in Mediterranean areas have been described in semi-arid environments (Maestre et al 2003), forests (Valladares & Guzman 2006) and Dehesa ecosystems (Gallardo et al. 2000), however, to our knowledge, comparisons of small-scale spatial patterns of different environmental variables in different landscape units are lacking.

In this study we quantified the spatial pattern of environmental variables at microsite scale in three landscape units of a Mediterranean mountain region (Sierra Nevada National Park, SE Spain): native forest, reforestation stand and shrubland. Knowledge about the spatial relations among environmental variables, which potentially influence seedling establishment, under different ecological scenarios will contribute to determine optimal microsites for seedling establishment. We conducted, in each landscape unit, a multivariate characterization of ca. 1,000 microsites separated each meter with a spatial explicit design. Thus, the main objectives of this study were (1) to quantify the spatial pattern of environmental variables, (2) to compare the spatial pattern among different landscape units, and (3) to describe spatial relationships among environmental variables. For these proposals, we used spatial analysis by distance indices (SADIE) (Perry 1998, Perry et al. 1999).

Material & Methods

Study area

The present study was conducted inside the Sierra Nevada National Park, surrounding the Trevenque Peak area (Granada Province, SE Spain), during the years 2004 and 2005. The climate is Mediterranean mountain type, with hot dry summers and cold, snowy winters, and rainfall (879 mm year^{-1}, average 1990-2003), heaviest in autumn and spring. Summer precipitation is highly variable across years (Fig. 1), with “wet summers” (higher than average, as 2004 was) and “dry summers” (lower than average, as 2005 was). The bedrock is calcareous and the predominating soils are regosols and cambisols (Delgado et al 1989; see also Castro et al 2005 for more information about the area). This protected area is composed by a mosaic landscape with three principal landscape units located between 1600-1900 m a.s.l.: shrubland, reforestation stand and native forest. The native forest (37º 04’ 54’’ N, 3º 28’ 17’’ W, 1680 m. a. s. l) was composed mainly by *Pinus sylvestris* var. *nevadensis* Christ. mixed with other trees such as *Taxus baccata* L. or *Acer opalus* subsp. *granatense* Boiss., and a dense shrubby understory composed by different fleshy-fruited shrub species (*Berberis vulgaris* subsp. *australis* Boiss., *Crataegus monogyna* Jacq., *Juniperus communis* L., and *Lonicera xylosteum* L.). Reforestation stand (37º 04’ 33’’ N, 3º 28’ 18’’ W, 1790 m. a. s. l) were sites contained *Pinus sylvestris* L. and *Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco, with densities of 521 individuals ha^{-1}. Shrubland (37º 04’ 50’’ N, 3º 27’ 50’’ W, 1825 m. a. s. l) was a post-fire area dominated by *Crataegus monogyna* Jacq., *Prunus ramburii* Boiss., *Salvia lavandulifolia* Vahl., and *Erinacea anthyllis* Link, with widely scattered trees.

Sampling design

In each landscape unit (native forest, reforestation and shrubland), 961 samplings points were selected in a 30 x 30 m plot at 1 m intervals. To achieve a spatial explicit design, X and Y coordinates of each sampled point were determined using a total station (model DTM-332, Nikon,, Tokyo, Japan). At each sampled point the following environmental variables were measured in a circular plot 0.30 m in diameter, following the “plant’s eye–view” approach (Turkington & Harper, 1979): 1) average soil compaction, 2) depth of the maximum soil–compaction value, 3) light availability, 4) soil moisture, 5) depth of the litter layer, 6) cover of herbaceous species, 7) stone and moss cover, 8) woody debris cover, 9) shrub cover, and 10) percentage of micro slope, a soil surface microtopograpy parameter. Soil compaction was measured using a Penetrologger penetrometer (Eijkelcamp, Giesbeek, The Netherlands). This device provides a profile describing the variation of soil compaction with depth at each point sampled. From these profiles, two variables relevant for rooting capacity, and thus for seedling establishment (Gómez–Aparicio et al 2005), were determined: the average compaction over the profile (MPa), and the depth of maximum compaction (cm). Light availability (hereafter, GSF) was quantified with hemispherical photography. Photographs were taken at each sample point at ground level using a horizontally levelled digital camera (CoolPix 5000, Nikon, Tokyo, Japan) and aimed at the zenith, using a fish–eye lens of 180º field of view (FCE8, Nikon). To ensure homogeneous illumination of the canopy and a correct contrast between canopy and sky, all photographs were taken before sunrise, after sunset, or during cloudy days. The images were analysed using Hemiview canopy analysis software version 2.1 (1999, delta–T Devices Ltd, Cambridge, United Kingdom). The software estimates the Global site factor (GSF), defined as the proportion of diffuse and direct radiation for clear–sky conditions at our study site (Rich, 1990). GSF is a continuous variable ranging from 1 (open sky) to 0 (complete obstruction). We measured soil moisture (in volumetric water content, VWC), measured along the first 20 cm depth (with a TDR mod 100; Spectrum Technologies, Inc., Plainfield, IL, USA) in four periods: at the middle of spring 2004, 2005, and at the end of summer 2004, 2005 (before autumn rainfalls). The depth of the litter layer was measured by inserting a metal ruler down to the soil surface. The different percentages of cover were visually estimated by dividing the circular plot into four hypothetical sections to ensure more accurate measurements. Soil surface mycrotopography was measured using the total station (see above), which determined in each sampled point, relative altitude (relative Z coordinate) with respect to the lowest sampling point in each landscape unit. From the relative Z coordinate data, percentage of micro slope was calculated in each sampled point, having into account relative Z of surrounding sampled points and. This parameter was obtained using ArcGis v9 (ESRI, Redlands, CA, USA).

Data analysis

We analysed the environmental variables using spatial analysis by distance indices (SADIE), a method designed for determine quantitatively the spatial pattern of a given variable and spatial co-variation between two variables (for a complete description, see Perry 1998, Perry et al 1999, Perry & Dixon 2002). We used different indices produced by SADIE: the index of aggregation (I_a) provides information on the overall spatial pattern of each environmental variable. It is cumpled if $I_a > 1$, random if I_a is close to one, and regular if $I_a < 1$. The index of clustering (v) measures the degree of clustering of the data into patches (areas of high values of target variable) and gaps (areas of low values); when date are contoured in a two-dimensional map, it show their spatial distribution. Sampled points within patches have values of v (by convention v_i) greater than 1.5, whereas those within gaps have values of v (by convention v_j) less than –1.5.

The overall spatial association index (X) measures if two variables are spatially associated, dissociated or not related between them, in fact, this index is the correlation coefficient between the values of v of two variables (Perry & Dixon 2002). Thus, sampling points where indices of both variables show a patch or a gap will contribute strongly and positively to the correlation, while those where one set shows a patch and the other a gap contribute strongly and negatively. Sampling points with small values of v will contribute weakly to the correlation. SADIE analyses were performed using the programs freely downloaded in (http://www.rothamsted.ac.uk/pie/sadie/)

In order to compare environmental heterogeneity among landscape units, we used the index of aggregation (I_a) of the environmental variables measured and tested the differences with PERMANOVA, a semi–parametric analysis of variance approach (Anderson 2001). This approach allows the testing of the simultaneous responses of a dataset to one or more factors in an ANOVA experimental design on the basis of any distance measure using permutation methods (see Anderson, 2001 and McArdle & Anderson, 2001 for details). PERMANOVA analysis were performed using the program PERMANOVA 1.6 (Anderson, 2005).

In addition, we used cluster indices (v) of each sampled point to make contour maps of the different landscape units and visually appreciate patch or gaps in different variables. Contour maps were performed with SURFER 8.0 (Golden Software, Boulder, Colorado, USA).

Results

Quantifying and comparing spatial patterns

Among landscape units, most of environmental variables studied showed a cumpled spatial pattern ($I_a > 1$) (Table 1). Aggregation indices were higher in native forests and reforestation stand than shrubland (Fig. 1). Average soil compaction had the highest I_a values in the three landscape unit studied (Table 1). Light availability in native forest had a higher I_a value in comparison to reforestation and shrubland (Fig 2). In the “wet summer”, soil moisture heterogeneity of native forest and reforestation increased from the spring to the end of the summer, while heterogeneity of reforestation stand and shrubland increased from the spring to the end of the summer in the “dry summer” (Fig.

3). Microslope percentage was cumpled in native forest and shrubland having the highest I_a values in native forest (Table 1, Figure 4).

Spatial relationships among environmental variables

In native forest, overall spatial association (X index) between light availability and soil moisture measures was found (Table 2A). Patches of soil moisture spatially coincided with patches of light. At the same time, shrub cover was spatially dissociated with soil moisture and light availability, whereas water availability was spatially associated with herbaceous cover (except for soil moisture in summer 2005). On the other hand, areas with high average soil compaction spatially coincided with gaps of litter depth (areas with low values). In addition, spatial associations of different soil moisture measurement were found (Table 2A). In general, micro slope percentage had relatively low values of spatial covariation indices (X) with other variables although a spatial association between micro slope and litter depth was found, patches of low microslopes percentage (flatter areas) spatially coincided to deeper litter layers.

In the case of reforestation stand, there were no spatial relation between light availability and different soil moisture measurements, however, as native forest, spatial associations of different soil moisture measurement were found (Table 2B). Average soil compaction was spatially related to soil moisture measurement being the highest overall spatial association indices (X) in 2004 measurements. Herbaceous cover was also spatially related to soil moisture in 2004 and average soil compaction. On the contrary, patches of herbaceous cover spatially coincided with gaps of stone and moss cover. In the same way, areas with higher values of litter cover coincided with lower values of debris cover. As native forest, micro slope of reforestation plot had relatively low covariation inidex values (X), however, there were a spatial dissociation between micro slope and soil compaction and between micro slope and herbaceous cover. Additionally, spatial association between microslope and stone and moss cover was found (Table 2B).

Shrubland was the landscape unit with the low spatial co-variation among environmental variables studied (Table 2C). Interestingly, shrub covers were spatially dissociated with herbaceous cover and, patches of debris cover coincided with areas with higher soil moisture values in summer 2005. As opposite of reforestation plot, there was a spatial association between micro slope and herbaceous cover (Table 2C).

Discussion

Quantifying and comparing spatial patterns

In the study area, spatial heterogeneity at landscape scale can be appreciated (Matias *et al. submitted*). In addition, within landscape units, aggregated spatial patterns of environmental variables have been the general trend. Other Mediterranean areas, spatial patterns of different variables have revealed the same results (Gallardo et al. 2000, Valladares & Guzmán 2006, Maestre et al 2003), however, contrasting with these previous works, this study showed that small-scale spatial heterogeneity can depend of landscape units studied. Thus, shrubland had the lowest aggregation index values indicating a reduced microsites range attributable to low structural complexity such a shrub patches and open interspaces (Gomez-Aparicio et al. 2005). On the contrary, native forest and reforestation responded to a higher structural complexity: trees, shrubs, woody debris, generating a wider microsites spectrum.

Spatial relationships among environmental variables

Overall, complex spatial association and dissociation patterns among variables was found in all landscape units studied. In native forest occurred a counterintuitive trend:

areas with higher levels of soil moisture spatially coincided to areas with higher light availability, and lower levels of soil moisture spatially coincided to areas with higher shrub cover levels. Lower levels of water availability under shaded condition is a phenomenon known as “shade drought” which have been observed in a Mediterranean California shrub (Valladares & Pearcy 2002) and, according to these authors, it is caused by greater competition for water in the understory, provoking a soil water depletion in this areas. Joint to this study, similar results have been found in temperate forests (Abrams & Mostoller 1995), however, to our knowledge, there no were evidences of shade drought at Iberian Mediterranean areas to this study. In principle, this result could contradict to empirical evidences of facilitation effects of shrubs and trees on seedlings in Mediterranean environments (Castro *et al*. 2004, Gómez-Aparicio *et al* . 2004), however, soil moisture measurements were done at the first 20 cm in the soil layer and most of woody facilitated seedlings have deeper root systems (Silva & Rego 2004, Guerrero-Campo et al 2006). Thus, woody seedlings are facilitated by shrubs avoiding water competition in upper layers.

On the other hand we also found some areas with higher light and water availability and herbaceous cover was spatially associated to soil moisture, indicating that herbaceous layer avoided soil water evaporation with no interferences in our soil moisture measurements which would be deeper than herbaceous root systems.

Under native forest and reforestation plots, we found that patches and gaps of soil moisture were spatially coincident in the four soil moisture measurements, 2004 and 2005 in spring and summer (Table 2A, 2B, Fig. 3) suggesting that microsites with good water conditions were constants across temporal variation.

In general, contrary to expected, micro slope was lower spatial association / dissociation with other variables, however, there were interesting relationships, for example micros slope-litter depth spatial association or microslope-soil moisture spatial dissociation in native forest and micro slope-stone and moss cover spatial association in reforestation and shrubland. Ability to derive spatial position of environmental variables from micro-topography measurements can be essential for optimising regeneration and restoration programmes.

In conclusion, quantitative small-scale spatial relationships among environmental variables should be an issue of great potential applications in restoration programmes, so demanded in Mediterranean scenarios (Terradas 2001, Jordano et al. 2002, Zamora 2002), i. e., selecting for planting or sowing “safe sites” (*sensu* Harper 1977) detected by relatively simple measurements.

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Table 1: Index of aggregation (I_a) values and and level of significance (*: $P < 0.05$) describing the spatial pattern of environmental variables studied in three different landscape units. It is cumpled if $I_a > 1$, random if I_a is close to one, and regular if $I_a < 1$.

Variables	Units	Native forest	Reforestation	Shrubland
Average soil compaction	MPa	10.7 *	7.1 *	5.6 *
Depth of the maximum soil-compaction value	cm	2.71 *	2.3 *	2.1 *
Light availability	GSF	6.2 *	2.6 *	1.7 *
Soil moisture spring 04	% VWC	3.7 *	3.1 *	1.6 *
Soil moisture summer 04	% VWC	5.2 *	3.8 *	1.3 *
Soil moisture spring 05	% VWC	2.9 *	2.8 *	1.5 *
Soil moisture summer 05	% VWC	2.5 *	3.3 *	3.1 *
Depth of the litter layer	cm	3.9 *	6.9 *	1.6 *
Cover of herbaceous species	%	2.8 *	4.3 *	5.2 *
Stone and moss cover	%	0.88	1.9 *	1.7 *
Woody debris cover	%	2.3 *	2.6 *	1.9 *
Shrub cover	%	3.1 *	1.3 *	2.4 *
Micro slope	%	7.1 *	0.9	3.8 *

Table 2: Overall co-variation among variables studied in native forest (A) , reforestation (B) and shrubland (C). The level of significance (*, $P < 0.05$) is indicated. Av C, average soil compaction, D v M, depth of the maximum soil–compaction value, Light Av, light availability, So Mo Sp 04, soil moisture 2004' spring, So Mo Su 04, soil moisture 2004' summer, So Mo Sp 05, soil moisture 2005' spring, So Mo Su 05, soil moisture 2005' summer, Li D, depth of the litter layer, H Cov, cover of herbaceous species, St Moss Cov, stone and moss cover, Deb Cov, woody debris cover, Sh Cov shrub cover, and Micro slope, percentage of micro slope. See Table 1 for units.

A)

Variables	D v M	Light Av	So Mo Sp 04	So Mo Su 04	So Mo Sp 05	So Mo Su 05	Li D	H Cov	St Moss Cov	Deb Cov	Sh Cov	Micro slope
Av C	-0.04	-0.18*	-0.07	-0.16*	0.003	0.05	-0.37*	-0.04	0.17*	-0.09	0.03	-0.05
D v M		0.10*	0.09*	0.16*	0.15*	0.04	-0.02	-0.04	-0.13*	0.03	-0.03	-0.05
Light Av			0.40*	0.49*	0.36*	0.28*	-0.09	0.22*	-0.14*	-0.03	-0.33*	-0.12
So Mo Sp 04				0.54*	0.52*	0.37*	-0.12*	0.32*	0.08	-0.12*	-0.20*	-0.17*
So Mo Su 04					0.53*	0.46*	-0.10*	0.21*	-0.12*	-0.08	-0.34*	-0.16*
So Mo Sp 05						0.41*	-0.17*	0.27*	-0.0007	-0.15*	-0.20*	-0.15*
So Mo Su 05							-0.24*	0.09	-0.07	0.002	-0.34*	-0.04
Li D								-0.07	-0.002	0.13*	0.12*	0.24*
H Cov									0.10	-0.30*	-0.07	-0.17
St Moss Cov										-0.34*	0.07	0.007
Deb Cov											-0.08	0.16
Sh Cov												-0.05

B)

Variables	D v M	Light Av	So Mo Sp 04	So Mo Su 04	So Mo Sp 05	So Mo Su 05	Li D	H Cov	St Moss Cov	Deb Cov	Sh Cov	Micro slope
Av C	0.21*	-0.08	0.37*	0.28*	0.18*	0.19*	-0.19*	0.40*	-0.24*	-0.05	0.20*	-0.35*
D v M		0.03	0.14*	0.05	0.1*	0.03	0.08*	0.21*	-0.17*	-0.02	0.03	-0.12*
Light Av			0.03	-0.04	0.05	0.06	0.17*	-0.13*	0.08	0.04	-0.10	0.05
So Mo Sp 04				0.46*	0.42*	0.41*	-0.21*	0.38*	-0.24*	-0.02	0.06	-0.19*
So Mo Su 04					0.45*	0.35*	-0.24*	0.32*	-0.12*	-0.02	0.11	-0.09
So Mo Sp 05						0.38*	-0.10*	0.22*	-0.11*	-0.08*	0.02	-0.04
So Mo Su 05							-0.13*	0.21	-0.11*	-0.006	-0.04	-0.14*
Li D								-0.10*	0.007	0.18*	-0.05	0.02
H Cov									-0.44*	0.13*	0.16*	-0.33*
St Moss Cov										-0.24*	-0.03	0.32*
Deb Cov											0.07	-0.12*
Sh Cov												-0.04

C)

Variables	D v M	Light Av	So Mo Sp 04	So Mo Su 04	So Mo Sp 05	So Mo Su 05	Li D	H Cov	St Moss Cov	Deb Cov	Sh Cov	Micro slope
Av C	0.23*	0.19*	-0.003	0.03	0.04	0.09*	-0.12*	0.04	-0.05	0.04	-0.16*	-0.15*
D v M		-0.06	-0.04	0.04	0.07	-0.04	0.15*	0.12*	-0.12*	0.05	-0.16*	-0.03
Light Av			-0.03	-0.06	-0.04	0.15*	-0.26*	-0.01	0.08*	0.007	-0.23*	-0.08
So Mo Sp 04				0.10*	0.14*	0.13*	-0.05	0.05	0.03	0.10*	-0.11*	0.01
So Mo Su 04					0.23*	-0.01	0.07	0.05	-0.06	0.03	0.05	0.01
So Mo Sp 05						-0.05	0.08	-0.07	-0.05	-0.05	0.06	-0.06
So Mo Su 05							-0.14*	-0.12*	0.01	0.34*	-0.11	-0.08*
Li D								0.09*	-0.18*	-0.03	0.20*	0.07
H Cov									-0.13	0.35*	-0.36*	0.33*
St Moss Cov										0.08*	-0.06	0.22*
Deb Cov											-0.28*	0.03
Sh Cov												0.03

Appendix: mean ± SE of environmental variables studied in each landscape unit (n =961).

Variables	Units	Native forest	Reforestation	Shrubland
Average soil compaction	MPa	3 ± 0.03	2.04 ± 0.02	1.28 ± 0.02
Depth of the maximum soil-compaction value	cm	36.81 ± 0.40	31.48 ± 0.45	22.15 ± 0.31
Light availability	GSF	0.15 ± 0.002	0.13 ± 0.001	0.76 ± 0.01
Soil moisture spring 04	% VWC	35.30 ± 0.19	32.86 ± 0.20	19.28 ± 0.14
Soil moisture summer 04	% VWC	9.23 ± 0.08	11.15 ± 0.09	5.68 ± 0.05
Soil moisture spring 05	% VWC	7.93 ± 0.07	11.30 ± 0.09	8.06 ± 0.06
Soil moisture summer 05	% VWC	4.58 ± 0.04	5.47 ± 0.06	3.98 ± 0.03
Depth of the litter layer	cm	53.74 ± 0.84	52.81 ± 0.92	17.74 ± 0.46
Herbaceous cover	%	18.86 ± 0.80	4.86 ± 0.32	7.72 ± 0.42
Stone and moss cover	%	0.15 ± 0.04	1.37 ± 0.22	8.16 ± 0.43
Woody debris cover	%	16.21 ± 0.43	14.09 ± 0.31	0.54 ± 0.12
Shrub cover	%	7.99 ± 0.44	3.73 ± 0.32	30.72 ± 0.93
Micro slope	%	28.23 ± 0.26	19.17 ± 0.19	21.78 ± 0.25

Figure 1: Aggregation index values (I_a) (mean ± SE) at the three landscape units studied. Different letters indicate significant differences ($P < 0.05$, post hoc comparisons, PERMANOVA).

Figure 2: Maps of GSF cluster indices (v) at the three landscape units studied. Dash lines indicate variable gaps ($v < -1.5$, areas with aggregated low values) and solid lines indicates patches ($v > 1.5$, areas with aggregated high values).

Figure 3: Maps of soil moisture cluster indices (v) at the three landscape units in four measurement times. Dash lines indicate variable gaps ($v < -1.5$, areas with aggregated low values) and solid lines indicates patches ($v > 1.5$, areas with aggregated high values).

Figure 4: Soil surface micro topography (z relative coordinates, m) at the three landscape units studied

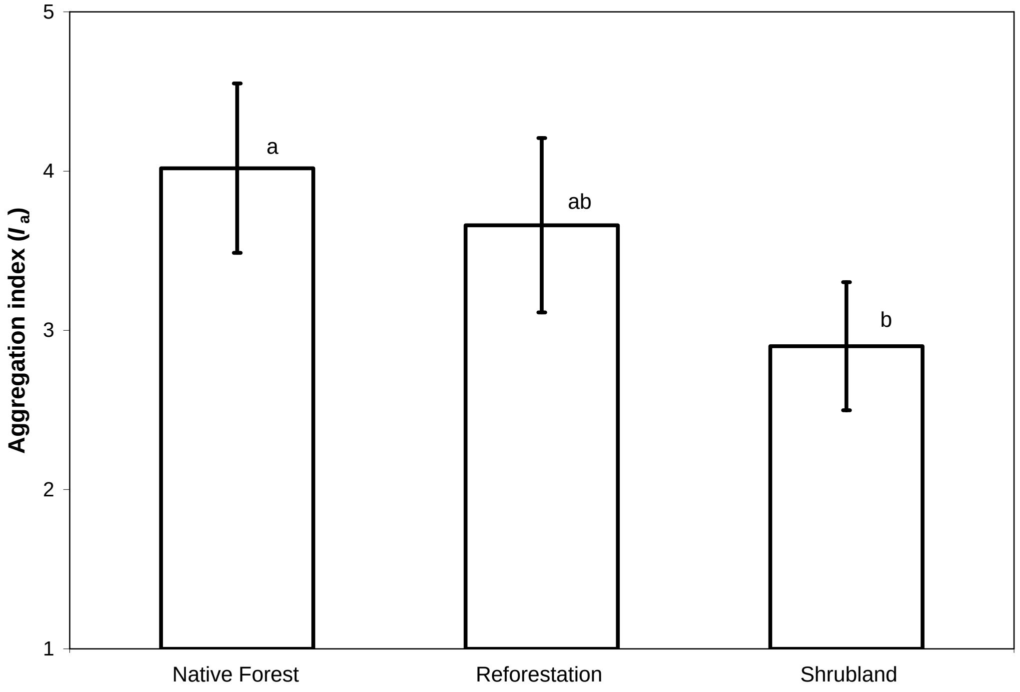
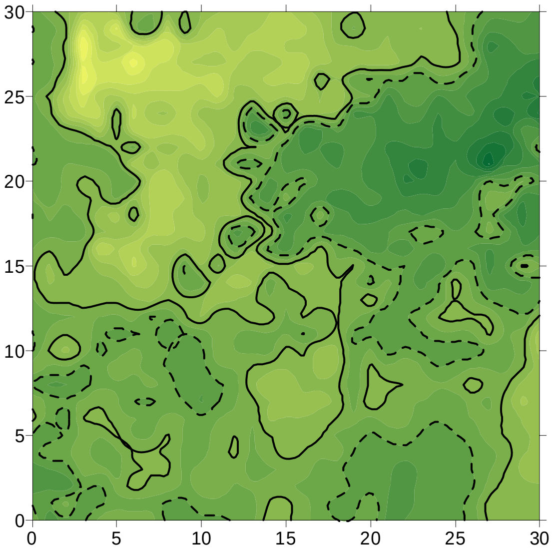
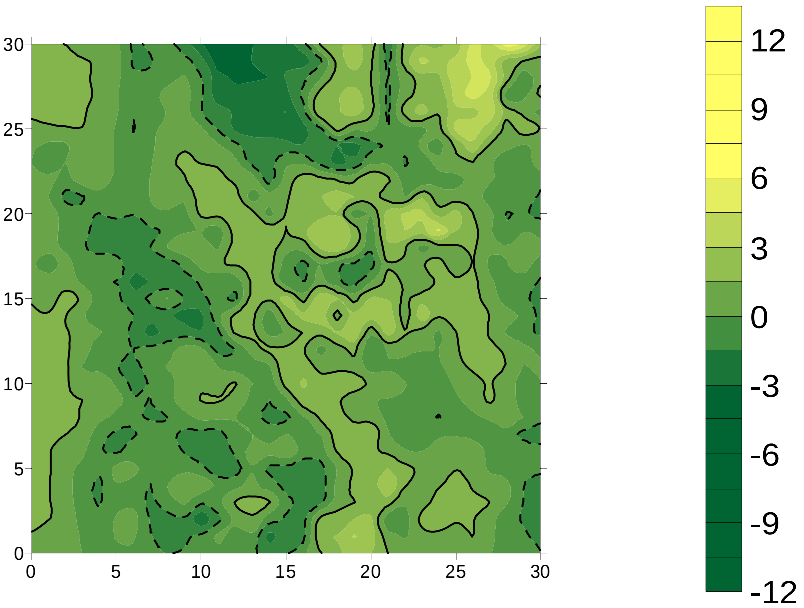

5
4
3
2
1
Aggregation index (I_a)
a
ab
b
Native Forest
Reforestation
Shrubland

Figure 1

Native Forest



Reforestation



Shrubland

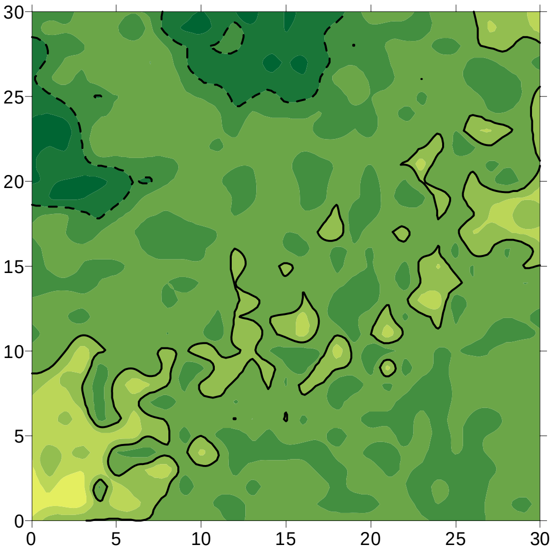


Figure 2

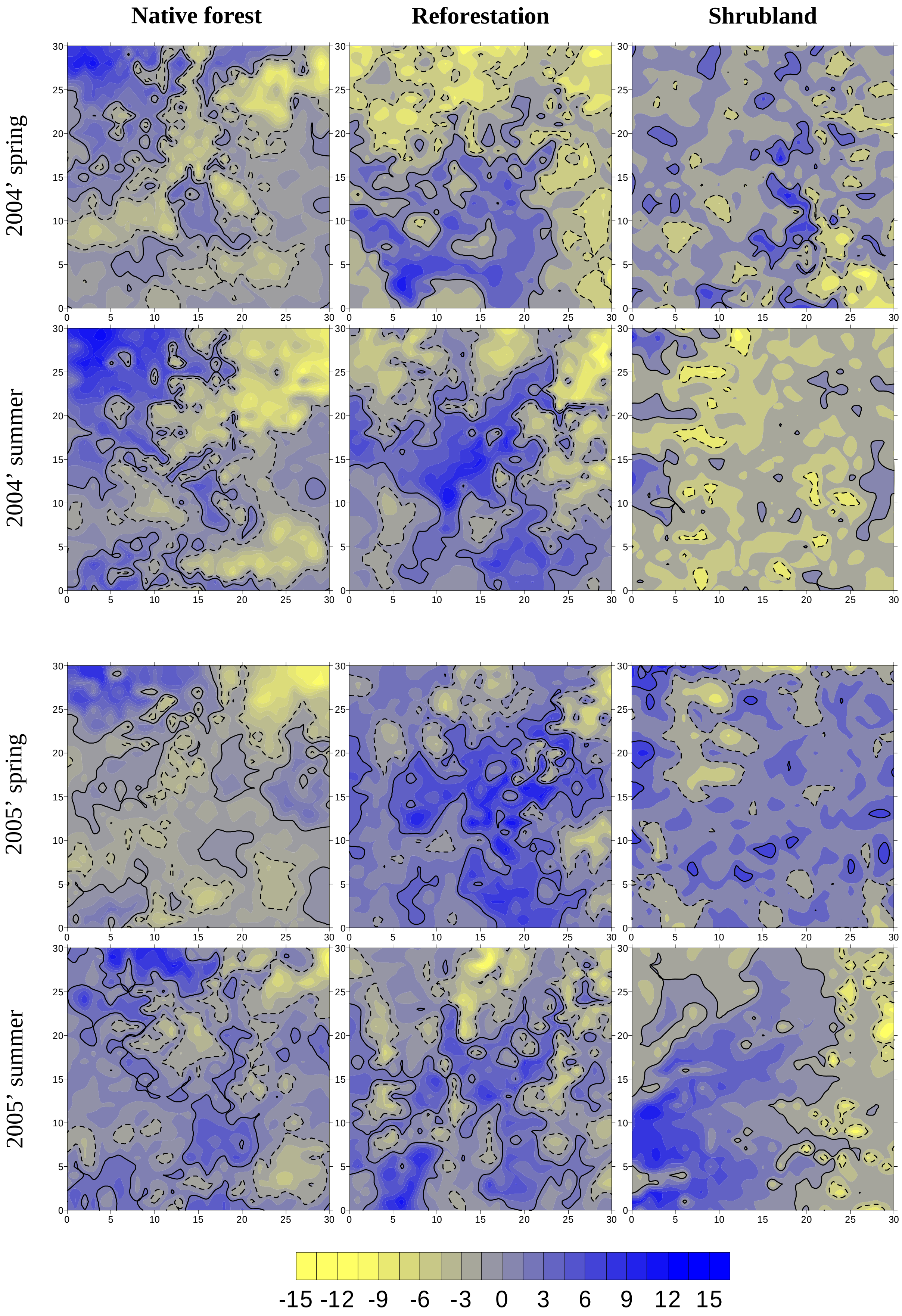
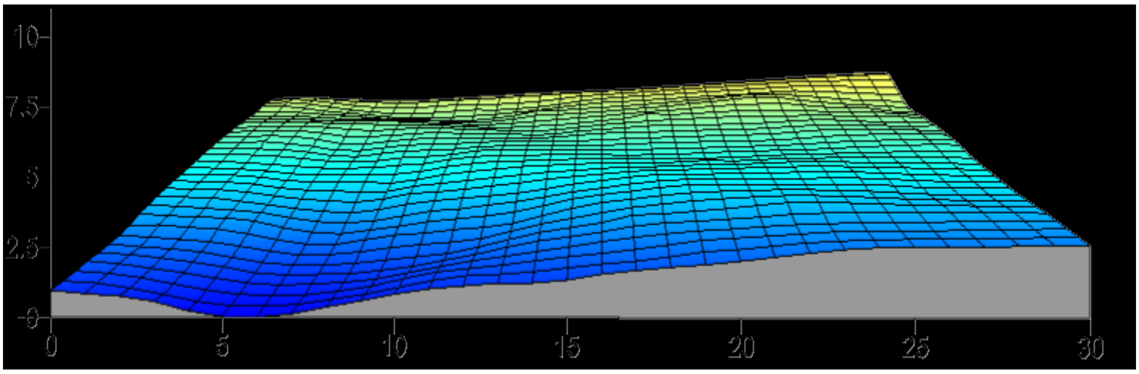
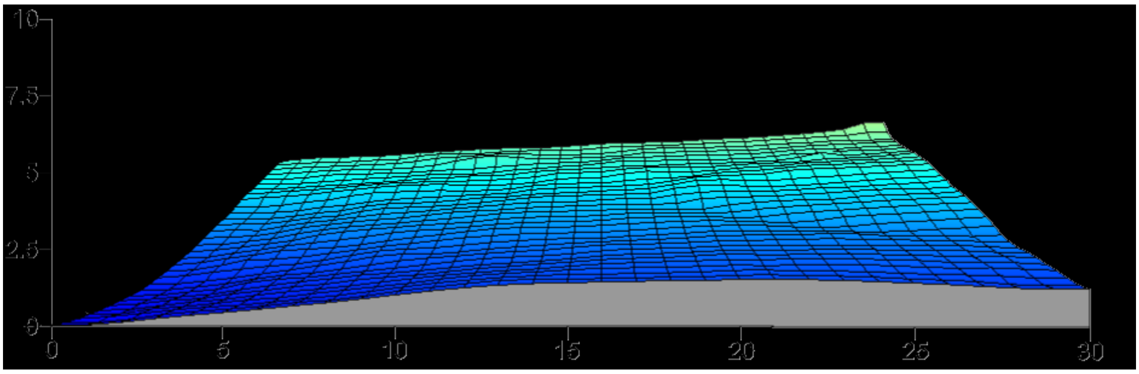

Native forest
Reforestation
Shrubland
2004’ spring
2004’ summer
2005’ spring
2005’ summer
0
5
10
15
20
25
30
-15 -12 -9 -6 -3 0 3 6 9 12 15

Figure 3

Native forest



Reforestation



Shrubland

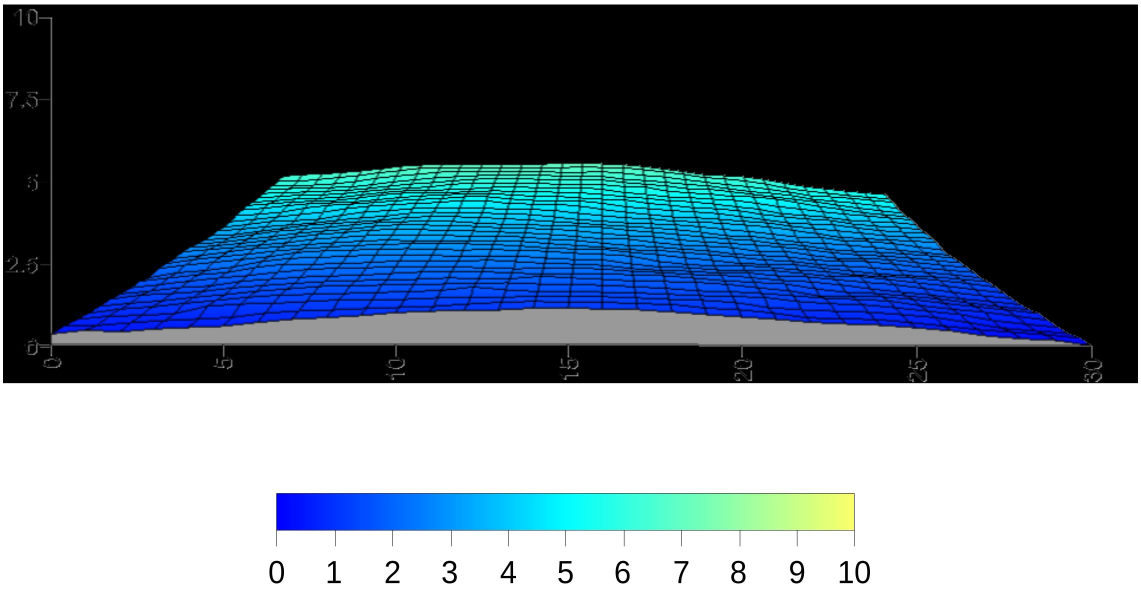


Figure 4

Capítulo 2: (en castellano) Heterogeneidad ambiental a pequeña escala y patrones espaciales de supervivencia de especies de leñosas en áreas de montaña mediterránea (Sierra Nevada, SE Península Ibérica)

Heterogeneidad ambiental a pequeña escala y patrones espaciales de supervivencia de especies de leñosas en áreas de montaña mediterránea (Sierra Nevada, SE Península Ibérica)

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RESUMEN

Los bosques de las montañas mediterráneas presentan una fuerte heterogeneidad espacial a diferentes escalas de observación. En Sierra Nevada (SE de la península Ibérica), el paisaje forestal está constituido por tres tipos básicos de rodales (bosque autóctono, pinar de repoblación y matorral pionero). Dentro de estos rodales, las variables ambientales pueden distribuirse heterogéneamente, determinando una supervivencia diferencial de plántulas de especies arbóreas. Para caracterizar con precisión el entorno inmediato de las plántulas, elegimos una escala de observación metro a metro y ubicamos en cada uno de los rodales un área de estudio o malla de 30 x 30 m con nodos separados cada metro, lo que hacen un total de 961 puntos por malla. En cada uno de estos nodos se sembraron semillas de dos especies arbóreas (*Quecus ilex* L. subsp. *ballota* (Desf.) Samp. *y Sorbus aria* (L.) Crantz.) y se anotaron diferentes variables ambientales para relacionar la variación espacial de la supervivencia de plántulas con los patrones espaciales de estas variables. Se utilizó la metodología SADIE para cuantificar la heterogeneidad espacial de las variables y el método de partición de la variación para relacionar la supervivencia con la heterogeneidad espacial. Con este método, básicamente podemos conocer qué parte de la variación es explicada por las variables ambientales, independientemente del patrón espacial y qué parte de la variación es explicada por la estructura espacial de éstas variables. La mayoría de las variables presentaron una distribución agregada en el espacio, indicando una heterogeneidad ambiental a pequeña escala. Los patrones espaciales de supervivencia de plántulas dependieron de algunas de las variables ambientales evaluadas, aunque una parte significativa de la variación se debe a la estructura espacial de las mismas. Estos resultados conectan la heterogeneidad espacial de factores ambientales con la respuesta de las plántulas en áreas de montaña mediterránea a una escala de resolución espacial muy fina, y pueden ser de gran ayuda a la hora de optimizar los programas de restauración en bosques de montaña mediterránea.

Palabras clave: Heterogeneidad ambiental, patrón espacial, supervivencia, restauración

INTRODUCCIÓN:

La heterogeneidad es la complejidad resultante de las interacciones entre la distribución de los factores ambientales y la respuesta diferencial de los organismos a esos factores (Milne 1991). Por tanto, y según esta definición, los organismos viven en hábitats que son altamente heterogéneos tanto en el espacio como en el tiempo (Stewart *et al*., 2000).

Esa heterogeneidad se puede apreciar claramente en los sistemas mediterráneos, particularmente en áreas de montaña mediterránea donde las condiciones ambientales son altamente variables en el tiempo y en el espacio (Blondel & Aronson, 1999). Temporalmente, esa heterogeneidad se puede observar en la gran variación existente entre años de la precipitación estival (meses junio, julio y agosto) (área del Trevenque, Sierra Nevada, datos propios). Espacialmente, la heterogeneidad presente en los ambientes mediterráneos puede evaluarse a distintas escalas: desde el ámbito regional a la unidad de paisaje. En la montaña mediterránea coexisten escenarios ecológicos contrastados a escala local debido a su compleja orografía, altas altitudes y clima impredecible (Blondel & Aronson, 1999).

Estudios recientes han proporcionado resultados sólidos que apoyan la importancia de incluir la heterogeneidad ambiental en los estudios de la dinámica de la regeneración de la vegetación (Beckage & Clark, 2003; Maestre *et al*, 2003). Esto resulta de gran importancia, debido a la perdida masiva de bosque nativo ocurrida en la cuenca mediterránea a causa de una historia milenaria de sobreexplotación (Blondel & Aronson, 1999). La cubierta forestal del área mediterránea no supera el 9-10 %, y en la Península Ibérica sólo el 0.2 % puede ser considerado bosque natural o seminatural (Marchand, 1990). La reforestación ha sido la técnica tradicional usada con el objetivo de recuperar parte de esa cubierta forestal. Pero en áreas mediterráneas, las reforestaciones padecen altas tasas de mortalidad temprana (Garcia-Salmeron, 1995), que las convierten en poco provechosas tanto en términos ecológicos como económicos. De aquí la importancia de incorporar la heterogeneidad ambiental en los proyectos de restauración de áreas degradadas mediterráneas y de regeneración del bosque autóctono mediterráneo. El periodo más crítico para el éxito de las reforestaciones en áreas mediterráneas es el primer verano después de la plantación. En el ciclo natural de regeneración de cualquier especie leñosa mediterránea, la fase de plántula suele ser la más limitante para el establecimiento, ya que esta es muy sensible ante cualquier circunstancia adversa. Por lo tanto, el estudio de la influencia de la heterogeneidad en esa fase de la planta resultaría provechoso.

