UNIVERSIDAD DE CÓRDOBA

Establecimiento de cuatro especies de *Quercus* en el sur de la Península Ibérica. Factores condicionantes

M. Victoria González Rodríguez

TITULO: *Establecimiento de cuatro especies de Quercus en el sur de la Península Ibérica. Factores condicionantes*

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TÍTULO DE LA TESIS: Establecimiento de cuatro especies de Quercus en el Sur de la Península Ibérica. Factores condicionantes

DOCTORANDO/A: M. Victoria González Rodríguez

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

El trabajo presentado evalúa distintas fases del establecimiento de cuatro especies de Quercus, cuya regeneración está fuertemente limitada. Los resultados obtenidos son muy interesantes desde un punto de vista básico como aplicado para la gestión y conservación de los bosques ibéricos. Victoria González ha realizado un trabajo encomiable, ya que ha realizado numerosos experimentos tanto en invernadero como en campo, que han requerido una fuerte dedicación. Durante el desarrollo de su tesis ha aprendido numerosas técnicas que comprenden: análisis de suelos, crecimiento y morfología de plantas, análisis estadísticos, etc. Por otro lado, ha desarrollado la capacidad de interpretación de los resultados, de plasmar estos resultados en publicaciones científicas y en sacarle los aspectos aplicados de su investigación. Todos los capítulos de su tesis están publicados o en vías de publicación.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 21 de julio de 2010

Firma del/de los director/es

Rafael Vill

Rafael M Navarro

Fdo.: RAFAEL VILLAR MONTERO Fdo.: RAFAEL M NAVARRO

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Prof. Dr. Juan Fernández Haeger, Director en funciones del Departamento de Botánica, Ecología y Fisiología Vegetal, informa que el presente trabajo de investigación desarrollado por Dña. M. Victoria González Rodríguez bajo la supervisión de los Prof. Dr. Rafael Villar Montero y Rafael M. Navarro Cerrillo reúne todos los requisitos necesarios para aspirar al Título de Doctor por la Universidad de Córdoba.

Córdoba, 19 de julio de 2010

Rafael Vill
Rafael Navarro

Juan Fernández Haeger   Rafael Villar Montero   Rafael M. Navarro Cerrillo

Esta tesis doctoral ha sido realizada gracias a una beca FPI-MEC (BES-2006-13059), dentro de los proyectos DINAMED (Dinámica del bosque mediterráneo en un escenario de cambio global)(CGL2005-05830-C03) e INTERBOS (Interacciones ecológicas y Cambio Global en el bosque mediterráneo) (CGL2008-04503-C03-01). Plan Nacional de I+D+i Ministerio de Ciencia e Innovación del Gobierno de España.

A mis padres, Rosa María y Claudio

“Cuando observo un bosque que quiero comprar, talar o hipotecar, no veo el bosque sino sus relaciones con mi querer.
Pero si no quiero nada de él y sólo dirijo mi mirada a su verde profundidad, entonces es bosque, naturaleza y vegetación.
Entonces es hermoso.”
(Herman Hesse)

“Si puedes mirar, ve.
Si puedes ver, repara.”

Epígrafe de Ensayo sobre la ceguera (José Saramago)

INDICE

Gran parte de los resultados de esta tesis se encuentran publicados en: Villar, R., González-Rodríguez, V., Alameda, D., Quero, J.L., Navarro-Cerrillo, RM. 2010. Regeneración de varias especies de Quercus. Factores limitantes para su establecimiento. En: Quero, JM (ed.) Investigación en el PN Sierra de Cardeña y Montoro (en prensa)

INTRODUCCIÓN GENERAL

La regeneración de los Quercus mediterráneos

La Península Ibérica, especialmente en su área mediterránea, se encuentra dominada por bosques de *Quercus*, tanto perennifolios como caducifolios. Los estudios paleobótánicos estiman que su expansión por el territorio se produjo en la era postglaciar a partir de las numerosas áreas de refugio de estas especies al pie de los macizos montañosos. Posteriormente, el hombre comenzó a influir sobre la vegetación, especialmante a partir del periodo neolítico (hace 7000 años), en el que las prácticas agropastorales se intensificaron (Costa et al, 1997). Desde entonces, la actividad humana ha sido uno de los mayores determinantes en la configuración del paisaje de la cuenca mediterránea, ya que la agricultura, los usos ganaderos y el aprovechamiento forestal han generado profundas transformaciones en los bosques originales. De esta forma, el paisaje que se observa actualmente es un mosaico de bosques intercalados entre matorrales, pastizales, cultivos agrícolas y formaciones adehesadas (Costa *et al*., 1997; Terradas, 1999).

A este escenario de alteraciones antropogénicas se unen las características propias del clima mediterráneo: fuerte sequía coincidente con el periodo de máximas temperaturas, suelos pobres en nutrientes y presencia de elementos perturbadores como la herbivoría y el fuego (Baraza et al., 2006; Lionello et al., 2006). Como resultado la regeneración de la vegetación mediterránea, especialmente la de las especies de crecimiento lento como son los *Quercus* (Villar et al., 2008), se encuentra fuertemente condicionada en todas sus fases (Jordano *et al*., 2008).

La regeneración es un proceso dinámico a través del cual se reclutan nuevos individuos en la población de adultos, compensando así las pérdidas por mortalidad natural (Harper, 1977). Este proceso supone una concatenación de eventos que pasan por la floración, la producción y la dispersión de frutos, la germinación, la emergencia y el establecimiento de plántulas, el crecimiento y el reclutamiento de adultos (Clark et al., 1999; Pulido, 2002) (Fig. 1).

Jordano et al. (2008) distinguen tres fases donde son más importantes las limitaciones: producción de semillas, dispersión de semillas y establecimiento de plántulas (o fase post-dispersiva), entendiendo por establecimiento el momento en el que la supervivencia de los nuevos individuos reclutados se estabiliza (lo que en el mediterráneo ocurre al final del primer o segundo verano después de la emergencia) (Jordano et al., 2008).

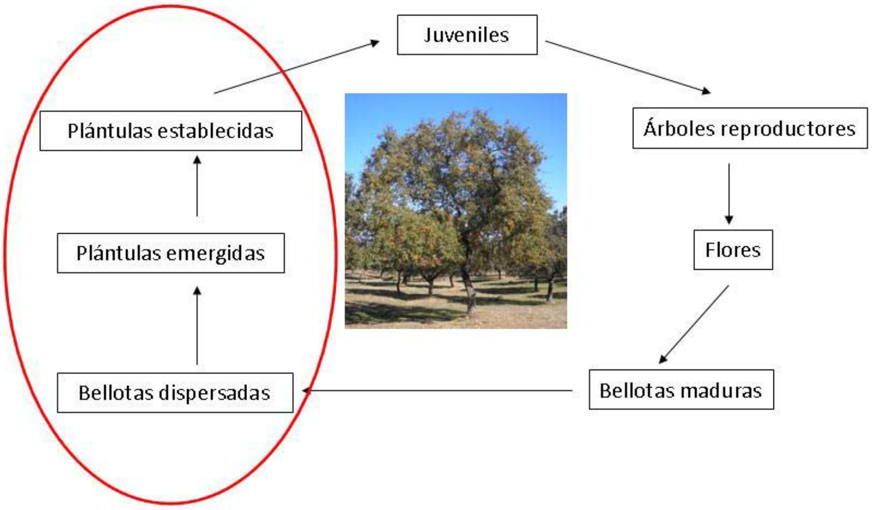

Figura 1. Esquema de las fases en la regeneración. El círculo rodea la fase de establecimiento o post-dispersiva (sensu Jordano et al., 2008), estudiada en esta tesis.

Fase de establecimiento o post-dispersiva: limitaciones

En las especies de *Quercus* la etapa de establecimiento es clave en el reclutamiento, que se encuentra en primer lugar fuertemente condicionado por la depredación de las bellotas dispersadas. Algunos estudios en el mediterráneo han mostrado tasas de desaparición de bellotas de hasta el 100% (Pérez-Ramos y Marañón, 2008) Entre los consumidores más habituales de bellotas en los bosques mediterráneos se encuentran los ungulados silvestres, jabalíes, micromamíferos y diversas aves. Las características propias de la bellota, como pueden ser la especie a la que pertenece o su tamaño, así como su posición en el espacio, determinan la posibilidad de escapar a la depredación (Gómez, 2004b; Pons y Pausas, 2007a).

Otras fases críticas en el establecimiento son la emergencia, crecimiento y supervivencia tras el primer verano, ya que en estos momentos las semillas y las plántulas recién emergidas son más vulnerables a las condiciones del medio circundante y a los recursos como la luz, el agua y los nutrientes (Grubb, 1977; Harper, 1977; Kitajima y Fenner, 2000), cuya disponibilidad puede variar en pocos metros (Gallardo *et al.*, 2000; Gallardo, 2003, Gómez *et al*., 2004; Quero, 2006). Además, estos factores presentan relaciones complejas entre si (Sack y Grubb, 2002; Gallardo, 2003; Marañón *et al*., 2004), generando todo un mosaico de micrositios con condiciones y recursos diferentes que condicionan el establecimiento de las plántulas. Esta gran heterogeneidad espacial puede influir en la diversidad, estructura y composición de los ecosistemas (Terradas, 2001; Maestre, 2006).

Las condiciones y recursos de un mismo micrositio pueden ser favorables en alguna fase del establecimiento y limitantes en otra. Por ejemplo, el riesgo de depredación de bellotas aumenta en las áreas de matorral donde los roedores concentran su actividad (Herrera, 1995; Pulido, 2002). Además, el estrato arbustivo compite con la plántula por la luz y los nutrientes, limitando así su crecimiento, de importancia para desarrollar raíces profundas y conseguir superficie fotosintética. En cambio, el sotobosque proporciona efectos facilitadores protegiendo contra los herbívoros y la fuerte evaporación de agua durante el periodo de sequía (Castro et al., 2002; Gómez-Aparicio et al., 2004). De hecho, numerosos estudios han definido los hábitats con condiciones intermedias de sombra - bajo árboles o arbustos de gran altura- como importantes nichos de regeneración para *Q. ilex* (Acacio et al., 2007; Puerta-Piñero et al., 2007; Gómez et al., 2004)

A toda esta enorme heterogeneidad espacial que se observa en los ecosistemas mediterráneos se añade la gran variabilidad temporal en los recursos y en las condiciones ambientales. Su importancia sobre el establecimiento ha sido ampliamente estudiada, ya que influye en el patrón de reclutamiento de nuevos individuos (Paynter et al, 1998; Castro et al, 2004; Quero 2007a; Herrero et al., 2008), especialmente en los climas mediterráneos y semi-áridos donde el agua es un recurso muy limitante (Milton, 1995; Veenendaal et al., 1996; Puerta-Piñero et al., 2007).

Peso semilla y factor materno: importancia para el establecimiento

Las posibilidades exitosas de transición entre unas fases y otras del establecimiento pueden verse afectadas no sólo por los factores externos sino también por las características intrínsecas. Una de ellas es el tamaño de la semilla, ya que representa la cantidad de reservas disponibles durante las primeras fases de vida, teniendo así consecuencias importantes sobre otros atributos de las plántulas y, por tanto, sobre el establecimiento. Muchos estudios han comprobado que las especies con semillas más grandes confieren a la plántula ventajas durante la fase de reclutamiento, como son mayores tasas de emergencia y de supervivencia (Vázquez, 1998; Gómez, 2004a; Moles y Westoby, 2004; Baraloto et al., 2005; Urbieta et al., 2008a), especialmente en ambientes adversos como zonas de sombra intensa (Leishman y Westoby, 1994; Saverimuttu y Westoby, 1996) o con suelos pobres en nutrientes (Milberg y Lamont, 1997).

Los *Quercus* presentan un amplio rango en el peso de sus semillas (Vázquez, 1998; Bonner, 2003, Ramírez-Valiente et al., 2009), que viene en primer lugar determinado por la especie. A su vez, se encuentra una variabilidad importante a nivel intraespecífico, de forma que distintas poblaciones e incluso individuos de la misma población pueden desarrollar bellotas de mayor o menor tamaño (Ramírez y Gómez, 1982; Ducousso et al., 1993; Bonfil, 1998; Gómez, 2004a). Otros estudios han mostrado diferencias en el porcentaje de humedad, la composición química o las tasas de germinación entre bellotas de la misma especie (Tilki y Alptekin, 2005; Fernández-Rebollo et al, 2008), y esta variabilidad también se refleja en otros caracteres morfológicos y fisiológicos de las plántulas como pueden ser la resistencia a la sequía o los caracteres foliares (Leiva y Fernández-Alés, 1998; Bruschi et al. ,2003; González-Rodríguez y Oyama, 2005; López de Heredía y Gil, 2006; Sánchez-Vilas y Retuerto, 2007) Así, tanto la población como el propio individuo dentro de una misma población (factor materno) pueden determinar no sólo el tamaño de la semilla, sino otras características relacionadas con el establecimiento. Aunque se ha estudiado la variabilidad en rasgos relacionados con semillas y plántulas de distintas poblaciones de *Quercus* (Tilki y Alptekin, 2005; Fernández-Rebollo et al., 2008; Ramírez-Valiente et al., 2009), los trabajos orientados a evaluar estas diferencias a nivel intrapoblacional son todavía escasos.

Necesidad de aplicar los conocimientos sobre regeneración natural a la restauración y gestión forestal.

Del estudio de los procesos que determinan la estructura y funcionamiento de los ecosistemas se deberían desprender recomendaciones prácticas encaminadas a mejorar las estrategias de manejo y restauración forestal. A partir del conocimiento generado en los últimos años, aspectos como la biodiversidad o la conectividad de los ecosistemas se han comenzado a tener en cuenta en las políticas de gestión ambiental (Noss, 1990; Franklin, 1993). De la misma forma, el estudio de la regeneración natural debería ofrecer pautas para una adecuada gestión de la vegetación.