Es vital en estos estudios tener en cuenta la influencia que pueda tener la escala en la heterogeneidad. La heterogeneidad frecuentemente va asociada a la escala en que se mide; esto quiere decir que los procesos e interacciones que se aprecian a distintas escalas de observación pueden no coincidir. La consecuencia principal de esta afirmación es que los resultados obtenidos de una cuestión ecológica en particular pueden depender fuertemente de la escala a la cual el estudio es llevado a cabo (Turner *et al.*, 2001). Por lo tanto, es importante realizar aproximaciones espacio-temporales a distintas escalas, para completar el entendimiento de los procesos e interacciones ecológicas. Debido a eso, se han realizado estudios de regeneración de plantas y estructura de las poblaciones en ambientes mediterráneos a distintas escalas de observación, desde escalas que engloban el área de distribución geográfica de una especie, hasta nivel de rodal (Gómez-Aparicio *et al.*, 2005b, a). Ya que las condiciones ambientales pueden variar a muy pequeña escala (Gómez-Aparicio *et al*, 2005a), el mejor diseño para entender como los factores ambientales determinan las probabilidades de establecimiento de especies leñosas es el denominado “plant’s eye view” (Turkington & Harper, 1979), que se centra en la estructura del hábitat que inmediatamente rodea a la plántula (Collins & Good, 1987; Gibson & Good, 1987; Collins, 1990; McArthy & Facelli, 1990). En pocas ocasiones se ha explorado la heterogeneidad espacial a pequeña escala y sus consecuencias en la regeneración (Maestre *et al.*, 2003).

En este estudio hemos analizado la heterogeneidad ambiental a escala de micrositio mediante la evaluación de distintas variables ambientales en tres unidades de paisaje diferentes: matorral pionero, repoblación de pino silvestre (*Pinus sylvestris* L.) y bosque autóctono de pino silvestre nevadense (*Pinus sylvestris* L. subsp. *nevadensis* (H. Christ) Heywood). Para analizar esta heterogeneidad espacial a escala de micrositio, se delimitó una malla de 30 x 30 metros en cada unidad de paisaje con 1 metro de resolución. Con el fin de relacionar esa heterogeneidad con los patrones de supervivencia de plántulas de leñosas, se sembraron semillas de serbal (*Sorbus aria* L. Crantz.) y bellotas de encina (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.) en cada malla. Nuestra hipótesis general es que la escala de micrositio determina el patrón espacial de supervivencia de las plántulas, y los objetivos específicos de este estudio son los siguientes: 1) cuantificar los patrones espaciales a pequeña escala de las variables ambientales en las tres unidades de paisaje; 2) evaluar la relación entre esas variables y el establecimiento de las plántulas de las especies objeto de estudio; 3) contrastar el efecto de la heterogeneidad a pequeña escala en las plántulas frente a la variabilidad a gran escala; 4) discutir la importancia de la heterogeneidad ambiental para optimizar los programas de restauración en bosques de montaña mediterránea.

MATERIAL Y MÉTODOS:

Área de estudio

El presente estudio se llevo a cabo en Sierra Nevada durante los años 2004 y 2005. Sierra Nevada posee un clima mediterráneo, caracterizado por una fuerte sequía estival. La precipitación anual es de 846.5±55.7 mm (media para la serie temporal 1991-2002), pero la precipitación estival (considerada como la suma de la precipitación caída en los meses de junio, julio y agosto) es de tan sólo 47.3±5.5 (media para la serie temporal 1991-2002). La precipitación varía fuertemente entre años, pudiendo identificarse “años húmedos” (o con precipitación superior a la media), y “años secos” (o con precipitación inferior a la media). Adicionalmente, el reparto intra-anual de la precipitación puede variar de un año a otro.

El estudio se realizo en tres unidades de paisaje diferentes en el entorno del Trevenque: matorral pionero, repoblación de pino silvestre y bosque autóctono de pino silvestre nevadense.

El matorral pionero se encuentra en la zona conocida como Loma de los Panaderos (N 37º 04’ 50’’ W 3º 27’ 50’’) situada a 1825 m con sustrato calizo y suelos poco desarrollados y muy pedregosos. En 1983 un incendio arrasó la zona, creando un vacío de vegetación de unas 8 hectáreas que hoy día constituye un mosaico de matorrales y parches de suelo desprovistos de vegetación. La especie dominante por su cobertura es *Salvia lavandulifolia* Vahl, también aparece *Ononis aragonensis* Asso y diversas especies de arbustos espinosos caducifolios como *Prunas ramburiii* Boiss, *Crataegus monogyna* Jacq., *Beriberis vulgaris* L. subsp. *australis* (Boiss) Heywood y distintas especies del género *Rosa* Tourn. Ex L. De la cobertura arbórea de pino silvestre nevadense y repoblado que cubría la zona antes del incendio sólo quedan algunos individuos aislados.

La repoblación está situada muy cerca del Jardín Botánico de la Cortijuela (perteneciente a la Red de Jardines Botánicos de Andalucía), a una altura de 1787 m (N 37º 04’ 33’’ W 3º 28’ 18’’). El suelo está formado mayoritariamente por cambisoles cálcicos y regosoles calcáricos (Delgado *et al.*, 1989). El estrato árboreo está formado mayoritariamente por pino silvestre, aunque tiene algún pie de pino laricio (*Pinus nigra* J. F. Arnold). La repoblación tiene unos 50 años, y una alta densidad de pies. En el sotobosque podemos encontrar diferentes especies de leñosas: *Sorbus aria*, *Crataegus monogyna*, *Lonicera xylosteum* L., *Cotoneaster granatensis* Boiss. , *Prunus ramburii*, *Quercus ilex* , *Quercus pyrenaica* Willd., etc.

El bosque autóctono esta situado en la zona del Trevenquillo (un subpico del Trevenque) a 1684 m (N 37º 04’ 54’’ W 3º 28’ 17’’). El suelo dominante esta formado en este caso por regosoles calcáricos y rendzinas (Delgado *et al.*, 1989). El dosel arbóreo esta formado por *Pinus sylvestris* subsp. *nevadensis* con un sotobosque rico en especies leñosas: *Crataegus monogyna*, *Lonicera xylosteum*, *Rubus ulmifolius* Schott, *Prunus ramburii*, *Quercus ilex* , *Rosa canina* L., etc. Esta parcela es sin duda la mejor conservada, por tanto la utilizaremos como sistema de referencia.

Diseño de muestreo:

La plántula esta influenciada por las condiciones del micrositio donde germina, que viene definido como la zona que inmediatamente la rodea. Para recoger esa influencia del micrositio, hemos escogido una escala de observación de metro a metro. En cada parcela o rodal (matorral pionero, repoblación y bosque autóctono) delimitamos una malla o área de estudio de 30 x 30 metros con nodos separados cada metro. Eso nos da un total de 961 puntos por unidad de paisaje. Cada malla fue protegida por un cercado para evitar el pisoteo por ganado y poder centrarnos así en los factores a nivel de micrositio. Este diseño nos ha permitido realizar el estudio a pequeña escala.

En cada uno de los 961 puntos se midieron cada una de las variables ambientales recogidas en este estudio. De esta manera, obtenemos un diseño espacialmente explícito, en la que cada coordenada de la malla lleva asociado un valor de las variables. Las variables ambientales medidas en nuestro estudio son las siguientes:

- Humedad del suelo. Medida con un sensor TDR ("Time Domain Reflectometry", Field Scout TDR 100, Spectrum Technologies, Inc. USA). En las mallas de la repoblación y del bosque autóctono utilizamos varillas de 20 cm, mientras que en el caso del matorral pionero tuvimos que usar las de 10 cm debido a que el suelo pedregoso del lugar hacia imposible el uso de las de 20 cm.
- Cobertura de especies leñosas. Cuantificamos la cobertura de especies leñosas (%) en un radio de 15 cm alrededor del nodo.

- Cobertura de hojarasca de leñosas. Cuantificamos la cobertura de hojarasca de leñosas (%) en un radio de 15 cm alrededor del nodo.
- Cobertura de herbáceas. Cuantificamos la cobertura de especies herbáceas (%) en un radio de 15 cm alrededor del nodo.
- Cobertura de hojarasca de herbáceas. Cuantificamos la cobertura de hojarasca de herbáceas (%) en un radio de 15 cm alrededor del nodo.
- Cobertura de ramas. Cuantificamos la cobertura de ramas, piñas y otros restos de madera (%) en un radio de 15 cm alrededor del nodo.
- Cobertura de rocas y musgo. Cuantificamos la cobertura de rocas y musgos (%) en un radio de 15 cm alrededor del nodo.
- Profundidad de hojarasca. Medimos la profundidad de hojarasca en cuatro puntos diferentes en un radio de 15 cm alrededor del nodo (en los cuatro cuadrantes de la circunferencia imaginaria centrada en el nodo). Calculamos la media de esos cuatro valores. La profundidad de hojarasca se midió en centímetros.
- Compactación media. Medimos la compactación del suelo en cada nodo mediante el uso del penetrómetro (Penetrologger penetrometer, Eijkelkamp Agriserch Equipment, Giesbeek, The Netherlands). Este aparato proporciona un perfil que describe la variación de la compactación del suelo con la profundidad en cada punto de muestreo. De estos perfiles, se desprenden dos variables relevantes para la capacidad de formar raíces y, por tanto para el éxito del establecimiento (Gómez-Aparicio *et al*. 2005a). Una de las variables es la compactación media de cada perfil (MPa).

- Profundidad a la que la compactación es máxima. Esta es la otra variable que se deriva de las medidas realizadas con el penetrómetro, la profundidad a la cual se encuentra la compactación máxima (cm).
- Disponibilidad de luz. Cuantificamos la disponibilidad de luz en cada malla mediante fotografía hemisférica, una técnica ampliamente aceptada para el estudio de las condiciones de luz del sotobosque (Roxburgh & Nelly, 1995). La comparación de distintos métodos ha revelado la exactitud que proporciona el uso de la fotografía hemisférica para la descripción de la disponibilidad de luz del sotobosque particularmente en sitios heterogéneos con gran cantidad de claros (Bellow & Nair, 2003). Tomamos las fotografías en cada nodo a nivel del suelo (para captar de esta manera la luz que les llega a las plántulas) con una cámara digital nivelada horizontalmente (CoolPix 5000, cámara digital, Nikon, Tokio, Japón) apuntando al cenit, usando un objetivo de ojo de pez de 180º de visión de campo. Para asegurarnos una iluminación homogénea del dosel arbóreo y un correcto contraste entre el dosel y el cielo, tomamos todas las fotografías antes del amanecer, después de la puesta del sol o durante días nublados. Analizamos las fotografías digitales con el programa Hemiview canopy analysis software version 2.1 (1999, delta-T Devices Ltd, Cambridge, United Kingdom). El programa estima el parámetro denominado GSF (Global Site Factor), definido como la proporción de radiación directa y difusa en cada punto de muestreo, considerando las condiciones lumínicas en nuestro área de estudio (Rich, 1990). GSF es una variable continua que oscila entre 0 (cielo abierto) y 1 (obstrucción completa del cielo).

En 2004 y en 2005 sembramos semillas de dos especies arbóreas formadoras de ecosistemas de montaña mediterránea en las tres mallas de estudio: encina (*Quercus ilex)* y serbal (*Sorbus aria)*. Cada año sembramos semillas de cada una de las especies en una submuestra homogénea de 300 nodos en cada malla, en el caso de la encina una bellota por nodo, y del serbal 3 semillas que se introdujeron en la tierra a pocos centímetros de la superficie. En el caso de las bellotas sembradas en la malla del matorral pionero, dispusimos unas rejillas de aluminio encima del nodo para protegerlas de los depredadores de semillas (*Apodemus sylvaticus* principalmente). Los individuos que germinan en primavera se denominan plántulas y después de superar el primer verano y el invierno pasan a denominarse juveniles.

Análisis de datos:

Para cuantificar la heterogeneidad espacial de las variables ambientales medidas utilizamos el método SADIE (Spatial Analysis by Distance Indices), técnica desarrollada por Joe Perry y colaboradores en la Estación Experimental de Rothamsted (UK), que se basa en la utilización de índices de distancia. Su sencilla base matemática y estadística junto con los escasos requerimientos de la estructura de datos, hacen de SADIE un asequible método para el análisis espacial de los datos ecológicos. SADIE no exige requerimientos tales como la estacionariedad (los datos deben estar normalmente distribuidos), el isotropismo (el patrón debe mostrar la misma intensidad en todas direcciones), la equidistancia (los datos deben estar espaciados de manera regular) o el efecto borde (el tamaño y la forma del área de estudio afectan a la capacidad de los estadísticos para estimar el patrón espacial), ya que los resultados están condicionados a la heterogeneidad presente en los mismos (Bell, 1998).

Una variable puede presentar en el espacio un patrón agregado, regular o aleatorio. La base matemática que usa SADIE para evaluar el patrón espacial de una variable es una estima de la distancia mínima en el espacio, D, requerida para obtener la regularidad, esto es, que los distintos valores de la variable estudiada alcancen el valor promedio en todas las posiciones del espacio. Para calcular esta distancia, SADIE utiliza un algoritmo que optimiza el flujo de transporte desde zonas con valores altos de la variable hasta zonas con valores bajos de la variable. Para evaluar si el valor D obtenido con nuestros datos (D_{obs}) difiere de la aleatoriedad, SADIE realiza un test de permutaciones donde los valores de la variable son distribuidos al azar en el espacio. Este test se repite varios cientos o miles de veces, calculándose D para cada una de las permutas y obteniéndose así su distribución de frecuencias. La división del valor observado, D_{obs}, por el valor medio, D_{perm}, obtenido a partir de las permutaciones genera un índice de agregación, I_a. El índice de agregación describe el patrón espacial de los datos de la variable: el patrón espacial es agregado, cuando $I_a > 1$, aleatorio si $I_a = 1$ y regular si $I_a < 1$. La significación estadística de D (p_a) puede obtenerse calculando qué proporción de valores de D en la distribución de frecuencias tiene un valor igual o mayor al valor observado. Para más información sobre cómo se obtiene el índice I_a consultar el tutorial sobre SADIE, que puede descargarse gratuitamente en la siguiente dirección:

http://www.rothamsted.ac.uk/pie/sadie/SADIE_downloads_tutorial_page_5_5.php

Otro de los puntos fuertes de la técnica SADIE es que proporciona información local mediante la detección de agregados locales de una variable en el área de estudio. Esto se consigue mediante el cálculo del índice de agrupación *(ν)* en cada posición muestreada, que cuantifica el grado en el que cada valor de la variable en su posición, contribuye al patrón espacial general de los datos. Si se considera una unidad

A donante que tiene un flujo de unidades hacia *n* unidades receptoras, la distancia media de flujo, Y_A, se calcula teniendo en cuenta la magnitud y la distancia del flujo desde *A* a *n*. Y_A es un buen indicador de la agregación porque tiende a ser mas elevado para una unidad que forma parte de una mancha (zona con valores altos de la variable) que para una que tiene un valor de la variable elevado pero que esta rodeada por unidades vecinas con valores bajos. Sin embargo, $\mathrm{Y_A}$ es dependiente de la escala a la que las distancias son medidas, del valor de cobertura y de su localización respecto a otras unidades. Para evitar esto, se calcula ν a partir de $\mathrm{Y_A}$, que es adimensional y tiene en cuenta estas características. Más detalles sobre los cálculos pueden encontrarse en Perry et al., (1999*)*. Valores de ν mayores que 1,5 o menores que –1,5 indican la presencia de una mancha o de un claro (zona con valores bajos de la variable) respectivamente, mientras que aquellos cercanos a 1 indican una distribución aleatoria de esa unidad (Perry et al., 1999). Como disponemos de un valor de ν por cada posición muestreada en nuestra área de estudio, podemos visualizar las manchas y claros mapeando los valores de ν mediante interpolación lineal. Esto se llevo a cabo mediante el programa informático Surfer (Golden Software Surfer 8.2, Golden Software, Inc. USA).

Para evaluar la relación existente entre los patrones espaciales de la supervivencia y los de las variables ambientales se utilizó la regresión logística. Este tipo de regresión estima la probabilidad de que la supervivencia ocurra en función de los valores que tomen el conjunto de variables ambientales medidas (Hosmer & Lemeshow, 1989). La formulación del modelo de regresión se expresa en términos de la razón de probabilidades, que es el cociente entre la probabilidad del estado 1 (supervivencia) frente al estado 0 (mortalidad) (Martinez Arias, 1999):

$$\frac{P(Y=1)}{1-P(Y=1)} = \frac{e^{b0+\sum bjXij} / (1+e^{b0+\sum bjXij})}{1/(1+e^{b0+\sum bjXij})} = 1 + e^{b0+\sum bjXij}$$

donde b_0 es un término constante, b_j son los pesos de las variables predictoras incluidas en el modelo (j=1, 2,…,p) y Xij son los valores i-ésimo en el predictor j-ésimo. Si se transforman logarítmicamente los dos términos de la ecuación, se obtiene un modelo similar al de la regresión lineal múltiple:

$$\ln [p/(1-p)] = b_0 + b_1 X_{i1} + b_2 X_{i2} + \ldots\ldots\ldots + b_p X_{ip}$$

El modelo selecciona el conjunto de variables que predice de forma óptima los cambios en la razón de probabilidades utilizando el método de máxima verosimilitud (“maximum likelihood estimation”), que maximiza la probabilidad de que un proceso, en este caso la supervivencia, ocurra.

Se ha utilizado el método de Borcard *et al.* (1992) para evaluar que importancia tienen las variables ambientales medidas como controladoras del patrón espacial de la supervivencia de las dos especies arbóreas elegidas en nuestra zona de estudio, y la importancia relativa de estas frente a otras variables no evaluadas. En dicho método las coordenadas donde se encuentran las plántulas son consideradas como una variable sobre la que se pueden realizar análisis estadísticos. La base de este análisis es que, cuando se estudian las causas de la variación de un fenómeno ecológico, la estructura espacial de los datos puede actuar como una variable sintética de los procesos que la han generado (Borcard *et al*, 1992). Con el método de Borcard *et al.* (1992) se consigue una partición de la variación de la supervivencia de las plántulas (variable dependiente), en dos matrices: la denominada matriz X, formada por las variables ambientales; y la matriz W, constituida por variables espaciales derivadas de la combinación lineal de las coordenadas de las plántulas.

Mediante esta técnica se divide la variación de la supervivencia de las plántulas en cuatro fracciones: variación explicada por las variables ambientales independientemente de la estructura espacial (a), variación explicada por la estructura espacial de las variables ambientales (b), variación explicada por las variables espaciales independientemente de las variables ambientales (c) y variación que no es explicada ni por las variables ambientales ni por las espaciales (d). Para calcular estas fracciones se llevan a cabo las siguientes regresiones (Legendre & Legendre, 1998): una regresión logística de la supervivencia utilizando la matriz X como variables explicativas, que extrae la fracción a + b; una regresión logística de la supervivencia utilizando la matriz W como variables explicativas, que extrae la fracción b + c; y una regresión logística de la supervivencia utilizando las matrices X y W como variables explicativas, que extrae la fracción a + b + c. Se utilizó la R^2 de Nagelkerke para cuantificar la proporción de variación explicada por cada fracción (Nagelkerke, 1991). Finalmente, las fracciones se obtuvieron de la siguiente forma (Legendre & Legendre, 1998):

- Fracción *a*: (a + b + c) – (b + c)
- Fracción *b*: (a + b) – a
- Fracción *c*: (a + b + c) – (a + b)
- Fracción *d*: 1- (a + b + c)

Para poder extraer todos los gradientes aparte de los lineales cuando se analizan los datos, la matriz W se formó con las coordenadas de las plántulas y por todos los términos de un polinomio de tercer grado obtenido a partir de estas coordenadas (Borcard *et al*, 1992). Previamente, se centraron las coordenadas en sus respectivas medias, para reducir la multicolinealidad entre las variables (Legendre & Legendre, 1998).

Se utilizó el estadístico χ^2 para evaluar el ajuste de los modelos conseguidos mediante regresión logística, el cual compara la hipótesis nula de que todos los coeficientes excepto la constante son cero (Norŭsis, 1997). Se realizó una selección hacia atrás de las variables (“stepwise selection”) con el fin de seleccionar las variables que mas contribuyeron a explicar la supervivencia de las plántulas (Norŭsis, 1997).

Previamente a la regresión logística se realizó un análisis para detectar la multicolinealidad entre las distintas variables incluidas en las dos matrices. Se realizaron regresiones múltiples por separado para cada variable ambiental, usando está como variable dependiente y al resto como independientes. El mismo procedimiento fue aplicado en el caso de la matriz W. Se utilizó el factor de inflado de la varianza (FIV) entre las distintas variables como indicador de la multicolinealidad, calculándose con la siguiente fórmula (Etxeberría, 1999):

$$\mathrm{FIV} = 1 / 1 - R^2_i$$

donde R^2_i es el coeficiente de determinación múltiple entre la variable cuya multicolinealidad se está calculando y el resto de variables de la matriz. El FIV fue en todos los casos inferior a 4.5 y 8 para las matrices X y W respectivamente, indicando la ausencia de una multicolinealidad importante (Chatterjee & Price, 1999).

Las regresiones logísticas y múltiples se realizaron con el paquete estadístico SPSS 12.0.

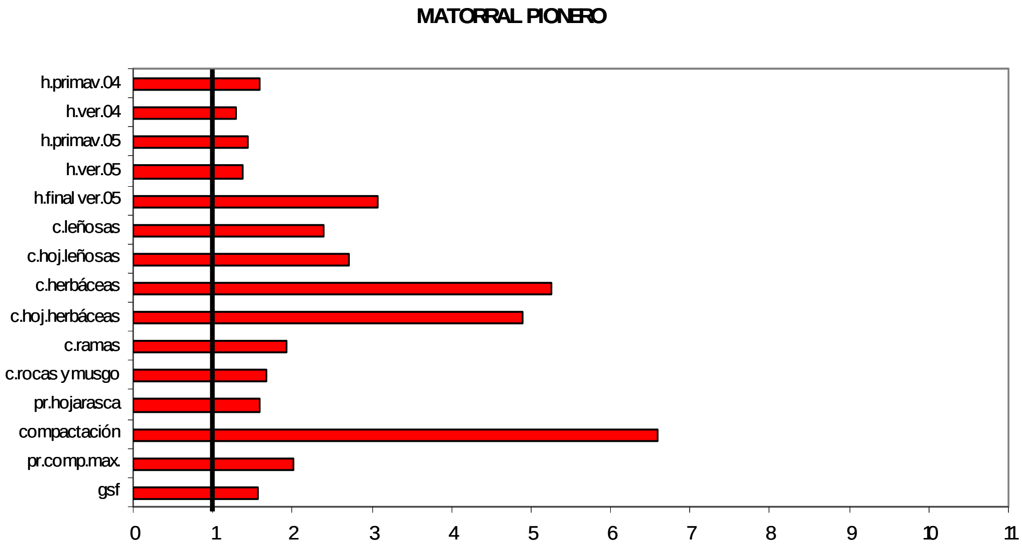
RESULTADOS

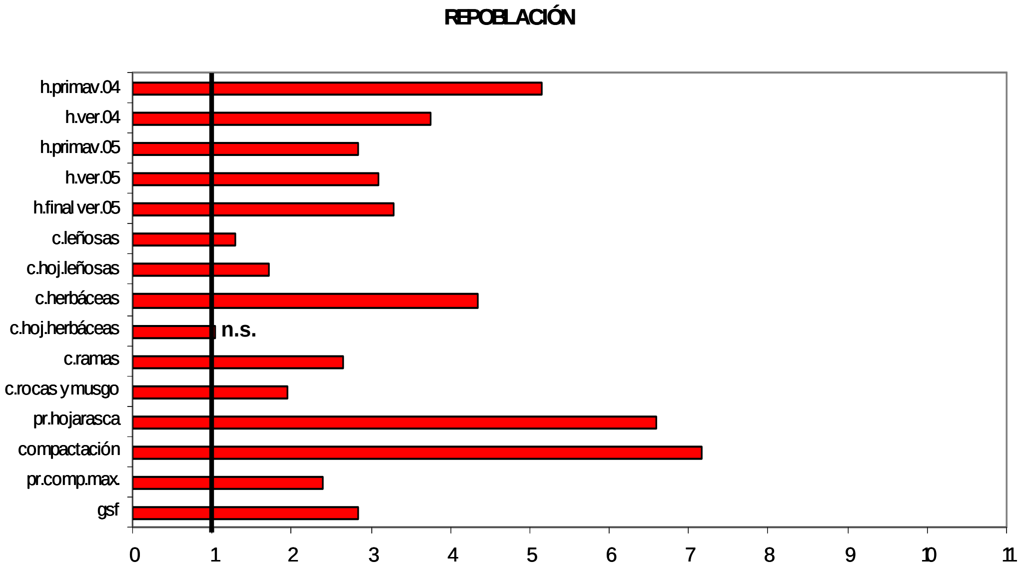
La mayoría de las variables ambientales presentaron un patrón espacial agregado (Figura 1). Todos los valores de I_a fueron significativos ($p_a<0.05$), excepto la cobertura de hojarasca de herbáceas en el caso de la repoblación y la cobertura de rocas y musgo en el caso del bosque autóctono. Por lo tanto, las tres parcelas estudiadas presentan una fuerte heterogeneidad ambiental a pequeña escala.

Respecto a los datos de supervivencia, en la malla del matorral pionero no pudimos obtener ninguna información valida debido a los serios problemas que tuvimos con los depredadores de semillas (*Apodemus sylvaticus*).

En el 2004, en las mallas de bosque autóctono y la repoblación hubo una gran supervivencia de las plántulas de las dos especies después del verano, sobretodo en la repoblación que rozó el 100 % de las emergidas (98.01 % para el serbal y 99.40 % para la encina) (Figura 2). En la malla de bosque autóctono los valores fueron más bajos (64,81 % serbal y 80.18 % encina).

En el 2005, la supervivencia registrada tomo valores muy diferentes a los del año anterior. En cuanto a los juveniles (las plantas sembradas el año anterior), en la parcela del bosque autóctono la supervivencia fue inferior al 50 % (14.91 % para el serbal y 42.73 % para la encina), y en la repoblación en cambio superior (84.23 % serbal y 63.33 % encina). Las plántulas que germinaron de las semillas sembradas en el 2005 tuvieron una alta tasa de mortalidad ya que la supervivencia fue en todos los casos inferior al 25 %. De hecho, en la parcela del bosque autóctono no quedo ninguna plántula viva de las dos especies, y en la repoblación la supervivencia del serbal fue insignificante (3.8 %). La encina presentó la supervivencia más alta en este caso (20 %) (Figura 2).

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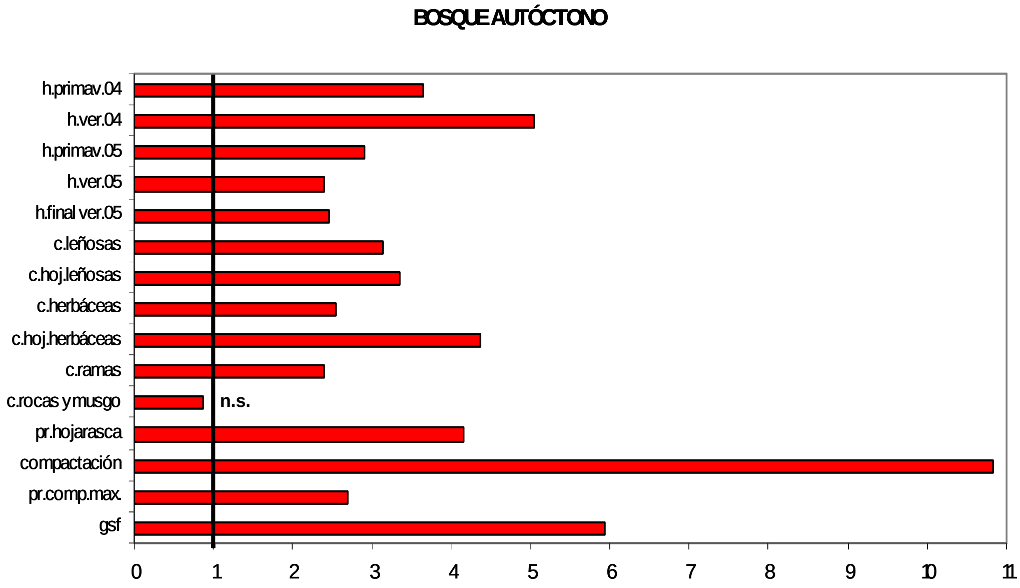
BOSQUE AUTÓCTONO

Figura 1. Indices de agregación (I_a) para cada una de las variables ambientales en las tres unidades de paisaje. n.s. = no significativo; h.primav.=humedad primavera; h.ver.=humedad verano; h.final ver.=humedad final verano; c.leñosas=cobertura leñosas; c.hoj.leñosas=cobertura hojarasca leñosas; c.hebáceas=cobertura herbáceas; c.hoj.herbáceas=cobertura hojarasca herbáceas ; c.ramas=cobertura reamas; c. rocas y mugo=cobertura rocas y musgo; pr.hojarasca=profundidad de hojarasca; compactación=compactación media; pr.comp.max=profundidad a la que la compactación es máxima; gsf=Global Site Factor (disponibilidad lumínica).

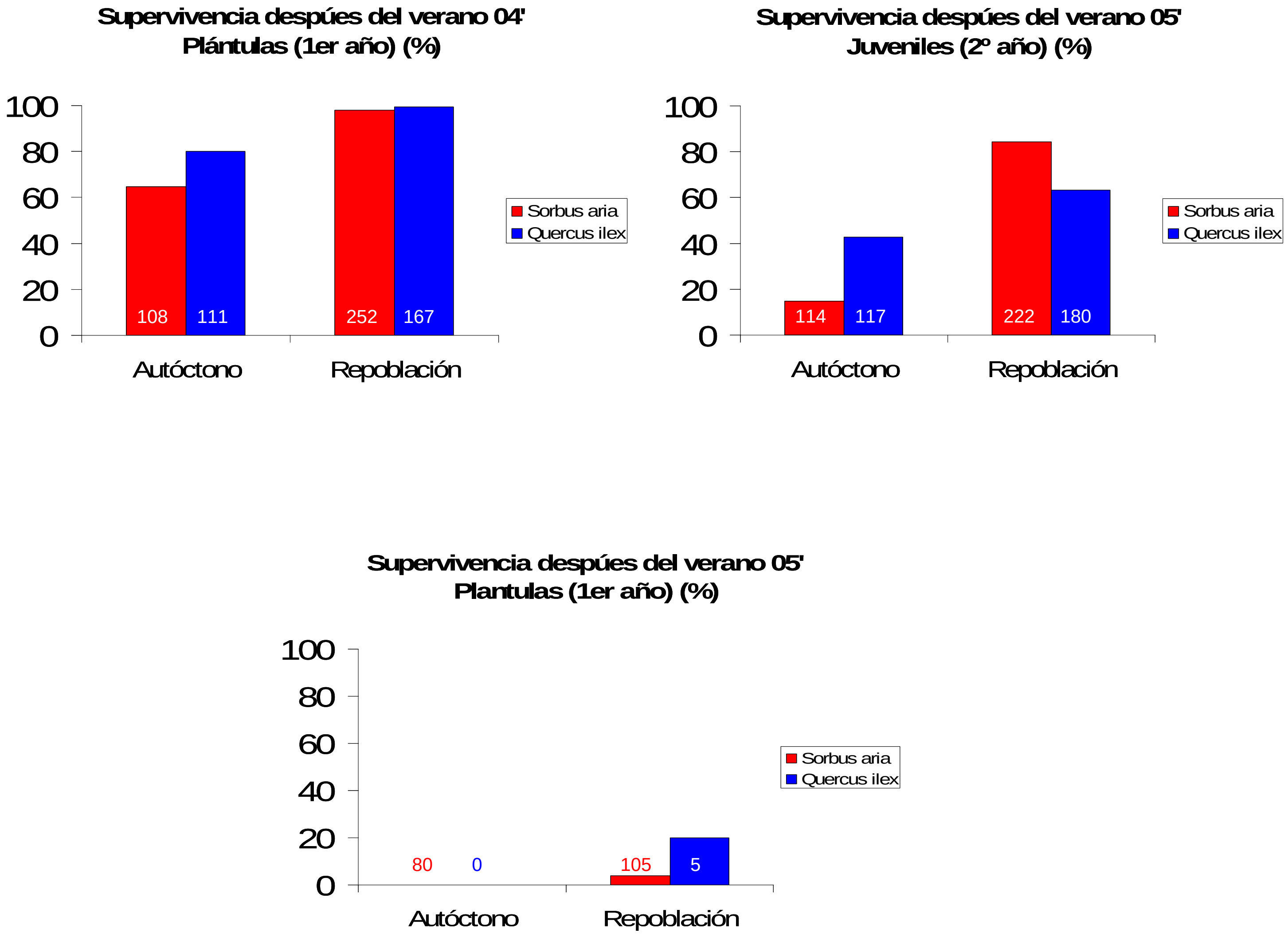

Figura 2. Porcentaje de plántulas y juveniles de encina (*Quercus ilex*) y serbal (*Sorbus aria*) que sobrevivieron después de los veranos del 2004 y 2005 en el bosque autóctono y en la repoblación. Los números de las barras indican el número total de plántulas emergidas o juveniles vivos antes del verano.

En el año 2005, cuando se registraron los porcentajes bajos de supervivencia, sólo cayeron 8 mm de lluvia en los meses de junio, julio y agosto (Figura 3) (Datos del Jardín Botánico de la Cortijuela) y la temperatura media esos meses fue más alta que en 2004. En cambio en el verano del 2004 la precipitación media mensual alcanzó un mínimo de 9 mm (en junio cayeron unos 23mm). El punto de toma de estos datos se puede considerar representativo de la zona de estudio donde se encuentran ubicadas las tres mallas.

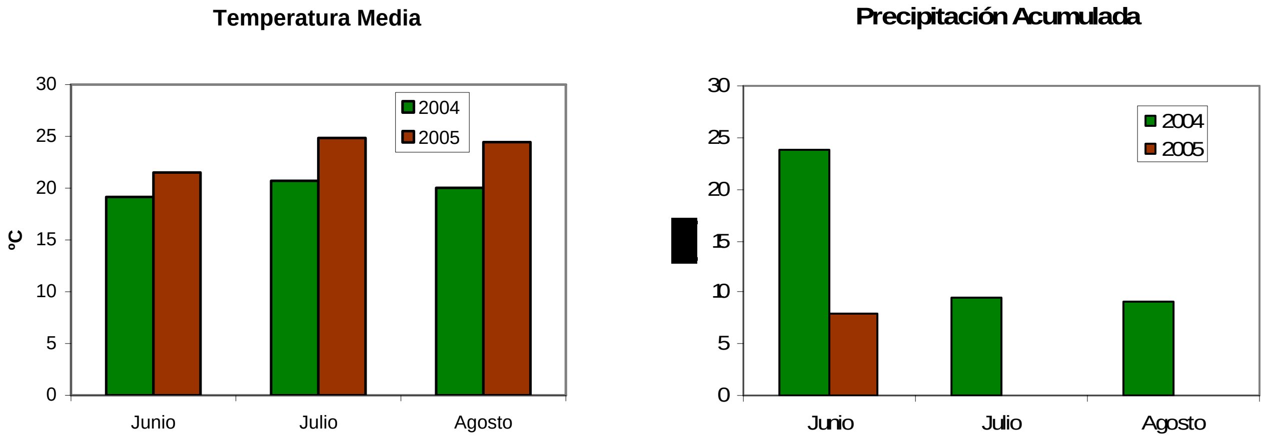

Figura 3. Datos metereológicos recogidos en el Jardín Botánico de la Cortijuela los veranos (meses de junio, julio y agosto) del 2004 y 2005. En el gráfico de la izquierda se presenta la temperatura media mensual y en el de la derecha la precipitación mensual acumulada.

En cuanto a los patrones espaciales de la supervivencia, en el caso de las plantas sembradas en 2004 podemos observar un incremento de la agregación del patrón de supervivencia después del verano del 2005 en todos los casos estudiados, excepto en el de la encina en la repoblación (Figura 4). En la Figura 5 se puede observar como se agregan las manchas de supervivencia de la encina en el bosque autóctono.

Las plántulas del 2005 sufrieron una tasa de mortalidad tan alta que sólo pudimos calcular el índice de agregación del patrón espacial de supervivencia para el caso del serbal en la repoblación, que fue de 0.90, el valor más bajo obtenido.

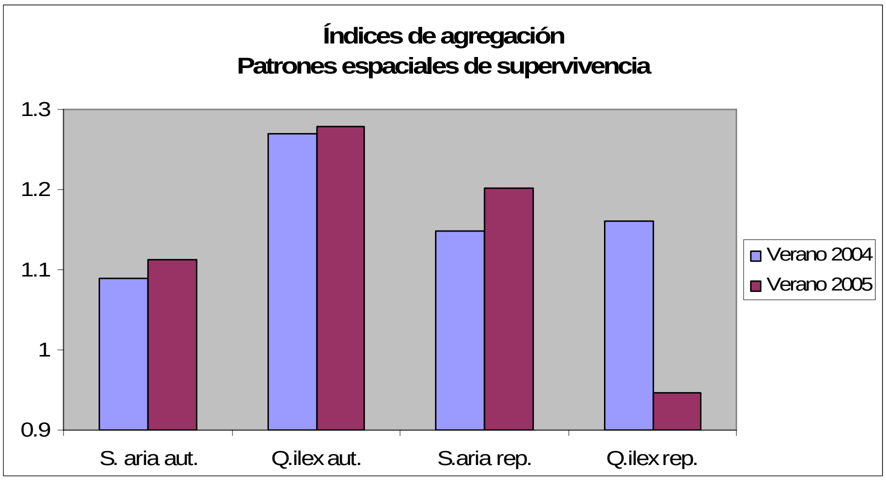

Figura 4. Índices de agregación (I_a) de los patrones espaciales de supervivencia de los individuos sembrados en 2004, después de los veranos del 2004 y del 2005.

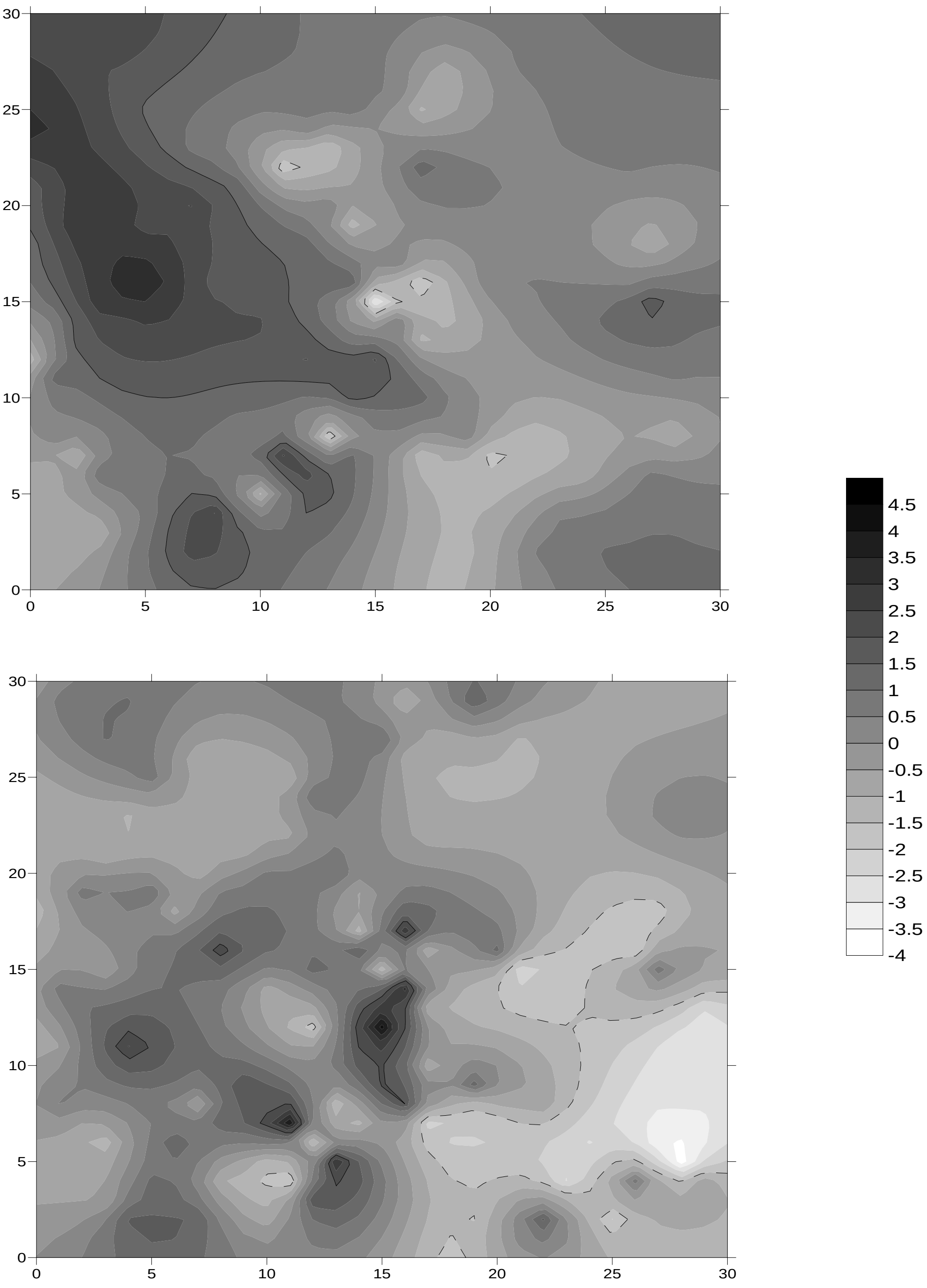

Figura 5. Mapas elaborados a partir de los índices de agrupación (v) del patrón espacial de la supervivencia. Las coordenadas x están representadas horizontalmente y las coordenadas y verticalmente. Las zonas oscuras delimitadas por línea continua indican manchas de supervivencia ($v > 1.5$) y las zonas claras delimitadas con línea discontinua indican claros de supervivencia ($v <$ 1.5). a) Representación del patrón espacial de la supervivencia de plántulas de encina (*Quercus ilex)* en el bosque autóctono después del verano del 2004 b) Representación del patrón espacial de la supervivencia de juveniles de encina (*Quercus ilex)* en el bosque autóctono después del verano del 2005.

Realizamos el método de la partición de la variación sólo en el caso de los juveniles, ya que las plántulas del 2004 tuvieron tasas de mortalidad muy bajas y las del 2005 muy altas. La fracción *a* (la explicada por las variables ambientales independientemente de la estructura espacial) fue mayor en la malla del bosque autóctono que en la repoblación (Figura 6). En el bosque autóctono esta fracción fue mayor en el caso del serbal (19,8 %) que en el de la encina (15.6 %). La variación de la supervivencia explicada por la estructura espacial de las variables ambientales (*b*) resulto ser del 17. 5% en el caso del serbal en el bosque autóctono, mientras que en el resto de casos fue muy baja. La variación explicada por la variables espaciales (*c*) fue similar en todos los casos, alrededor del 10 %, excepto en el de la encina en la parcela repoblada. La variación explicada por variables no medidas oscilo entre el 52 % y el 90 %. Por lo tanto, fue en la malla del bosque autóctono donde mas variación de la supervivencia explicaron las variables ambientales (fracción *a* + *b*).

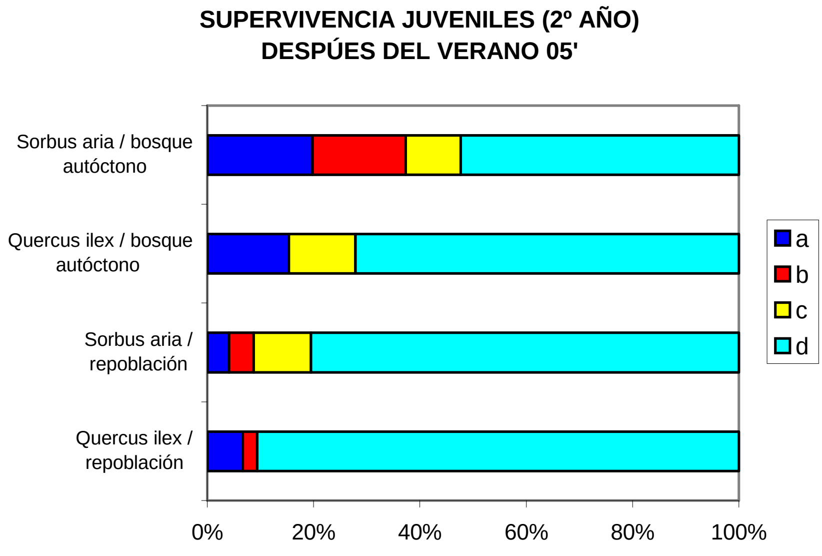

Figura 6. Resumen de la partición de la variación de la supervivencia de los juveniles sembrados en 2004 despúes del verano del 2005. Las fracciones son las siguientes: a) variación explicada por las variables ambientales independientemente de la estructura espacial; b) variación explicada por la estructura espacial de las variables ambientales; c) variación explicada por las variables espaciales independientemente de las variables ambientales; y d) variación que no es explicada ni por las variables ambientales ni por las espaciales.

De entre las variables ambientales que más contribuyeron a explicar la supervivencia de los juveniles, encontramos que la humedad de finales de verano en el 2005 aparece en todos los casos, excepto en la repoblación en el caso de la encina (Tabla 1). En este último caso aparece la humedad medida a mediados de verano en 2005. Esto confirma la importancia de la humedad estival del suelo en la supervivencia. La cobertura de ramas y de las diferentes hojarascas también aparece en más de un caso, así como la disponibilidad lumínica (GSF).

Tabla 1. Lista de las variables que más contribuyen a explicar la supervivencia de los juveniles sembrados en el 2004 despúes del verano del 2005. Pr.comp.max.= Profundidad a la que la compactación es máxima. GSF=Global Site Factor (disponibilidad lumínica).

	Variables ambientales
***Sorbus aria* / bosque autóctono**	Hojarasca de herbáceas, Cobertura de ramas, Humedad final de verano 05', GSF y Pr.comp.max.
***Quercus ilex* / bosque autóctono**	Hojarasca de leñosas, Cobertura de ramas y Humedad final de verano 05'.
***Sorbus aria* / repoblación**	Humedad final de verano 05' y Cobertura de ramas.
***Quercus ilex* / repoblación**	Humedad verano 05', GSF y Cobertura de leñosas.

DISCUSIÓN:

La gran mayoría de las variables ambientales evaluadas presentaron patrones espaciales agregados en los tres tipos de rodales estudiados: matorral pionero, repoblación y bosque autóctono. Algunas de ellas con índices de agregación elevados como la compactación media, la cobertura de especies herbáceas o la humedad del suelo medida en diferentes momentos de la época estival. Por lo tanto, podemos asegurar que existe una gran heterogeneidad ambiental a escala de micrositio en zonas de montaña mediterránea.

Hay que resaltar la gran influencia que ejerce la variabilidad ambiental a gran escala, ya que esta puede hacer desaparecer la influencia de la heterogeneidad ambiental a pequeña escala. Las duras condiciones ambientales del verano del 2005, altas temperaturas y escasas precipitaciones, hicieron imposible calcular los índices de agregación de los patrones de supervivencia de las plántulas germinadas ese año debido a la alta tasa de mortalidad sufrida o propiciaron que esos patrones no fueran agregados sino regulares. Las que germinaron en 2004, presentaron índices de agregación mas elevados el año siguiente seguramente como consecuencia de la sequía de ese año. Estos individuos soportaron las condiciones duras del verano del 2005 gracias a que en la fase de plántula gozaron de buenas condiciones ambientales: precipitaciones más abundantes y repartidas y temperaturas más bajas. Es decir que los juveniles en el verano de 2005 nos presentan una situación similar a la que tendría un grupo de plántulas en un verano no tan duro como el del 2005, ni tan benigno como el del 2006. Por lo tanto, el incremento de los índices de agregación de la supervivencia de las plantas sembradas en 2004 después del verano del 2005, nos lleva a pensar que contra más alejado de los extremos estén las condiciones ambientales a gran escala mas influencia tendrá la heterogeneidad ambiental a pequeña escala. En el caso de la encina en la repoblación el índice de agregación de la supervivencia bajo después del verano del 2005 en comparación con el calculado después del verano del 2004. Esto pudo deberse a que la encina es una especie que soporta muy bien la sequía y su patrón espacial pudo deberse a otros factores que los evaluados en este estudio como corroboran los resultados de la partición de la variación. Como consecuencia de todo lo explicado, podemos afirmar que el patrón espacial de la supervivencia es agregado cuando las variaciones ambientales a gran escala lo permiten.

Hemos dicho que los patrones espaciales se agregaron seguramente a causa de la sequía. La sequía hace que el grado de exigencia de una plántula o un juvenil respecto a un micrositio aumente. Con presencia de sequía el micrositio deberá de ser más húmedo o recibir menos radiación directa o durante menos horas. Es decir que las condiciones adversas hacen que "afloren" los mejores micrositios para las plántulas y juveniles, no sólo en cuestión de radiación y de humedad como hemos comentado anteriormente, también micrositios que tengan valores de compactación adecuados o de otras variables ambientales. Con el método de partición de la variación hemos comprobado la importancia que tiene la humedad del suelo en la supervivencia de las plántulas y juveniles, así como otras variables que están relacionadas con la humedad como la cobertura de hojarasca de herbáceas, de leñosas o la cobertura de ramas. Los sitios con mucha hojarasca suelen ser más secos (obs. pers), en cambio la cobertura de ramas puede que retenga algo de humedad. La importancia de la disponibilidad lumínica en la supervivencia de los juveniles también está respaldada por el método de la partición de la variación.

Las variables ambientales y su estructura espacial explican más variación de la supervivencia en el bosque autóctono que en la repoblación, indicando que el bosque autóctono es un escenario ecológico más heterogéneo que la repoblación, por lo tanto con más micrositios potenciales para el reclutamiento de plántulas. Es posible que en la repoblación se expresen más algunos de los factores no evaluados, como pueden ser los factores fisiológicos, ya que la repoblación parece un ambiente más homogéneo.

Finalmente, los resultados de este estudio pueden ser de gran ayuda para mejorar los programas de restauración de áreas mediterráneas degradadas. Primero, prediciendo los eventos o variaciones de las condiciones ambientales a gran escala para así poder evitarlas y realizar los trabajos de restauración los años con mejores condiciones. Y segundo identificando parches con valores de variables ambientales apropiadas para las plántulas y juveniles, con lo que se optimizaría el rendimiento de plantaciones y/o siembras. La humedad de suelo y la disponibilidad lumínica serían los indicadores abióticos ideales para este cometido. Estos dos factores además de ser de vital importancia para el desarrollo de las plantas, contribuyen como ya hemos visto (junto a su estructura espacial) en una parte importante a explicar la supervivencia de los juveniles. Para desarrollar un método óptimo habría que detectar el rango de los valores de esas dos variables dentro del cual las plántulas y juveniles sobrevivan y crezcan. Para no tener que realizar múltiples mediciones en las zonas a restaurar sería ideal relacionar estos indicadores abióticos con algún indicador biótico. Lo ideal sería que el indicador biótico fuese de amplia distribución en las montañas mediterráneas, para ampliar lo más posible su área de aplicación. Por lo tanto, la heterogeneidad ambiental a pequeña escala puede ser una herramienta de gran valor para la restauración.

CONCLUSIONES:

Las variables ambientales presentaron patrones espaciales agregados a pequeña escala, demostrando la gran heterogeneidad ambiental existente a escala de micrositio. Esta heterogeneidad ambiental afecta a la supervivencia de las plántulas o juveniles solo cuando la variabilidad ambiental a gran escala lo permite. Las relaciones espaciales entre las variables ambientales y los patrones de supervivencia pueden usarse para optimizar los programas de restauración: prediciendo las variaciones ambientales a gran escala y escogiendo los mejores años para realizar las actuaciones; y mapeando parches con valores de las variables ambientales apropiadas para las plántulas y juveniles.

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4. BLOQUE II: AISLANDO FACTORES IMPORTANTES: RESPUESTAS ECOFISIOLÓGICAS A LA LUZ Y EL AGUA.

Capitulo 3: (en ingles) Seed mass effect in four Mediterranean *Quercus* species (*Fagaceae*) growing in contrasting light environments. (en revision en *American Journal of Botany*)

SEED MASS EFFECT IN FOUR MEDITERRANEAN *QUERCUS* SPECIES (FAGACEAE) GROWING IN CONTRASTING LIGHT ENVIRONMENTS[1]

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Three different hypotheses have been proposed to explain the functional relationship between seed mass and seedling performance: the reserve effect (larger seeds retain more proportion of reserves), the metabolic effect (larger-seeded seedlings have slower relative growth rate) and the seedling size effect (larger seeds produce larger seedlings). Here, we tested these hypotheses in four Mediterranean *Quercus* species growing under different light conditions (3, 27 and 100 % available radiation). Evidence for two out of three hypotheses have been found in this study, however, none of the four species matched the hypotheses at the same time. The reserve effect was not met by any species, the metabolic effect by two species (*Q. ilex* and *Q. pyrenaica*) and the seedling size effect by all species. Apart of the species factor, light availability significantly affected the seed size relationships. For two species (*Q. ilex* and *Q. canariensis*) a seedling size effect was found for all the different light conditions. However, for the other two species (*Q. suber* and *Q. pyrenaica*) a seedling size effect was found only in the case of the lowest light availability (3 %). In all species, stronger correlations of seed-seedling mass occurred with decreasing light availability confirming that oak seedlings growing in low light availability depend more on their seed reserves. A causal model connecting the three hypotheses is presented. We found that, in general, larger seeds produce larger seedlings and in only few cases no seed-seedling mass relationship is found.

Key words: acorn mass; Fagaceae; light availability; Mediterranean oaks; Quercus; seed-seedling relationships; seed size; RGR.

Seed size is one of the most important traits influencing the germination (Milberg et al., 2000; Pearson et al., 2002), emergence (Castro, 1999), growth and survival of seedlings (Seiwa, 2000; Baraloto et al., 2005). Three different mechanisms have been proposed to explain the functional seed-seedling relationships (Westoby et al., 1996; Leishman et al., 2000). i) The reserve effect predicts that, after germination, larger seeds retain a greater proportion of their seed reserves. Thus, the availability of an extra amount of reserves in the large cotyledons enhances the chances for the seedling to establish successfully under adverse environmental conditions by mobilizing reserves for growth, maintenance, and repair (García-Cebrián et al., 2003; Green and Juniper 2004a). ii) The metabolic effect predicts that there is a negative relationship between seed size and relative growth rate (RGR) during the early stages (Cornelissen et al., 1996; Saverimuttu and Westoby, 1996; Wright and Westoby, 1999). A higher SLA, lower DNA content and smaller cell size are thought to contribute to the growth advantage of small-seeded seedlings (Marañón and Grubb, 1993) whereas in larger-seeded seedlings the growth disadvantage is compensated by their higher initial plant mass. iii) The seedling size effect proposes that larger seeds produce larger seedlings, which are more robust and better able to escape size-dependent mortality. Larger seedlings have a larger shoot to overtop neighboring seedlings and capture more light (Foster, 1986), form deeper and more extensive roots to capture more soil water during the dry season (Metcalfe and Grubb, 1997; Lloret et al., 1999; Poorter and Hayashida, 2001), and can emerge from deeper soil layers than smaller ones (Bond et al., 1999).

Since the publication of the seminal paper by Westoby et al. (1996) where the three seed size hypotheses were presented, most studies have analyzed the effect of seed size on seedling performance by comparing different species with contrasting life-history traits (Saverimuttu and Westoby, 1996; Poorter and Rose, 2005). In this type of studies, the main objective is to explain the seed-seedling relationship from an evolutionary point of view (Wright et al., 2000; Westoby et al., 2002). However, species not only vary in their seed size, but also in many other traits, potentially confounding functional relationships with correlated variation due to life history. For instance, small-seeded species tend to be light-demanding, and generally have photosynthetic-type cotyledons, a high SLA and LAR whereas large-seeded species tend to be shade-tolerant and have the opposite suite of traits (Kitajima, 1996; Poorter and Rose, 2005; Zanne et al., 2005). A within-species analysis may therefore show more clearly the functional significance of seed size, although few studies have taken such approach (but see Ke and Werger, 1999, Green and Juniper, 2004a).

Seed size variation is thought to be important for the establishment success of seedlings under different ecological conditions. Species with considerable intraspecific variation in seed size may therefore enhance their establishment success in a heterogeneous environment. This might especially be the case for *Quercus* which show considerable interspecific (Aizen and Patterson 1990) and intraspecific (Leiva and Fernandez-Alés 1998) variation in seed size.

The effect of seed size on seedling performance is highly variable, and depends on environmental conditions such as light availability (Leishman and Westoby, 1994; Rose, 2000; Poorter and Rose, 2005), nutrient availability (Jurado and Westoby, 1992), and damage by herbivores (Harms and Dalling, 1997). For instance, Rose (2000) found for eight rainforest tree species that seedling survival was closely and positively correlated to seed size in low irradiance whereas the relationship became weak or insignificant in high irradiance.

Light has a highly spatial and temporal heterogeneity at the understorey ground level (Valladares, 2001) and many studies have demonstrated its importance for the regeneration niche of Mediterranean woody species (Sack, 2004, Gómez-Aparicio et al., 2006). We predicted that seedlings growing under deep shade, where carbon gain is reduced (Quero et al., 2006), will depend more strongly on their acorns.

The objective of this study is to evaluate the functional importance of seed size within four Mediterranean *Quercus* species. Specifically, four questions were addressed: (i) do larger seeds retain proportionally more reserves, which may be advantageous to overcome potential hazards? (the reserve effect); (ii) is the seed mass negatively related with RGR of the seedling? (the metabolic effect); (iii) do larger seeds result in a larger initial seedling biomass? (the seedling size effect) and iv) do these relationships change with light availability?

The three hypotheses have been postulated as possible mechanisms to tolerate different environmental hazards (Westoby et al., 1996). Here we present a conceptual framework that connects the three hypotheses.

MATERIAL AND METHODS

Experimental design— For this study four oak species were selected that are typical of Mediterranean forest in south Spain: two evergreen species [*Quercus suber* L. and *Quercus ilex* ssp. *ballota* (Desf.) Samp.], and two deciduous species (*Quercus canariensis* Willd. and *Quercus pyrenaica* Willd.) (Table 1). The four species have storage-type cotyledons, thus avoiding the problem of comparing species with different type of cotyledons (Kitajima, 1996, Green and Juniper, 2004a). Seeds were collected during autumn 2002 in the south of Spain (Table 1) and stored on a moist substrate at 5 ºC until sowing. Single acorns were weighted individually for their fresh mass. A subsample of each species was oven-dried at 70º C for at least 48 h and weighed for the dry mass; then initial seed dry mass was estimated for each individual from the seed fresh mass, using species-specific regression equations (Table 1). The experiment was carried out in a greenhouse of the University of Córdoba, Spain (at 37º 51' N, 4º 48' W and altitude of 100 m. a. s. l.) On December 2002, acorns were separately sown in cylindrical pots (50 cm height by 10.5 cm diameter) of 3.9 liters volume. A large pot size was used to allow for extensive root growth. Pots contained a mixture of 2/3 sand and 1/3 peat. The air temperature was maintained between 15 and 25 ºC.

Seedlings were subjected to three light levels: 1) high-irradiance treatment (HI), receiving the total radiation inside the greenhouse; 2) medium-irradiance treatment (MI), covered by a light green screen, and representing 27 % of available radiation inside the greenhouse; and 3) low-irradiance treatment (LI), covered by a dense green cloth representing 3 % of available radiation. The mean ± SE of the photosynthetic active radiation measured every day in January, 2003 around noon was 281 ± 28 (HI), 76 ± 9 (MI) and 9 ± 1 (LI) µmol of photons m^{-2} s^{-1}. These light levels are typical for light levels in large forest openings (HI treatment), below individual trees (MI), and under deep shade of the forest understorey (LI) (Marañón et al., 2004). Each light treatment was imposed using a shade frame (150 x 120 x 200 cm) and replicated 4 times. Light quality, red: far red ratio (R: FR ratio), was measured with a light sensor (SKR 110, Skye Instruments, Llandrindod Wells, UK). In the LI treatment, the value of R: FR ratio was 0.25 ± 0.004 which is comparable to the R: FR measured in dense forest microhabitats (0.28 ± 0.03, t-test, $P = 0.31$); while in the other two treatments (HI and MI) the R: FR was 1, similar to the corresponding natural habitats (open areas and below trees, respectively).

Once the seedlings emerged (the exact date was annotated for each seedling), a drip-irrigation system was inserted in the pots, to assure that water was not a limiting factor. After ca. 50 days (mean 51, range 48-55) of growth, over sixteen seedlings of each species per light treatment were harvested. We harvested after 50 days, because field studies have shown that seedlings complete their spring growth within 50 days (Quero et al., 2003). Individual seedlings were carefully extracted from each pot and the soil was gently washed from the roots. Plants were separated into acorn remnant, leaves, stem, and roots. Dry mass of the plant organs was determined after oven-drying at 70º C for at least 48 h. The used seed reserve was calculated as the difference between the estimated initial acorn dry mass and the remnant acorn dry mass at the moment of the harvest. The relative growth rate (RGR, rate of biomass growth per unit plant biomass) was calculated as the difference between the Ln of seedling biomass minus Ln used seed reserve (Steege et al., 1994), and divided by the growth period.

Statistical analysis— A two-way ANOVA was used to evaluate whether there were differences in seed mass among four species and three light treatments. When significantly different, a post-hoc test (Tukey's Honestly Significant Difference test for Unequal N) was carried out to evaluate differences in initial seed mass within light treatments and within species. Data were arcsine- or log-transformed before to analysis to satisfy the assumptions of normality and homocedasticity (Zar, 1984). The programme STATISTICA v 6.0 (Statsoft, Inc., Tulsa, OK, USA) was used for statistical analyses.

Within each species, seed-seedling relationships were explored among light treatments using standard major axis (SMA) regressions (Legendre, 2000), because the emphasis on this study was on calculating the intra-specific allometric slopes and their 95 % confidence limits to compare within the three light treatments and against a null model of slope 1 or 0 (depending of the hypothesis, see below).

To fulfill the reserve effect (larger seed retain more proportion of reserves) two conditions should be met: 1) the slope of log used reserve versus log seed mass should be significantly lower than 1, and 2) the slope of log seedling mass versus log seed mass should be significantly higher than that of log used reserve versus log seed mass because the functional meaning of these effect is that retained reserves has to be translated to seedlings for better performance (for more details, see Green and Juniper, 2004a). To meet the metabolic effect (larger seeds have slower relative growth rate) the slope of RGR versus log seed mass should be significantly lower than zero. To accept the seedling size effect (larger seeds produce larger seedlings) the slope of log seedling versus log seed mass should be significantly higher than zero. SMA regressions were done for each species considering the different light treatments and performed using the (S)MATR package of Falster et al. (2003). SMA slopes for different light treatments were compared against each other using routines found in the (S)MATR package.

A causal model connecting the three hypotheses— The hypothesis of the reserve effect has been studied with the relationship between the used reserve and seed mass (see Fig. 1A), which can be parameterised as:

$$\text{Ln (used reserve)} = a_1 + b_1 * \text{Ln (seed mass)} \qquad \text{(eq. 1)}$$

where a_1 is the intercept and b_1 is the slope. If b_1 is lower than 1, then the condition 1 of the reserve effect hypothesis is accepted.

The hypothesis of the metabolic effect has been contrasted using the relationship between RGR and seed mass (see Fig. 1B), which has been parameterised as:

$$\text{RGR} = a_2 + b_2 * \text{Ln (seed mass)} \qquad \text{(eq. 2)}$$

If b_2 is lower than 0, then the metabolic effect hypothesis is accepted.

The hypothesis of seedling size effect has been studied with the relationship between seedling biomass and seed mass, and it has been parameterised as:

$$\text{Ln (seedling biomass)} = a_3 + b_3 * \text{Ln (seed mass)} \qquad \text{(eq. 3)}$$

If b_3 is higher than 0, then the seedling size effect hypothesis is accepted.

The relative growth rate (RGR) has been defined as:

$$\text{RGR} = [\text{Ln (seedling biomass)} - \text{Ln (used reserve)}] / T \qquad \text{(eq. 4)}$$

where T is the time of growth. Rearranging the equation 4 we have:

$$\text{Ln (seedling biomass)} = \text{Ln (used reserve)} + (\text{RGR} * T) \qquad \text{(eq. 5)}$$

In eq. 5, if we replace Ln (used reserve) from the equivalent in eq. 1 and RGR from the equivalent in eq. 2, we have:

$$\text{Ln (seedling biomass)} = [a_1 + b_1 * \text{Ln (seed mass)}] + [a_2 + b_2 * \text{Ln (seed mass)}] *T$$

Rearranging the equation:

$$\text{Ln (seedling biomass)} = (a_1 + a_2 * T) + (b_1 + b_2 *T) * \text{Ln (seed mass)} \qquad \text{(eq. 6)}$$

Which is similar to eq. 3, where $a_3 = (a_1 + a_2 * T)$ and $b_3 = (b_1 + b_2 *T)$

Note that we used Ln in the causal model instead of Log_{10} , because RGR is calculated using Ln, although the slopes of the relationships based in Ln or Log_{10} are the same.

Because we are interested in the slope of the relationship between seedling biomass and seed mass (b_3), we can say that this slope depends on the slope of used reserve against seed mass (b_1) and the slope of RGR against seed mass (b_2) and also of the time of growth. As we are interested in the prediction of Y from given values of X, a linear regression is more appropriate than SMA regression (Falster et al., 2003). For that, we use for the connection of the three hypotheses the slope calculated from the linear regressions. To test graphically the connection between the three hypotheses, three dataset were simulated for the reserve effect using eq. 1 ($b_1 > 1$, $b_1 = 1$ and $b_1 < 1$)

and three dataset were simulated for the metabolic effect using eq. 2 ($b_2 > 0$, $b_2 = 0$ and $b_2 < 0$), (Fig. 3A, 3B). Then, we used these datasets to calculate 9 dataset resulting from different combinations of b_1 and b_2 slopes, and b_3 slopes were checked according to eq. 6 (Fig 3C). Simulations were done using Excel 2000 (Microsoft®).

RESULTS

Differences in seed mass among Quercus species**—** Mean values of the different variables measured in the four *Quercus* species in the three light treatments are presented in Appendix S1. There were significant differences among *Quercus* species in the initial seed mass (ANOVA, $P < 0.05$). *Q. canariensis* and *Q. ilex* had smaller acorns (about 2 g of dry mass) whereas *Q. suber* and *Q. pyrenaica* had larger acorns (about 4 g of dry mass). Within species, there was a 5-fold difference in seed mass (Table 1). There was no bias in assigning different seed mass among light treatment (ANOVA, $P = 0.33$). However, there was a significant species-light interaction, because inadvertently smaller acorns of *Q. pyrenaica* were selected for the high-light treatment (Appendix S1).

Reserve effect**—** For all species and light treatments, the initial seed mass was positively related with the seed reserves used during the 50 days of growth (Fig. 1A). However, each species used their seed reserves differently in relation to seed size and in some cases there was an effect of light availability. *Q. suber* in HI was the only species that met condition 1 of the reserve effect, because it showed a decrease of the use of reserves as the seed size increased (SMA slope 0.74, marginally significant lower than 1, $P = 0.06$; Fig. 1A). To fulfil condition 2, the slope of log seedling mass versus log seed mass should be significantly higher than that of log used reserve versus log seed mass. In this case (*Q. suber* in HI), condition 2 of reserve effect was not satisfied since there was no significant seedling-seed mass relationship hence, slope was likely to be close to 0 . On the other hand, two species (*Q. ilex* in HI and LI, and *Q. pyrenaica* in HI and MI) increased the use of reserves as the seed size increased (Fig. 1A, SMA slope significantly higher than 1) whereas *Q. canariensis* had a SMA slope that did not differ from 1 and that was similar across all light treatments.

Metabolic effect–– For two species (*Q. suber* and *Q. canariensis*) there were no significant relationships between RGR and seed mass for the three light treatments, while for *Q. ilex* in HI and LI and *Q. pyrenaica* in HI and MI there was a negative relationship (accepting the metabolic effect hypothesis for these light treatments; Fig. 1B). *Q. ilex* and *Q. pyrenaica* showed higher slopes of RGR-seed mass relationship with increasing light but slopes were significantly different only for *Q. ilex* ((S)MART test statistic = 8.36, *P* = 0.003; Fig. 1B).

Seedling size effect— After 50 days growth the seedling biomass was positively affected by seed mass for all species (Fig. 1C), in agreement with the seedling size effect although it was dependent on light availability. For *Q. ilex* and *Q. canariensis*, positive seedling-seed mass relationships were found for the three light treatments (Fig. 1C) and the slopes for light treatments were similar [(S)MART test statistic = 1.14, *P* = 0.58 for *Q. ilex*; (S)MART test statistic = 1.41, *P* = 0.49 for *Q.canariensis*]. In contrast, *Q. suber* and *Q. pyrenaica* only had a significant and positive relationship between seedling biomass and seed mass under deep shade. For all species a stronger correlation seed-seedling mass was found in deep shade, while this relationship became weaker or disappeared at higher light levels. Across species, there was a general trend of increasing the correlation coefficient with a decrease in light availability (Fig. 2).

Causal model connecting the three hypotheses**—** From Material and Method section, we reach the conclusion that

Ln (seedling biomass) = $(a_1 + a_2 * T) + (b_1 + b_2 *T)$ * Ln (seed mass) (eq. 6)

which is similar to equation 3

Ln (seedling biomass) = $a_3 + b_3$ * Ln (seed mass) (eq. 3)

where $a_3 = (a_1 + a_2 * T)$ and $b_3 = (b_1 + b_2 *T)$

being a_1 and b_1 the coefficients for the reserve effect hypothesis [Ln (used reserves) vs. Ln (seed mass)] and a_2 and b_2 the coefficients for the metabolic effect hypothesis [RGR vs. Ln (seed mass)].

We can estimate the slope of seed-seedling mass relationship (b_3; eq. 3 and 6) if we know b_1, b_2 and the time of growth (*T*), in our case 50 days. We made different simulations considering the different possible values of the slopes b_1 and b_2 and as reference values; we also take into account the observed values of the slopes b_1 and b_2 of our data.

For the reserve effect, three main results are possible: slope > 1 (Fig 3A, case a, for example *Q. ilex* and *Q. pyrenaica*, follow this pattern, Fig. 1A), slope = 1 (Fig 3A case b, for example the case of *Q. canariensis*, Fig. 1A) and slope < 1 (Fig 3A case c, for example the case of *Q. suber*, Fig. 1A).

For the metabolic effect three main results are possible: slope = 0, (Fig 3B, case 1, for example the case of *Q. suber*, Fig. 1B), slope < 0 (Fig 3B, case 2, for example the cases of *Q. ilex* and *Q. pyrenaica*, Fig. 1B) and slope > 0 (Fig 3B, case 3, no any case in our study). The combinations of these possible results generate nine simulations of Ln seedling biomass-Ln seed mass (the seedling size effect) (Fig. 3C). The slopes of the linear regression Ln seedling mass-Ln seed mass (b_3) are the same as those calculated as $b_1 + b_2 * T$ from the Ln used reserve-Ln seed mass (slope b_1) and RGR-Ln seed mass (slope b_2) linear regressions. We can see that in most cases (eight of the nine combinations), a positive relationship between seedling biomass and seed mass exists, therefore the seedling size effect hypothesis is accepted. Only in one case (c-2, Fig. 3C) the resulting relationship of seedling-seed mass was lost (where the slope of used reserve-seed mass is lower than 1 and a negative slope of RGR-seed mass exists). Therefore we can conclude that, in general, bigger seeds produce bigger seedlings and only in some cases, there is no relationship between seed mass and seedling biomass.

One way to check the connection between the hypotheses with our data is to estimate the slopes of Ln seedling biomass-Ln seed mass (as $b_1 + b_2 * T$) and contrast them with the observed slopes of these relationships (b_3). We have only four cases in which there were significant correlations in both of Ln used reserve-Ln seed mass and RGR-Ln seed mass (*Q. ilex* HI, *Q. ilex* LI, *Q. pyrenaica* MI and *Q. pyrenaica* LI, Fig. 1). For that, a proper evaluation of the connection of the three hypothesis is only done with these four cases because a significant and linear relationship between X and Y exists. If the slopes of Ln seedling-Ln seed mass are calculated using the slope b_1 (equation 1) and b_2 (equation 2) and assuming a T of 50 days we obtained a estimated slope of Ln seedling-Ln seed mass, and this slope is compared with the slope of the regression line obtained with the observed data (Ln seed mass and Ln seedling biomass). The results of the estimated slopes of Ln seedling-Ln seed mass are strongly correlated with the slopes from the observed data Ln seedling-Ln seed mass (Pearson correlation, $r = 0.99$, $P < 0.01$), which can be considered as a proof of the connection of the three hypotheses.

DISCUSSION

In this study, we evaluated three hypotheses concerning the influence of initial seed mass on seed reserve use and seedling traits within four congeners growing under contrasting light conditions. We evaluated whether larger seeds retain more reserves than smaller ones ("the reserve effect"), whether seed mass is negatively related to RGR ("the metabolic effect") and whether larger seeds result in a larger seedling mass ("the seedling size effect"). Overall, seed mass had highly significant effects on seed and seedlings traits, but this effect depended on species and light conditions.

Reserve effect— The reserve effect hypothesis postulates that 1) larger seeds retain a larger proportion of their reserves and 2) the slope of the seedling biomass-seed mass relationship is significantly higher than the slope of the used reserve versus seed mass (Green and Juniper, 2004a). In this way, the reserves can be mobilized later on to support the seedlings during periods of carbon deficit, for example when they are growing in deep shade. Only one out of four species tested (*Q. suber* in full light, HI) met condition 1 of this hypothesis, with larger proportion of their reserves retained in large seeds. Interestingly, this is the largest seeded species, which can easily set some reserve aside for future hazards, especially in high irradiance levels. However, condition 2 was not satisfied by this species. Therefore, in our experiment no reserve effect was found in any *Quercus* species. Similarly, Green and Juniper (2004a) found that within species, the seed reserve effect was rare (only seven out of 22 Australian rainforest species tested had a seed reserve effect). In contrast, we found in two species (*Q. ilex* and *Q. pyrenaica*) that larger seeds spent proportionally more reserves than smaller ones (Fig. 1A). This unexpected result has not been found in other intra-specific studies (Green and Juniper, 2004a). A possible explanation would be that seedling from larger seeds invest more biomass in roots (i.e., they have a larger root mass fraction; data not shown) and less in photosynthetic tissue. Seedlings from bigger seed may therefore depend more strongly on their seed reserves, as they have a relatively smaller photosynthetic tissue.

All *Quercus* species retained a surprisingly large part (between 40 to 60 %, Appendix S1) of their initial seed reserves by the end of the experiment. The question is whether those reserves can be mobilized later on in case of stress or disturbance events such as herbivore damage. Greenhouse and field experiments have shown that *Quercus* seedlings indeed can re-sprout after stem removal (Harmer, 1999; Kullberg and Wellander, 2003; Kabeya and Sakai, 2005), but the ability to re-sprout is independent of seed size (Erniwati, 2006), probably because the remaining reserves are more than sufficient to re-sprout once. Maybe after repeated clipping, such a seed size effect would have shown up.

Metabolic effect— The metabolic effect hypothesis predicts a negative relationship between seedling relative growth rate and seed size (Shipley and Peters, 1990; Marañón and Grubb, 1993). If plants have a slower RGR and a slower metabolic rate, then seed resources could be consumed more slowly, which implies that seedlings can rely for a longer period of time on their seed reserves (Green and Juniper, 2004a). This hypothesis was confirmed for only two species (*Q. ilex* and *Q. pyrenaica*). For these species the relationship between RGR and seed size became stronger with an increase in irradiance. Similarly, Poorter and Rose (2005) found in a between-species study that the relationship between RGR and seed size has a stronger slope under high-light conditions, when the small-seeded (pioneer) species can realize their full growth potential, whereas the large-seeded (shade-tolerant) species have a low inherent growth rate.

Seedling size effect**—** The seedling size effect hypothesis proposes that larger seeds produce larger seedlings, which are more robust and better able to escape size-dependent mortality. In our experiment, such a relationship is found for species in at least one light level, in line with the findings of other studies (Bonfil, 1998; Ke and Werger, 1999; Rey et al., 2004; Baraloto et al., 2005). However, other studies attribute this positive relationship within species to other traits indirectly associated with seed size, such as genetic variability of the maternal plant (Castro, 1999). In our study, light availability was another source of variation: *Q. ilex* and *Q. pyrenaica* met the seedling size hypothesis in all light treatments whereas for the other two species, a positive relationship between seedling and seed mass was only found in deep shade. Moreover, in all species a strong correlation between seed mass and seedling biomass was found in deep shade, but this relationship became weaker or disappears at higher light levels (Fig. 2). Strong correlations between seed and seedling biomass can be expected in deep shade (Fig. 2), where seedlings have low photosynthetic rates (Quero et al., 2006) and depend mostly on seed reserves for their growth. Under intermediate and optimal light conditions seedling growth becomes more autotrophic, and hence, genotypic differences become more important determinants of intraspecific variation in seedling growth and mass. Across species this pattern is consistent with other studies that demonstrate better performance of larger-seeded species (as these *Quercus* are) under deep shade conditions (Saverimuttu and Westoby, 1996; Poorter and Rose, 2005).

A causal model connecting the three hypotheses— The three hypothesis have been postulated as possible mechanisms to tolerate different hazards (Westoby et al., 1996), but up to now there have not been any attempt to connect them. Here we present a conceptual framework to connect the three hypotheses, and to evaluate till what extent seed size affect the seedling biomass of the species.

The principal result of the proposed causal model is that there are high possibilities to find a seedling size effect, as it has been found in many studies (Bonfil, 1998; Castro, 1999; Ke and Werger, 1999; Rey et al., 2004; Baraloto et al., 2005). This model gives the mathematical explanation of this general result. The relationship between seedling biomass and seed mass depends on the slope of used reserve against seed mass, the slope of RGR against seed mass and on the duration of the growth period. Only in some cases, it is more difficult to find a seedling size effect, for example, when RGR is strongly and negatively related to seed mass and the proportion of used reserves decrease with seed mass. In our study, there are some species that show a strongly negative relationship of RGR with seed mass (for example *Q. pyrenaica* in HI and MI) and according with our model, in these cases there was not any seedling size effect.

Another prediction of our model is that the time of growth can have an effect on the seed-seedling relationships and this depends also of the relationship of RGR with seed mass (the slope b_2). According to our model the slope of seed-seedling mass (b_3) is equal to $b_1 + b_2*T$. If b_2 is zero, the time of growth will have little effect on b_3, however, if b_2 is negative, increasing the time of growth, the value of b_3 will decrease. From the literature it has been generally found that the slope b_2 (the RGR-seed mass slope) is zero or negative (see Poorter and Rose, 2005 and our study), which implies the seedling seed effect would disappear with time. This has been found in other studies, for example

Poorter and Rose (2005) have found that that the strength of the correlation between growth parameters and seed mass declines over time, and disappears after 1-4 years. Also Castro (1999) found in *Pinus sylvestris* L. that after one growing season the seed mass had no effect on seedling performance.

Ecological implications— How can we translate these experimental results to the field? The Mediterranean *Quercus* forests are characterized by a high level of environmental perturbation (drought years, fires; Aschman, 1973, Ojeda, 2001) and a high level of herbivory on acorns and seedlings (Gómez et al., 2003; Zamora et al. 2004). In this scenario, the observed effects of seed size on seedling traits would confer advantages to *Quercus* seedlings in four different ways: 1) independency in front of unpredictable environmental conditions and soil characteristics for germination and establishment (Puerta-Piñero et al., 2006); 2) providing larger seedling with longer roots to escape summer drought (Metcalfe and Grubb, 1997; Lloret et al., 1999); 3) re-sprouting after herbivory by retaining a substantial part of their initial seed reserves (Green and Juniper, 2004b); 4) facilitating the establishment of the reserve-rich seeds in the shade because acorns are mainly dispersed by jays and rodents mostly to shady environments (Bosema, 1979; Gómez, 2003).