El paisaje actual de la península se encuentra tan fragmentado que en ocasiones la sucesión natural se encuentra colapsada y la comunidad vegetal ha perdido la capacidad de recuperarse por sí sola (Costa, 2006; Acacio et al., 2007), haciéndose necesarias actuaciones concretas de restauración. La reforestación con especies de *Quercus* presenta muchas dificultades debido a sus bajas tasas de crecimiento y supervivencia, especialmente si las condiciones del primer periodo de sequía son muy estresantes (Navarro-Cerrillo et al., 2005) Además, no parece existir un acuerdo sobre el mejor método de repoblación para estas especies. Por un lado, la siembra directa de semillas presenta ventajas como un menor coste económico (Bullard et al., 1992) y la posibilidad de desarrollar sistemas radiculares más profundos que permitan acceder al agua durante la sequía estival (Lloret et al., 1999; Maestre et al., 2003). Sin embargo, la producción de plantas en viveros evita la depredación de la semilla, factor muy limitante en la siembra directa (Madsen y Löf, 2005; Birkedal y Löf, 2007; Pérez-Ramos y Marañón, 2008). Además, las características de las plantas procedentes de vivero (edad, tamaño, condiciones de cultivo) también pueden influir en la supervivencia en campo. Por tanto, la complejidad de factores que interactúan y repercuten en el éxito de una repoblación es tal que se necesitan más estudios que comparen diversas técnicas en ambientes concretos.

Ya sea para su siembra o para el cultivo en vivero, el tamaño de la semilla es un factor a tener en cuenta. Una práctica muy empleada es la selección de árboles madre productores de bellotas más grandes que puedan originar plántulas más robustas (Vázquez, 1998). Como ya se ha comentado antes, plántulas procedentes de distintos árboles pueden presentar otras diferencias a nivel morfológico y fisiológico que repercutan en su establecimiento, siendo otro factor a tener en cuenta a la hora de seleccionar semillas. Sin embargo, la selección de semillas de un número limitado de árboles madre puede llevar a plantaciones donde la variabilidad genética se vea fuertemente reducida (Rajora, 1999; Burgarella et al., 2007). Aparentemente, la persistencia de especies de amplia distribución como son los *Quercus* no está amenazada, sin embargo muchas poblaciones de las áreas marginales del sur, consideradas reservorios de diversidad genética intraespecífica, corren el riesgo de desaparecer debido a su pequeño tamaño o situación de aislamiento (López de Heredía y Gil, 2006). Parece por tanto necesario llegar a un equilibrio entre la selección de material adecuado y el mantenimiento de la diversidad de los recursos genéticos.

Habitualmente en los proyectos de reforestación se presta más atención a factores relativos la calidad de la planta o la procedencia de las semillas, sin tener en cuenta que las condiciones del medio pueden cambiar en pocos metros, condicionando así el establecimiento de las especies. El reconocimiento de esta heterogeneidad espacial y temporal es especialmente importante en las actuaciones llevadas a cabo en un ambiente tan variable como es el mediterráneo (Zamora, 2002; Valladares y Gianoli, 2007).

En conclusión, la optimización de las acciones de restauración pasa por conocer mejor los procesos subyacentes a la regeneración natural, la búsqueda de técnicas de bajo costo e impacto ambiental, la mejora de las técnicas de cultivo en vivero y la consideración explicita de la heterogeneidad ambiental y temporal inherente a los ecosistemas.

OBJETIVOS Y ESTRUCTURA DE LA TESIS

Los conceptos de regeneración y establecimiento tienen diferentes acepciones y en ocasiones pueden confundirse, haciéndose necesaria la aclaración del uso que de ellos se hace en esta tesis. Como ya se ha indicado anteriormente, la regeneración se refiere al proceso de reclutamiento de nuevos individuos en una población, comprendiendo el ciclo completo desde la producción de semillas hasta la formación de plantas adultas. La fase de establecimiento se entiende como aquella en la que la supervivencia de los nuevos individuos reclutados se estabiliza, que en condiciones mediterráneas ocurre un año o dos después de la emergencia (Jordano et al., 2008) (Fig.2). Los contenidos de esta tesis abarcan por tanto las limitaciones a esta fase dentro del ciclo de regeneración, también llamada fase post-dispersiva (Jordano et al., 2008).

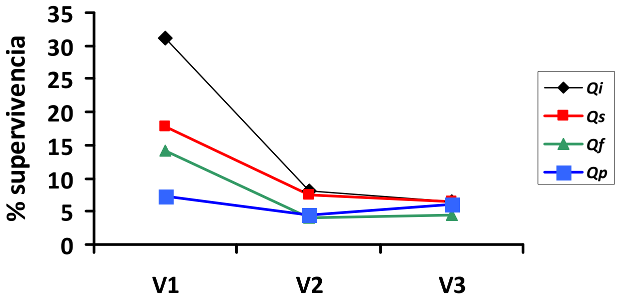

Figura 2. Se muestran los porcentajes de supervivencia de las plantas no regadas del capítulo 4 después del primer (V1), segundo (V2) y tercer verano (V3) tras la emergencia. Se observa como a partir del segundo año la supervivencia se estabiliza. (*Qi*: Q. ilex; *Qs*: Q. suber; *Qf*: Q. faginea; *Qp*: Q. pyrenaica)

En la figura 3 se resumen los principales aspectos tratados en esta tesis. El objetivo general es el estudio de los factores que condicionan el establecimiento de cuatro especies del genero *Quercus*: quejigo (*Q. faginea* Lam.), encina (*Q. ilex* L. subsp *ballota* (Desf.) Samp.), roble (*Q. pyrenaica* Willd.) y alcornoque (*Q. suber* L.), muy abundantes en los bosques mediterráneos del sur de la Península Ibérica.

Este objetivo general se puede desglosar a su vez en varios objetivos específicos:

1) Evaluar la importancia del tamaño de la semilla y del árbol madre en las distintas fases del establecimiento.

2) Explorar la heterogeneidad espacial a pequeña escala en diversos factores ambientales (luz, humedad del suelo y producción de herbáceas), y evaluar la influencia de estos factores en el patrón espacial y temporal de la emergencia, supervivencia y éxito del reclutamiento.

3) Comparar el efecto de distintos micrositios en las tasas de depredación, emergencia, crecimiento y supervivencia, y determinar qué condiciones y recursos explican estas diferencias.

4) Comparar los resultados de las distintas fases de establecimiento en cuatro especies de *Quercus* que difieren en su morfología y longevidad foliar.

5) Comparar el éxito de distintos métodos de repoblación (siembra y plantación) y calidad de planta (diferentes edades de planta de vivero) en el establecimiento temprano de dos especies del genero *Quercus*.

6) Generar información básica sobre las limitaciones ambientales durante la fase de establecimiento en la regeneración natural de especies del genero *Quercus* en el P.N. Sierra de Cardeña y Montoro, donde se han realizado gran parte de los experimentos, que pueda ser de utilidad práctica para los gestores del parque.