In this study, evidence has been found for two out of the three hypotheses, the metabolic and the seedling size effect. According to the causal model, bigger seeds produce bigger seedlings in most of cases evaluated. These hypotheses have been confirmed within species, suggesting that functional relationships underlie the observed patterns. The strongest correlations between seed size and seedling biomass were found in the shade, indicating that in low light the seedlings depend more on their seed reserves.

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Table 1. Oak species included in the experiment (nomenclature follows Amaral, 1990), the frequency in southern Spain (calculated from 12,572 records in the National Forest Inventory, Urbieta et al., 2004) origin of seed, regression equations to calculate initial acorn dry mass (DM) from initial acorn fresh mass (FM) (N=40-66 per species), R^2 of the regressions, and the mean (± SE), range, and coefficient of variation (CV) of initial seed dry mass used in this experiment (N = 47-48 per species).

Species	Frequency in southern Spain (%)	Origin of seeds	Regression equations	N	R^2	Acorn dry mass		
						Mean (g)	Range (g)	CV (%)
Quercus suber L.	15.8	Sierra del Aljibe (SE Spain)	DM = 0.32 + 0.61 * FM	66	0.94	3.89 ± 0.14	1.74-5.89	23
Quercus ilex ssp. *ballota* (Desf.) Samp	50.8	Sierra Nevada (SW Spain)	DM = - 0.29 + 0.73 * FM	50	0.99	1.78 ± 0.09	0.39-3.03	23
Quercus canariensis Willd.	2.4	Sierra del Aljibe (SE Spain)	DM = 0.13 + 0.55 * FM	66	0.95	1.76 ± 0.09	0.65-3.76	30
Quercus pyrenaica Willd.	0.4	Sierra de Cardeña (S Spain)	DM = - 0.70 + 0.66 * FM	40	0.93	3.60 ± 0.20	1.34-6.75	32

Appendix S1: Mean ± S.E. values of variables analysed for *Quercus* seedlings in different light treatments: HI: high irradiance, MI: medium irradiance, and LI: low irradiance. Specific leaf area (SLA) was calculated as the quotient between leaf area and leaf dry mass. Leaf area ratio (LAR) was calculated as the total area of leaves divided by the total seedling dry mass. The seedling biomass allocation — root mass fraction (RMF), stem mass fraction (SMF), and leaf mass fraction (LMF) — were calculated as the dry mass of root, stem and leaves, respectively, divided by the total seedling dry mass. For the rest of variables, see the text (M & M section). In general, there were sixteen replicates per treatments, exceptions are indicated between parentheses.

Species	Light		Initial seed dry mass (g)	Used reserve (g)	Remained reserve (%)	Seedling dry mass (g)	RGR ($g\ g^{-1}\ day^{-1}$)	SLA ($m^2\ kg^{-1}$)	LAR ($m^2\ kg^{-1}$)	RMF (g/g)	SMF (g/g)	LMF (g/g)
Q. suber	HI		4.00 ± 0.23	2.27 ± 0.1	41.9 ± 2.0	1.2 ± 0.07	-0.0059 ± 0.0004	19.27 ± 0.59	4.98 ± 0.28	0.64 ± 0.01	0.10 ± 0.0	0.26 ± 0.01
	MI		3.92 ± 0.22	2.29 ± 0.1	40.7 ± 1.9	1.08 ± 0.05	-0.0067 ± 0.0005	23.17 ± 0.71	5.17 ± 0.29	0.67 ± 0.02	0.10 ± 0.0	0.23 ± 0.01
	LI	(n = 15)	3.75 ± 0.29	1.97 ± 0.18	42.5 ± 2.2	0.96 ± 0.09	-0.0066 ± 0.0002	28.78 ± 1.22	4.73 ± 0.49	0.72 ± 0.02	0.12 ± 0.01	0.17 ± 0.01
Q. ilex ssp. *ballota*	HI		1.8 ± 0.14	0.99 ± 0.08	45.4 ± 2.0	0.66 ± 0.05	-0.0028 ± 0.0008	11.72 ± 0.45	3.33 ± 0.18	0.59 ± 0.01	0.13 ± 0.01	0.29 ± 0.02
	MI		1.82 ± 0.14	0.98 ± 0.08	46.5 ± 1.8	0.59 ± 0.04	-0.0039 ± 0.0005	12.99 ± 0.65	3.86 ± 0.29	0.57 ± 0.02	0.13 ± 0.01	0.3 ± 0.02
	LI		1.73 ± 0.2	0.94 ± 0.11	46.0 ± 1.7	0.55 ± 0.05	-0.0037 ± 0.0005	17.18 ± 0.78	3.84 ± 0.33	0.61 ± 0.02	0.16 ± 0.02	0.23 ± 0.02
Q. canariensis	HI	(n = 17)	1.87 ± 0.12	1.07 ± 0.08	42.3 ± 1.2	0.51 ± 0.08	-0.0083 ± 0.0015	20.44 ± 1.45	6.59 ± 0.61	0.56 ± 0.02	0.12 ± 0.01	0.32 ± 0.02
	MI	(n = 15)	1.43 ± 0.13	0.78 ± 0.08	41.3 ± 1.3	0.45 ± 0.1	-0.0078 ± 0.0016	20.75 ± 1.12	7.16 ± 0.44	0.52 ± 0.02	0.12 ± 0.01	0.35 ± 0.02
	LI		1.96 ± 0.17	1.16 ± 0.1	40.4 ± 1.4	0.39 ± 0.05	-0.0094 ± 0.001	29.13 ± 2.35	8.61 ± 0.72	0.57 ± 0.01	0.13 ± 0.01	0.3 ± 0.02
Q. pyrenaica	HI		2.67 ± 0.24	1.11 ± 0.13	59.6 ± 2.0	1.75 ± 0.09	0.0047 ± 0.0011	18.36 ± 0.31	3.87 ± 0.26	0.72 ± 0.02	0.07 ± 0.01	0.21 ± 0.01
	MI		3.82 ± 0.32	1.82 ± 0.18	53 ± 1.6	1.71 ± 0.12	-0.0002 ± 0.0008	23.71 ± 1.28	3.63 ± 0.31	0.77 ± 0.02	0.07 ± 0.01	0.16 ± 0.01
	LI		4.3 ± 0.32	1.92 ± 0.17	55.2 ± 2.0	1.49 ± 0.12	-0.0021 ± 0.0005	29.78 ± 0.77	2.21 ± 0.32	0.83 ± 0.02	0.1 ± 0.01	0.07 ± 0.01

Figure legends

Figure 1. Used seed reserve, relative growth rate (RGR) and seedling biomass after ca. 50 days of growth *vs.* log initial seed mass in four *Quercus* species (species are ordered from lowest to the highest mean seed mass). Pearson correlation and significance are indicated as: a $P < 0.1$; *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$. The standardised major axis regression (SMA) lines are given when they are significant: grey line for high irradiance (HI), dotted line for medium irradiance (MI) and black line for low irradiance (LI). The slope of the SMA regression are indicated (S) and their significance against the null model ($S = 1$ for the reserve effect and $S = 0$ for the metabolic effect and the seedling size effect).

Figure 2. Pearson correlation coefficients for seed mass-seedling mass relationship after ca. 50 days of seedling growth for the four oak species in three light conditions (3, 27 and 100%). *Q. ilex* ssp. *ballota* (Δ), *Q. canariensis* (□), *Q. suber* (◊) and *Q. pyrenaica* (○). Black symbols indicate significant correlations ($P < 0.05$). Thick line indicates 2nd order polynomial regression ($y = 0.78 - 0.0102 * x + 5.8 * 10^{-5} * x^2$).

Figure 3. Simulations of the causal model. (A) a, b and c represent possible contrasting results of the used seed reserve-seed mass relationship (the reserve effect). (B) 1, 2 and 3 represent possible contrasting results of the RGR-seed mass relationship (the metabolic effect). (C) the results of the seedling biomass-seed mass relationship (seedling size effect) depending of the combinations of a, b and c with 1, 2 and 3.

Figure 1

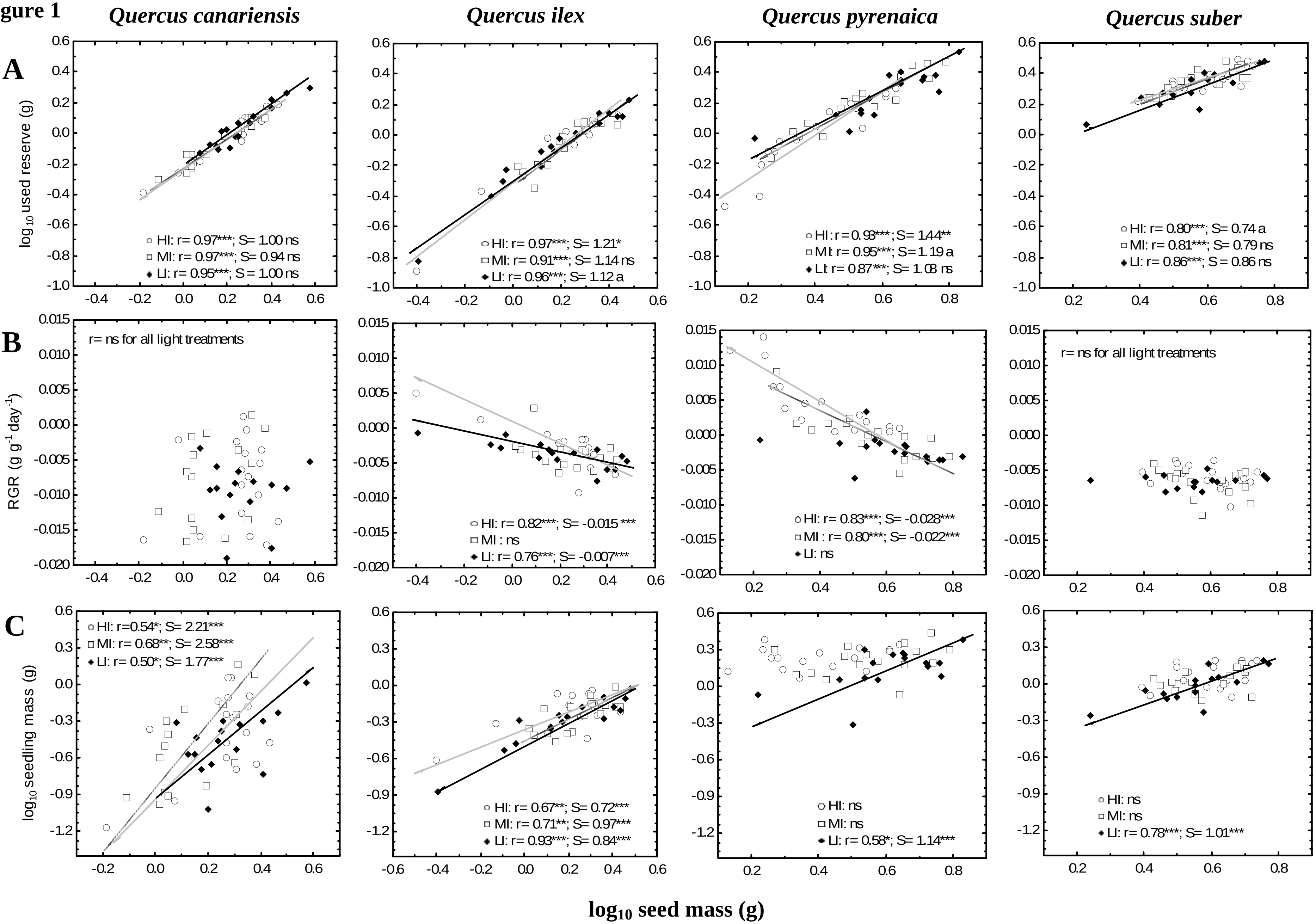
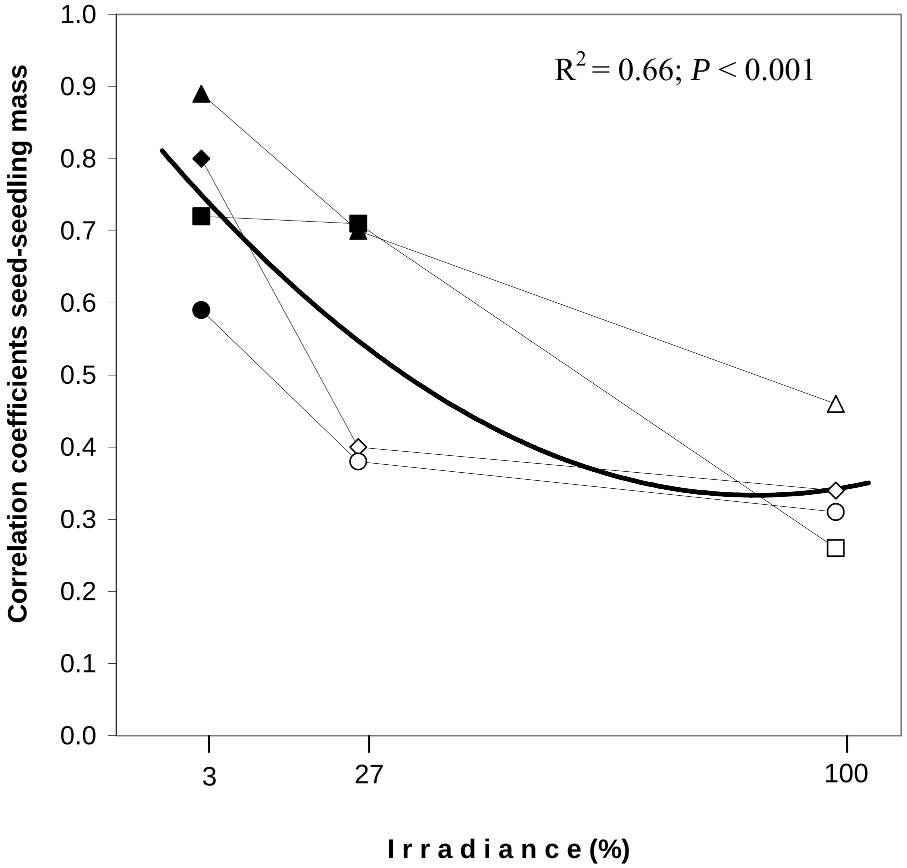
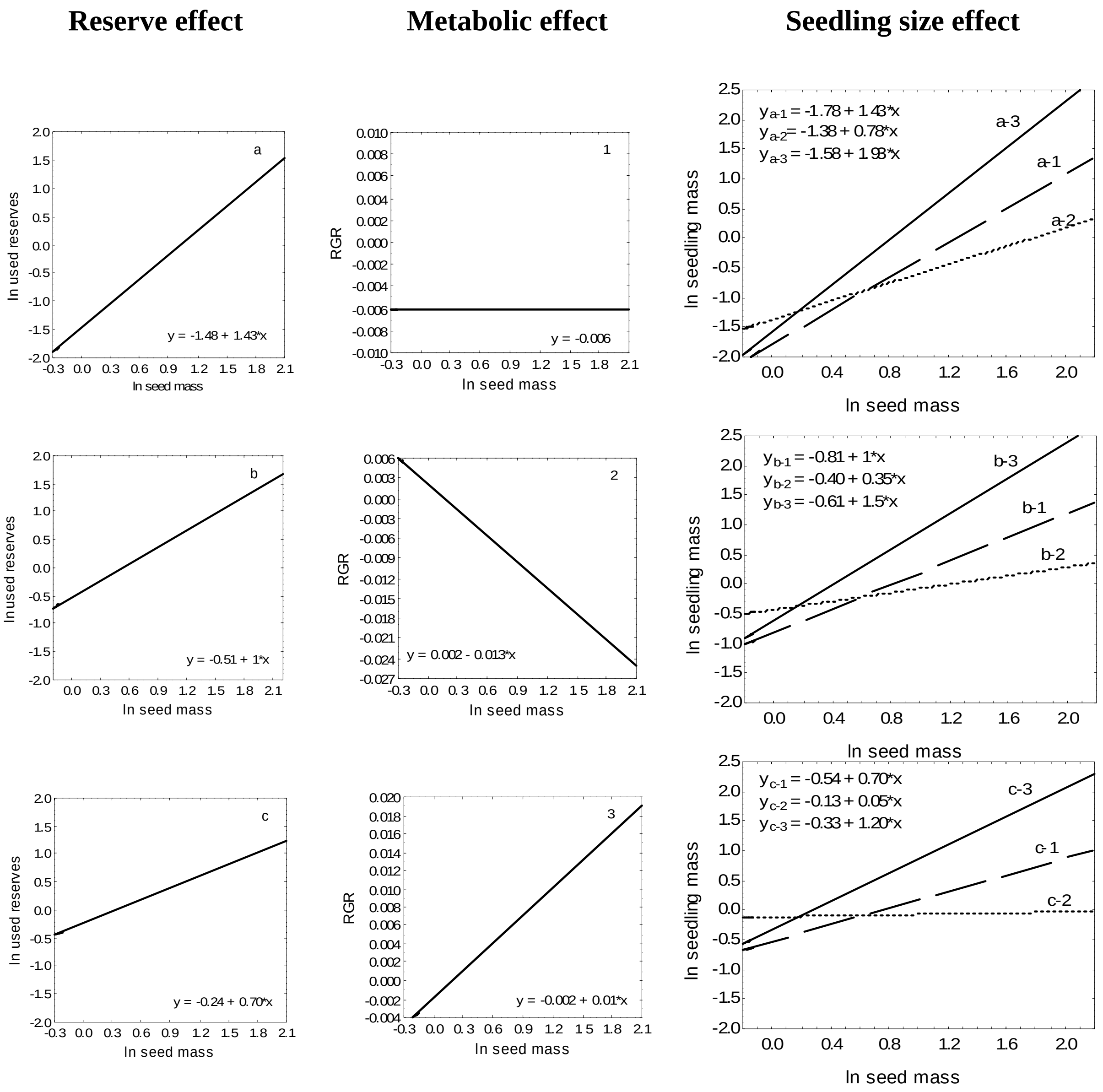

Quercus canariensis
Quercus ilex
Quercus pyrenaica
Quercus suber
A
B
C
log10 used reserve (g)
RGR (g g-1 day-1)
log10 seedling mass (g)
log10 seed mass (g)
HI: r= 0.97***; S= 1.00 ns
MI: r= 0.97***; S= 0.94 ns
LI: r= 0.95***; S= 1.00 ns
HI: r= 0.97***; S= 1.21*
MI: r= 0.91***; S= 1.14 ns
LI: r= 0.96***; S= 1.12 a
HI: r= 0.93***; S= 1.44**
MI: r= 0.95***; S= 1.19 a
LI: r= 0.87***; S= 1.08 ns
HI: r= 0.80***; S= 0.74 a
MI: r= 0.81***; S= 0.79 ns
LI: r= 0.86***; S = 0.86 ns
r= ns for all light treatments
HI: r= 0.82***; S= -0.015 ***
MI : ns
LI: r= 0.76***; S= -0.007***
HI: r= 0.83***; S= -0.028***
MI : r= 0.80***; S= -0.022***
LI: ns
r= ns for all light treatments
HI: r=0.54*; S= 2.21***
MI: r= 0.68**; S= 2.58***
LI: r= 0.50*; S= 1.77***
HI: r= 0.67**; S= 0.72***
MI: r= 0.71**; S= 0.97***
LI: r= 0.93***; S= 0.84***
HI: ns
MI: ns
LI: r= 0.58*; S= 1.14***
HI: ns
MI: ns
LI: r= 0.78***; S= 1.01***

Figure 2



R² = 0.66; P < 0.001
Correlation coefficients seed-seedling mass
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3
27
100
Irradiance (%)

Figure 3


Reserve effect
Metabolic effect
Seedling size effect
a
y = -1.48 + 1.43*x
ln used reserves
ln seed mass
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-0.3
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
1
y = -0.006
RGR
ln seed mass
0.010
0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
-0.006
-0.008
-0.010
y_a-1 = -1.78 + 1.43*x
y_a-2 = -1.38 + 0.78*x
y_a-3 = -1.58 + 1.93*x
a-3
a-1
a-2
ln seedling mass
ln seed mass
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
0.0
0.4
0.8
1.2
1.6
2.0
b
y = -0.51 + 1*x
ln used reserves
ln seed mass
2
y = 0.002 - 0.013*x
RGR
ln seed mass
0.006
0.003
0.000
-0.003
-0.006
-0.009
-0.012
-0.015
-0.018
-0.021
-0.024
-0.027
y_b-1 = -0.81 + 1*x
y_b-2 = -0.40 + 0.35*x
y_b-3 = -0.61 + 1.5*x
b-3
b-1
b-2
ln seedling mass
ln seed mass
c
y = -0.24 + 0.70*x
ln used reserves
ln seed mass
3
y = -0.002 + 0.01*x
RGR
ln seed mass
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-0.002
-0.004
y_c-1 = -0.54 + 0.70*x
y_c-2 = -0.13 + 0.05*x
y_c-3 = -0.33 + 1.20*x
c-3
c-1
c-2
ln seedling mass
ln seed mass

Capitulo 4: (en ingles) Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses (publicado en *New Phitologist*, 2006; 170: 819-834)

Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses

Suggested running title: *Interactions of shade and drought on Quercus seedlings*

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Summary

- We investigated the physiological and structural leaf responses of seedlings of two evergreen and two deciduous *Quercus* species, grown in a greenhouse and subjected to contrasted conditions of light (low, medium and high irradiance) and water (continuous watering versus two-months drought).
- The impact of drought on photosynthetic rate was strongest in high irradiance, while the impact of shade on photosynthetic rate was strongest with high water supply, contradicting the Smith & Huston's hypothesis of allocation trade-off.
- Multivariate causal models were evaluated using d-sep method. The model that best fitted the dataset propose that the variation in specific leaf area affects photosynthetic rate and leaf nitrogen concentration, and this trait determines stomatal conductance, which also affects photosynthetic rate.
- Shade conditions seemed to ameliorate, or at least not aggravate, the drought impact on oak seedlings, therefore, drought response on leaf performance depend of light environment.

Key-words: deciduous; evergreen; leaf traits; Mediterranean oaks; photosynthesis; nitrogen; specific leaf area; water-use efficiency.

Abbreviations:

Φ (quantum yield, no units); θ (curvature, no units); Area (leaf area, cm^2); A_{area} (photosynthetic rate per area, μmol CO_2 m^{-2} s^{-1}); A_{mass} (photosynthetic rate per mass, nmol CO_2 g^{-1} s^{-1}); C_{area} (carbon content per area; g C m^{-2}); C_{mass} (carbon concentration, mg g^{-1}); Chl index (chlorophyll index, no units); Ci/Ca (ratio internal versus external CO_2 concentration); gs_{area} (stomatal conductance per area, mmol H_2O m^{-2} s^{-1}); gs_{mass} (stomatal conductance per mass, mmol H_2O g^{-1} s^{-1}); LCP (light compensation point, μmol photons m^{-2} s^{-1}); LSP (light saturation point, μmol photons m^{-2} s^{-1}); N_{area} (nitrogen content per area; g N m^{-2}); N_{mass} (nitrogen concentration, mg g^{-1}); R_{area} (respiration rate per area, μmol CO_2 m^{-2} s^{-1}); R_{mass} (respiration rate per mass, nmol CO_2 g^{-1} s^{-1}); PNUE [photosynthetic nitrogen-use efficiency, μmol CO_2 (mol N)$^{-1}$ s^{-1}]; SLA (specific leaf area, cm^2 g^{-1}); WUE [water-use efficiency, μmol CO_2 (mmol H_2O)$^{-1}$]

Introduction

Light and water are main resources affecting leaf traits, regulating plant growth and survival, and determining the distribution of plants at global scale. The functional response of seedlings to the combination of shade and drought involves biochemical, physiological, and structural changes at the leaf and whole-plant level (Holmgren, 2000; Sack & Grubb, 2002; Sack, 2004; Aranda *et al.*, 2005). Some hypotheses predict that under limiting light availability (primary limitation), the shortage of another resource such as water should have less impact on plant performance (Canham *et al.*, 1996). In addition, shade by the tree canopy has indirect effects, such as reducing leaf and air temperatures, vapour pressure deficit, and oxidative stress, that would alleviate the drought impact on seedlings in the understorey (Holmgren, 2000). In fact, empirical evidence of facilitation effects of shrubs and trees on seedlings in the understorey in Mediterranean environments has been widely documented (Castro *et al.*, 2004a; Gómez-Aparicio *et al.*, 2004). A contrary hypothesis predicts that deep shade will aggravate the stress imposed by drought, based on the proposed trade-off mechanism that shaded plants allocate more to shoot, and to leaf area, than to root, thereby diminishing the ability to capture water (Smith & Huston, 1989). In fact, some studies have found a higher impact of water stress on shaded plants (Abrams & Mostoller, 1995; Valladares & Pearcy, 2002). A third group of hypotheses posits that the effects of shade and water-shortage are independent, that is, their impacts are orthogonal (Sack & Grubb, 2002; Sack, 2004).

In woody species, there is a suite of leaf traits associated to leaf life span. Deciduous species tend to achieve higher photosynthetic and respiration rates and higher stomatal conductance, and have higher N concentration in the leaf, compared with related evergreen species (Reich *et al.*, 1992; Villar *et al.*, 1995; Reich *et al.*, 1997;

Takashima *et al.*, 2004; Wright *et al.*, 2004). In Mediterranean environments, deciduous species tend to be more abundant in habitats with greater availability of water and nutrients, where the overstorey canopy is denser. Hence, it would be expected that seedlings of deciduous species are more shade-tolerant and water-demanding. In contrast, evergreen species tend to dominate in habitats that are drier and poorer in nutrients, where the overstorey canopy is sparse. We would therefore expect that seedlings of evergreen species are more tolerant to drought but not necessarily to shade.

One way to understand plants function is to explore leaf-trait relationships in different environmental conditions, but most studies have discussed simple bivariate relationships. In order to develop a quantitative model of plant functioning relating to gas exchange, it would be necessary to move to multivariate relationships to be investigated by causal model (Meziane & Shipley, 2001). These authors proposed a model in which SLA was the forcing variable directly affecting both leaf N and net photosynthetic rate. Leaf N then directly affects photosynthetic rate, which in turn affects stomatal conductance. This model was found to agree with several datasets (Meziane & Shipley, 2001). Up to now, these models have not been applied to datasets with limiting light and water conditions, as are typical of Mediterranean forest.

We have designed an experiment with controlled conditions of light and water to investigate the physiological and structural leaf traits responses of tree seedlings to six combinations of light (three levels) and water (two levels). Four species of the same genus (*Quercus*) differing in leaf life span, were selected: two evergreens and two deciduous. Thus, we compared deciduous and evergreen species under the same genus, including the phylogeny in the design and data analysis.

There are some specific questions to investigate plant responses to different light x water scenarios: Are shade and drought impacts on seedlings positive, negative or independent? Do species or functional groups (evergreen versus deciduous) respond differently? Which physiological and structural leaf traits are most affected by the combined stress? What are the functional relationships among those variables? The answers to these questions would help to understand the functioning of plants and their implications for the species distribution in nature.

Materials and Methods

Experimental design

Acorns of four oak species, major components of Mediterranean forest, △*Quercus suber* L. and *Quercus ilex* ssp. *ballota* (Desf.) Samp., (evergreen), *Quercus canariensis* Willd., and *Quercus pyrenaica* Willd. (deciduous) ⊶ were collected in the South of Spain. At landscape scale, the evergreen species tend to occupy drier habitats than the deciduous species at each site, although the regional ranges overlap (see Table 1 for more details). Single acorns were weighted individually and sown (in December 2002) in cylindrical pots of 3.9 litres volume (50 cm height, 10 cm diameter), thereby avoiding as much as possible interference during root growth. Pots contained a mixed soil of 2/3 sand and 1/3 peat. Ten g of a slow-release fertiliser (Plantacote® Pluss NPK: 14-9-15) were added at the middle of the experiment. The experiment was carried out in a greenhouse of the University of Córdoba (Spain, 37º 51’ N, 4º 48’ W; at an altitude of 100 m. a. s. l.) with an automatic irrigation system and regulation of air temperature.

Oak seedlings were subjected to three light levels: 1) high-irradiance treatment (HI), receiving available radiation inside the greenhouse; 2) medium-irradiance treatment (MI), covered by a light green screen, 27 % of available radiation; and 3) deep-shade or low-irradiance treatment (LI), covered by a dense green cloth, 3 % of available radiation. Each light treatment was imposed using a shade frame (150 x 120 x

200 cm) and replicated 4 times; therefore there were 12 shade frames in total. Each of the 4 species and the 2 levels of watering were set up within each shade frame, each by one plant in a single pot. The experimental light treatments simulated the field conditions in the forest understorey, distinguishing three types of microhabitat: open (HI), under single tree cover (MI), and under shrub and tree cover (LI) (Marañón *et al.*, 2004). The mean ± S.E. of the photosynthetic active radiation measured (with EMS7, canopy transmission meter, PP-system, UK) at midday on May 28, 2003, for each light treatment was 760 ± 150 (in HI), 187 ± 27 (in MI), and 23 ± 2 (in LI) μmol of photons m^{-2} s^{-1} respectively. Light quality (R:FR ratio, measured with sensor SKR 110, Sky Instrument, UK) was different from 1 only in LI, but this value (0.25 ± 0.004) was similar to that for dense forest microhabitat (0.28 ± 0.03, t-test, $P = 0.31$).

Pots were watered weekly during the first stage of the experiment. Once the seedlings emerged (January-February, 2003), a drip-irrigation system was inserted in the pots. Four months after sowing (at the end of April 2003), half of the pots stopped receiving any watering (LW, low-water treatment) while the other half was kept continuously moist (HW, high-water treatment). LW simulated a typical Mediterranean-climate situation of seasonal drought, compared with a continuously moist one (HW) with reduced or no drought. During the experiment, we measured soil moisture (in volumetric water content, VWC), measured along the first 20 cm depth (with a TDR mod 100, Spectrum Technologies, Inc.) each ca. three days, in a subsample of five pots under different light and water treatments. Pots under LW decrease their water content similarly for the three light treatments (Table 2A; repeated measures ANOVA, $P = 0.17$). At the same time of photosynthetic measurements (end of July 2003, ca. two months after stopping irrigation), we measured VWC of each pot. For each water treatment, there were no differences in water content between the pots of different species or between the three different light treatments at the end of the experiment (Table 2B). The mean ± S.E. values in July 2003, were 13.20 ± 0.20% (for HW treatment) and 2.96 ± 0.13% (for LW). The later value was very similar to those found under field conditions at the end of the drought period (Gómez-Aparicio *et al.*, 2005).

Physiological and structural measurements

Photosynthesis response to irradiance was measured in mid-height fully expanded leaf of, in general, six plants per species and treatment combination. The measurements were done in the four different shade frames (replicates) for each light treatment to avoid pseudoreplication. We used a gas-exchange portable analyser (Ciras-2, PP-System, UK). The instrument was adjusted to have constant conditions of CO_2 concentration (360 ppm), flow (150 $cm^3\ min^{-1}$), and leaf temperature (25 ºC) inside the leaf chamber. Photosynthetic rate was measured at ten light intensities of PAR obtained by using a quartz halogen light unit coupled to leaf chamber following the order 1000, 1300, 1500, 800, 600, 400, 200, 100, 50, and 0 $\mu mol\ m^{-2}\ s^{-1}$ (Figure 1), to reduce the equilibrium time required for stomatal opening and photosynthesis induction (Kubiske & Pregitzer, 1996). Each leaf was kept for one minute at the same light intensity into the leaf chamber; net assimilation rate, transpiration rate, and intercellular CO_2 concentration were recorded three times, and the average value at each light intensity was calculated. Net CO_2 assimilation rates (A) were plotted against incident PAR, and the resulting curve was fitted by the nonrectangular hyperbola model of Thornley (1976):

$$A(I) = \frac{\Phi I + A_{max} - \sqrt{(\Phi I + A_{max})^2 - 4\theta I A_{max}}}{2\theta} - R_d \qquad (1)$$

where A is the photosynthetic rate, I the photosynthetic active radiation (PAR), **Φ** the apparent quantum yield, A_{max} the maximum light saturated assimilation rate, R_d the dark respiration rate, and θ the "bending degree" or curvature. Parameters of the model were calculated by the non-linear estimation module (Statistica v 6.0). The variance explained by the model was very high (mean r^2 values of 0.98 ± 0.03). Despite its methodological importance, this value is rarely given, and comparison with other studies is difficult. Using this formula, by definition, the maximum photosynthetic rate is obtained at the infinite light intensity, and then overestimated. Therefore, we recalculated A_{max} (hereafter, A_{area}) assuming a PAR of 2000 μmol m^{-2} s^{-1}, the approximate maximum value for that season and latitude (Castro *et al.*, 2004b; Rey-Benayas *et al.*, 2005). The light saturation point (LSP) was calculated as the lowest value of PAR for which photosynthesis reached 90% of $A_{area.}$ Water-use efficiency (WUE) values were calculated as A_{area}/gs_{area} ratio (Cavender-Bares & Bazzaz, 2000) and photosynthetic nitrogen-use efficiency (PNUE) as A_{mass}/ N concentration (Field & Mooney, 1986).

In the same leaves, a chlorophyll index was measured using a CCM-200 (Optic Science, USA), which works similarly to SPAD (Minolta) and readings are well correlated with chlorophyll content. Then, leaves were collected and scanned, and the area was measured with an image analyser (Image Pro-Plus v 4.5 Media Cybernetic, Inc). They were oven-dried (at 80 ºC for at least 48 hours) and weighed. The specific leaf area (SLA) was calculated as the ratio between the leaf area and its dry mass. Leaves were ground with N liquid in an agate mortar, and analysed for N and C concentration using an elemental analyser (Eurovector EA 3000, EuroVector SpA. Italy).

The level of response to the variation of each factor (light and water) was estimated by the indices $Response_{light}$ and $Response_{water}$ respectively, ranging from 0 to 1. The index of response was calculated as the difference between the maximum and the minimum mean values, divided by the maximum mean value. Although other authors called this the plasticity index (PI) (Valladares *et al.*, 2000a), we have preferred the neutral term “Response” firstly, because in the case of water treatment, the seedlings had to adjust to a seasonal drought and were not acclimated from the beginning of the experiment, and secondly, because we did not control possible genetic variability.

Statistical analyses

Mean (± S.E.) values of the 20 variables of seedling leaf performance, for each *Quercus* species and irradiance and water treatment, are shown in Appendix 1. To avoid pseudoreplication, we calculate the mean values of the different variables for each light treatment replicates. These mean values were used to test the differences among species and the effects of light and water treatments on each variable by three-way ANOVAs (species, light, and water as source factors) with Type III sums of squares. Previously, ANCOVA was explored considering the seed mass as covariable; seed mass did not significantly affect leaf traits of six-month-old seedlings ($P > 0.05$ in all cases), then we present here only the ANOVA results for simplicity. A similar ANOVA procedure was used to explore the differences between deciduous and evergreen species, using leaf habit as factor instead of species. When the difference was significant, a multiple comparison of means test (post hoc Unequal N Tukey's Honestly Significant Difference test) was carried out. Prior to ANCOVA and ANOVA, data were square-root-, arcsine-, or log-transformed to satisfy the normality and homocedasticity assumptions (Zar, 1984). Leaf-trait relationships were studied by Pearson’s correlation analyses between pairs of variables, separating watered and drought conditions. The program Statistica v 6.0 was used for statistical analyses.

In order to explain the empirical patterns of direct and indirect covariation between variables, a multivariate analysis was carried out to test for causal models linking changes in main leaf traits (SLA and nitrogen content) with physiological performance (photosynthetic rate and stomatal conductance), following Shipley's d-sep method (Shipley, 2000). Significance was fixed at the 0.05 level throughout the study. In order to control the inflation of type I error derived from repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was applied to repeated test tables throughout the paper. The FDR was controlled at the 5% level using a standard step-up procedure (see García, 2004). However, when testing multiple path models, we got an estimate for the expected number of erroneously accepted null hypotheses (type II errors), while controlling the FDR at the 5% level (see Ventura *et al* 2004). This approach allowed us to focus the attention on those accepted models which had a low probability of being type II errors.

Results

Combined effects of shade and drought

The reduction in the availability of light and water imposed structural changes in the leaves of oak seedlings and affected their physiological performance (Figs. 1 and 2). Most variables showed strong interactions of light and water effects (as demonstrated by the ANOVAs, Table 3 and Fig. 2), reflecting that the drought impact on the physiological and structural traits of seedlings was highly significant under HI and MI but negligible under LI. Some exceptions were SLA and nitrogen concentration (Fig. 2).

Photosynthetic rate and stomatal conductance of the four oak species were similar along the three irradiance levels in HW (Fig. 2A, B). However, these traits decreased with irradiance under the LW. WUE (ratio between these traits) showed differences in water treatments, being higher in LW. However, PNUE decreased in LW as whole (Table 3).

Leaves of oak seedlings grown under LI had higher SLA (Fig. 2C) and were richer in nitrogen (Fig. 2D).

Differences among *Quercus* species

Leaf structural traits were characteristic of each species and showed significant differences in the ANOVAs (see species as factor in Table 3; Appendix 1). For example, leaf area varied across the species (54% of variance explained) and SLA showed statistical differences among each of the four *Quercus* species (30% of variance), with the rank *Q. ilex* < *Q. suber* < *Q. pyrenaica* < *Q. canariensis* (Fig. 2C).

Fewer physiological features varied across the *Quercus* species (only 6 out of 13; Table 3). For example, A_{mass} differed among species (22% of variance; deciduous *Q. pyrenaica* and *Q. canariensis* had higher values than evergreen *Q. ilex* and *Q. suber*) (Fig. 2A). In general, the effects of shade and/or drought on physiological variables were higher than the inter-specific variation [for example, LCP was highly affected by light (38% of variance), but varied only slightly across species (1% of variance)] (Table 3).

Differences between functional groups

Leaf traits of seedlings were related to the leaf habit. When the seedlings of deciduous species (*Q. pyrenaica* and *Q. canariensis*) were grouped and compared by

ANOVAs with the evergreen species (*Q. ilex* and *Q. suber*), all seven leaf structural traits showed significant differences (Appendix 1). Seedlings of deciduous species had higher leaf area, SLA (Fig. 2C), and N_{mass} (Fig. 2D), but lower Chl index (Appendix 1).

Differences in life span also predicted some variation in seedling physiological performance (significant ANOVAs for 5 out of 13 variables). Seedlings of deciduous species had higher A_{mass} (19% of variance), R_{mass} (13% of variance), PNUE (13% of variance), and stomatal conductance (3% of variance) than evergreens. There were no apparent differences between deciduous and evergreen seedlings in WUE.

Responses to variation of light and water

There was a high variation in the degree of response to light *versus* that to water, for the 20 variables measured (Fig. 3). Results for the four species were averaged to show the general response pattern. The response to light ($Response_{light}$) had a mean value of about 0.35 for the 20 variables, with a wide variation among them (Figure 3). The structural water-induced response of leaf traits was very low (mean $Response_{water}$ of 0.07), while the general physiological response was relatively high (mean $Response_{water}$ of 0.35) (Figure 3). Some variables had relatively persistent values even for stressed seedlings (low response traits). Among the variables exhibiting high response, some were highly affected by shade ($Response_{light} > 0.5$) but not affected by drought; the most remarkable example is SLA. In contrast, other leaf traits had high response in drought-affected seedlings ($Response_{water} > 0.5$), but were more independent of shade stress; the best example here is the gs_{area} and A_{area}.

Causal links among leaf structural traits and physiological performance

A diverse correlation patterns were revealed among leaf structural traits and physiological variables. These relationship patterns were similar for the four oak species between different variables shown in the four oak species (test of Homogeneity of slopes model, $P > 0.05$ for all cases; data not shown). In many cases, correlations between leaf traits differed depending on the water treatment (44% of bivariate relationships were different, Table 4). A_{mass} and R_{mass} were significantly correlated in both drought and watered conditions (Table 4). A_{mass} was also correlated with gs_{area}, under drought and water treatments (Fig. 4D).

In some cases, leaf structural traits can be used as predictors of physiological performance. N_{mass} was a good predictor of gs_{area}; but only for drought-affected seedlings (Fig. 4C). The specific leaf area (SLA) was a good predictor for several physiological activities. Seedlings of higher SLA tended to have higher photosynthetic rate (Fig. 4B), higher N_{mass} (Fig. 4A), and lower LCP and LSP (Table 4). The instantaneous water-use efficiency (WUE) was negatively correlated with the instantaneous photosynthetic nitrogen-use efficiency (PNUE) for watered seedlings, but not when affected by drought (Table 4). The SLA of drought-affected seedlings (unlike watered ones) was significantly correlated with PNUE. WUE was not correlated with SLA for either of the water treatments.

The results of the multivariate analyses (d-sep test) of causal models linking leaf traits (SLA and N_{mass}) and physiological functions (A_{mass} and gs_{mass}) are shown in Table 5 and Figure 5. Model D was accepted by the whole dataset and most of the different light and water treatments. According to this model, the variation in SLA affects A_{mass} and N_{mass}, and this trait determines gs_{mass}, which also affects photosynthetic rate. Model F, which best fitted the datasets in the study by Meziane &

Shipley (2001), was also accepted by most datasets in this experiment but did not fit the data of LI, and hence it was rejected for the combined dataset (Table 5).

Discussion

Are the impacts of shade and drought on seedlings, positive, negative or independent?

Most leaf traits showed strong interactions in their responses to light and water treatments (Table 3; Fig. 2), and hence their variation was not independent. We did find that oak seedlings grown under deep shade increased their SLA, but they did not necessarily have a lower physiological performance, in terms of net photosynthetic rate, stomatal conductance, or water-use efficiency, when subjected to drought, as would be expected from the trade-off hypothesis (Smith & Huston, 1989). On the contrary, under similar drought conditions, deep-shaded seedlings were able to achieve higher photosynthetic rate, stomatal conductance, and nitrogen concentration than seedlings under full light (Fig. 2). Moreover, under drought conditions, seedlings with higher SLA had higher A_{area} while lower R_{area}, indicating a higher positive carbon balance in these leaves (Table 4). The apparent alleviation of drought impact for seedlings growing in shade, demonstrated here under experimental conditions, could explain the pattern of higher seedling survival under shade of shrubs and trees (facilitation effect), commonly observed in Mediterranean forests (e.g., Castro *et al.*, 2004b; Gómez-Aparicio *et al.*, 2004; Marañón *et al.*, 2004).

Other studies have also found structural and physiological evidence supporting the hypothesis of shade as lessening the drought stress on seedlings of woody species (Holmgren 2000; Prider & Facelli, 2004, Duan et al. 2005). On the contrary, plants under high irradiance, when subjected to water stress, suffer a more drastic reduction in net photosynthesis, and can be more predisposed to photo-inhibition, in comparison with plants in the shade (see references in Holmgren, 2000); although sunflecks can cause severe photo-inhibition in shaded leaves (Valladares & Pearcy, 2002). However, Sack & Grubb (2002) and Sack (2004) found that the effect of shade and drought showed orthogonal impacts (no interactions) on final dry mass, relative growth rate, and biomass allocation on seedlings of different species. The authors proposed that seedlings are able to tolerate both shade and drought by developing plant features conferring reduced demand for light and/or water (see references in Sack & Grubb, 2002).

In contrast, there are studies showing negative responses to combined shade and drought conditions for *Quercus* species. In a controlled experiment, *Quercus suber* seedlings grown in shade were less efficient in developing physiological mechanisms of water tolerance in particular, osmotic adjustment and effective control of water loss (Aranda *et al.*, 2005). This have been found in field studies with other woody species (Valladares & Pearcy, 2002).

These contrasting results indicate that, physiological and structural mechanisms involved in the integrated responses of the tree seedlings to shade and drought strongly depend on plant functional type.

Do species or functional groups (evergreen *versus* deciduous) respond differently?

Seedlings of the deciduous species here (*Q. pyrenaica* and *Q. canariensis*) differed in leaf structure (higher values for leaf area, SLA, and nitrogen, but lower chlorophyll concentrations) and in physiological activities (higher values of photosynthetic and respiration rates, stomatal conductance, and PNUE), in comparison with seedlings of evergreen oaks (*Q. ilex* and *Q. suber*) subjected to the same conditions of light and water. A similar trend in structural and physiological differences between seedlings, associated to the contrasted leaf habit (deciduous *versus* evergreen) of adults, has been documented for other Mediterranean species (Villar *et al.*, 1995; Villar & Merino, 2001). Within the same genus *Quercus*, Takashima *et al.* (2004) found that the PNUE in evergreen species was lower than in deciduous ones; in evergreen oak seedlings the allocation of N to photosynthesis was smaller, while that to cell walls was greater, in order to acquire leaf toughness.

In general, leaf traits of seedlings of deciduous species allow them to achieve a higher relative growth rate than that of seedlings of congeneric, evergreen species (Antunez *et al.*, 2001; Ruiz-Robleto & Villar, 2005).

Which physiological and structural leaf traits are most affected by the combined stress?

Leaf response to irradiance was very variable, in both structural and physiological traits (Fig. 3). For example, shade induced a relatively high variation in the key leaf trait SLA for all four oak species (mean $\text{Reponse}_{\text{light}}$ of 0.6), similar to the light-induced plasticity values found for evergreen tropical shrubs (16 *Psychotria* species, mean of 0.4; Valladares *et al.* 2000b). The ability to respond to light by modifying leaf structural traits may confer shade tolerance by increasing light-capture efficiency (Valladares *et al.*, 2002b). At the same time, the relatively high responsiveness of leaf physiology may also indicate a tolerance to high irradiance (Valladares *et al.*, 2002a).

Drought induced a relatively low response in structural leaf traits but a high one in physiological traits (Fig. 3). In this experiment, we have simulated the Mediterranean-climate seasonal drought predictably occurring few months after seedling emergence. When drought stress becomes more severe, first-year seedlings, grown under varied irradiance conditions, have already finished their growth, and therefore have low ability to modify structural leaf traits, which usually have a large ontogenetic component. However, they show a high physiological responsiveness to optimise photosynthesis/transpiration ratios under drought conditions.

What are the functional relationships among variables?

Because bivariate relationships are unsuccessful to make causal inferences, we have tested several causal models of multivariate links among structural (SLA and N_{mass}) and physiological (A_{mass} and gs_{mass}) leaf traits (Figure 5) and accepted one of them (model D) as the best fitted to the experiment results. According to this model, there is a direct causal relationship of SLA with dry mass concentration of cytoplasmic constituents, including nitrogen, which in turn affects stomatal conductance. Assuming that stomatal behaviour is regulated to maximise water-use efficiency, then the passive process of gas exchange across the stomata would result in the net photosynthetic rate (Meziane & Shipley 2001). In addition, the model proposes a direct causal relationship of SLA with A, not mediated by leaf N. One explanation is that the accumulation of non-structural carbohydrates will decrease SLA and also reduce photosynthesis (Meziane & Shipley, 2001). Another explanation is that self-shading of chloroplasts in the lower part of thicker leaves (with lower SLA) will decrease the net carbon fixation on a leaf-mass basis (Reich *et al.*, 1999). Thus, there is a complex multivariate link among these three leaf traits: the ratio of leaf area to mass (SLA) is balanced with the amount of organic leaf nitrogen per mass (N_{mass}) to maximise photosynthesis rate (A_{mass}) mediated by stomatal conductance (gs_{mass}) and hence optimising loss of water by transpiration, so important in Mediterranean environments.

Ecological significance

The four Mediterranean oak species studied here share a general syndrome of leaf traits that can be suited to a "reduced demand for resources" (Sack *et al.*, 2003), as well as part of a "conservative resource-use strategy" (Valladares *et al*., 2000a). Although in the physiological literature these traits are usually considered adaptations to the dry Mediterranean climate, most probably they are ancestral traits of Tertiary subtropical oaks, which allowed them to be sorted in when the climatic change imposing the seasonal drought typical of Mediterranean climate became established about 3.5 My ago (Herrera, 1992).

Within that general "Mediterranean oak syndrome", there are inter-specific differences in the seedling responses to light and water. The changes in structural leaf traits of leaf area, SLA, and concentrations of N and C, and the physiological performance of photosynthetic and respiration rates, and nitrogen efficiency (PNUE), were the most-affected by the species factor in this experiment. These leaf traits are associated to the plant's physiological response to the abundance of resources, and determine their growth and survivorship (Lambers & Poorter, 1992; Wright *et al*., 2004). For example, the seedlings of *Q. pyrenaica* showed the highest values of A_{area}, A_{mass} gs_{area}, gs_{mass}, leaf area, and PNUE, compared with the other three oak species. These leaf traits would favour seedling growth in nutrient-rich and mesic habitats, but they may confer less tolerance to drought (see species distribution in Table 1).

Mediterranean drought, at all levels of light, is a problem for the seedling in terms of avoiding water loss and maintaining carbon uptake, and therefore of biomass gain. On the other hand, deep shade in the closed forest understorey environment, independently of water availability, can be a limiting factor in maintaining a positive carbon balance. In this experiment, the shade conditions seemed to ameliorate, or at least not aggravate, the drought impact on oak seedlings, therefore, drought response on leaf performance depend of light environment.

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Table 1. Oak species included in the experiment (nomenclature follows Amaral, 1990), their leaf life span, frequency in southern Spain (calculated from 12572 records in the National Forest Inventory), and range of precipitation where they were recorded (data from the National Meteorological Institute; Urbieta *et al.* 2004).

Species	Origin of seeds	Functional group	Frequency in S Spain (%)	Precipitation (mm)	
				Mean	Range
Quercus canariensis Willd.	Sierra del Aljibe (SE Spain)	Deciduous	2.4	1073	628-1338
Quercus ilex ssp. *ballota* (Desf.) Samp	Sierra Nevada (SW Spain)	Evergreen	50.8	668	268-1366
Quercus pyrenaica Willd.	Sierra de Cardeña (S Spain)	Deciduous	0.4	773	604-990
Quercus suber L.	Sierra del Aljibe (SE Spain)	Evergreen	15.8	839	489-1366

Table 2. A) Soil water content (measured with TDR) at the beginning, middle and end of the experiment (mean ± SE) in a subsample of pots under the six light and water combinations. B) Results of the three-way ANOVA for the effects of water supply (W), irradiance treatments (I), and species (S), and their interactions (df = degrees of freedom; MS= mean squares) at the end of the experiment for all pots where photosynthetic measurements were done. HI: high irradiance; MI: medium irradiance; LI: low irradiance (see methods for details).

A)		Combined Treatments					
		HIGH WATER			LOW WATER		
	Time (days)	LI	MI	HI	LI	MI	HI
Soil water content (%)	0	13.8 ± 0.6	12.4 ± 0.6	12.0 ± 0.6	13.1 ± 0.6	10.3 ± 0.6	11.1 ± 0.6
	30	11.8 ± 0.5	11.0 ± 0.5	11.0 ± 0.5	6.6 ± 0.5	5.2 ± 0.5	3.6 ± 0.5
	60	12.8 ± 0.4	13.2 ± 0.4	13.2 ± 0.4	3.2 ± 0.4	2.4 ± 0.1	2.2 ± 0.1

B)

3-way ANOVA results	Factor	df	MS	P
	Water (W)	1	3053	<0.001
	Irradiance (I)	2	69.02	0.408
	Species (S)	3	66.14	0.461
	W x I	2	16.70	0.804
	W x S	3	6.98	0.965
	I x S	6	32.96	0.856
	I x W x S	6	7.63	0.996
	Error	111	76.38	

Table 3. Results of the three-way ANOVAs for some structural and physiological leaf traits, according to the factors oak species (S), and light (L) and water (W) treatments. The proportion of the explained variance (SS_x/ SS_{total}) and the level of significance (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001) for each factor and the interactions are indicated; those values not remaining significant after controlling the false discovery rate are underlined. R^2 is the proportion of total variance absorbed by the model. See text (Abbreviations section) for the full name of variables and units.

Leaf traits	Factors			Interactions				R^2
	Species	Light	Water	L x W	S x L	S x W	S x L x W	
Structural traits								
Area	53.8 ***	12.1 ***	0.0	1.3	2.1	1.4	2.0	72.8
SLA	29.9 ***	63.7 ***	0.0	0.1	0.8	0.4	0.5	95.3
N_{mass}	14.5 ***	39.8 ***	5.5 ***	4.9 **	3.5	0.4	5.3	73.9
C_{mass}	50.8 ***	1.0	$\underline{2.8^{*}}$	0.3	3.4	6.0 *	2.2	66.5
N_{area}	29.0 ***	44.4***	3.5 ***	$\underline{1.8^{*}}$	2.1	1.7	2.2	84.6
C_{area}	34.1 ***	59.2 ***	0.0	0.1	0.9	0.6	0.4	95.3
Chl index	31.9 ***	5.2 *	6.7 **	6.5 **	3.4	4.3	4.4	62.5
Physiological traits								
Φ	3.2	7.5 *	19.6 ***	7.3 *	4.7	2.3	5.9	50.5
θ	4.9	6.6 *	1.6	9.3 **	14.4 *	4.4	9.1	50.3
LCP	0.9	38.2 ***	0.2	5.4 *	2.7	7.7 *	5.4	60.5
LSP	0.3	18.1 ***	10.1 ***	6.4 *	9.1	4.2	5.2	53.5
A_{area}	5.8 ***	5.9 ***	37.0 ***	23.4 ***	2.3	4.0 **	2.3	80.7
R_{area}	3.2	18.2 ***	16.7 ***	0.2	5.7	3.0	5.4	47.5
gs_{area}	7.4***	2.0 *	46.0 ***	24.2 ***	0.5	3.3 **	1.6	84.8
A_{mass}	21.9 ***	14.2 ***	21.5 ***	14.4 ***	1.9	5.0 **	2.9	81.7
R_{mass}	17.9 ***	3.4	12.5 ***	0.7	5.6	3.5	5.0	48.6
gs_{mass}	19.2 ***	7.8 ***	30.8 ***	15.6 ***	0.2	4.6 **	2.1 *	80.3
WUE	2.4	3.0	19.9 ***	12.6 ***	7.8	8.6 **	5.6	59.9
PNUE	17.5 ***	6.1**	24.1 ***	13.7 ***	1.5	7.6 ***	$\underline{6.0^{*}}$	76.6
Ci/Ca	2.1	$\underline{5.1^{*}}$	9.0 ***	10.7 **	13.5 ***	9.0 ***	6.3	55.7

Table 4. Pearson's correlation coefficients for some structural and physiological traits. Bold and normal letters represent high- (HW) and low- (LW) water treatments respectively. The level of significance (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001) is indicated; those values not remaining significant after controlling the false discovery rate are underlined. See text (Abbreviations section) for the full name of variables and units.

controlling the false discovery rate are underlined. See text (Abbreviations section) for the full name of variables and units.

		A_{mass}	gs_{mass}	PNUE	R_{mass}	C_{mass}	N_{mass}	SLA	WUE	LSP
LCP	HW	-0.16	-0.05	-0.07	0.61 ***	-0.02	-0.18	-0.53 **	-0.36 *	0.59 ***
	LW	-0.76 ***	-0.75 ***	-0.75 ***	0.05	0.24	-0.56 ***	-0.61 ***	0.07	0.30
LSP	HW	0.18	0.38 *	0.36 *	0.41 **	-0.15	-0.33 *	-0.49 **	-0.41 *	
	LW	-0.28	-0.17	-0.30 *	-0.15	0.20	-0.11	-0.31 *	-0.30	
WUE	HW	-0.15	-0.62 ***	-0.28	-0.19	0.16	0.22	0.28		
	LW	-0.09	-0.27	-0.03	-0.22	-0.17	-0.26	-0.12		
SLA	HW	0.46 **	0.24	0.14	0.26	-0.40 *	0.71 ***			
	LW	0.74 ***	0.74 ***	0.66 ***	0.56 ***	-0.43 **	0.80 ***			
N_{mass}	HW	0.36 *	0.15	-0.11	0.34 *	-0.21				
	LW	0.74 ***	0.78 ***	0.61 ***	0.49 **	-0.25				
C_{mass}	HW	-0.57 ***	-0.57 ***	-0.51 **	-0.35 *					
	LW	-0.43 **	-0.39 *	-0.44 **	-0.30					
R_{mass}	HW	0.38 *	0.42 **	0.25						
	LW	0.45 **	0.43 **	0.40 **						
PNUE	HW	0.89 ***	0.83 ***							
	LW	0.98 ***	0.94 ***							
gs_{mass}	HW	0.85 ***								
	LW	0.97 ***								

Table 5. Probabilities under the null hypothesis that the data accord with each of the six proposed models, for each of the treatment conditions and combinations using the d-sep method. HI: high irradiance; MI: medium irradiance; LI: low irradiance or deep shade. HW: high water; LW: non-watered (drought). Models that would be rejected at the 5% level on a per-test basis are shown in bold. However those whose values remained significant after controlling the False Discovery Rate (FDR) at the 5% level, following the Ventura *et al.* (2004) criteria, are underlined.

	Model type					
Treatment	**A**	**B**	**C**	**D**	**E**	**F**
HI HW	**0.02258**	**0.01406**	0.66464	**0.01595**	0.81801	0.36896
HI LW	0.66877	0.41394	0.74490	0.48724	0.07526	0.50847
MI HW	0.46033	0.52570	0.79599	0.34195	0.72478	0.67319
MI LW	0.45570	0.45980	0.57111	0.66589	0.64803	0.32076
LI HW	**0.00745**	**0.01084**	0.09345	0.23975	0.16812	0.11715
LI LW	**0.01414**	**0.00251**	0.07707	0.42238	0.06243	0.02955
HI	0.78200	0.14879	0.98447	0.11576	**0.00618**	0.95464
MI	0.48803	0.47054	0.26774	0.93449	**0.01079**	0.13347
LI	**0.00001**	**0.00000**	**0.00623**	**0.02383**	**0.01079**	**0.01376**
HW	0.14121	0.05122	0.07750	0.6833	0.08963	0.19061
LW	**0.02473**	**0.00759**	0.52332	0.10452	**0.01823**	0.39736
HI + MI	0.44674	0.09157	0.46729	0.35493	**0.00001**	0.22299
Combined	**0.00044**	**0.00000**	**0.00017**	0.17411	**0.00000**	**0.00356**

Figure legends

Figure 1. Evolution of the photosynthetic rate (mean values and S.E. bars) with increasing irradiance (light curves), for seedlings (e.g. *Quercus pyrenaica*) cultivated in high (100 %, **A**), medium (27 %, **B**), and low (3 %, **C**) irradiance respectively. In each figure (of light conditions), seedlings under continuous irrigation treatment (HW, black circle) are distinguished from seedlings subjected to drought (LW, white circle).

Figure 2. General variation in leaf traits (see meaning of abbreviation names in Abbreviations section) of oak seedlings (means and S.E. bars for replicates of the four *Quercus* species) in response to the six combinations of light and water treatments. Light levels are "Low irradiance" (LI, 3 %), "Moderate irradiance" (MI, 27 %), and "High irradiance" (HI, 100 %), and water levels are " High water" (HW, solid line) and "Low water" (LW, dashed line).

Figure 3. Bivariate diagram of the comparative response to light ($\mathrm{Response}_{\mathrm{light}}$) versus the response to water ($\mathrm{Response}_{\mathrm{water}}$), for physiological and structural leaf traits, averaged for the four oak species. Response was calculated as (maximum value – minimum value / maximum value). See abbreviation names for the leaf traits and units in Abbreviations section.

Figure 4. Correlations between leaf traits. (**A**) N concentration (N_{mass}) and (**B**) photosynthetic rate on mass basis (A_{mass}) versus specific leaf area (SLA). (**C**) Stomatal conductance on area basis (gs_{area}) versus N_{mass}. (**D**) A_{mass} versus gs_{area}. *Q. ilex* ssp. *ballota* (○), *Q. canariensis* (◊), *Q. suber* (□) and *Q. pyrenaica* (Δ). Values for seedlings grown under high-water conditions (black shapes) are distinguished from those grown under low-water conditions (white shapes).

Figure 5. Alternative multivariate models linking the specific leaf area (SLA), leaf nitrogen content on mass basis (N), net photosynthetic rate on mass basis (A), and stomatal conductance on mass basis (gs). Model D (framed) was the best fitted to the dataset of *Quercus* seedling responses to water and light treatments.

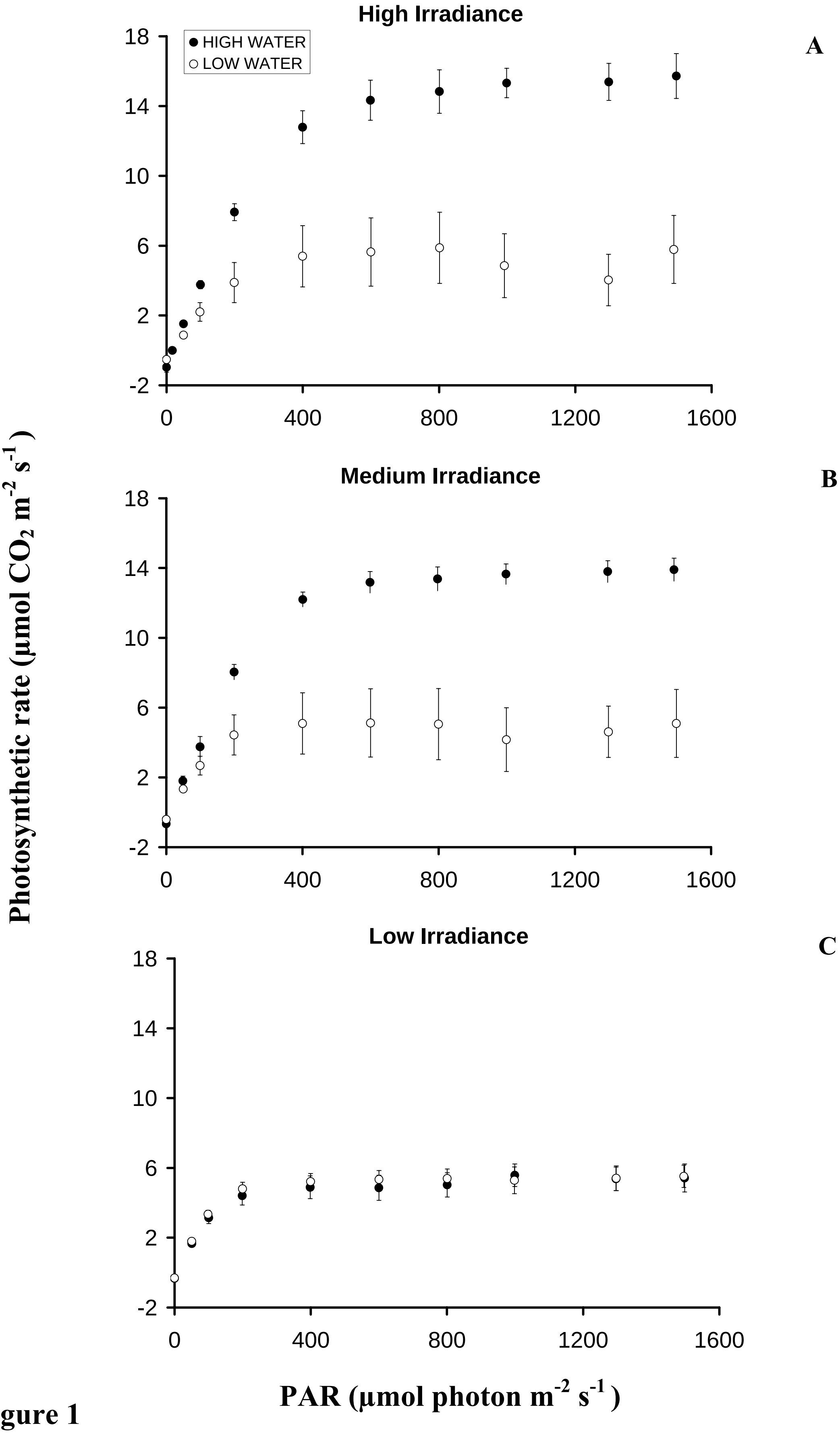

High Irradiance
A
● HIGH WATER
○ LOW WATER
Medium Irradiance
B
Low Irradiance
C
18
14
10
6
2
-2
0
400
800
1200
1600
Photosynthetic rate (µmol CO$_2$ m^{-2} s^{-1})
PAR (µmol photon m^{-2} s^{-1})

Figure 1

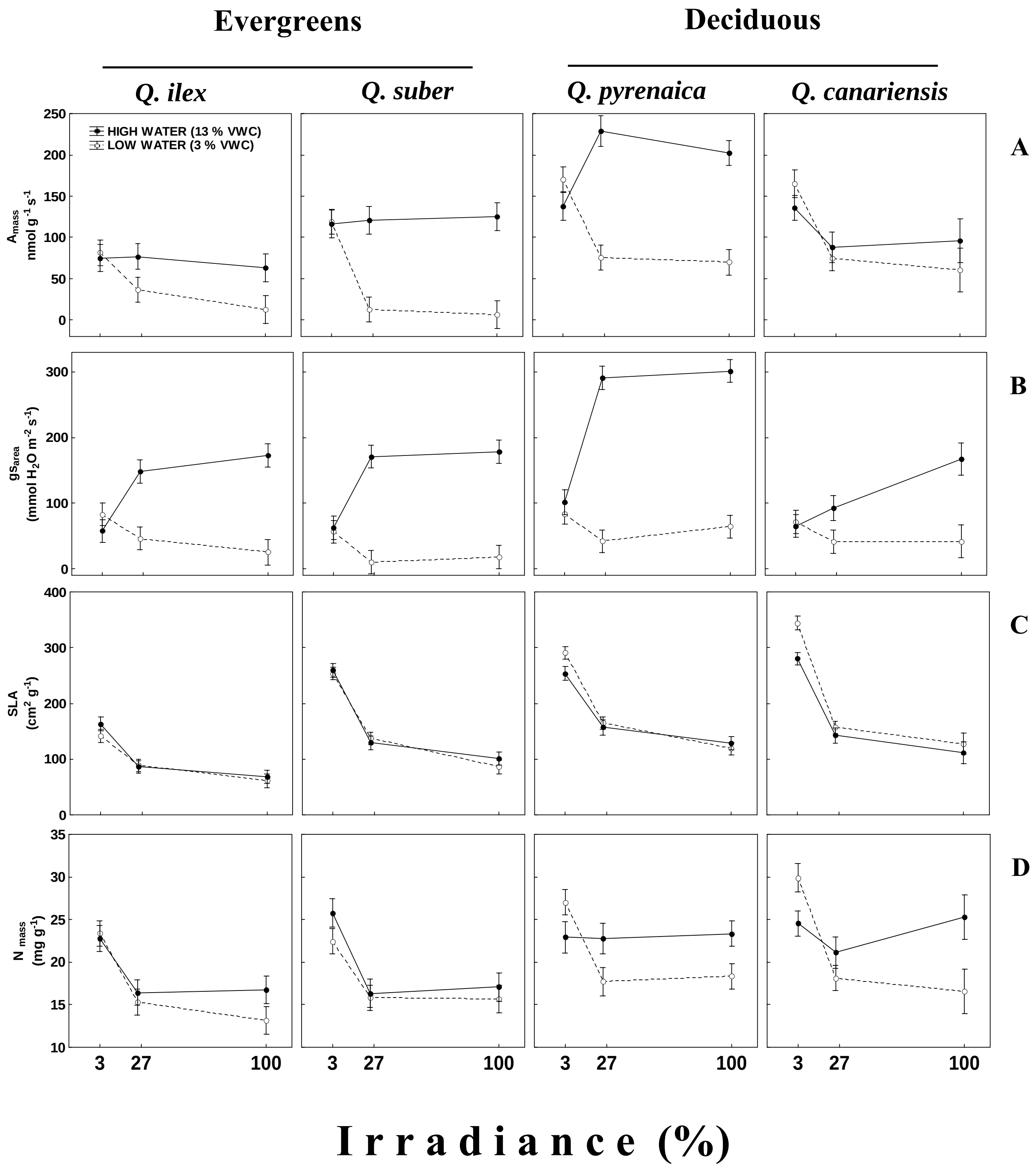
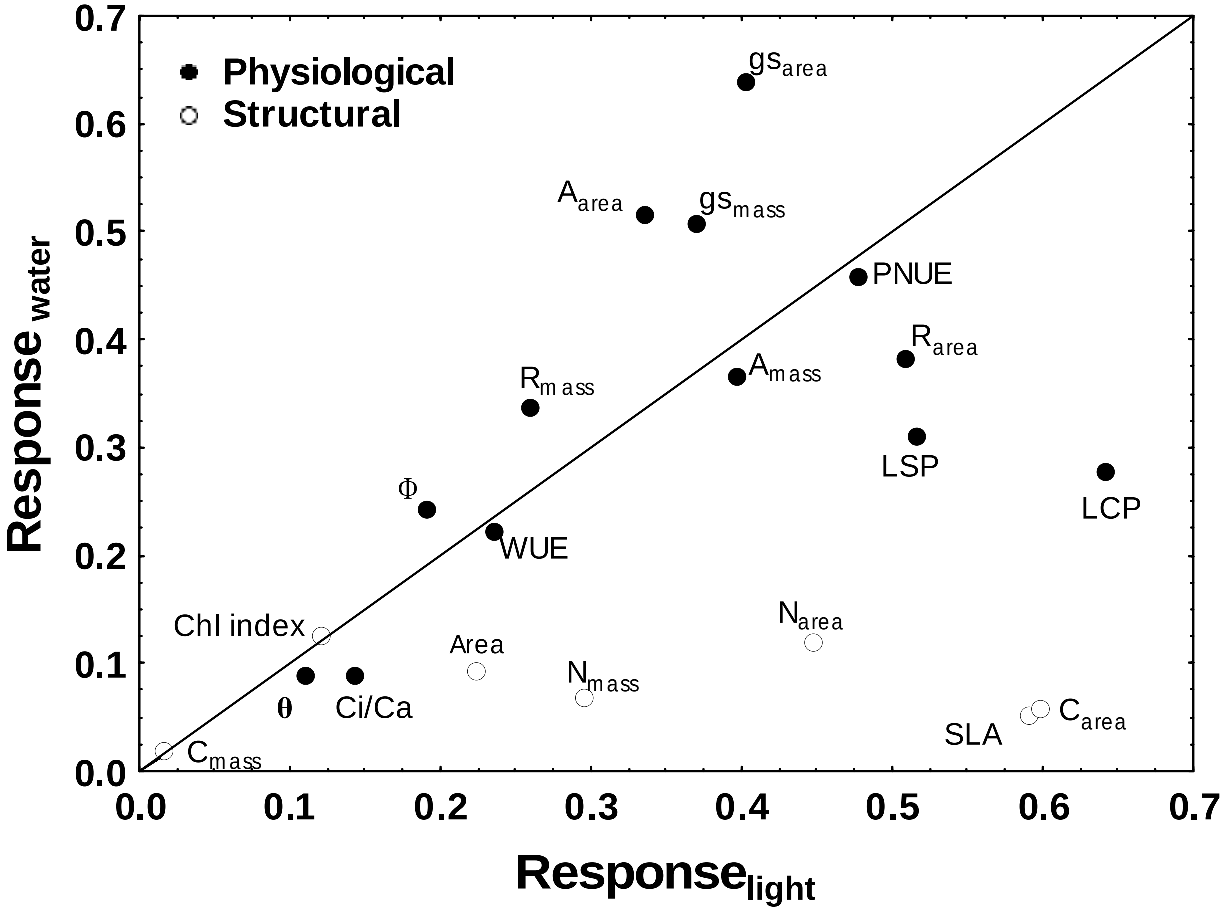

Evergreens
Deciduous
Q. ilex
Q. suber
Q. pyrenaica
Q. canariensis
HIGH WATER (13 % VWC)
LOW WATER (3 % VWC)
A
B
C
D
A_{mass} nmol g^{-1} s^{-1}
gs_{area} (mmol H_2O m^{-2} s^{-1})
SLA (cm^2 g^{-1})
N mass (mg g^{-1})
0
50
100
150
200
250
300
400
10
15
20
25
30
35
3
27
100
Irradiance (%)

Figure 2



Physiological
Structural
Response water
Response light
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
gs area
gs mass
A area
A mass
PNUE
R area
R mass
LSP
LCP
Φ
WUE
θ
Ci/Ca
Chl index
Area
N mass
N area
SLA
C area
C mass

Figure 3

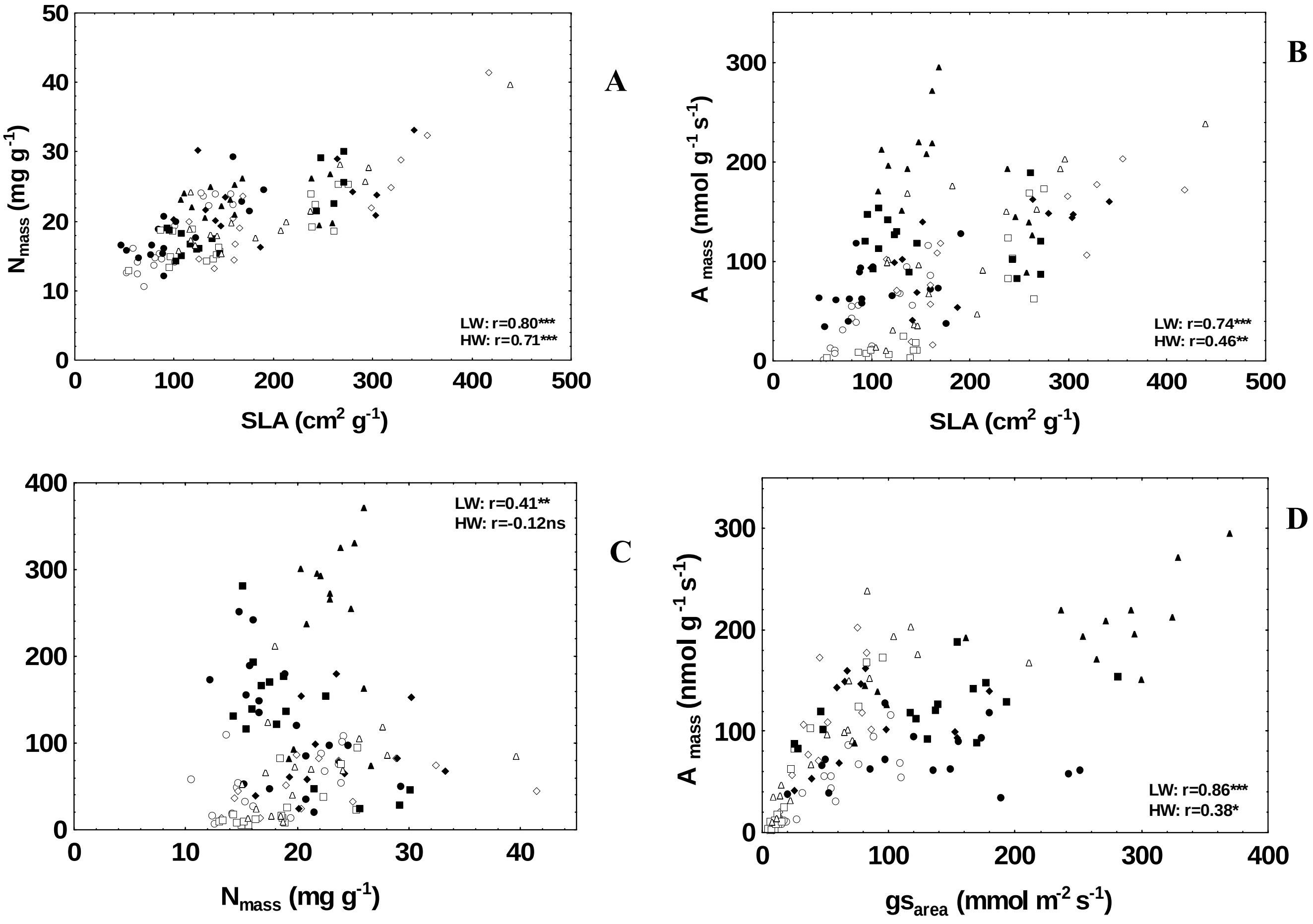

A
B
C
D
N_{mass} (mg g^{-1})
SLA (cm^2 g^{-1})
A $_{mass}$ (nmol g^{-1} s^{-1})
gs_{area} (mmol m^{-2} s^{-1})
LW: r=0.80***
HW: r=0.71***
LW: r=0.74***
HW: r=0.46**
LW: r=0.41**
HW: r=-0.12ns
LW: r=0.86***
HW: r=0.38*
0
10
20
30
40
50
100
200
300
400
500

Figure 4

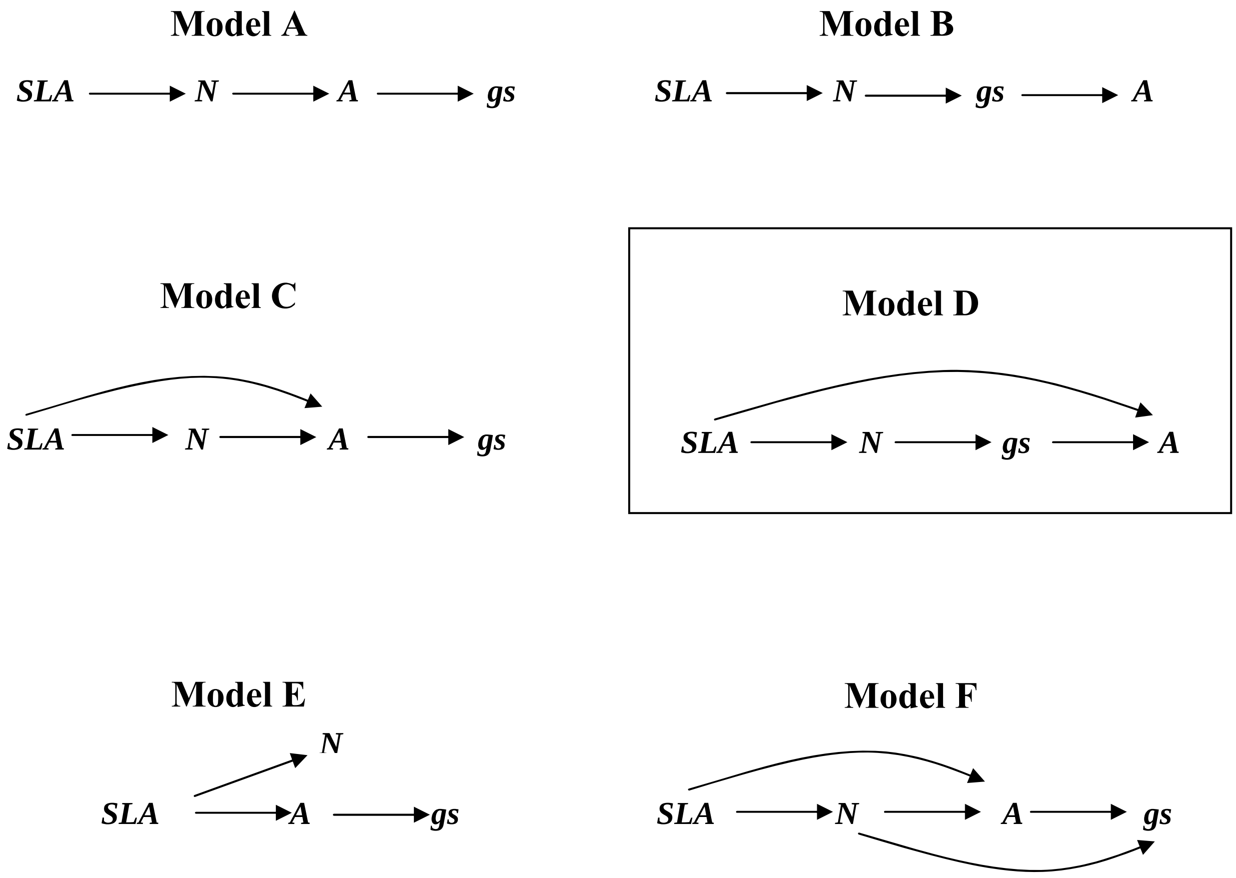

Model A
SLA
N
A
gs
Model B
SLA
N
gs
A
Model C
SLA
N
A
gs
Model D
SLA
N
gs
A
Model E
N
SLA
A
gs
Model F
SLA
N
A
gs

Figure 5

Appendix 1 : Mean ± S.E. values of structural and physiological leaf traits analysed for *Quercus* seedlings in different light and water treatments. Light treatments were HI: high irradiance, MI: medium irradiance, and LI: low irradiance. Water treatments were LW: low water, and HW: high water). For complete names of variables, see abbreviations section. In general, there were four replicates per treatments, exceptions are indicated between parentheses.