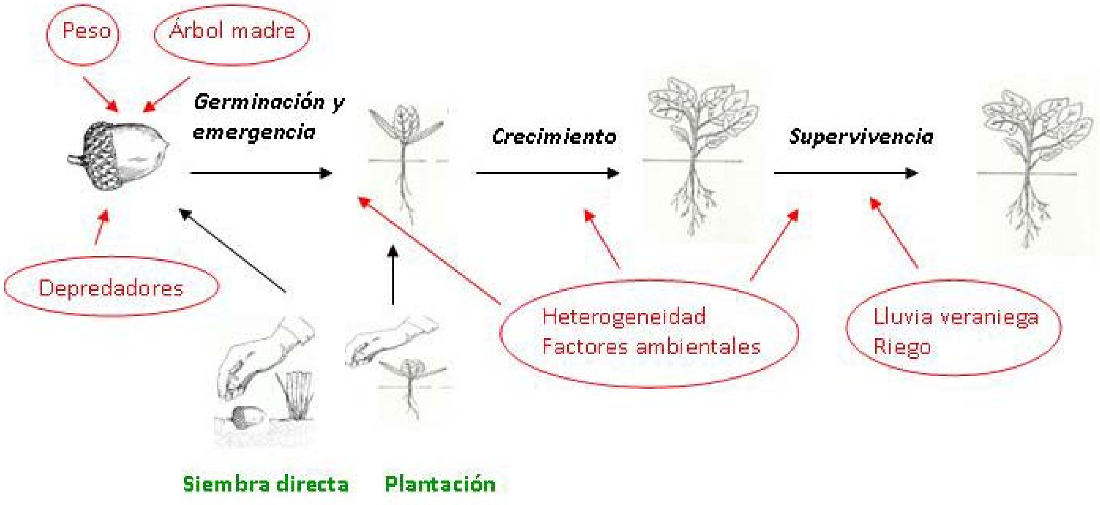

Figura 3. Esquema conceptual de los objetivos de estudio.

Para alcanzar estos objetivos se han realizado diferentes experimentos tanto en invernadero como en campo y se han organizado en capítulos en los que se tratan uno o varios de los objetivos simultáneamente (ver índice).

En el **capítulo 1** se estudia la fase de depredación post dispersiva de semillas como primer factor que condiciona el establecimiento, comparando las tasas de desaparición de bellotas de distinto tamaño en las 4 especies y en distintos microhábitats. De esta forma se contemplan los objetivos 1, 3, 4 y 6.

En el **capítulo 2** se exponen los resultados de un experimento de invernadero en el que se evalúan las relaciones entre el tamaño de la semilla y el uso de sus reservas, el crecimiento y el tamaño de plántula tanto a nivel inter como intra-específico. La variabilidad intra-específica se estudió recolectando bellotas en distintos árboles madre seleccionados en el P.N. Sierra de Cardeña y Montoro, abordando así los objetivos 1, 4 y 6.

El **capítulo 3** muestra los resultados de una siembra experimental con un diseño espacialmente explícito en el P.N. Sierra de Cardeña y Montoro. Se estudia la heterogeneidad espacial y temporal en el reclutamiento (emergencia, supervivencia y éxito en establecimiento) y crecimiento, así como sus factores condicionantes, alcanzando por tanto los objetivos 1, 2, 3, 4 y 6.

En el **capítulo 4** se analizan los micrositios más favorables al establecimiento mediante una siembra experimental P.N. Sierra de Cardeña y Montoro, y se explora el efecto del progenitor de origen como factor condicionante en la supervivencia y crecimiento temprano. De esta forma este capitulo contribuye a responder a las hipótesis planteadas en los objetivos 1, 3, 4 y 6.

El **capítulo 5** compara varios métodos de repoblación de encina y alcornoque en una antigua zona de cultivo, alcanzando los objetivos 1 y 5.

ASPECTOS NOVEDOSOS DE ESTA TESIS

El presente trabajo incluye aspectos novedosos o poco tratados en el estudio de la regeneración de *Quercus*:

1) *Estudio de la variabilidad intrapoblacional*. El análisis de los efectos del progenitor sobre el establecimiento realizado tanto en campo como en invernadero supone una de las aproximaciones más novedosas a nivel científico de esta tesis. Además, el efecto materno se ha analizado conjuntamente con otros factores (peso de la semilla y factores ambientales) a fin de comprobar las interacciones con los mismos.

2) *Estudio de los factores condicionantes del tiempo de emergencia*. Habitualmente el tiempo de emergencia ha sido estudiado como factor condicionante de la supervivencia (Fowler, 1988; Jones at al., 1997), sin embargo pocos trabajos evalúan los factores que pueden influir en el tiempo de emergencia (pero ver Merouani et al., 2001; Urbieta et al., 2008a)

3) *Estudio de crecimiento de plántulas en campo*. Aunque los experimentos en condiciones controladas proporcionan mucha información, es necesario contrastar sus resultados con los estudios de campo, donde son muchos los factores que influyen e interactúan. Debido a la dificultad metodológica, las medidas de crecimiento en campo suelen restringirse a altura o diámetro de tallo. En esta tesis se han medido otras variables como la biomasa aérea y subterránea, la tasa de crecimiento relativo (RGR) y el área específica foliar (SLA). Además se ha aplicado un diseño espacialmente explícito al estudio de algunas de estas variables para comprobar si el crecimiento presenta una distribución agregada en el espacio.

4) *Análisis de la regeneración de Quercus en el PN. Sierra de Cardeña y Montoro*. Los estudios sobre regeneración de *Quercus* en el parque son escasos y frecuentemente focalizados en una especie (*Q. pyrenaica*), dado que ésta se encuentra en los límites de su distribución y presenta problemas graves de regeneración. La aproximación multiespecífica y la combinación de experimentos que completan todas las fases de establecimiento suponen una valiosa fuente de información de interés para los gestores del parque.

5) *Estudio comparado de siembra y repoblación*. Esta disyuntiva aún no ha sido resuelta y los estudios realizados en la cuenca mediterránea son prácticamente inexistentes (ver Navarro et al., 2006).

MÉTODOS GENERALES

ÁREA DE ESTUDIO Y PARCELA EXPERIMENTAL

Los experimentos de campo de los capítulos 1, 3 y 4 se llevaron a cabo en el P. N. Sierra de Cardeña y Montoro (38° 21´ N, 3° 12´ W y 38° 87´ N, 3° 32´ W). Además, las semillas de las cuatro especies de *Quercus* utilizadas en todos los experimentos de esta memoria proceden de este parque. El P. N. Sierra de Cardeña y Montoro se localiza en Sierra Morena, al noreste de la provincia de Córdoba, limitando al norte con la provincia de Ciudad Real y al este con el P.N. Sierra de Andujar (Jaén) (Fig. 1). Este parque pertenece a la Red de Espacios Naturales Protegidos de Andalucía (RENPA), ocupando una superficie de 38.449 ha. Ha sido también declarado Zona de Especial Protección para las Aves (ZEPA) y Lugar de Interés Comunitario (LIC).