	Unit	*Quercus suber* **(Evergreen)**					
		HI		MI		LI	
		LW	HW	LW	HW (n=3)	LW	HW
Structural traits							
Area	cm^2	9.2 ± 0.6	10.8 ± 0.6	12.6 ± 0.6	12.1 ± 0.8	8.3 ± 0.5	9.9 ± 0.8
SLA	cm^2 g^{-1}	90.32 ± 6.61	101.62 ± 3.38	137.59 ± 3.17	131.01 ± 5.71	246.85 ± 6.84	259.54 ± 4.81
N_{mass}	mg g^{-1}	15.66 ± 1.09	17.21 ± 0.88	15.68 ± 0.67	16.54 ± 0.54	22.13 ± 1.05	26.34 ± 1.92
C_{mass}	mg g^{-1}	480.3 ± 4.2	446.1 ± 5.7	468.9 ± 4.4	455.9 ± 6.7	470.1 ± 3.3	456.7 ± 13.1
N_{area}	g m^{-2}	1.77 ± 0.19	1.71 ± 0.13	1.15 ± 0.06	1.27 ± 0.06	0.90 ± 0.04	1.02 ± 0.08
C_{area}	g m^{-2}	5.52 ± 0.58	4.40 ± 0.11	3.43 ± 0.12	3.50 ± 0.11	1.91 ± 0.06	1.76 ± 0.06
Chl index	-	19.2 ± 2.1	26.5 ± 2.9	24.4 ± 0.5	29.1 ± 1.8	24.4 ± 1.9	29.4 ± 2.5
Physiological traits							
Φ	-	0.0274 ± 0.0118	0.0470 ± 0.0029	0.0312 ± 0.009	0.0531 ± 0.003	0.0438 ± 0.0044	0.0450 ± 0.004
θ	-	0.545 ± 0.273	0.931 ± 0.023	0.766 ± 0.124	0.727 ± 0.107	0.927 ± 0.034	0.827 ± 0.089
LCP	µmol photon m^{-2} s^{-1}	45.2 ± 1.2	15.1 ± 3.7	27.0 ± 8.0	13.4 ± 1.5	6.5 ± 1.3	10.0 ± 1.7
LSP	µmol photon m^{-2} s^{-1}	735.0 ± 155.8	395.2 ± 74.4	337.2 ± 100.4	517.3 ± 129.3	155.1 ± 27.1	227.7 ± 74.0
A_{area}	µmol CO_2 m^{-2} s^{-1}	0.94 ± 0.25	12.72 ± 0.97	1.08 ± 0.32	10.5 ± 1.80	4.48 ± 0.40	4.29 ± 0.51
R_{area}	µmol CO_2 m^{-2} s^{-1}	0.56 ± 0.10	0.73 ± 0.19	0.37 ± 0.06	0.69 ± 0.06	0.26 ± 0.04	0.42 ± 0.05
gs_{area}	mmol H_2O m^{-2} s^{-1}	14.3 ± 4.3	166.0 ± 29.4	11.4 ± 2.3	179.7 ± 20.9	51.6 ± 11.9	56.1 ± 19.6
A_{mass}	nmol CO_2 g^{-1} s^{-1}	6.9 ± 1.5	126.8 ± 9.2	14.8 ± 4.2	115.7 ± 13.7	111.1 ± 11.4	121.6 ± 23.7
R_{mass}	nmol CO_2 g^{-1} s^{-1}	4.6 ± 1.1	7.7 ± 2.2	5.1 ± 0.9	8.5 ± 0.6	6.6 ± 1.1	10.7 ± 1.0
gs_{mass}	mmol H_2O g^{-1} s^{-1}	0.10 ± 0.02	1.78 ± 0.42	0.16 ± 0.03	2.07 ± 0.15	1.28 ± 0.31	1.72 ± 0.78
WUE	µmol CO_2 (mmol H_2O)$^{-1}$	76.6 ± 8.2	80.1 ± 7.0	10.6 ± 2.3	57.8 ± 7.0	96.5 ± 11.8	93.7 ± 13.9
PNUE	µmol CO_2 (mol N)$^{-1}$ s^{-1}	5.97 ± 1.26	104.68 ± 13.04	13.58 ± 4.20	98.72 ± 13.87	70.89 ± 5.52	67.50 ± 17.00
Ci/Ca	-	0.62 ± 0.03	0.52 ± 0.02	0.49 ± 0.01	0.63 ± 0.04	0.51 ± 0.05	0.53 ± 0.05

Appendix 1 (continued)

	Unit	*Quercus ilex* ssp. *ballota* **(Evergreen)**					
		HI		MI		LI	
		LW (n=3)	HW	LW	HW	LW	HW
Structural traits							
Area	cm^2	7.9 ± 0.8	8.4 ± 0.3	8.6 ± 0.9	8.0 ± 0.3	7.3 ± 1.1	5.5 ± 0.4
SLA	$cm^2 g^{-1}$	61.00 ± 1.27	64.64 ± 8.98	91.68 ± 5.01	86.91 ± 1.35	142.74 ± 6.64	166.74 ± 8.86
N_{mass}	$mg g^{-1}$	13.65 ± 1.31	16.57 ± 0.33	15.68 ± 1.25	15.74 ± 1.58	23.31 ± 0.42	23.60 ± 2.04
C_{mass}	$mg g^{-1}$	481.8 ± 1.9	473.8 ± 5.5	477.6 ± 2.1	469.6 ± 5.3	460.4 ± 3.4	462.8 ± 5.3
N_{area}	$g m^{-2}$	2.27 ± 0.24	2.72 ± 0.35	1.71 ± 0.11	1.82 ± 0.19	1.65 ± 0.08	1.45 ± 0.13
C_{area}	$g m^{-2}$	7.98 ± 0.16	7.88 ± 1.13	5.26 ± 0.29	5.42 ± 0.07	3.25 ± 0.17	2.84 ± 0.17
Chl index	-	35.6 ± 3.0	34.5 ± 2.7	32.2 ± 1.9	36.2 ± 4.2	39.7 ± 3.8	32.5 ± 2.6
Physiological traits							
Φ	-	0.0163 ± 0.0051	0.0470 ± 0.0081	0.0413 ± 0.0033	0.0524 ± 0.0054	0.0502 ± 0.0025	0.0404 ± 0.0018
θ	-	0.805 ± 0.157	0.892 ± 0.065	0.906 ± 0.053	0.874 ± 0.053	0.801 ± 0.049	0.896 ± 0.025
LCP	$\mu mol\ photon\ m^{-2} s^{-1}$	24.3 ± 1.9	27.2 ± 2.9	19.0 ± 4.5	15.1 ± 4.1	6.4 ± 1.2	14.6 ± 4.0
LSP	$\mu mol\ photon\ m^{-2} s^{-1}$	325.0 ± 65.1	508.0 ± 122.0	158.8 ± 41.4	497.3 ± 99.0	294.7 ± 36.2	233.3 ± 33.0
A_{area}	$\mu mol\ CO_2\ m^{-2} s^{-1}$	1.98 ± 0.25	9.39 ± 1.50	3.56 ± 1.33	9.23 ± 1.15	6.10 ± 0.65	4.43 ± 0.27
R_{area}	$\mu mol\ CO_2\ m^{-2} s^{-1}$	0.46 ± 0.18	1.20 ± 0.26	0.76 ± 0.17	0.79 ± 0.19	0.31 ± 0.05	0.57 ± 0.15
gs_{area}	$mmol\ H_2O\ m^{-2} s^{-1}$	25.7 ± 4.3	170.1 ± 12.3	38.4 ± 15.5	152.2 ± 8.4	85.8 ± 8.8	61.8 ± 13.5
A_{mass}	$nmol\ CO_2\ g^{-1} s^{-1}$	12.7 ± 1.9	59.4 ± 9.2	30.7 ± 10.4	80.5 ± 10.7	87.5 ± 11.2	81.0 ± 16.2
R_{mass}	$nmol\ CO_2\ g^{-1} s^{-1}$	2.9 ± 1.2	8.1 ± 2.8	7.1 ± 1.9	6.8 ± 1.6	4.5 ± 0.7	8.9 ± 2.6
gs_{mass}	$mmol\ H_2O\ g^{-1} s^{-1}$	0.16 ± 0.03	1.03 ± 0.15	0.33 ± 0.12	1.3 ± 0.08	1.22 ± 0.14	1.19 ± 0.33
WUE	$\mu mol\ CO_2\ (mmol\ H_2O)^{-1}$	86.8 ± 18.1	58.2 ± 10.0	89.6 ± 7.7	67.1 ± 3.9	71.9 ± 4.6	83.7 ± 14.0
PNUE	$\mu mol\ CO_2\ (mol\ N)^{-1} s^{-1}$	14.01 ± 3.55	49.63 ± 6.80	28.83 ± 10.71	74.49 ± 13.75	52.70 ± 6.62	47.53 ± 8.7
Ci/Ca	-	0.56 ± 0.08	0.64 ± 0.05	0.54 ± 0.04	0.60 ± 0.02	0.60 ± 0.02	0.56 ± 0.06

Appendix 1 (continued)

	Unit	*Quercus canariensis* **(Deciduous)**					
		HI		MI		LI	
		LW (n=3)	HW (n=3)	LW	HW (n=3)	LW(n=3)	HW
Structural traits							
Area	cm^2	11.2 ± 0.6	9.5 ± 0.8	12.6 ± 1.8	13.6 ± 5.4	11.7 ± 0.5	9.1 ± 1.2
SLA	$cm^2\ g^{-1}$	127.81 ± 13.07	111.57 ± 11.98	154.83 ± 9.98	145.76 ± 5.69	339.72 ± 23.48	281.32 ± 8.23
N_{mass}	$mg\ g^{-1}$	16.57 ± 3.37	25.26 ± 4.95	18.38 ± 1.90	21.91 ± 1.56	29.08 ± 3.92	24.60 ± 1.66
C_{mass}	$mg\ g^{-1}$	451.4 ± 7.6	445.9 ± 11.4	451.4 ± 2.4	443.12 ± 7.14	445.1 ± 5.3	436.8 ± 9.1
N_{area}	$g\ m^{-2}$	1.34 ± 0.4	2.24 ± 0.2	1.18 ± 0.07	1.50 ± 0.05	0.85 ± 0.05	0.88 ± 0.08
C_{area}	$g\ m^{-2}$	3.58 ± 0.43	4.03 ± 0.33	2.9 ± 0.21	3.04 ± 0.07	1.32 ± 0.07	1.59 ± 0.09
Chl index	-	16.5 ± 0.9	30.1 ± 2.2	25.6 ± 1.4	28.0 ± 1.4	24.1 ± 1.4	24.2 ± 3.9
Physiological traits							
Φ	-	0.0342 ± 0.0104	0.0436 ± 0.0025	0.0325 ± 0.0048	0.0466 ± 0.032	0.0478 ± 0.0020	0.0448 ± 0.0062
θ	-	0.308 ± 0.308	0.744 ± 0.144	0.927 ± 0.029	0.760 ± 0.105	0.929 ± 0.036	0.896 ± 0.027
LCP	$\mu mol\ photon\ m^{-2}\ s^{-1}$	29.7 ± 4.6	20.5 ± 4.2	16.4 ± 4.3	16.0 ± 1.8	6.6 ± 1.5	9.1 ± 4.1
LSP	$\mu mol\ photon\ m^{-2}\ s^{-1}$	651.8 ± 256.3	619.7 ± 129.5	240.5 ± 49.2	430.4 ± 0.1	167.03 ± 47.7	222.9 ± 38.3
A_{area}	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	3.71 ± 2.58	10.54 ± 1.86	5.14 ± 0.74	7.45 ± 1.75	4.85 ± 0.77	5.08 ± 0.43
R_{area}	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	0.77 ± 0.28	0.84 ± 0.10	0.43 ± 0.06	0.63 ± 0.02	0.31 ± 0.07	0.33 ± 0.09
gs_{area}	$mol\ H_2O\ m^{-2}\ s^{-1}$	41.7 ± 22.4	174.3 ± 21.0	46.5 ± 11.4	125.4 ± 54.3	64.1 ± 16.0	66.8 ± 5.2
A_{mass}	$nmol\ CO_2\ g^{-1}\ s^{-1}$	60.8 ± 40.9	96.1 ± 3.1	79.6 ± 13.0	105.0 ± 34.4	155.0 ± 25.0	143.7 ± 8.8
R_{mass}	$nmol\ CO_2\ g^{-1}\ s^{-1}$	11.1 ± 4.2	10.7 ± 2.9	6.8 ± 1.3	9.0 ± 0.2	11.8 ± 3.7	9.1 ± 2.9
gs_{mass}	$mmol\ H_2O\ m^{-2}\ s^{-1}$	0.59 ± 0.39	1.71 ± 0.18	0.73 ± 0.21	1.78 ± 0.94	1.97 ± 0.48	1.89 ± 0.09
WUE	$\mu mol\ CO_2\ (mmol\ H_2O)^{-1}$	68.8 ± 16.2	60.6 ± 2.8	105.5 ± 13.9	69.3 ± 13.2	78.8 ± 7.0	78.4 ± 3.0
PNUE	$\mu mol\ CO_2\ (mol\ N)^{-1}\ s^{-1}$	46.22 ± 25.18	55.08 ± 9.05	60.35 ± 5.43	65.66 ± 17.49	76.17 ± 10.65	81.93 ± 6.22
Ci/Ca	-	0.64 ± 0.09	0.62 ± 0.02	0.46 ± 0.06	0.60 ± 0.04	0.59 ± 0.03	0.59 ± 0.01

Appendix 1 (continued)

	Unit	*Quercus pyrenaica* **(Deciduous)**					
		HI		MI		LI	
		LW	HW	LW	HW	LW	HW (n=3)
Structural traits							
Area	cm^2	13.0 ± 2.2	16.5 ± 2.4	20.1 ± 2.2	18.3 ± 1.8	12.1 ± 1.5	14.0 ± 2.5
SLA	$cm^2\ g^{-1}$	117.54 ± 4.69	123.63 ± 7.04	160.11 ± 8.96	158.63 ± 3.10	281.52 ± 29.14	255.15 ± 6.29
N_{mass}	$mg\ g^{-1}$	17.75 ± 1.20	22.54 ± 0.96	17.71 ± 0.81	22.85 ± 1.25	25.95 ± 2.93	22.92 ± 0.25
C_{mass}	$mg\ g^{-1}$	429.2 ± 11.4	439.7 ± 6.3	433.8 ± 0.9	437.2 ± 11.1	438.2 ± 6.8	440.6 ± 9.8
N_{area}	$g\ m^{-2}$	1.52 ± 0.07	1.85 ± 0.12	1.10 ± 0.07	1.44 ± 0.08	0.92 ± 0.02	0.92 ± 0.02
C_{area}	$g\ m^{-2}$	3.68 ± 0.19	3.61 ± 0.20	2.69 ± 0.17	2.76 ± 0.03	1.63 ± 0.14	1.76 ± 0.10
Chl index	-	19.2 ± 2.9	24.3 ± 1.7	23.0 ± 1.6	31.4 ± 1.5	29.0 ± 3.5	25.4 ± 3.4
Physiological traits							
Φ	-	0.0372 ± 0.008	0.0523 ± 0.0007	0.0310 ± 0.0047	0.0529 ± 0.0039	0.0451 ± 0.0059	0.0643 ± 0.0092
θ	-	0.980 ± 0.011	0.824 ± 0.057	0.974 ± 0.023	0.906 ± 0.026	0.797 ± 0.097	0.615 ± 0.069
LCP	$\mu mol\ photon\ m^{-2}\ s^{-1}$	19.7 ± 1.9	23.4 ± 3.6	11.6 ± 4.3	17.9 ± 6.7	9.6 ± 3.1	14.5 ± 7.0
LSP	$\mu mol\ photon\ m^{-2}\ s^{-1}$	293.8 ± 93.3	621.8 ± 54.7	153.2 ± 53.2	434.1 ± 21.8	294.1 ± 53.7	378.4 ± 46.6
A_{area}	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	4.69 ± 2.04	15.22 ± 1.27	4.00 ± 1.35	13.76 ± 0.22	5.51 ± 0.62	5.36 ± 0.80
R_{area}	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	0.55 ± 0.03	1.19 ± 0.17	0.32 ± 0.10	0.85 ± 0.24	0.38 ± 0.06	0.85± 0.38
gs_{area}	$mol\ H_2O\ m^{-2}\ s^{-1}$	52.7 ± 29.3	293.9 ± 10.7	34.6 ± 18.0	274.8 ± 33.4	86.1 ± 7.7	101.2 ± 11.2
A_{mass}	$nmol\ CO_2\ g^{-1}\ s^{-1}$	57.8 ± 26.8	187.2 ± 17.8	65.3 ± 23.8	234.1 ± 18.2	163.2 ± 24.8	135.6 ± 16.6
R_{mass}	$nmol\ CO_2\ g^{-1}\ s^{-1}$	6.6 ± 0.6	14.7 ± 2.1	5.3 ± 2.0	14.1 ± 4.5	11.3 ± 1.4	22.0 ± 10.3
gs_{mass}	$mmol\ H_2O\ g^{-1}\ s^{-1}$	0.67 ± 0.40	3.67 ± 0.34	0.58 ± 0.32	4.49 ± 0.44	2.53 ± 0.35	2.56 ± 0.23
WUE	$\mu mol\ CO_2\ (mmol\ H_2O)^{-1}$	108.0 ± 8.6	52.0 ± 5.0	147.2 ± 17.7	52.2 ± 4.7	63.8 ± 3.8	56.4 ± 4.8
PNUE	$\mu mol\ CO_2\ (mol\ N)^{-1}\ s^{-1}$	43.87 ± 18.12	115.63 ± 7.07	61.15 ± 26.96	143.31 ± 5.42	87.20 ± 9.58	87.99 ± 15.83
Ci/Ca	-	0.45 ± 0.04	0.62 ± 0.03	0.31 ± 0.06	0.63 ± 0.02	0.65 ± 0.02	0.68 ± 0.03

Capítulo 5: (en ingles) Growth and biomass allocation under limiting light and water in seedlings of four *Quercus* species

Growth and biomass allocation under limiting light and water in seedlings of four *Quercus* species

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Abstract

We have investigated the effects of light and water treatments on final biomass, relative growth rate (RGR) and its components, in seedlings of four *Quercus* species differing in leaf habit (deciduous and evergreen). Plants were grown 192 days in three levels of irradiance (100%, 27%, and 3% of incident radiation) and two levels of watering. For three species there was still a positive effect of seed mass on final biomass. Contrary to what it was expected, evergreen species showed higher final biomass, but there was a big difference within the leaf habit. Both shading and drought reduced seedling growth rates, and considering all species there was a significant interaction between light and water: the drought treatment had a major impact for seedlings under full irradiance, but had a negligible effect for seedlings under deep shade. The variation in RGR, among species and treatments, was explained by differences in net assimilation rate (NAR), but neither by the leaf area ratio (LAR) nor by the specific leaf area (SLA). There was only a small variation in biomass allocation to leaves, stems and roots, across shade and drought treatments, indicating relatively low plasticity for these oak species during the seedling stage. Seedling RGR was not correlated to maximum photosynthetic rate but it was strongly correlated with the net carbon balance scaled up from leaf level light-response curves indicating the potential to predict seedling growth responses in diverse light and water treatments from leaf-level gas exchange measurements.

Keywords: growth analysis, Mediterranean oak, biomass allocation, carbon balance

Introduction

Light and water are two main resources for plants and are responsible of patterns of growth and survival between individuals or species. For example, Poorter (2001) found in six rain forest tree species that relative growth rate (RGR) increased with irradiance, mainly because of an increase in net assimilation rate (NAR). Similarly, Montgomery & Chazdon (2002), Montgomery (2004) and Sánchez et al. (2006) found a positive effect of light on growth in tree seedlings, although plants did not increase RGR linearly with light and showed maximum response at intermediate irradiance levels. Light availability also affect the biomass allocation. In general, plants respond to limiting light increasing specific leaf area (SLA, the leaf area / mass ratio) and LAR (leaf area ratio) (Hladka & Priwitzer 2005, Gratani et al 2006, Sánchez et al. 2006) and decreasing root mass fraction (RMF) (Montgomery & Chazdon 2002, Sánchez et al. 2006). Species show contrasting responses to light which may explain the different abundance of seedlings and saplings in different microsites (Montgomery & Chazdon, 2002).

Water availability has also showed an effect on growth and survivorship on early stages. For example, Matthes & Larson (2006) in a 18-year field study showed that drought was the principal cause of mortality on *Thuja occidentalis*-individuals and this effect decreased with increasing plant size. Moreover, Caspersen and Kobe (2001) found that, when species were sensitive to drought, bigger plants increased survival probabilities. Recently, studies of plant performance under different water conditions has become a worldwide need in a global change context (Maestre & Reynolds 2007). Droughts are occurring with increasing frequency and severity in Mediterranean areas (Piñol et al. 1998; De Luis et al. 2001; Peñuelas et al. 2002) and global change climate scenarios predict for the 21st century an increase of drought period and a decrease in rainfall of about 20% (Gitay et al. 2002).

In nature, both resources may change in the space independently or be associated in a positive or negative way, so many combinations of levels of light and water may appear

(Quero 2006). In addition, in Mediterranean areas and also in other biomes, heterogeneity of these light and soil water levels may appear in a very small scale (meters or cm) (Maestre et al. 2003, Quero 2006, Valladares & Guzmán 2006), which has been proposed as a mechanism of coexistence of different species (Beckage & Clark 2003, Valladares 2003).

Recently, there has been an increasing interest on the interactive effect of light and water on plants, specifically the effect of low light conditions together with low water availability. Plant responses to these two limiting resources appears to be contrary (i.e. low RMF under low light but high RMF under low water, Prider & Facelli 2004). These responses have been studied across levels of biological organization, from leaves (Kao & Forseth, 1991) to community levels (Zavala & Bravo, 2005). However, results of shade and drought experiments have shown controversial results.

Some studies have shown negative effects of shade under drought conditions (Smith & Huston, 1989; Aranda et al., 2005). Valladares & Pearcy (2002) found that plant water potential reduction in drought environments was more severe in understory than in open habitats due to greater competition in the shade. Similar results were reported by Abrams & Mostoller (1995). On the contrary, other studies have shown a positive effect of shade under drought conditions (Canham *et al.*, 1996; Holmgren, 2000). Quero et al. (2006), at leaf-level, have found a stronger impact of drought for plants in high irradiance than for plants in deep shade. We noted that for slow-growing Mediterranean tree seedlings in deep shade, a low impact of drought on leaf photosynthetic rate and on RGR might be anticipated, due to the drought-tolerance of these species, and to the low evaporative demand under deep shade. If these hypotheses are valid, then for slow-growing species, a interaction of shade and drought may be observed, such that seedlings in high irradiance would be far more impacted by drought in both RGR and total growth than seedlings in deep shade. Finally, in other studies the effects of light and water conditions on growth have been found to be independent, this is, for a given degree of RGR reduction in shade under optimal water conditions, a water shortage will lead to an additive reduction in RGR (Sack & Grubb, 2002; Sack, 2004).

Validation of each group of hypotheses seems to be dependent of a wide array of causes as the irradiance and soil moisture ranges evaluated (Aranda et al 2005), growth variables measured (Quero et al., 2006), species (Prider & Facelli, 2004) or phenotypes (Valladares et al., 2005) studied.

Relative growth rate (RGR) is considered as a variable that summarize the metabolic response of plants to different resources. Differences in RGR may be due to the morphological traits (SLA, LAR or biomass allocation) or to the physiological activities (photosynthetic and respiration rates or NAR). Studies comparing different species have found that in many cases a simple parameter as SLA may explain up to 80 % of variance in RGR (Poorter and Remkes 1990; Antunez et al., 2001; Ruiz-Robleto & Villar 2005). Surprisingly, maximum photosynthetic rate did not seem to be an explanatory factor of RGR (Poorter, Remkes & Lambers 1990, Sims et al. 1994, but see Reich et al. 1992 Montgomery 2004) . However, recently the paradigm that differences in RGR are mainly explained by SLA is somehow under question. Shipley (2002) has found that the factor explaining the differences in RGR may be different depending of the light conditions (i.e. under low light conditions RGR was explained by SLA, but under high light conditions RGR was explained by NAR. Interestingly, studies under field conditions (where light levels are in general higher than in controlled conditions) RGR was explained by NAR (Montgomery & Chazdon, 2002; Villar et al. 2005).

We carried out a greenhouse experiment with controlled conditions of light and water to investigate growth and biomass allocation of tree seedlings to six combinations of light (three levels) and water (two levels). Our first aim was to determine the effects of different conditions of light and water on total biomass, RGR and biomass allocation of four *Quercus* species and to know which are the main morphological and physiological traits that drive the variation in RGR under those conditions.

Species with big seeds depends in their initial stages of development of the seed reserves. *Quercus* species show a relative high seed mass and a wide range. Also, these four species used differently their seed reserves, with an effect of light availability on the way of using them (Quero et al, submitted). We have also modelised the use of the reserve and predicted that the importance of seed mass on seedling biomass should decrease with time. Our second aim was therefore to determine the effect of seed mass on final biomass and other related variables and if the seed mass effect may change under different light and water conditions.

Seedling performance in terms of growth characteristics could be also influenced by leaf habit (evergreen vs. deciduous). Comparing to evergreen species, deciduous ones tend to growth faster (Reich et al. 1992; Cornelissen et al 1996; Antúnez et al., 2001; Ruiz-Robleto & Villar 2005). Then, it is expected than deciduous species should be more affected by limiting conditions of light and water. In the present study, two evergreen and two deciduous species of *Quercus* were selected to allow comparison within a phylogenetic context. Then, our third aim was to know if leaf habit has any effect on growth and biomass allocation and if leaf habit respond differently to light and water conditions.

Although there are many studies on light and water effect on plants, there are not many attempts in which whole-plant responses are compared to leaf level responses. In a previous paper (Quero et al. 2006) we have analyzed the responses of structural and physiological variables at leaf level and here we analyzed the response at whole plant level. Therefore, our four aim was to derive the ability to scale up from photosynthetic leaf responses described in the previous paper (Quero et al., 2006) to carbon gain at whole-seedling scale. The ability to scale up from short-term measurements of net leaf photosynthetic rate to whole-plant RGR (Nobel 1984) is essential for a predictive ecology in Mediterranean areas and to predict forest dynamics under global change scenarios.

Material & Methods

Experimental design

Acorns of four oak Mediterranean species — the evergreens *Quercus suber* L. and *Quercus ilex* subsp. *ballota* (Desf.) Samp., and the deciduous *Quercus canariensis* Willd. and *Quercus pyrenaica* Willd. — were collected in forests of South Spain (nomenclature follows Amaral, 1990). Single acorns were weighted individually and sown (on December 2002) in pots of 3.9 litres volume (50 cm height, 10.5 cm diameter) thereby avoiding as much as possible interference during root growth. An acorn subsample of each species was oven-dried at 70º C for at least 48 h and weighed for the dry mass; then initial seed dry mass was estimated for each individual from the seed fresh mass, using species-specific regression equations (Quero et al. submitted). The experiment was carried out in a greenhouse of the University of Córdoba (Spain, 37º 51' N, 4º 48' W; at an altitude of 100 m. a. s. l.) with an automatic irrigation system and regulation of air temperature.

Oak seedlings were subjected to six combinations of light and water treatments (three levels of light and two levels of water). We used a split-plot design with three light treatments: high-irradiance (HI) receiving full light (no shade frame; 100% irradiance), and medium-irradiance (MI; 27% irradiance) and low-irradiance (LI; 3% irradiance) imposed by using shade frames (150 x 120 x 200 cm), and all replicated 4 times, making a total of 12 "shade-frame" blocks. Each of the four oak species and the two levels of watering were randomly arranged within each shade-frame block, using one plant in each pot. Water treatments were imposed in late April 2003: for half of the pots no more water was provided (LW, low-water treatment) while the other half were kept continuously moist (HW, high-water treatment). Low-water treatment simulated a typical Mediterranean-climate situation of seasonal drought where soil moisture values were similar to those found at the end of the experiment (Gómez-Aparicio et al. 2005). Light levels simulated the field conditions in the forest understorey where oak seedlings grow (Marañón et al. 2004). For more details about origin of seeds, characteristics of the substrate, levels of shade and drought treatments, see Quero *et al.* (2006).

Two harvests of seedlings were carried out; the first one on late April 2003 (seedlings 4-months old), before the beginning of the drought treatment, and the second one on late July 2003 (seedlings 7-months old). In the first harvest, 15-17 seedlings per species and light treatments were harvested, and in the second harvest, 12-16 seedlings per species, light and water treatment combination were sampled, distributing evenly among the 12 shade-frame blocks. During each harvest, individual seedlings were carefully extracted from each pot and their roots were washed off the soil. Immediately the plant was separated into leaves, stems and roots. Leaves were scanned and leaf area was measured using an image analyser software (Image Pro-Plus v 4.5 Media Cybernetic, Inc). Dry mass of the plant parts was determined after oven-drying at 70º C for at least 48 h. Specific leaf area (SLA) was calculated as leaf area/ dry mass. Root mass fraction (RMF), stem mass fraction (SMF), and leaf mass fraction (LMF) were calculated as the dry mass of root, stem and leaves, respectively, and divided by the total seedling dry mass. Acorns remains were not considered in the calculations.

Shade and drought effect at the end of experiment

For each species, effects of shade and drought at the end of experiment (second harvest, seven-months old seedlings) were tested with General Linear Models, considering the initial seed dry mass as covariable and light and water treatments (as well as for shade-frame) as categorical factors, on total growth (final biomass at the seven months), SLA, LAR and biomass allocation. As shade frame has not any effect on any variable measured, for clarity we re-analyzed the data without considering shade frame, avoiding high-order interactions and to increase statistical power (Zar 1996). Furthermore, we analyzed the data considering the four species together and including species as a factor. In addition, to determine whether leaf habit has any effect on the variables measured we analyzed the data including leaf habit [evergreen (*Q. ilex* and *Q. suber*) and deciduous (*Q. canariensis* and *Q. pyrenaica*)]. Before the analysis, data were square-root-, arcsine-, or log-transformed to satisfy the normality and homoscedasticity assumptions (Zar, 1996). Statistical analysis were performed with the software STATISTICA v 6.0 (Statsoft, Inc., Tulsa, OK, USA). In order to control the inflation of type I error derived from repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was applied to repeated test. The FDR was controlled at the 5% level using a standard step-up procedure (see García, 2004).

Growth components calculations

Relative growth rate (RGR) and their components (NAR, LAR, SLA, LMF, SMF and RMF) during the two and a half months of growth were calculated following the classical approach (see Evans 1972), using the method of Hunt et al. (2002; http://aob.oxfordjournals.org/cgi/content/full/90/4/485/DC1) which incorporates the improvements described by Hoffmann and Poorter (2002). Pearson’s correlation analysis was performed separating the two water treatments (LW and HW) to check relationships between RGR and growth components in the two water treatments.

Calculations of net carbon gain

To scale up from leaf-level to whole-plant level responses, net photosynthetic carbon assimilation was estimated using the model of Thornley (1976):

$$A(I) = \frac{\Phi I + A_{max} - \sqrt{(\Phi I + A_{max})^2 - 4\theta I A_{max}}}{2\theta} - R_d \qquad (1)$$

where A is the photosynthetic rate, I the photosynthetic active radiation (PAR), Φ the apparent quantum yield, A_{max} the maximum light saturated assimilation rate, R_d the dark respiration rate, and θ the "bending degree" or curvature. The light curve parameters (Φ, A_{max}, θ, and R_d) were reported from Quero et al., (2006) in each individual seedling. We measured 3 to 6 leaves for species, light and water treatment. During the experiment daily variation in photosynthetic photon flux density (**I**) outside the greenhouse was measured each hour with a photosynthetic active radiation sensor. Percentage of available radiation inside of the greenhouse was calculated using hemispherical photography (**AR**). Photographs were taken at aboveground seedling level using a horizontally-leveled digital camera (CoolPix 995 digital camera, Nikon, Tokyo, Japan) and aimed at the zenith, using a fish-eye lens of 180º field of view (FCE8, Nikon). Hemispherical photographs were analysed with Hemiview canopy analysis software ver. 2.1 (1999, delta-T Devices, Cambridge, UK). Two of the light treatments were done using a green screen with different level of light transmittance (**LT**) (27 % of available radiation for MI and and 3 % for LI). The photosynthetic active radiation (PAR) received for each individual were calculated as **I x AR x LT** for each hour during April 22 to July 16. Leaf net carbon gain was calculated using the photosynthetic light curve (eq. 1) and the PAR received each hour. Leaf R_{area} values were considered constant and were apply to the carbon gain during the night and we did not consider root and stem respiration rates assuming these potential errors on calculations. Values of leaf net carbon gain were averaged for the whole period of growth. Resulting leaf net carbon gain values was multiplied by LAR values (of the final harvest) per individual seedling to transform data from leaf area basis to whole plant mass basis, assuming the fact that leaves could differ within seedlings. This estimate of net assimilation was correlated with the relative growth rate (RGR) in each treatment. Additionally, RGR values per species, light and water treatments were correlated with A_{max} data from Quero et al. (2006).

Results

Seed mass effect

After seven months of growth, there was still an effect of initial seed mass in final biomass (Table 1, Fig. 1). Comparing the four species, the influence of initial seed mass decrease as final biomass increase from about 11 % for *Q. canariensis* (the species with lowest final biomass) to null effect for *Q. pyrenaica* (the species with highest biomass). Interestingly, correlations between seed mass and final biomass were higher at low irradiance (LI) than the others light treatments (MI and HI, Fig. 1). However, this result did not occur in *Q. pyrenaica*, the only species with null effect of seed mass after seven months of growth, but in contrary it showed a negative relationship between seed mass and final biomass in low light treatment (Fig. 1D).

The patterns of the relationship between final biomass and seed mass were very similar for the water and drought treatment (Fig. 1), and the general tendencies were the same.

Light and water effects on total growth

Seedling biomass was different among oak species, being larger (higher total biomass) the seedlings of *Q. suber* and *Q. pyrenaica* (Fig 2 A). Light had the strongest effect on final biomass for all species (ranged from 64 to 86 % of variance explained), increasing the biomass with a increase in light (Table 1; Fig. 2 A). However, water reduction has a very small effect on seedling biomass (3.4 % for *Q. pyrenaica* to 0 % for *Q. canariensis*). For *Q. suber* there was a significantly light and water interaction because almost no water-induced variation on seedling biomass under deep shade conditions was found (Table 1; Fig. 2 A). Considering all species, there was also a significantly light and water interaction although very low proportion of variance was explained (0.6 %). Factors "species" and "light" also showed a significant interaction, mainly due to seedlings of *Q. canariensis* which showed different patterns respect to the other three oak species, mainly due to no water-induced variation across light treatments (Table 1; Fig. 2 A), although this interaction was lost after controlling FDR correction. The final biomass of seedlings was reduced by drought under full light conditions (Fig. 2 A), but negligibly under low irradiance.

Considering leaf habit as factor, its effect on final biomass was significant but weak in the model, having the evergreen species higher final biomass (Table 1). Light had again the strongest effect (57 % of explained variance) and interestingly the effect acorn mass was considerably stronger compare to the model including species as factor (24 % vs. 4 % of explained variance, Table 1). Light and water interaction was also significant, although it explained low variation.

Shade and drought impact on relative growth rate and their components

Reduction of light and water availabilities produced a decrease in the seedling relative growth rates (Fig. 2 B). In particular, RGR reduction was very strong under limiting light (from 58 % in *Q. canariensis* to 99 % in *Q. pyrenaica*) and weaker under drought conditions (from 0 % in *Q. canariensis* to 38% in *Q. pyrenaica*), although having interactions with light: droughted seedlings showed a RGR decline under full light but a negligible reduction under deep shade (Fig. 2 B).

Seedlings of the four oak species showed different responses to changes in light and water conditions (Fig. 2 B). *Q. canariensis* showed strongest tolerance of shade, with higher RGR maintained under intermediate and deep shade, followed by *Q. suber*. Respect to the effect of drought, *Q. pyrenaica* showed a negligible RGR when experiencing drought under deep shade (Fig. 2 B).

The response of net assimilation rate (NAR) to light was similar to that of RGR (Fig. 2 C), and thus very strongly reduced under low irradiance (about 92 %). On average, droughted plants showed a decrease of about 18 % in NAR relative to well watered plants; as for RGR, the impact of drought on NAR was negligible under deep shade. In contrast, the leaf area ratio

(LAR) increased at lower irradiance (Fig. 2 C), and was in general unaffected by drought (Fig. 2 C; Table 1). LAR is the product of the specific leaf area (SLA) and the leaf mass fraction (LMF). Oak seedlings responded to shade by increasing SLA (Fig. 2 D; Table 1), while LMF remained relatively constant (Fig. 3 A; Table 1). Under deep shade, oak seedlings allocated relatively more biomass to stems (Fig. 3 B; Table 1), but less to roots (Fig. 3 C) than in full light. The two evergreen species (*Q. suber* and *Q. ilex*) increased the allocation to roots under drought conditions (Fig. 3 B; Table 1).

Relationships of RGR and growth components

Considering all species and treatments, variations in RGRs were explained mainly by changes in NAR, but not by modifications of LAR (Table 2, Figs. 4A and 4B). Most of the LAR variation was directly explained by SLA (r = 0.64, $P < 0.01$), and also by LMR (r = 0.48, $P < 0.05$; Table 2). Specific leaf area was negatively correlated with NAR (r = - 0.68, $P < 0.001$) and with RGR (r = - 0.64, $P < 0.001$, Fig. 4C). Seedling RGR was negatively correlated with the biomass allocation to stems, but there was no relation with allocation to leaves or to roots (Table 2). The allocations to stems and to leaves were negatively correlated with allocation to roots (Table 2, Fig. 4D), and allocation to leaves was positively related to allocation to stems.

Considering the two water treatments apart, the results were very similar for drought and water treatments (data not shown), with the exception that under drought conditions, the relationships between RGR and SLA and SMF were not significant.

Leaf carbon balance and seedling growth

Seedling RGR was not correlated with maximum photosynthetic rate on an area basis (Fig. 5A) and on mass basis (data not shown). However, RGR was positively related with average carbon balance at leaf level, either in area or mass basis (Fig. 5B).

Discussion

Seed mass effect

Initial seed mass has been recognized as crucial factor influencing seedling growth (Ke & Werger, 1999; Poorter & Rose, 2005). In general, a positive effect of seed mass on seedling growth was observed, even after seven months from germination. However, although the four species are close related, the use of reserves differ. For example, in *Q. pyrenaica* there is no any effect of seed mass on seedling biomass under any conditions of light and water, which may be due to a lower use of the seed reserves by this species (Quero et al, submited). Surprisingly, the effect of seed mass on seedling biomass was very high considering the leaf habit (Table 1, seed mass explain about 25 % of variance in seedling biomass). However, this may be due to the fact than within each functional group there is one species with small seed mass and another with high seed mass (i.e. within evergreens *Q. ilex* has small seed mass, but *Q. suber* has big seed mass and within deciduous *Q. canariensis* and *Q. pyrenaica* had small and large seed mass) (Quero et al. submitted).

A meta-analysis reported by Poorter & Rose (2005) showed that the strength of seed-seedling correlation may disappeared over 1-4 years. Castro (1999) in *Pinus sylvestris* (a species with small seed mass, 0.009 g), did not found seed mass effect on seedlings after one growing season. Quero et al. (submited) developed a model that predict a decreasing effect of seed mass with time. The effect of the seed mass on seedling biomass during the first 50 days of growth was about 1 (Quero et al, submited). On the contrast, the calculated effect of seed mass on seedling biomass after 7 months was lower as it was expected. The seed reserves were used mostly during the first period of development (the first 50 days of growth) where the plant has mostly a heterotrophic growth (seed reserves are spent between 40 to 60 % depending of the species and light environment) (Quero et al. submitted). However, our results shows that the remaining seed reserves have also some effect on seedling biomass for longer periods (seven months or more).

On other hand, larger seedlings from larger seeds could have considerable advantages than smaller ones on the face of Mediterranean drought summers (i.e. larger root system to capture more soil water). Positive effect of seedling size in relation to survival have been reported by Caspersen & Kobe (2001) in temperate forests, however, a contrary effect is expected in Mediterranean areas (Marañón et al. 2004), since larger seedlings will have more transpiration surface. One way to avoid this negative effect is to allocate more biomass to root, as the four studied *Quercus* species do (Fig. 3). Therefore, higher RMF allows capture water more efficiently (Metcalfe and Grubb, 1997) and, at the same time, avoids loss water under environments with high evaporative demand.

This dependence of the seedling on the initial seed reserve was stronger under shaded conditions (Fig. 1) as reported by Paz & Martínez-Ramos (2003). Under deep shade conditions seedling growth becomes more heterotrophic and cotyledon reserve becomes more important than under intermediate and optimal light conditions, where genotypic differences can determine intraspecific variation in seedling growth and mass. These low light conditions are typical in understory of mediterranean forest and therefore having a high seed mass may confer advantages under these conditions.

Light and water effects on total growth, relative growth and their components

Most studies that have looked at drought impacts on plants at multiple irradiances have found a significant impact of drought even in deep shade (Valladares & Pearcy 2002). In this study that was not found, probably due to the relatively slow growth of these Mediterranean tree seedlings. However, this experiment focuses on a situation that must occur relatively commonly —i.e., the drought that impacts in high irradiance but not in deep shade must occur during short droughts, specially with slow growing species. Using these findings we can generalize further from the models for shade x drought interaction described in Sack & Grubb (2002) and Sack (2004). In those studies, the applied drought had a significant impact on RGR in deep shade, and it was proportional to the impact in high irradiance. These results scaled up to a stronger impact of drought on total growth in high irradiance than in deep shade. In this study, slow growing species in deep shade did not experience an impact of drought on RGR or on final mass. Thus, the impact of drought on RGR or final mass in deep shade will be less than that in high irradiance if the plant grows slowly, if the drought is short-lived, or if the shade is especially deep—or, when it does impact in deep shade, it will have an impact proportional to that in high irradiance. This hypothesis covers a wide range of shade x drought combinations, and predicts that plants are protected from short-term drought by deep shade, and in longer-term drought, the impact of drought will be proportional across irradiances. This new model, supported by our data and previous studies, stands in contrast to the trade-off model of Smith & Huston, which predicts a stronger impact of drought in deeper shade, and in contrast to the 'interplay' model of Holmgren & Huston, which predicts an especially strong impact of drought in deep shade and in high irradiance, but a lesser impact in moderate shade.

Concerning to growth components, seedling RGR is the product of a ''physiological'' function, the NAR, and a morphological trait, the LAR. At the same time, LAR can be factored into the biomass allocated to the leaves, LMF, and the leaf area constructed with that biomass, SLA (Poorter & Remkes 1990). In our study, RGR was strongly related with NAR but no with LAR, suggesting that oak seedlings respond to light and water conditions by adjusting physiological activities more than modifying leaf morphology (Quero et al. 2006). The relative importance of LAR or NAR on determining plant RGR, can vary depending on the conditions of light (Shipley 2002), temperature (Loveys et al. 2002) or the time scale used (Villar et al. 2005).

RGR did not increase linearly with light (i.e. from 27 % to 100% of light there are not an increase in RGR). Sanchez et al. (2006) with 8 Mediterranean woody species found similar results. Although we found an increase in NAR with light intensity, there was a decrease in

LAR and SLA with light, which may explain the constant RGR under different light conditions.

Differences in leaf habit

The evergreen species had higher biomass than the deciduous ones. These results are opposite to commonly cited literature (Reich et al. 1992, Cornelissen et al 1996, Antúnez et al. 2001, Ruiz-Robleto & Villar 2005). However, there is a high variation in final biomass within each functional group (Fig. 1A). Although we studied only four con-generic species, categorical division of leaf habit can be questioned as it has already been at worldwide spectrum (Wright et al 2004).

Deciduous species have a higher SLA, but they invest lower biomass in leaves (and more to roots), which determine a similar LAR between deciduous and evergreens. Comparing to evergreen species, deciduous ones tend to growth faster (Antúnez et al., 2001, Reich et al. 1992, Cornelissen et al 1996, Ruiz-Robleto & Villar 2005) because they have higher stomatal conductance, photosynthetic and respiration rates, leaf nitrogen concentration and SLA (Reich et al., 1992, 1997; Villar et al., 1995; Takashima et al., 2004). However, comparisons taking into account the phylogeny, had found that in phylogenetically independent contrast there are not always a difference in RGR between deciduous and evergreens (Antúnez et al., 2001, Ruiz-Robleto & Villar 2005), as it has been found in our study.

Scaling up from leaf to whole plant level

As our findings, lack of correlation of an instantaneous maximum photosynthetic rate (A_{max}) and an integrative variable of growth as RGR is relatively frequent in literature (Poorter, Remkes & Lambers 1990, Sims et al. 1994). A_{max} is measured in individual leaves under saturating light conditions, but this saturating light conditions are not always met by the whole shoot. However, a more integrative estimate of carbon gain at leaf level was positively correlated with the relative growth rate (RGR) at whole-plant level. Similarly, (Montgomery 2004) found a positive correlation between Amax and RGR in three tropical woody species.

Taking into account that we only estimated the mean carbon gain by the leaf without considering the stem and root respiration, the correlation with RGR is very high, which can indicate the potential to predict seedling growth responses in diverse light and water treatments from leaf-level gas exchange measurements.

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Table 1. Results of two-way (individual species) and three-way (all species' data and leaf habit) ANCOVAs for final biomass, specific leaf area (SLA), leaf area ratio (LAR), leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) of 7-month-old seedlings (n=12-16) according to the light (L) and water (W) treatments and oak species (S) for all species' data. Initial acorn weight was used as covariable. The proportion of the explained variance (SS_x/ SS_{total}) and the level of significance (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$) for each factor and the interactions are indicated; those values not remaining significant after controlling the false discovery rate are underlined. R^2 is the proportion of total variance absorbed by the model.

Species	Factor	Final Biomass	SLA	LAR	LMF	SMF	RMF
Q. suber	Seed mass	3.8***	0.6	1.8**	2.4	1.0	0.0
	Light (L)	86.0***	87.0***	77.3***	15.2***	27.1***	18.8***
	Water (W)	1.2***	0.1	2.5**	14.9***	14.7***	21.1***
	L x W	1.3**	0.1	0.9	4.3	2.5	0.6
	R^2	92.3	87.7	82.5	36.9	45.3	40.5
Q. ilex	Seed mass	8.1***	1.1	0.4	0.3	1.0	0.0
	Light (L)	84.1***	77.2***	62.5***	7.2*	23.6***	4.9
	Water (W)	0.5*	0.1	3.0**	15.6***	2.3	12.3***
	L x W	0.4	0.1	1.6	8.0*	7.2*	18.0***
	R^2	93.1	78.5	67.5	31.0	34.1	35.2
Q. canariensis	Seed mass	10.8***	1.8*	1.8*	0.7	0.2	0.1
	Light (L)	63.7***	70.1***	59.8***	13.3**	21.5***	32.3***
	Water (W)	0.0	0.4	0.8	6.0*	0.0	2.9
	L x W	0.7	3.6**	3.9*	1.6	7.2*	3.0
	R^2	75.2	75.9	66.4	21.5	28.9	38.3
Q. pyrenaica	Seed mass	0.6	0.4	1.8	1.5	0.0	0.1
	Light (L)	77.9***	52.3***	47.0***	0.4	59.4***	43.0***
	Water (W)	3.4***	1.2	1.1	0.0	0.1	0.0
	L x W	0.4	0.3	1.3	3.3	0.1	1.4
	R^2	82.3	54.1	51.1	5.3	59.5	44.4
All species	Seed mass	3.7***	0.6**	1.1***	0.3	0.0	0.0
	Species (S)	7.3***	29.9***	14.5***	49.5***	9.7***	32.6***
	Light (L)	71.9***	48.2***	50.9***	1.8**	28.3***	12.9***
	Water (W)	0.5**	0.3*	0.5*	2.6***	1.1**	4.7***
	S x L	0.7*	0.8	1.6**	1.9*	5.3***	2.0*
	S x W	0.3	0.1	1.2**	1.2*	2.2**	2.3***
	L x W	0.6**	0.1	0.6*	0.7	1.8**	1.9***
	S x L x W	0.1	0.4	1.1	1.5	1.2	2.9*
	R^2	85.0	80.3	71.5	59.5	49.7	59.4
Leaf habit	Seed mass	24.3***	1.0**	10.2***	25.2***	0.8*	15.1***
	Leaf habit (H)	0.4**	19.3***	0.1	18.9***	5.9***	15.0***
	Light (L)	57.3***	47.0***	47.2***	1.8**	33.3***	12.5***
	Water (W)	0.4**	0.2	0.5*	1.8***	1.4**	3.7***
	H x L	0.3	0.5	0.9*	0.8	1.5*	1.0*
	H x W	0.1	0.1	0.6*	0.4	1.3**	1.3**
	L x W	0.4*	0.0	0.5	0.5	1.7**	1.7**
	H x L x W	0.0	0.3	0.1	1.0*	0.3	1.4*
	R^2	83.1	68.3	60.1	50.4	46.1	51.7

Table 2. Pearson's correlation coefficients between RGR and growth components for all species and light and water treatments. The level of significance (*: P<0.05; **: P<0.01; ***: P<0.001) is indicated; those values not remaining significant after controlling the false discovery rate (FDR) are underlined. Full name and units: relative growth rate (RGR, mg g^{-1} day^{-1}), net assimilation rate (g cm^{-2} day^{-1}), leaf area ratio (LAR, m^{2} kg^{-1}), specific leaf area (m^{2} kg^{-1}), leaf mass fraction (LMF, g g^{-1}), stem mass fraction (SMF, g g^{-1}), root mass fraction (RMF, g g^{-1}).

	NAR	LAR	SLA	LMF	SMF	RMF
RGR	0.85 ***	-0.35 ns	-0.64 ***	0.21 ns	-0.48 *	0.02 ns
NAR		-0.64 **	-0.68 ***	-0.11 ns	-0.65 **	0.32 ns
LAR			0.64 **	0.48 *	0.71 ***	-0.62 **
SLA				-0.31 ns	0.42 *	0.07 ns
LWR					0.56 **	-0.95 ***
SWR						-0.77 ***

Legends of the figures

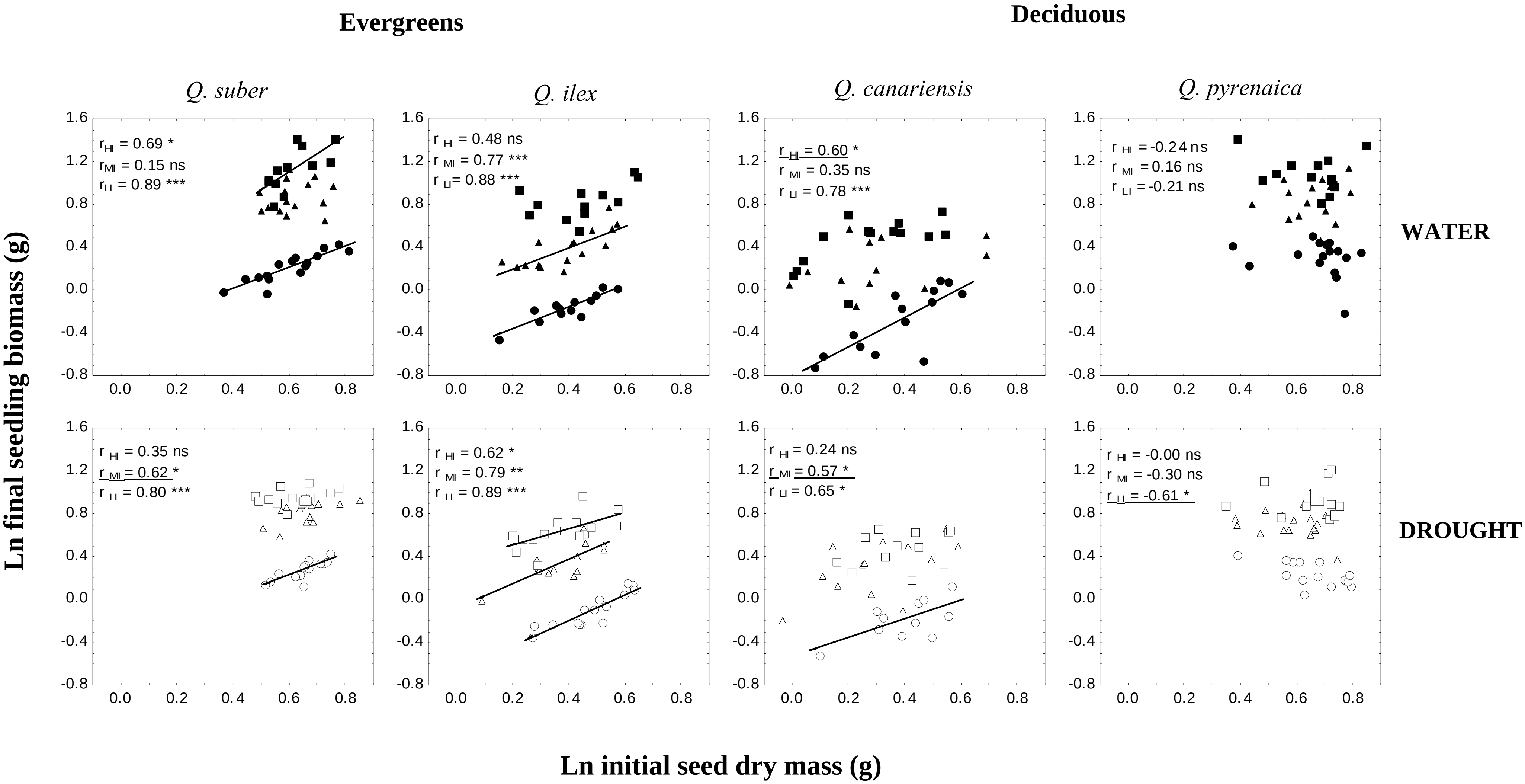
Figure 1: Final seedling biomass in relation to initial seed mass after seven months of growth for the four species. Squares, high irradiance treatment (HI); triangles, medium irradiance treatment (MI); circles, low irradiance treatment (LI). Values for seedlings grown under high-water conditions (closed symbols) are distinguished from those grown under low-water conditions (open symbols). The level of significance (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$) is indicated; those values not remaining significant after controlling the false discovery rate (FDR) are underlined.

Figure 2. Mean values and S.E. of (A) final seedling biomass (after seven months of growth), (B) RGR (relative growth rate), (C) NAR (net assimilation rate), (D) LAR (leaf area ratio) and (E) SLA (specific leaf area), under three irradiance levels: low (LI, 3 %), moderate (MI, 27 %) and full light (HI, 100 %), and two water treatments: continuously moist (closed symbol) or drought (open symbols), for seedlings of four oak species.

Figure 3. Mean values and S.E. of biomass allocation to leaves (A, LMF), stems (B, SMF), and roots (C, RMF), under three irradiance levels: low (LI), moderate (MI) and full light (HI), and two water treatments: continuously moist (closed symbol) or drought (open symbols).

Figure 4. Correlations between relative growth rate (RGR) and their components, RGR-NAR (A), RGR-LAR (B), RGR-SLA (C) and correlation between LMF-RMF (D). Squares, high irradiance treatment (HI); triangles, medium irradiance treatment (MI); circles, low irradiance treatment (LI). Values for seedlings grown under high-water conditions (closed symbols) are distinguished from those grown under low-water conditions (open symbols).

Figure 5. (A) Correlations between relative growth rate (RGR) and maximum photosynthetic rate (A_{max}; data reported by Quero et al. 2006). (B) Relationships between the seedling relative growth rates, for each oak species and light and water treatments, with the average carbon gain at leaf level, calculated using the light curve parameters, and the estimated total radiation reaching the leaf during the studied growth period. Mean and S.E. values are presented. Squares, high irradiance treatment (HI); triangles, medium irradiance treatment (MI); circles, low irradiance treatment (LI). Values for seedlings grown under high-water conditions (closed symbols) are distinguished from those grown under low-water conditions (open symbols).


Evergreens
Deciduous
Q. suber
Q. ilex
Q. canariensis
Q. pyrenaica
WATER
DROUGHT
Ln final seedling biomass (g)
Ln initial seed dry mass (g)
r HI = 0.69 *
r MI = 0.15 ns
r LI = 0.89 ***
r HI = 0.48 ns
r MI = 0.77 ***
r LI = 0.88 ***
r HI = 0.60 *
r MI = 0.35 ns
r LI = 0.78 ***
r HI = -0.24 ns
r MI = 0.16 ns
r LI = -0.21 ns
r HI = 0.35 ns
r MI = 0.62 *
r LI = 0.80 ***
r HI = 0.62 *
r MI = 0.79 **
r LI = 0.89 ***
r HI = 0.24 ns
r MI = 0.57 *
r LI = 0.65 *
r HI = -0.00 ns
r MI = -0.30 ns
r LI = -0.61 *
1.6
1.2
0.8
0.4
0.0
-0.4
-0.8
0.0
0.2
0.4
0.6
0.8

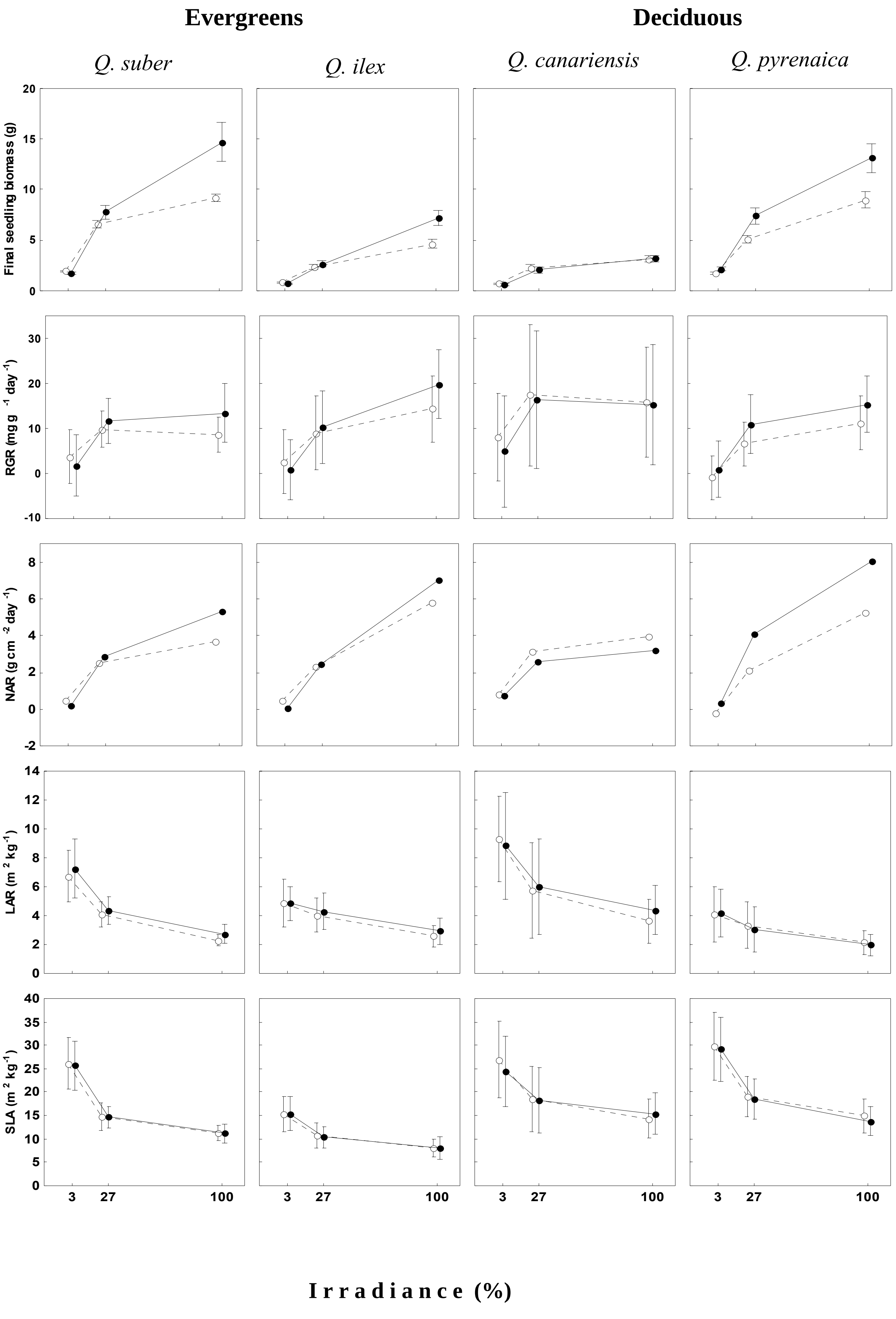

Evergreens
Deciduous
Q. suber
Q. ilex
Q. canariensis
Q. pyrenaica
Final seedling biomass (g)
0
5
10
15
20
RGR (mg g -1 day -1)
-10
0
10
20
30
NAR (g cm -2 day -1)
-2
0
2
4
6
8
LAR (m 2 kg-1)
0
2
4
6
8
10
12
14
SLA (m 2 kg-1)
0
5
10
15
20
25
30
35
40
3
27
100
Irradiance (%)

Figure 2

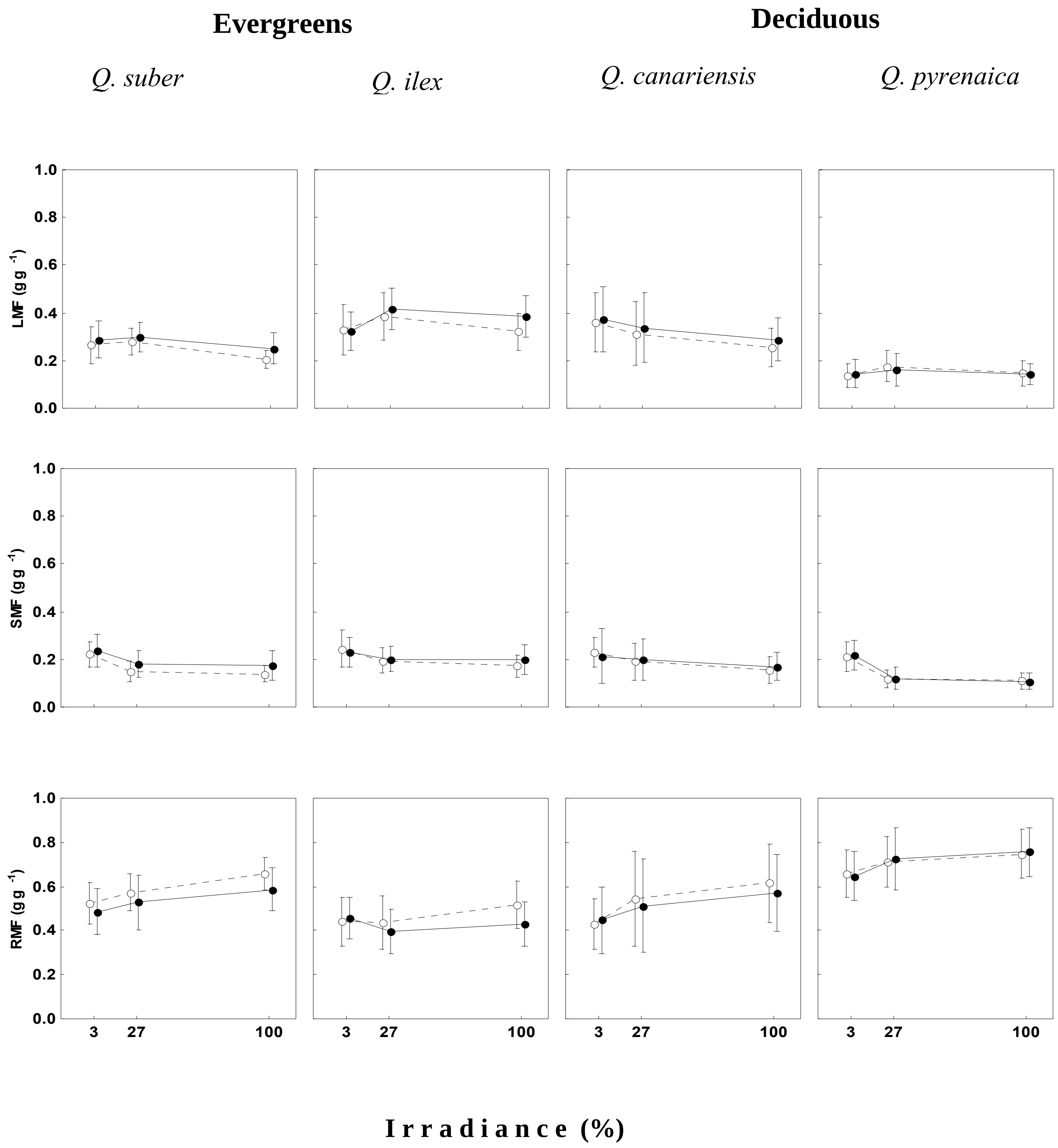

Evergreens
Deciduous
Q. suber
Q. ilex
Q. canariensis
Q. pyrenaica
LMF (g g -1)
SMF (g g -1)
RMF (g g -1)
0.0
0.2
0.4
0.6
0.8
1.0
3
27
100
I r r a d i a n c e (%)

Figure 3

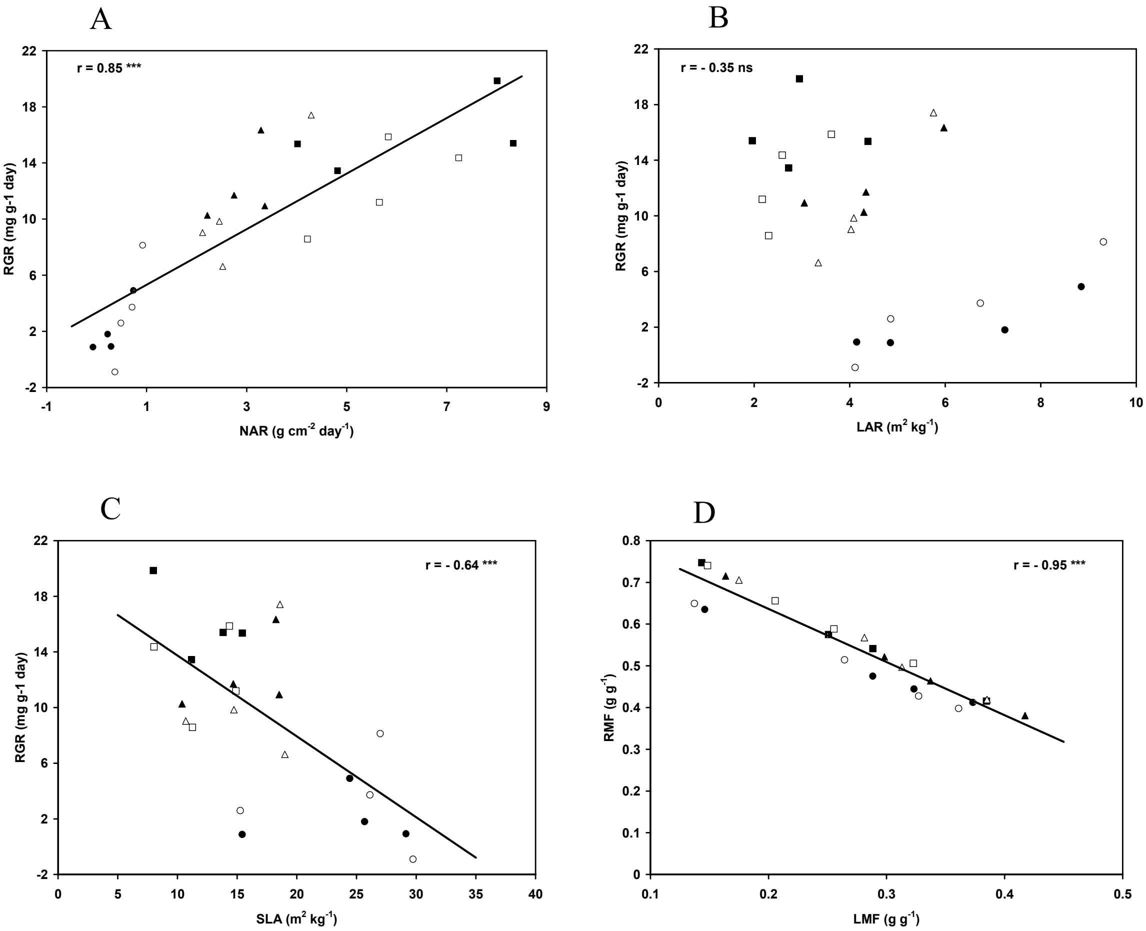

A
r = 0.85 ***
RGR (mg g-1 day)
NAR (g cm^{-2} day^{-1})
-2
2
6
10
14
18
22
-1
1
3
5
7
9
B
r = - 0.35 ns
RGR (mg g-1 day)
LAR (m^{2} kg^{-1})
0
2
4
6
8
10
C
r = - 0.64 ***
RGR (mg g-1 day)
SLA (m^{2} kg^{-1})
0
5
10
15
20
25
30
35
40
D
r = - 0.95 ***
RMF (g g^{-1})
LMF (g g^{-1})
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.1
0.2
0.3
0.4
0.5

Figure 4

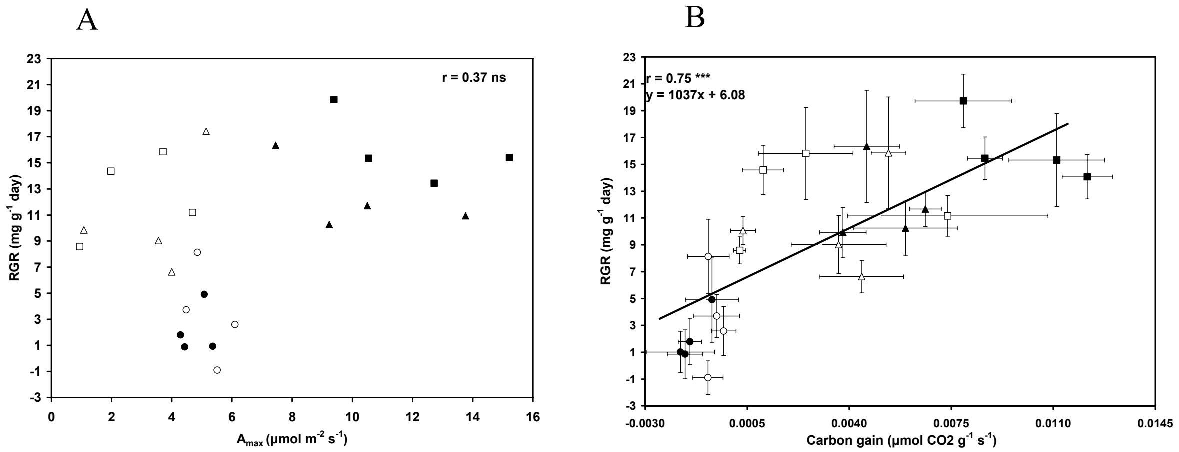

A
B
r = 0.37 ns
RGR (mg g-1 day)
23
21
19
17
15
13
11
9
7
5
3
1
-1
-3
0
2
4
6
8
10
12
14
16
Amax (µmol m-2 s-1)
r = 0.75 ***
y = 1037x + 6.08
RGR (mg g-1 day)
-0.0030
0.0005
0.0040
0.0075
0.0110
0.0145
Carbon gain (µmol CO2 g-1 s-1)

Figure 5

5. BLOQUE III: LOS FACTORES AMBIENTALES EN CONDICIONES NATURALES: EL NICHO DE REGENERACIÓN

Capitulo 6: (en inglés) Shifts in the regeneration niche of an endangered tree (*Acer opalus* ssp. *granatense*) during ontogeny: using an ecological concept for application

Shifts in the regeneration niche of an endangered tree (*Acer opalus* ssp. *granatense*) during ontogeny: using an ecological concept for application

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Number of words: 5,283.

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Summary

Most of our knowledge regarding ontogenetic niche shifts in plants has been derived from studies involving only two or unconnected life stages. Approaches covering a broader range of different life stages are still needed to fully understand the implications of ontogenetic niche shifts for plant regeneration dynamics. We investigated ontogenetic shifts in the endangered Mediterranean tree species *Acer opalus* ssp. *granatense* (*A. opalus*) comparing the environmental characteristics of individuals of different ages with those of a random sample of available microsites. In addition, since herbivory pressure could be a limiting factor, herbivory damage was quantified among stages. Differences on environmental characteristics between individual plants and randomly located points accentuated with age, suggesting that the regeneration niche of *A. opalus* shifts during ontogeny, undergoing a contraction. The presence of shrubs and adult trees, the depth of the litter layer, and herbivory pressure were the main factors influencing these changes. *A. opalus* reaches and germinates in all available microhabitats, but saplings establish and grow only in a subset of those available, which represent temporal changes in tolerance to extrinsic factors. Old saplings establish under the canopy of shrubs, far away from tree cover that could block light required in the oldest stage. Therefore, temporal changes in the nature and strength of plant–plant interactions are also occurring. The ecological concept of niche shifts determines the microsites with higher probabilities of long–term sapling survival of *A. opalus*: shrub cover involves an array of environmental changes crucial for the successful establishment of *A. opalus* saplings under stressful Mediterranean conditions, from microhabitat amelioration to herbivory protection.

Keywords: *Acer opalus* spp. *granatense*, Mediterranean mountain, ontogenetic shift, regeneration niche, tree regeneration.

Introduction

To a large extent, the abundance and distribution of species are determined during early regeneration stages, when seedlings and saplings are most vulnerable to their immediate environment (Kitajima & Fener, 2000; Zoladeski & Maycock, 1990). Thus, it is not surprising that the so–called “regeneration niche” (characteristics of the environment at the time of establishment; Grubb, 1977) is crucial for understanding the composition, structure, and dynamics of plant communities in a variety of environments (Silvertown, 2004). Recent studies suggest that the regeneration niche changes over the life cycle of individuals (Chase & Leibold, 2003; Miriti, 2006). These changes, known as ontogenetic niche shifts (Parrish & Bazzaz, 1985), have been widely explored in animal ecology (e.g. Amundsen, Bohn, Popova, Staldvik, Reshetnikov et al., 2003; Post, 2003). However, knowledge regarding ontogenetic niche shifts in plants is much more limited. The studies available have analysed only two life stages, often unconnected, such as seeds and seedlings (Schupp & Fuentes, 1995), seedlings and mature trees (Cavender–Bares & Bazzaz, 2000), or juveniles of different ages (Espelta, Riba & Retana, 1995). Approaches involving a broader range of different life stages are still needed to understand fully whether ontogenetic niche shifts drive the spatial distribution and successional dynamics of plant communities (Grubb, 1977).

Plants do not actively seek ideal habitats, but they experience distinct differences in tolerances to extrinsic factors at different developmental stages (Parrish & Bazzaz, 1985; Franco & Nobel 1989; Miriti, 2006), causing changes in niche dimension. A niche expansion occurs when species requirements are stricter in earlier stages, whereas a niche contraction would indicate that requirements are stricter in later stages of development.

Parrish & Bazzaz (1985) considered niche expansion the most common situation for plants, since species requirements were usually narrow at the germination stage and broader at the seedling and adult stages. Contrary to this prediction, niche contraction could limit the establishment of a plant by restricting it to a subset of environmental characteristics within the potential scenarios that can be occupied by the species.

The demographic responses of different stages to abiotic and biotic conditions will be expressed in different ways (e.g. differential mortality rates of seedlings, and differential growth or reproductive allocation of larger plants, Parrihs & Bazzaz, 1985; Miriti, 2006). Such ontogenetic constraints are often critical in understanding the whole dynamic of a plant population in which a particular stage needs specific condition not shared by other life stages. An alternative paradigm to the significance of ontogenetic changes is that shifting conditions alternatively favour positive or negative interactions among plants different in size (Callaway, 1995; Holmgren, Scheffer & Huston, 1997; Holzapfel & Mahall, 1999). Whatever the case, proper knowledge of ontogenetic niche changes is essential from both a basic and applied standpoint in order to increase our understanding of the processes underlying niche shifts as well as to establish appropriate management and restoration plans that consider the microsites where the species has higher probabilities of long–term survival.

The Mediterranean tree *Acer opalus* ssp. *granatense* (Boiss.) Font Quer and Rothm (hereafter *A. opalus*) is an ideal species to evaluate ontogenetic niche shifts because: (i) the wind dispersal of its seeds facilitates the spatial distribution and emergence of *A. opalus* seeds in a wide range of microhabitats (Gómez–Aparicio, Gómez & Zamora 2005a); (ii) its architectural attributes, with growth scars on the main stem, facilitate accurate age determinations of saplings (see Taylor & Aarsen, 1989 and Boerner & Brinkman, 1996 for *Acer saccharum* Marsh.). Furthermore, Mediterranean forests constitute an excellent model to analyse ontogenetic niche shifts, given that juveniles of woody species have a delayed growing period due to climatic stress (Zamora, Gómez, Hódar, Castro & García, 2001; Castro, Zamora, Hódar, Gómez & Gómez–Aparicio, 2004a; Castro, Zamora & Hódar 2006), and therefore the transition from seedling to established larger saplings is delayed in comparison to tropical or temperate forests.

Here, we report results from a study designed to evaluate whether *A. opalus* undergoes ontogenetic niche shifts, from seedlings to old saplings. Both the seed–dispersion and seedling–emergence demographic stages of *A. opalus* are strongly connected, because seedling spatial distribution is a mirror of seed–rain spatial distribution (Gómez–Aparicio et al., 2005a). Furthermore, old *A. opalus* saplings have a very high survival probability over time (Gómez–Aparicio, 2004), and hence the distribution and abundance of old saplings provide a representative sampling of future adult trees. For these reasons, our ontogenetic study of *A. opalus*, from seedling to established old saplings, offers an adequate picture of the entire life cycle in this species (from seed dispersal to adult stages). Specifically, we seek to answer the following questions: (i) Do ontogenetic niche shifts occur in *A. opalus*? (ii) If so, do they reflect niche expansion or contraction among stages (*sensu* Eriksson, 2002)? (iii) What variables drive such shifts? For the answers, we followed the “plant’s eye–view” approach (Turkington & Harper, 1979), conducting a multivariate characterization of the environment in the immediate vicinity of individuals of different ages.

Materials and methods

Natural history and study site

A. opalus is an endemic Iberian–Mauritanian species with a patchy geographical distribution within the Iberian Peninsula (Gómez–Aparicio, Zamora & Gómez, 2005c). Its populations appear usually in areas where Mediterranean summer drought is ameliorated, such as in north–facing slopes, in shady ravines or in the surroundings of riverbanks (Costa, Morla & Sainz, 1998). It has been catalogued as "vulnerable" by the IUCN (2000) and is included in the recent Red List of Threatened Vascular Plants of Andalusia (Blanca, Cabezudo & Hernández–Bermejo, 2000). Seed dispersal spans September to December and seedlings emerge during the next spring (April–June).

The study was conducted during spring 2004 in the Sierra Nevada National Park (Barranco del Espinar, 1700 to 1950 m. a. s. l.; 37º06'N, 3º21'W; Granada, SE Spain). The vegetation of this native mixed mountain forest is dominated by trees (*Pinus sylvestris* L. var. *nevadensis* Christ., *Acer opalus* ssp. *granatense* and *Taxus baccata* L.), with a dense shrub understorey dominated by fleshy–fruited shrubs (*Juniperus communis* L., *Juniperus sabina* L. and *Berberis hispanica* Boiss. and Reut.) and non–fleshy–fruited (*Ononis aragonensis* Asso., *Genista versicolor* Boiss. and *Erinacea anthyllis* Link.) shrubs (see García, Zamora, Hódar, Gómez & Castro, 2000 for more information). The climate is typical of Mediterranean mountains: mean annual rainfall of 879 mm (average 1990–2003) with hot, dry summers and cold, snowy winters. The bedrock is calcareous and soils are regosols and cambisols (Delgado, Delgado, Párraga, Gámiz, Sánchez et al., 1989).

Field sampling

For this study, *A. opalus* individuals were grouped in 3 age classes: "seedlings", that emerged in the spring of the study, still with cotyledons; "young saplings" (from 2 to 5 years old), and "old saplings" (from 6 to 10 years old). Average height (means ± SE) was 3.5 ± 0.15, 6.5 ± 0.35, and 32.9 ± 18.4 cm for seedlings, young saplings, and old saplings, respectively. Age was determined by counting growth scars on the main stem (Gómez–Aparicio et al., 2005c). *A. opalus* individuals were sampled using twelve 25–m transects randomly distributed throughout the study site. In each transect, random points were generated (in order to compare them to those points with *A. opalus* individuals) by using haphazard number lists of orientations (0–359º) and distances (0–7 m) every 5 m (at meters 0, 5, 10, 15, 20 of the transect for seedlings, meters 1, 6, 11, 16, 21 for young saplings, and meters 2, 7, 12, 17, 22 for old saplings). Plants were selected for analysis by locating the nearest *A. opalus* seedling or sapling to each of these random points. Sampling did not have spatial dependence since distances between individuals and random points did not differ among age classes (ANOVA, $F_{2\ 177} = 1.65$, $P = 0.177$). A total of 60 random points and 60 *A. opalus* individuals per age class were selected for measurements, resulting in 360 points measured.