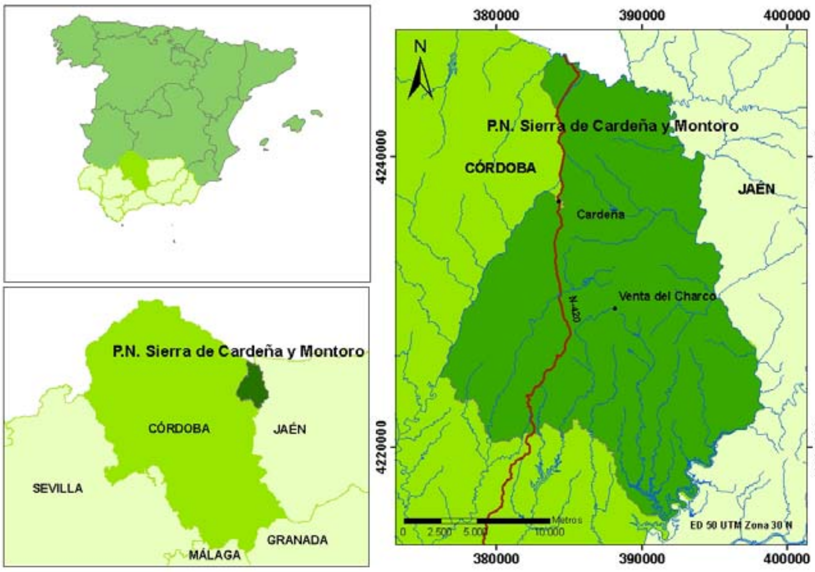

Figura 1. Localización del P.N. Sierra de Cardeña y Montoro

La geología dominante está formada por materiales graníticos de la era primaria, y los principales tipos de suelos que se pueden encontrar, ácidos y poco evolucionados, son los Cambisoles, Regosoles y Leptosoles. Todos los cursos de agua pertenecen a la cuenca del Guadalquivir, siendo los ríos Yeguas y Arenoso los dos principales cursos de agua. La altitud media se sitúa en los 750 m oscilando entre los 200 y 800 m, con pendientes entre el 10 y 25%. El clima es mediterráneo con un ombroclima sub-húmedo. La media anual de temperatura es de 15,3 °C con grandes oscilaciones entre -1 y 40°C. (Quero, 2007b) y con aproximadamente 56 días de heladas entre los meses de Noviembre y Abril. Las precipitaciones oscilan entre los 570 y 970 mm al año. Sin embargo, existe una fuerte variabilidad interanual de la precipitación. Por ejemplo, en los años agronómicos 94-95, 98-99 y 04/05 las precipitaciones fueron de menos de 400 mm (Fig. 3). Este hecho, junto con la variación en el reparto temporal de la precipitación a lo largo del año (Fig 2), puede tener un fuerte impacto sobre la ecofisiología y los procesos demográficos de las plantas leñosas mediterráneas (Quero 2007a).

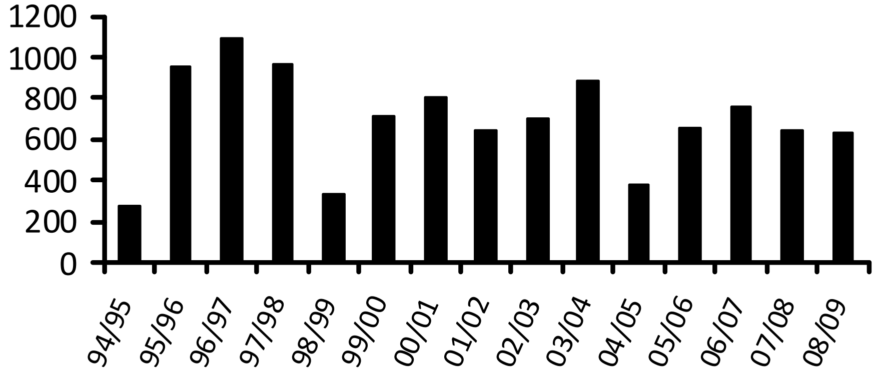

Figura 2. Precipitación anual acumulada (L m^{-2}) durante los últimos años (año agrícola) en el P.N. Sierra de Cardeña y Montoro. Datos proporcionados por la dirección del Parque.

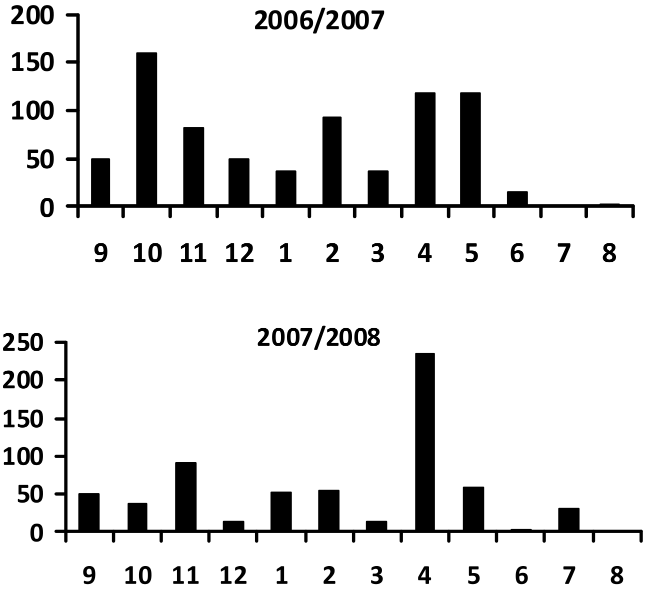

Figura 3. Precipitaciones mensuales (L m^{-2}) tomadas en el Centro de Visitantes del P.N. Sierra de Cardeña y Montoro correspondientes a los años agrícolas 2006/2007 y 2007/2008, en los que tuvieron lugar gran parte de los experimentos de esta tesis. Datos proporcionados por la dirección del Parque.

La vegetación existente se encuadra en el piso bioclimático mesomediterráneo, y sus paisajes son el resultado de la interacción entre los aprovechamientos humanos y la configuración natural del territorio, comprendiendo áreas de dehesa, repoblaciones, olivar, bosques de ribera y monte mediterráneo. Las especies mejor representadas en el estrato arbóreo pertenecen a distintas especies de género *Quercus*, pudiéndose encontrar también acebuches (*Olea europaea* L. var. *sylvestris* Brot.) y repoblaciones de distintas especies de pinos (*Pinus pinaster* Aiton*, Pinus pinea* L. *y Pinus canariensis* Sweet). Entre las especies de matorral destaca el enebro (*Juniperus oxycedrus* L.), madroño (*Arbutus unedo* L.), lentisco (*Pistacea lentiscos* L.) y durillo (*Viburnum tinus* L.). En cuanto a la fauna, se encuentran descritas numerosas especies tanto de invertebrados como de vertebrados, muchas de ellas catalogadas por organismos internacionales para la conservación o en la Directiva Hábitats, destacándose tres de las especies de grandes vertebrados ibéricos más amenazadas: el lince (*Lynx pardinus*), el lobo (*Canis lupus*) y el águila imperial ibérica (*Aquila adalberti*). El lince ibérico, catalogado como especie "en peligro crítico de extinción" es la especie emblema del parque, y desde el año 2002 se vienen realizando una serie de actuaciones para su conservación gracias el proyecto Life subvencionado por la Unión Europea. Entre ellas se incluyen acciones para la mejora del hábitat y de las poblaciones de conejo, como la instalación de varios cercados de cría y alimentación suplementaria.

Respecto a los usos y aprovechamientos humanos, destacan aquellos que tienen lugar en las dehesas (un 40% de la superficie del parque), fundamentalmente de tipo ganadero. La actividad cinegética también es importante en la zona, y en menor medida los usos agrícolas (Quero, 2007b; Quero y Villar, 2009).