At each sampled point (with and without plants), the following environmental variables were measured in a circular plot 0.30 m in diameter, for seedlings and young saplings, or 0.50 m for old saplings, according to class size: 1) average soil compaction, 2) depth of the maximum soil–compaction value , 3) light availability, 4) distance to the nearest *A. opalus* mature tree, 5) depth of the litter layer, 6) number of woody species present, 7) cover of herbaceous species, 8) moss cover, 9) stone cover, 10) tree cover and

11) shrub cover. Soil compaction was measured using a Penetrologger penetrometer (Eijkelcamp, Giesbeek, The Netherlands). This device provides a profile describing the variation of soil compaction with depth at each point sampled. From these profiles, two variables relevant for rooting capacity, and thus for seedling establishment (Gómez–Aparicio, Valladares, Zamora & Quero, 2005b), were determined: the average compaction over the profile (MPa), and the depth of maximum compaction (cm). Light availability (hereafter, GSF) was quantified with hemispherical photography. Photographs were taken at each sample point at ground level using a horizontally levelled digital camera (CoolPix 5000, Nikon, Tokyo, Japan) and aimed at the zenith, using a fish–eye lens of 180º field of view (FCE8, Nikon). To ensure homogeneous illumination of the canopy and a correct contrast between canopy and sky, all photographs were taken before sunrise, after sunset, or during cloudy days. The images were analysed using Hemiview canopy analysis software version 2.1 (1999, delta–T Devices Ltd, Cambridge, United Kingdom). The software estimates the Global site factor (GSF), defined as the proportion of diffuse and direct radiation for clear–sky conditions at our study site (Rich, 1990). GSF is a continuous variable ranging from 1 (open sky) to 0 (complete obstruction). The distance to the nearest adult *A. opalus* tree was measured with a laser distance–meter (model Disto lite, Leica Geosystem, Heerbrugg, Switzerland). The depth of the litter layer was measured by inserting a metal ruler down to the soil surface. The different percentages of cover were visually estimated by dividing the circular plot into four hypothetical sections to ensure more accurate measurements. Since *A. opalus* can undergo increasing risk of herbivory with age (Gómez–Aparicio et al. 2005c), yearly herbivore damage was recorded in each individual and age class as the percentage of

consumed twigs. Because herbivory damage was marginal in seedlings and young saplings (2 individuals out of every 60 in each age class), we explored differences in herbivory among microsites only in old saplings.

Data analysis

To evaluate whether the environmental variables measured differed between age classes (seedlings, young saplings and old saplings) and point type (with and without *A. opalus* individuals), the semi–parametric multivariate analysis of variance approach of Anderson (2001) was used (hereafter PERMANOVA). This approach allows the testing of the simultaneous responses of a multivariate dataset to one or more factors in an ANOVA experimental design on the basis of any distance measure using permutation methods (see Anderson, 2001 and McArdle & Anderson, 2001 for details). In addition to PERMANOVA analyses, we conducted a canonical analysis of principal coordinates (PCO) to identify the particular environmental variables responsible for the multivariate patterns observed (see Anderson & Willis, 2003 for a detailed account of the method). In brief, this method performs a principal–coordinate analysis of a multivariate matrix using a dissimilarity measure of choice to provide a series of m orthonormal axes. A canonical discriminate analysis is then conducted on the first m axes. The canonical axes resulting from this ordination are derived from linear combinations of variables that maximize the differences between the different experimental groups (in our case the 6 groups resulting from each combination of point type and age class). The first two axes were correlated with the environmental variables measured using the Spearman correlation coefficient; variables with highest absolute correlations will likely determine the differences between points with and without *A. opalus* individuals, as well as between microsites occupied by individuals of different age classes. Bonferroni correction was applied to be conservative in detection of significance from multiple pair–wise correlation analyses. PERMANOVA and PCO analyses were performed using the programs PERMANOVA 1.6 (Anderson, 2005) and CAP (Anderson, 2004), respectively (both can be freely downloaded from http://www.stat.auckland.ac.nz/~mja/Programs.htm). For these analyses, we used the Bray–Curtis distance (appropriate for the datasets containing a miscellaneous mixture of variables and numerous zeros; Quinn & Keough, 2002) and 10000 permutations (permutation of raw data; Anderson & Ter Braak, 2003). Correlation analyses were conducted with the program SPSS 10.0 for Windows (SPSS Inc, Chicago, IL, USA).

To evaluate whether herbivory damage in old saplings differed among microsites, a one–way ANOVA was used. Microsites were grouped into four categories according to predominant cover: tree, palatable shrub (*Ononis aragonensis*), non–palatable shrub (*Juniperus* spp. and *Berberis hispanica*) and open. Palatability criteria were based on Baraza, Zamora & Hódar (2006).

Results

The environmental characteristics of the locations occupied by seedlings and saplings differed from those of the randomly located points (PERMANOVA, F = 3.30, $P = 0.0043$; Table 1). Moreover, there were significant differences among age classes (PERMANOVA, F = 4.49, $P = 0.001$). A marginally significant point type × age interaction was also found (PERMANOVA, F = 1.68, $P = 0.0711$). This interaction was explained by the fact that the environmental characteristics of the random points did not differ among age classes, but those of the points containing seedlings/saplings did (Table 2A). Differences in the environmental characteristics of points with and without plants increased with age, from non–significant in seedlings to highly significant in old saplings (Table 2B).

The first two PCO axes explained over 50% of the variation observed in the data, and clearly separated the points with and without *A. opalus* individuals, as well as those occupied by seedlings from those occupied by saplings (Fig. 1). Most environmental variables measured were correlated with the first two PCO axes (Table 3). As large sample sizes (our case, $n = 360$) are more likely to produce statistically significant results (Chow, 1988), we emphasize only those variables with correlation coefficients (ρ) ≥ 0.6. The first axis of this ordination was positively correlated with tree cover, and negatively with shrub cover (Table 3). The second axis was positively correlated with the depth of litter layer. These results suggest a gradient along axis 1, where the environment of seedlings is characterized by higher tree cover and lower shrub cover than that of young and old saplings, in this order. Axis 2 determined a gradient from decreased depth of litter layer in seedlings as compared to that found in young and old saplings (Fig. 1).

Herbivory damage in old saplings differed among microsites ($F_{3, 56} = 3.97$, $P < 0.05$) Saplings growing among non–palatable shrubs sustained less damage than those growing in open areas or under trees (Fig. 2).

Discussion

Three questions regarding the regeneration niche of the endangered tree *Acer opalus* ssp. *granatense* were considered: whether ontogenetic shifts occur, whether these changes showed expansion or contraction, and what variables drive these shifts. Our results suggest that niche shifts among the different demographic stages studied are occurring (Table 2A). In addition, we found differences in microsites between individual plants and randomly located points, which accentuated with age (Table 2B). The environment surrounding seedlings did not differ from that of random points, in agreement with previous results indicating that *A. opalus* seeds arrive and germinate in a wide array of microsites (Gómez–Aparicio et al. 2005a). However, microsites of *A. opalus* saplings were characterized by higher shrub and lower tree cover than those of randomly located points, especially in the oldest age class. Furthermore, old saplings underwent differential damage by herbivores, depending on the growing microhabitats (Fig. 2). All of these findings suggest that a contraction of the regeneration niche is taking place in *A. opalus* (i.e., niche requirements become more restrictive with age). Tree cover, shrub cover and litter depth, together with herbivory, were the principal factors governing these changes (Table 3, Fig. 2).

Concerning niche breadth, we found that differences between individuals plants and randomly located points accentuated with age (Table 2B), suggesting that the regeneration niche of *A. opalus* shifts during ontogeny, undergoing a contraction (i.e., niche requirements become more restrictive with sapling age). Wind–dispersed seeds of *A. opalus* reach and germinate in all available microhabitats, but saplings establish and grow only in a subset of those available. These results correspond to ontogenetic shifts in tolerance to extrinsic factors and/or resource use, and agree with previous studies showing that conditions for successful establishment are more stringent than those for successful germination (Turnbull, Crawley & Rees, 2000). A narrowing of niche through ontogeny can be considered a critical component of spatiotemporal dynamics (Miriti, 2006) acting as an ecological restriction on the observed abundance patterns of *A. opalus* populations.

Microsites occupied by *A. opalus* saplings were characterized by low tree and high shrub cover, especially in the oldest age class. Therefore, the regeneration niche of *A. opalus* occurs under the canopy of shrubs, far away from adult trees, a pattern found for most woody species in Mediterranean environments (Castro, Zamora, Hódar & Gómez, 2004b; Gómez–Aparicio, Zamora, Gómez, Hódar, Castro et al., 2004; Rey & Alcántara, 2000). Negative effects of adult trees on the establishment of woody plants have been previously reported (e.g., Maestre, Cortina, Bautista & Bellot, 2003; Li & Ma, 2003), suggesting an increase in asymmetrical competitive interactions between saplings (the weaker competitors) and the adult trees (the stronger competitors) with age for resources (Lorimer, 1984; Buckley, Sharik & Isebrands, 1998). However, these effects are species–specific (Reinhart, Maestre & Callaway, 2006) and depend on environmental conditions (Callaway, 1998, Maestre, Cortina & Bautista, 2004). The high shrub cover found in areas occupied by older individuals (Table 1), and the strong (and negative) correlation between this variable and the first PCO axis (Table 3), suggest that shrub cover is a crucial factor to ensure the establishment of *A. opalus* saplings (Gómez–Aparicio et al. 2005c). It is noteworthy that none of the individual abiotic factors was better than shrub cover for predicting *A. opalus* recruitment patterns. Furthermore, a protective effect of non–palatable, spiny shrubs against herbivory was discerned (Fig. 2, see also Baraza et al. 2006). These results were presumably because shrubs involve an array of environmental changes crucial for the success establishment of *A. opalus* saplings under stressful Mediterranean conditions, from microhabitat amelioration to herbivory protection. In this context, we propose a conceptual scheme that shows the principal factors driving the ontogenetic niche shifts of *A. opalus* (Fig. 3). After seed dispersal, there are no critical conditions for seed germination or early seedling establishment (Gómez–Aparicio et al. 2005a). However, in later stages, factors emerge to impose more restrictive conditions for establishment: i) deeper litter could favour soil–moisture retention, ii) shrub cover favours plant performance by: 1) alleviating stressful Mediterranean summer drought conditions for seedlings and young saplings and, 2) if shrubs are non–palatable and spiny, protecting old saplings from herbivory; iii) lastly, the tree cover could block light required in the oldest stage. These results indicate an ontogenetic shift in plant–plant interactions. Saplings of *A. opalus* experienced a positive interaction from shrubs (facilitation), but a negative interaction from adult trees (asymmetrical competition).

Ontogenetic niche shifts constitute an ecological concept largely unexplored when restoring degraded populations and ecosystems (Young, Petersen & Clary, 2005). We provide new empirical evidence that can be applied to optimise management and restoration programmes involving woody species. *A. opalus* populations in Mediterranean mountains show remnant dynamics (*sensu* Eriksson, 1996), and small populations are maintained by the longevity of a few old individuals (Gómez–Aparicio et al., 2005c). Effective restoration activities may broaden the regeneration niche through assisted establishment, since it is strongly needed to ensure viable populations of this species in many places (Gómez–Aparicio et al., 2004). This can be achieved by planting seedlings or sowing seeds of this tree in areas with the environmental characteristics of the microsites occupied by *A. opalus* saplings older than 5 years, i.e. selecting for planting or sowing microhabitats with a high non–palatable shrub cover and a deep litter layer, in addition to avoiding both bare ground soil and dense tree cover. These results reinforce the common pattern of nurse effects of shrubs observed in Mediterranean environments (Castro et al., 2004a; Gómez–Aparicio et al., 2004).

In summary, the spatial pattern of *A. opalus* establishment in the long term is not random, but limited to a small fraction of all microsites available. This pattern shifts ontogenetically, with the presence of shrubs and adult trees as well as the depth of the litter layer, together with herbivore pressure, as the main factors influencing this change. These niche characteristics are a subset of the overall suitable microsites for seed germination and seedling establishment (Fig. 3). In our study, ontogenetic shifts represent temporal changes both in tolerance to extrinsic factors and in the nature and strength of plant–plant interactions. As a result, *A. opalus* population fate is strongly dependent on availability of neighboring shrubs.

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Table 1. Values of the environmental variables measured across age classes (seedlings = 1 year old; young saplings = from 2 to 5 years old; and old saplings = from 6 to 10 years old) and point type (with and without *A. opalus* individuals) levels. Data represent means ± SE ($n = 60$).

	Units	Seedlings		Young saplings		Old saplings	
		With plants	Without plants	With plants	Without plants	With plants	Without plants
Average compaction	MPa	141.8 ± 8.9	122.4 ± 8.9	141.9 ± 10.7	141.8 ± 9.3	149.4 ± 8.8	132.4 ± 9.7
Depth of maximum compaction	cm	20.6 ± 1.3	23.3 ± 1.4	25.1 ± 1.3	22.7 ± 1.3	24.2 ± 1.4	23.4 ± 1.6
Global site factor	unitless	0.289 ± 0.025	0.340 ± 0.030	0.253 ± 0.022	0.325 ± 0.029	0.326 ± 0.027	0.345 ± 0.029
Herbaceous cover	%	10.6 ± 2.4	10.8 ± 2.8	12.5 ± 2.8	12.4 ± 2.7	10.4 ± 2.5	8.8 ± 2.7
Litter depth	mm	16.5 ± 1.4	17.2 ± 1.6	23.1 ± 1.5	16.9 ± 1.8	27.4 ± 1.9	18.8 ± 2.3
Moss cover	%	3.3 ± 1.5	5.2 ± 1.8	10.3 ± 3.0	4.9 ± 2.6	10.8 ± 3.0	5.4 ± 2.8
Distance to the nearest conespecific adult	m	8.5 ± 0.8	12.3 ± 1.2	10.9 ± 0.8	10.9 ± 0.8	11.3 ± 1.0	10.3 ± 0.8
Shrub cover	%	31.7 ± 4.9	40.6 ± 5.7	62.7 ± 5.4	46.0 ± 5.9	61.8 ± 4.8	43.1 ± 4.9
Stone cover	%	14.2 ± 2.9	11.7 ± 2.3	12.2 ± 2.4	13.4 ± 2.8	16.3 ± 2.9	20.6 ± 3.5
Tree cover	%	47.2 ± 6.4	51.5 ± 6.4	35.3 ± 6.2	36.5 ± 6.2	25.3 ± 5.5	38.2 ± 6.2
Number of individual of woody species	–	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	0.9 ± 0.2	2.2 ± 0.2	1.6 ± 0.2

Table 2. Pair–wise *a–posteriori* comparisons of the environmental characteristics (from Table 1) after a two–way, semi–parametric, MANOVA (PERMANOVA). A) Randomly located points and points containing an individual plant. B) Age classes (seedlings = 1 year old; young saplings = from 2 to 5 years old; and old saplings = from 6 to 10 years old). Points as follows: 1 = with an *A. opalus* individual; and 2 = randomly located (without an *A. opalus* individual).

A)

	Age class	t	*P* (perm)
Random located points	seedlings *vs*. young saplings	0.8374	0.6299
	seedlings *vs*. old saplings	1.1918	0.2105
	young saplings *vs*. old saplings	1.1624	0.2434
Individual plants	seedlings *vs*. young saplings	2.1229	0.0007
	seedlings *vs*. old saplings	2.8987	0.0001
	young saplings *vs*. old saplings	1.7868	0.0055

B)

	Points	t	*P* (perm)
Seedlings	1, 2	1.308	0.1283
Young saplings	1, 2	1.474	0.0560
Old saplings	1, 2	1.688	0.0117

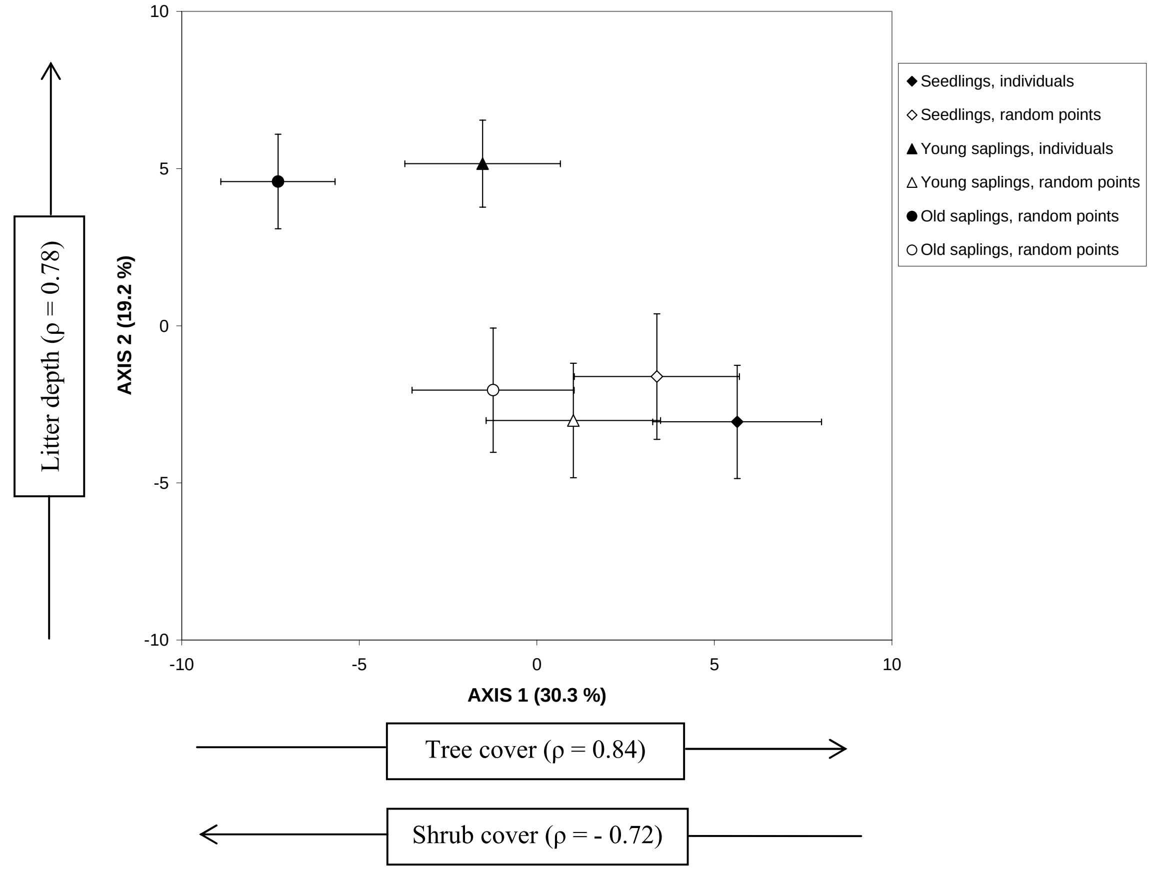
Table 3. Correlation matrix between the environmental variables evaluated and the two first axes from a Principal Coordinate Analysis. *P* values ≥ 0.0022 are not significant according to Bonferroni multiple comparison test.

	AXIS 1		AXIS 2	
Variables	Spearman's Rho	*P*–values	Spearman's Rho	*P*–values
Average compaction	0.035	0.502	-0.238	<0.001
Depth of maximum compaction	-0.147	<0.01	0.290	<0.001
Global site factor	-0.553	<0.001	-0.548	<0.001
Herbaceous cover	-0.097	0.065	-0.124	0.018
Litter depth	-0.074	0.161	0.780	<0.001
Moss cover	-0.263	<0.001	0.397	<0.001
Distance to the nearest conespecific adult	-0.338	<0.001	-0.173	<0.001
Shrub cover	-0.715	<0.001	0.441	<0.001
Stone cover	-0.133	0.011	-0.356	<0.001
Tree cover	0.841	<0.001	0.105	0.045
Number of individual of woody species	-0.500	<0.001	0.259	<0.001

Figure 1. Principal Coordinate Analysis of the environmental variables characterizing the sampling points with and without *Acer opalus* ssp. *granatense*. Age classes as follows: seedlings = 1 year old; young saplings = from 2 to 5 years old; and old saplings = from 6 to 10 years old. Data represent means ± SE ($n = 60$).

Figure 2. Herbivory damage in *Acer opalus* ssp. *granatense* old saplings (from 6 to 10 years–old), estimated as the percentage of consumed twigs, in different microhabitats according to predominate cover. Non–palatable shrubs (*Juniperus* and *Berberis*) are the dominant shrub species in the study area (Garcia et al. 2000). Palatability criteria were based on Baraza et al. (2006). Columns and error bars show means ± SE. Parenthetical numbers indicate sample size in each microhabitat (n = 60 as whole). Different letters show significant differences among microhabitats at $P < 0.05$ (HSD Tukey test for unequal n).

Figure 3. Conceptual scheme showing the principal factors driving the regeneration niche of *Acer opalus* ssp. *granatense* through ontogeny. Grey line indicate *A. opalus* niche contraction. Depth of litter layer is not critical during the seedling stage, but deeper litter, such as that generated by shrubs, could favour soil– moisture retention. Saplings are more affected by tree cover than seedlings because trees could block the light required in older stages. Shrub cover involves an array of critical environmental changes (amelioration and herbivory protection) for the successful establishment of *A. opalus* saplings under stressful Mediterranean conditions.



10
5
0
-5
-10
-10
-5
0
5
10
AXIS 1 (30.3 %)
AXIS 2 (19.2 %)
Litter depth (ρ = 0.78)
Tree cover (ρ = 0.84)
Shrub cover (ρ = - 0.72)
◆ Seedlings, individuals
◇ Seedlings, random points
▲ Young saplings, individuals
△ Young saplings, random points
● Old saplings, random points
○ Old saplings, random points

Figure 1

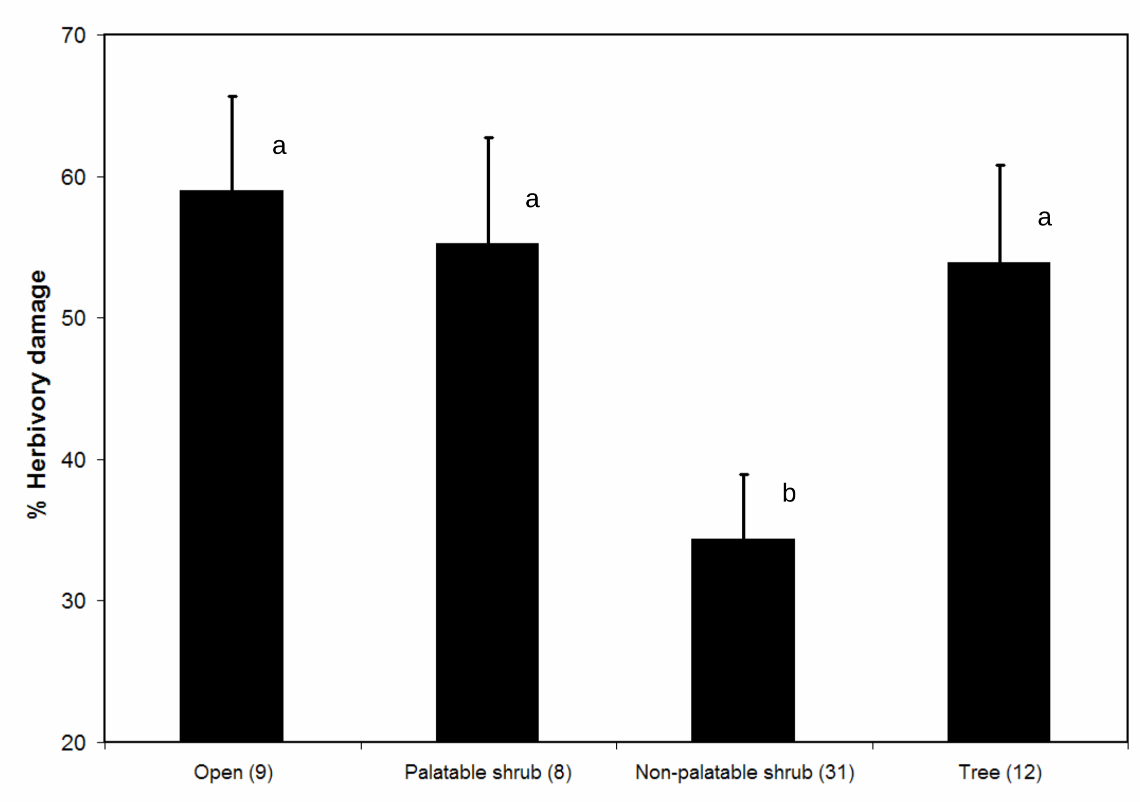
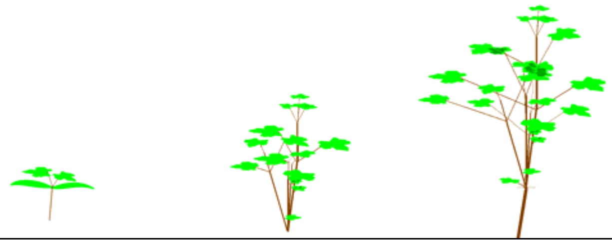
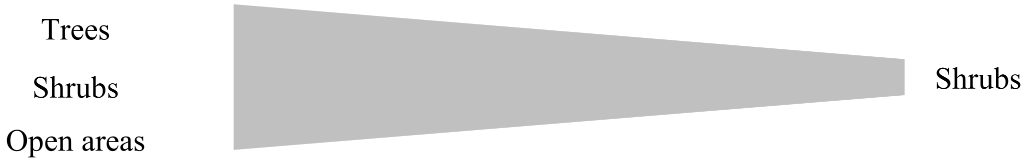

% Herbivory damage
70
60
50
40
30
20
a
a
b
a
Open (9)
Palatable shrub (8)
Non-palatable shrub (31)
Tree (12)

Figure 2



Class	Seedlings	Young saplings	Old saplings
Age (years)	1	2–5	6–10



Driving factors			
	_	Depth of litter layer	Depth of litter layer
	_	Tree cover	Tree cover
	_	Shrub cover	Shrub cover
	_	–	Herbivory pressure

Figure 3

6. DISCUSIÓN GENERAL

Los dos primeros capítulos de la presente memoria ponen de manifiesto la importancia de la heterogeneidad espacial para la supervivencia de las plantas en zonas de montaña mediterránea. Evidencias de heterogeneidad espacial a pequeña escala han sido encontradas en otros ambientes mediterráneos como el semiárido (Maestre et al. 2003) y dehesas (Gallardo et al. 2000), sin embargo, el papel de la heterogeneidad no ha sido explorado en zonas de mayor altitud y precipitación como las de este estudio. Los patrones espaciales de las variables estudiadas revelaron que el ambiente en el que una plántula puede potencialmente establecerse varía en el espacio a una escala de metros, que se agrupan en manchas y claros y que las variables covarían entre ellas, lo que genera un amplio abanico de micrositios. Sin embargo, de la diferente heterogeneidad de cada una de las unidades de paisaje estudiadas se desprende que la gama de micrositios fue reducida en el matorral pionero, una zona principalmente delimitada por manchas de vegetación leñosa y suelo desnudo. Por el contrario, el bosque autóctono y las repoblación respondieron a unas características mucho más complejas desde el punto de vista estructural: estrato arbóreo, estrato arbustivo, manchas de vegetación herbácea, restos de ramas, lo que genera que la gama de micrositios sea más compleja. La combinación de variables, así como su posición en el espacio, llega a determinar la supervivencia en los primeros meses de crecimiento de una plántula. Una vez la semilla ha llegado a uno de estos lugares y tras superar riesgos potenciales como la depredación pre- y post-dispersiva, comienza en el ciclo de este individuo una fase muy dependiente de las condiciones que inmediatamente le rodean (Harper 1977), por ejemplo, desde que la raíz inicia el crecimiento, la compactación del suelo (Verdú & García-Fayos et al 1996) ya determina que la plántula pueda explorar horizontes más profundos para capturar más agua cuando llegue la época seca (Lloret et al 1999). Como ya hemos visto, estos micrositios son un compendio de variables que se agregan en el espacio e interactúan de manera compleja generando o no, las condiciones óptimas para el establecimiento. De cara a la gestión del bosque, será todo un reto la detección de micrositios favorables para las plántulas, previamente a la ejecución de siembras y plantaciones y alejarse del manejo tradicional basado en el uso de maquinaria y plantaciones regulares.

En este primer bloque se ha utilizado como metodología básica en Ecología, el estudio de campo (capítulo 1). Esta aproximación nos ha revelado que ciertas variables pueden ser el agente causal de los patrones espaciales de supervivencia observados. Aunque los datos sugieren que la luz y el agua son los factores que mas han influido en la supervivencia, con la observación en campo, no puede probarse. Podría ocurrir que otros factores que varían con la disponibilidad de luz y agua, sean realmente los responsables de la relación observada. Es por ello que en el segundo bloque de la tesis, se utilizó una aproximación metodológica distinta, el experimento en condiciones controladas. Al diseñar este tipo de experimentos se intentan aislar los presuntos agentes causales, de cara a evitar posibles interferencias con otros factores como puede ocurrir en el campo, con lo que las evidencias encontradas son lo suficientemente sólidas como para validar las hipótesis o los postulados que se planteen. Así, los tres capítulos siguientes de la presente memoria, engloban un experimento en el que se aisló por un lado el factor luz (capítulo 3) y por otro, los factores luz y agua en diferentes combinaciones de los mismos, (capítulos 4 y 5). Incluso en estos experimentos de condiciones controladas, hay factores intrínsecos de difícil control real, aunque no estadístico. Tal era el caso referente al efecto del peso de semilla en las primeras fases de crecimiento de las especies estudiadas bajo diferentes condiciones de luz. En el capítulo 3, el peso de semilla fue utilizado como un factor más que, cruzado con los tratamientos de luz, generó unos resultados muy interesantes. La influencia del tamaño de semilla ha sido un tema muy tratado en la literatura científica sobre todo por el grupo de investigación australiano del Prof. Westoby (Leishman & Westoby 1994, Saverimuttu and Westoby 1996, Westoby et al 1996, Wright and Westoby, 1999) derivándose de éstos y otros trabajos las hipótesis planteadas en este capítulo, que explican la relación existente entre el peso de la semilla y su capacidad de contribuir al desarrollo de la plántula. A pesar de la enorme contribución en este campo, la mayoría de estos trabajos se han centrado en relaciones ínter-específicas con conclusiones de carácter filogenético y evolutivo, dejando a un lado las relaciones dentro de especie, que pueden ser de gran interés a la hora de evaluar el significado funcional del peso de semilla. Es por tanto un aspecto mucho menos estudiado y que unido al efecto de los distintos tratamientos de luz, el estudio adquiere un aspecto novedoso. El resultado principal obtenido en este estudio fue que las semillas más grandes determinaba plántulas con una mayor biomasa, aunque esta relación varío de acuerdo la disponibilidad de luz. En concreto, bajo sombra profunda, las correlaciones entre el peso de semilla y la biomasa de plántula fueron más altas. En el caso de las especies mediterráneas de *Quercus* estudiadas, cuyas plántulas viven en ambientes extremos o con una alta incidencia de perturbaciones, las bellotas más grandes le conferirán independencia frente a estos ambientes (Puerta-Piñero et al. 2006, Metcalfe and Grubb 1997, Lloret et al. 1999). De este planteamiento se desprende de inmediato la siguiente cuestión: entonces, ¿por qué existe tanta variación intra-específica dentro del género *Quercus*? Las ventajas de tener una bellota grande se asumen una vez salvada la depredación posdispersiva. Gómez (2004) alude a esta cuestión con la expresión “bigger is not always better”, es decir, las bellotas grandes en el contexto del ciclo de vida de una especie tiene más probabilidad de ser depredadas, por tanto, pueden existir presiones selectivas opuestas actuando sobre el tamaño de la semilla: negativa, durante la fase posdispersiva (mayor éxito de las semillas pequeñas) y positiva en la fase de establecimiento (mayor éxito de las plántulas emergidas de semillas grandes). Futuras investigaciones, por tanto, deberían profundizar en las probabilidades de transición de una fase demográfica a otra para calcular con exactitud las ventajas y desventajas de ser una semilla grande o pequeña.

Los **capítulos 4 y 5**, sin alejarse de otras cuestiones, se han centrado en los efectos interactivos de la luz y el agua sobre los mecanismos fisiológicos y morfológicos a nivel de hoja y plántula completa. La interacción entre estos factores es un fenómeno complejo sobre el que no existe una respuesta unificadora, incluso dentro del escenario mediterráneo, concerniente a sus efectos en las plántulas (Sánchez-Gómez 2006). Nuestros resultados a nivel foliar (capítulo 4) mostraron un fuerte efecto interactivo y positivo de la luz y el agua, promovido por un efecto beneficioso de la sombra en condiciones de sequía. Por el contrario, la interacción luz y agua a nivel de planta completa (capítulo 5) tuvo menor intensidad, por lo que los resultados de la interacción luz y agua, como otros autores han apuntado (Sánchez-Gómez et al. 2006), principalmente dependieron de la variable respuesta analizada. Por otra parte, es de destacar que tras 7 meses de crecimiento todavía hubo un efecto del peso de la semilla, como ya se demostró en el capítulo 3 (pero en los primeros 50 días de crecimiento). Otros autores han mostrado que los efectos de la semilla tienen entre 1 y 4 años de permanencia (Poorer & Rose 2005), aunque la intensidad del efecto irá disminuyendo con el tiempo.

Por otra parte, en el capítulo 5 se ha tratado de conectar las respuestas a nivel foliar (capítulo 4) con la respuesta a nivel de planta completa, un tema tratado con herramientas de modelización en ecosistemas mediterráneos (Zavala & Bravo 2005), pero del que hay escasas evidencias empíricas. En un principio, la relación entre la tasa máxima de fotosíntesis neta (A) y el crecimiento (RGR) fue inexistente como otros autores han demostrado (Poorter et al. 1990, Sims & Pearcy 1994). Sin embargo, tras integrar la asimilación de carbono con la disponibilidad lumínica total que tuvieron las hojas y por tanto determinar la ganancia media de carbono (GC), se encontró un relación positiva entre GC y RGR. Además, en sendos capítulos se han estudiado las respuestas de los diferentes habitos foliares en los que se incluían las especies objeto de estudio, perennifolia (*Q. suber* y *Q. ilex*) y caducifolia (*Q. canariensis* y *Q. pyrenaica*). En general, no hubo una clara diferenciación de los dos grupos funcionales, como de hecho, actualmente está en entredicho la separación clara de estos grupos funcionales (Wright et al 2004). En cuanto a las diferencias entre especies, se puede decir que las 4 especies de *Quercus* estudiadas en este experimento comparten características comunes tales como el uso conservativo de los recursos (Valladares et al. 2000), aunque dentro de este síndrome general, las especies muestran diferentes respuestas a la variación de la luz y el agua, las cuales pueden ser las responsables de la diferenciación de nicho (Lambers & Poorter 1992, Wright *et al*. 2004) en el escenario mediterráneo.

Pero en la aproximación utilizada en este bloque, la experimentación en condiciones controladas, los resultados no son directamente aplicables al campo. En la naturaleza, las plantas no sólo están influidas por las diferentes combinaciones de luz y agua, hay otros muchos factores que influyen desde abióticos (ambiente físico y químico que les rodea) como bióticos (la interacción con otras plantas, con los animales, etc). Es por ello que en el siguiente y último bloque de la presente memoria se salta de nuevo al campo con una tercera aproximación básica en la metodología ecológica, los experimentos naturales. La idea subyacente a esta aproximación es simplemente observar, con un protocolo de muestreo adecuado, lo que sucede en el campo. Este método fue el elegido para llevar a cabo el capitulo 6 de esta tesis. Hasta el momento, se ha tratado de investigar las diferentes respuestas de las plantas a la heterogeneidad ambiental, sin embargo, todavía no se ha caracterizado el microambiente en el que un individuo habita en condiciones naturales durante la fase de establecimiento, lo que puede ser definido como nicho de regeneración. Intentar comprender cómo las respuestas estudiadas en el bloque 2 pueden llegar a influir en los procesos demográficos era el objetivo de este último estudio. Resultó que el nicho de regeneración cambió a lo largo de las fases demográficas estudiadas, y que los requerimientos de la especie (*Acer opalus*) se hicieron más estrictos con la edad al sufrir el nicho una contracción. A medida que la especie iba avanzando en edad, los individuos quedaron relegados en micrositios caracterizados por una alta cobertura de matorral, profundidad de hojarasca, los cuales estaban alejados de los árboles. Por tanto, parece ser que los juveniles de más edad de esta especie se establecen sólo en una parte del ambiente lumínico que en edades más tempranas toleraban. Por supuesto, el matorral, aparte de la cantidad de luz adecuada para la supervivencia, le confirió menor demanda evaporativa en los meses de sequía críticos, siendo el agua por tanto también protagonista en este proceso demográfico. Adicionalmente, el matorral tuvo un efecto protector contra los herbívoros, con lo que luz, agua y herbivoría pueden considerarse como los factores que han regido el proceso demográfico de esta especie hasta su establecimiento.

EN DEFINITIVA, EN UNA COMUNIDAD DE BOSQUE MEDITERRÁNEO, LA AMPLIA HETEROGENEIDAD DEL MEDIO EN EL ESPACIO Y EN EL TIEMPO PERMITE QUE UNA MEZCLA DE ESPECIES QUE DIFIEREN EN SUS REQUERIMIENTOS DE REGENERACIÓN, YA SEA TAMAÑO DE SEMILLA, LUZ, AGUA U OTROS FACTORES, PUEDAN COEXISTIR EN SU PARTE DIFERENCIADA DE NICHO.

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7. CONCLUSIONES

1. Se encontró una alta heterogeneidad espacial a pequeña escala (metros) a lo largo de las tres unidades de paisaje estudiadas: bosque autóctono, repoblación y matorral pionero, aunque la heterogeneidad en el bosque autóctono y la repoblación fue mayor, lo que generó una amplia gama de micrositios donde las plántulas pueden establecerse.
2. La heterogeneidad espacial observada en las variables ambientales se vio reflejada en el patrón espacial de supervivencia de las especies estudiadas, siendo también agregado aunque sólo en el año en el que las condiciones estivales fueron mas extremas.
3. Dentro del abanico de variables ambientales estudiadas, la disponibilidad lumínica y la humedad del suelo explicaron en mayor medida la supervivencia de las especies.
4. Se encontró un fuerte efecto del peso de semilla sobre la biomasa final de la plántula tras 50 días de crecimiento. Las plántulas que crecieron bajo condiciones de sombra profunda fueron más dependientes de sus semillas.
5. De las diferentes hipótesis que explican las relaciones del peso de semilla con el desarrollo de la plántula se dedujo que las semillas más grandes generan plántulas de mayor biomasa, independientemente de otros atributos como la retención de reservas en la semilla o la tasa de crecimiento relativo. Este efecto de la semilla sobre la biomasa de la plántula desaparece con el tiempo.
6. A nivel de hoja, el estrés combinado de sombra y sequía fue positivo, es decir, la sombra profunda mitiga el efecto de la sequía. Los rasgos morfológicos a nivel foliar fueron característicos de cada especie, mientras que existió una menor variación entre especies de los rasgos fisiológicos de la hoja. Las especies

caducifolias mostraron diferencias en los rasgos morfológicos y fisiológicos a nivel foliar con respecto a las especies perennifolias.

7. Las variación de la luz provocó respuestas plásticas a nivel foliar tanto en variables morfológicas como fisiológicas. Por otra parte, la variación de la disponibilidad hídrica provocó una mayor respuesta plástica sólo en las variables fisiológicas.
8. Encontramos relaciones complejas entre las variables estudiadas a nivel foliar: el área específica foliar estaba relacionada positivamente con la concentración de nitrógeno en la hoja, lo cual determinaba un aumento en tasa máxima de fotosíntesis pero ésta a su vez estaba regulada por la conductancia estomática, para así optimizar la pérdida de agua por transpiración, tan importante en ecosistemas mediterráneos.
9. Tras 7 meses de crecimiento, el efecto del peso de semilla todavía era patente en la biomasa de las plántulas, aunque el uso de las reservas varió entre especies. La intensidad lumínica moduló esta relación siendo nuevamente las plántulas bajo sombra profunda las mas dependientes de sus semillas.
10. A nivel de planta completa, también se encontraron efectos positivos del estrés combinado de sombra y sequía, aunque disminuyó la intensidad de los mismos.
11. La ganancia de carbono calculada a partir de la asimilación a nivel de hoja estuvo relacionada con la tasa de crecimiento relativo de las plántulas. La capacidad de predecir respuestas de crecimiento integral a partir de medidas instantáneas de asimilación de carbono a nivel de hoja puede ser esencial para predecir la dinámica forestal en un escenario de cambio global.
12. Las especies pueden sufrir cambios ontogenéticos en su nicho de regeneración. Para el caso estudiado, las características del nicho sufrieron una contracción

encontrándose plántulas y juveniles de menor edad en una amplia gama de micrositios mientras que los juveniles de mayor edad quedaron relegados a micrositios de características más específicas.

13. Los factores que dirigieron estos cambios ontogenéticos fueron la profundidad de hojarasca, la cobertura arbórea y de matorral, así como la herbivoría por parte de ungulados.
14. Conocer las características del nicho de regeneración en juveniles de avanzada edad tiene un alto valor aplicado de cara a optimizar programas de manejo y restauración, ya que son los micrositios donde la especie tendrá mayor probabilidad de supervivencia a largo plazo.