El Plan Rector de Uso y Gestión del parque (P.R.U.G) contempla entre sus objetivos el mantener las masas de *Q. pyrenaica* y favorecer la evolución de las formaciones forestales de pinar de repoblación hacia formaciones con mayor presencia de frondosas autóctonas. En este sentido se han realizado en los últimos años repoblaciones y tratamientos silvícolas en las manchas de robledal del Parque (comunicación personal del director del Parque Natural). Con respecto a la naturalización de los pinares se han ido llevando a cabo actuaciones forestales como claras selectivas con el objetivo de favorecer la regeneración de las frondosas autóctonas y del paisaje característico del monte mediterráneo, fundamental para la conservación de numerosas especies clave (lince y águila imperial). Para estas acciones de naturalización de pinares se ha realizado un importante esfuerzo económico y humano, haciéndose necesarias las investigaciones y seguimientos que confirmen la eficacia de estas actuaciones.

Casi todas las investigaciones y actuaciones relacionadas con la regeneración de leñosas se han centrado en la población de *Quercus pyrenaica,* aunque también se encuentran estudios gracias a convenios de prácticas y acuerdos con la Universidad de Córdoba que incluyen otras especies de leñosas (ver relación de estudios en Quero, 2007b). Concretamente se ha realizado un seguimiento de la supervivencia de una serie de repoblaciones realizadas en taludes de carretera entre los años 1999 y 2001, que incluía a *Q. faginea* y *Q. ilex*, entre otras especies de leñosas (Quero et al., 2010).

Los experimentos realizados en esta tesis se han llevado a cabo en un cercado de exclusión de herbívoros (2 Ha) en la finca de propiedad pública “La Vegueta del

Fresno" (Fig. 4), clasificada en el Plan de Ordenación de los Recursos Naturales como zona de reserva (zona A), que exige el máximo nivel de protección.

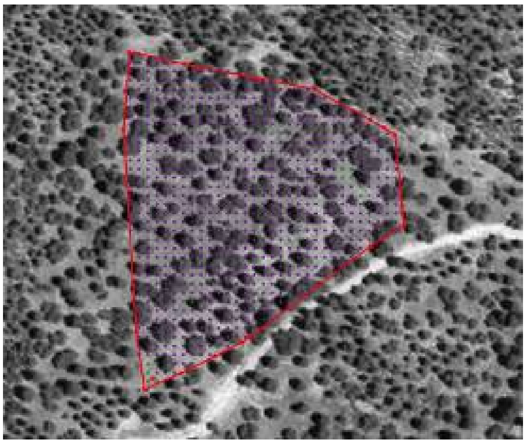

Figura 4. Foto aérea de la parcela de exclusión de herbívoros de La Vegueta. La línea indica el límite de la parcela y los puntos indican una distancia de 4 m.

La vegetación está formada por un bosque abierto de encina (*Q. ilex*) y algunos ejemplares de pino negral (*P. pinaster*). Los parches de matorral son escasos y predomina la jara pringosa (*Cistus ladanifer*), aunque también se encuentran algunos ejemplares de enebro (*Juniperus oxycedrus*) y tomillos (*Thymus sp*). Originalmente el cercado tenía el objetivo de ser una zona para cría de conejo silvestre, pero la recolonización de conejo no tuvo éxito y durante los años de trabajos experimentales no presentó ocupación por parte del mismo. El cercado supone la exclusión de grandes herbívoros que ocupan la zona, principalmente jabalí (*Sus scrofa*) y ciervo (*Cervus elaphus*). Parte del experimento del capítulo 1 se llevó a cabo en las zonas adyacentes al cercado experimental, de idénticas características al mismo salvo por la presencia de los dos grandes herbívoros antes mencionados y una menor abundancia de jaras.

ESPECIES DE ESTUDIO

Se han seleccionado cuatro especies de *Quercus* que difieren en su longevidad foliar: las perennifolias encina (*Q. ilex* ssp *ballota* (Desf.) Samp) y alcornoque (*Q. suber* L.), y las caducifolias o marcescentes quejigo (*Q. faginea* Lam.) y roble melojo *(Q. pyrenaica* Willd.). Estas especies coexisten en el P. N. Sierra de Cardeña y Montoro y tienen a su vez una amplia representación en el resto de la Península (Fig.5). Son especies típicamente mediterráneas en sentido amplio, presentando, sin embargo, distintas adaptaciones y requerimientos que les hacen coincidir ocasionalmente en el espacio, aunque ocupando nichos ecológicos bien diferenciados.

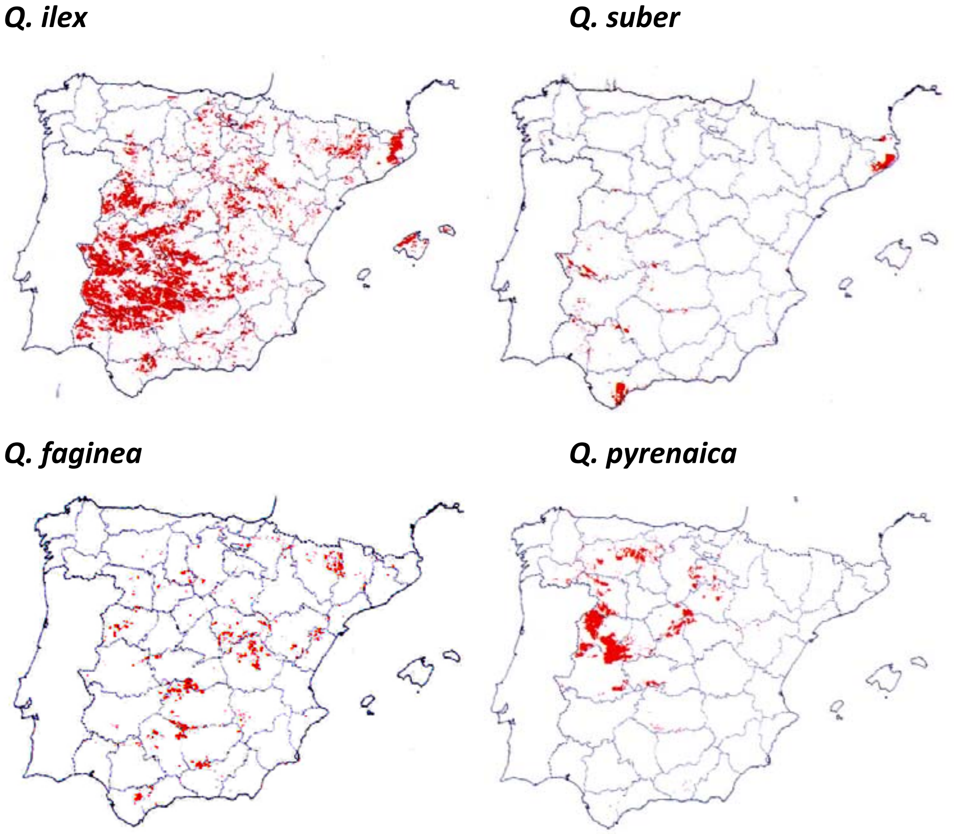
Q. ilex
Q. suber
Q. faginea
Q. pyrenaica

Figura 5. Mapas de distribución de las cuatro especies estudiadas. Tomado de Tuset y Sanchez (2004).

Las especies perennes (*Q. Ilex* y *Q. suber*) forman los bosques con mayor representación en la Península Ibérica, habiendo sido ampliamente favorecidos y aprovechados por el hombre a lo largo de la historia. Ambas especies presentan adaptaciones de tipo esclerófilo que les permiten soportar las grandes oscilaciones de temperatura, la sequía estival y la escasez de nutrientes propias de las áreas que ocupan. Algunas de estas adaptaciones son las cutículas gruesas para evitar pérdidas de agua, capas de esclerénquima reforzadas y pelos, escamas o ceras que reflejan la luz solar. La encina es una especie muy poco exigente con respecto a la humedad y tipo de suelo, que presenta gran plasticidad, encontrándose por ello descritas muchas variedades diferentes de la especie. El alcornoque sin embargo tolera peor las bajas temperaturas y su carácter calcífugo hace que se encuentre mejor representado en la parte oeste de la Península Ibérica (Castroviejo et al., 1987; Costa et al., 1997).

El quejigo se encuentra clasificado entre los robles de tipo marcescente, de carácter submediterráneo e indiferente a la naturaleza del sustrato. Es una especie más xerófitica que los robles y menos que la encina, encontrándose distribuida por toda la península, aunque al haber sido menos seleccionada por el hombre para la actividad ganadera, actualmente no es tan abundante como la encina (Castroviejo et al., 1987; Costa et al., 1997). El roble melojo es, de las especies estudiadas en esta memoria, la más exigente en humedad, necesitando precipitaciones entre los 650 y 1200 mm anuales. Se sitúa sobre suelos preferente ácidos, y es por ello más abundante en la mitad occidental de la Península, especialmente en el cuadrante noroeste (Castroviejo et al., 1987; Valdés et al, 1987; Costa et al., 1997).

Con respecto a su localización en el Parque, la encina es la especie con mayor presencia, estableciéndose en formaciones adehesadas con presencia de las otras especies de *Quercus* (alcornoque, quejigo y roble melojo) (Quero, 2007b), que ocupan zonas diferenciadas. Por ejemplo, es frecuente observar la presencia de *Q. faginea* en las proximidades de vaguadas por las que circula el agua temporalmente y, por tanto, hay mayor disponibilidad de recursos hídricos. El alcornoque (*Q. suber*) se encuentra en zonas de umbría o con mayor disponibilidad de agua en el suelo.

Es de destacar la población de roble melojo del Parque Natural de Cardeña, ya que es la única representación de dicha especie en la provincia de Córdoba. Actualmente se estima que el número total de individuos es inferior a los 15.000, encontrándose pequeñas manchas en las inmediaciones de arroyos y bordes de carreteras, así como formaciones adehesadas con regeneración escasa y pies muy envejecidos (López y Muñoz, 2010). Dichas formaciones debieron tener mayor representación en el pasado, y es posible que los superiores requerimientos hídricos de la especie, unidos al aparente endurecimiento del clima, la fuerte herbivoría y las actividades humanas sean los causantes de su regresión, aunque no existe una confirmación científica para estas observaciones (Quero, 2007b). Un alto porcentaje (63%) de la regeneración natural de la especie en la zona se da por brotes estoloniferos, siendo más probable la regeneración por semilla en los bordes de carretera (López y Muñoz, 2010).

MÉTODOS COMUNES EN TODOS LOS CAPÍTULOS

Con el objetivo de evitar repeticiones, a continuación se incluyen aquellos apartados metodológicos que son comunes a los cinco capítulos de la memoria.

Recogida de bellotas

La recolección de bellotas tuvo lugar en dos dehesas del P.N. Sierra de Cardeña y Montoro. Para los capítulos 2 y 4, se seleccionaron cinco progenitores para cada una de las especies estudiadas, y en el capítulo 4 se seleccionó uno por especie. Los progenitores de cada especie estaban localizados en la misma población, y se eligieron tratando de cubrir un amplio rango de tamaño de semilla. Para el resto de capítulos la recogida de semillas fue aleatoria en distintos árboles de las poblaciones escogidas. Las bellotas fueron recogidas entre los meses de octubre y diciembre, de acuerdo con el tiempo de fructificación de cada especie, y fueron almacenadas en cámara fría (2-5 °C) hasta el momento de inicio de cada experimento. Las bellotas que estaban perforadas por larvas de insectos o con síntomas de pudrición o ataque de hongos fueron descartadas.

Ecuaciones para la determinación del peso seco de las semillas

Para estimar el peso seco de la semilla y por tanto la cantidad de reservas de cada bellota, se construyeron predictores del peso de la semilla. Para ello se separó una submuestra de bellotas (entre 10 y 20) de cada uno de los progenitores o de las especies según el caso y se obtuvo su peso fresco individualmente. Posteriormente fueron secadas en estufa a 70 ºC durante al menos 48 h. Para cada semilla se separó el pericarpo de los cotiledones y se obtuvo el peso seco de cada una de las partes. A partir de los datos de peso seco, para cada progenitor se obtuvieron ecuaciones de regresión, de forma que fuera posible estimar con suficiente precisión el peso seco de los cotiledones a partir del peso fresco de las bellotas sembradas. En todos los casos los predictores tuvieron valores de $R^2 > 0,85$.

CAPÍTULO 1

Depredación post-dispersiva en cuatro especies de *Quercus*: importancia de la especie, tamaño de semilla y tipo de hábitat

Foto: Daniele Arnoldi

RESUMEN

La regeneración de los bosques de *Quercus* en la Península Ibérica está limitada por diferentes factores. Una de las fases más críticas en este proceso es la de depredación post-dispersiva. En este estudio se analiza la remoción de semillas de cuatro especies de *Quercus* (*Q. ilex subsp ballota, Q. suber, Q. faginea y Q. pyrenaica*) en el P.N. sierra de Cardeña y Montoro. Se analizó la influencia del tamaño de la semilla, la selección a nivel de especie y de micrositio (sombra de encina y claros) y el efecto de los cercados de exclusión de grandes herbívoros. Al final del experimento prácticamente el 100% de las semillas habían desaparecido, aunque la desaparición fue más rápida fuera del cercado de exclusión, donde al cabo de una semana habían desaparecido la mayoría de las semillas (80%). No se encontraron diferencias en la remoción fuera del cercado en relación al micrositio, pero sí a nivel de especie, ya que las bellotas de *Q. suber* y *Q. pyrenaica* fueron las primeras en desaparecer. Estas dos especies tuvieron las bellotas más grandes por lo que puede tratarse de selección por tamaño de semilla. En cambio, la remoción dentro del cercado no se vio influida por el tamaño de la semilla, pero sí por la especie y el micrositio. En general se encontró una mayor depredación de las semillas de *Q. ilex* y *Q. faginea* y se observó como la depredación fue mayor bajo cobertura de *Q. ilex* que en zonas de claro.

Palabras clave*: consumo post-dispersivo, exclusión, Quercus mediterráneos, regeneración natural, tamaño semilla*

ABSTRACT

In the Iberian Peninsula *Quercus* forest regeneration is limited by different factors, being post-dispersal predation is one of the most critical stages. In this study we analysed seed removal of four *Quercus* species in a conserved area in southern Spain. We analysed microsite (*Q. ilex* shade and open areas), seed mass and species selection both inside and outside a plot fenced to exclude large herbivores. At the end of the experiment almost 100% seeds had disappeared. Removal was faster in sites without exclusion where most seeds (80%) disappeared during the first week. In these sites, no differences were found in microsite selection, and *Q. suber* and *Q. pyrenaica* acorns disappeared first. As these species had the biggest acorns, this was probably the main cause of being removed faster. On the other hand, inside fenced plot acorns were selected by species and microsite, but not by seed mass. *Q. ilex* and Q. *faginea* acorns were removed faster, and predation was higher under *Q. ilex* shade.

Key words: *post dispersal consumption, exclusion, Mediterranean Quercus, natural regeneration, seed mass*

INTRODUCCIÓN

Las semillas de *Quercus*, al ser de gran tamaño, con abundantes sustancias de reserva y alto valor nutritivo (Vazquez, 1998), suponen una fuente importante de alimento para muchos consumidores (Jensen, 1985) y debido a ello la depredación post-dispersiva supone una fuerte limitación para el establecimiento (Herrera, 1995; Pulido y Díaz, 2005). En ambientes mediterráneos, los predadores más habituales de bellotas son los roedores (*Apodemus* sp.), ungulados silvestres como el ciervo (*Cervus elaphus*), corzo (*Capreolus capreolus),* o jabalí (*Sus scrofa*), ganado doméstico y diversas aves como las urracas (*Pica pica*) y arrendajos (*Garrulus glandarius*), (Shaw, 1968; Siscart et al., 1999; García et al., 2002; Gómez et al., 2003; Pons y Pausas, 2007b). Algunos estudios han mostrado tasas de desaparición de bellotas de hasta el 100% (Pérez-Ramos y Marañón, 2008). Un pequeño porcentaje de estas bellotas son almacenadas, generalmente por pequeños mamíferos o aves, y, si no se recuperan posteriormente, tienen oportunidad de germinar y emerger (Pulido and Díaz, 2005). Así, estos animales actúan como depredadores-dispersores. Por otro lado, depredadores como los ungulados y jabalíes suelen consumir las bellotas en el momento (Bonal y Muñoz, 2007; Muñoz et al., 2009), quedando en estos casos el reclutamiento de individuos totalmente colapsado. Debido a esto, los proyectos de restauración y de repoblación artificial deben tener en cuenta este factor y emplear métodos de exclusión para asegurar la permanencia de las semillas en el suelo (Madsen y Löf, 2005).

Debido a que los movimientos de los depredadores no suelen ser aleatorios, la localización de una bellota en el espacio condiciona sus probabilidades de escapar a la depredación (Ouden et al., 2005; Russo et al., 2006; Pons y Pausas, 2007a). Dicha posición viene a su vez determinada por procesos anteriores como son la lluvia de semillas o la misma dispersión por parte de los depredadores (García y Houle, 2005).

Gómez (2004a) propone la existencia de presiones de selección opuestas en lo referente al tamaño de la semilla. Por un lado las semillas grandes presentan muchas ventajas para un establecimiento exitoso, como mayores tasas de germinación y emergencia, así como una mayor probabilidad de supervivencia (Moles and Westoby, 2004; Baraloto et al., 2005; Urbieta et al., 2008a), teniendo sin embargo más probabilidad de ser consumidas. No obstante, esta selección por parte de los depredadores no está tan clara, y puede darse o no en función de la especie (Pons y Pausas, 2007c).

En los bosques mediterráneos es habitual que las especies de *Quercus* se encuentren formando bosques mixtos en los que coexisten varias especies simultáneamente (Costa et al., 1997). En presencia de varias fuentes de alimentos, los depredadores podrían escoger semillas de forma diferencial (Shimada, 2001). Pocos estudios evalúan la selección de semillas de varias especies a la vez en ambientes mediterráneos (pero ver Pons y Pausas 2007a; 2007b; 2007c). En los estudios en los que se ha estimado la preferencia sobre distintos tipos de semillas, se ha comprobado como la composición química es un factor importante en la selección por depredadores (Shimada y Saitoh, 2003).

Por otro lado, la heterogeneidad espacial y temporal en la disponibilidad de semillas y en las poblaciones de depredadores (Madsen y Löf, 2005), así como el gran número de factores que influyen en estas interacciones, requieren extender los estudios a más especies y áreas. Por ello, no se pueden extrapolar fácilmente unos resultados de una zona a otros y son necesarios estudios específicos en distintas zonas para evaluar la importancia de la depredación y los factores que la controlan.

El objetivo de este experimento fue cuantificar la remoción de bellotas de cuatro especies de *Quercus* (*Q. ilex subsp ballota*, *Q. suber*, *Q. faginea* y *Q. pyrenaica*) en el P.N. Sierra de Cardeña y Montoro, considerando las siguientes preguntas: 1) ¿Tienen las bellotas dentro de un cercado de exclusión de grandes herbívoros menor probabilidad de remoción? 2) ¿Existe selección de bellotas de mayor tamaño por parte de los depredadores? 3) ¿Existe una selección preferencial en función de la especie? 4) ¿Cuáles son los micrositios con más riesgo de depredación?

MÉTODOS

Área de estudio y especies

El área de estudio se describe en el apartado de métodos generales (paginas 21-26). Las especies con las que se hicieron los experimentos fueron *Q. ilex subsp ballota*, *Q. suber*, *Q. faginea* y *Q. pyrenaica*. La descripción de las especies se encuentra en el apartado de métodos generales (paginas 26-29).

En el parque se encuentran varios depredadores potenciales de las bellotas: jabalí (*Sus scrofa*), ciervo (*Cervus elaphus*), micromamíferos (*Mus spretus, Apodemus sylvaticus*) y aves como el arrendajo (*Garrulus glandarius*) o la urraca (*Pica pica*). En el área de estudio fuera del cercado de exclusión se encuentran abundantes signos de actividad de jabalíes (suelo removido, hozaduras).

Diseño experimental

La recolección y estima del peso seco de las bellotas se encuentran descritas en el apartado de métodos generales (página 29)

En noviembre de 2009 se numeraron con un punzón 250 bellotas por especie, se tomó el peso fresco individualmente, y se distribuyeron aleatoriamente en un total de 50 unidades experimentales con la siguiente combinación de factores: cercado de exclusión de herbívoros (si/no) y micrositio (bajo encina o claro). El cercado de exclusión de herbívoros ocupa una superficie aproximada de dos hectáreas y se encuentra cerrado con malla cinegética que no permite la entrada de grandes herbívoros (ver descripción más detallada en apartado de métodos). En total se situaron 20 unidades experimentales fuera del cercado (10 bajo encina y 10 en claro), y 30 unidades dentro del cercado (15 bajo encina y 15 en claro).

En cada unidad experimental se colocaron 4 bellotas (sin cúpula) por especie, divididas en 4 grupos separados entre ellos un metro. La posición de cada grupo se marcó con una varilla de bambú separada 20 cm del mismo para evitar efectos de atracción. En total de colocaron 800 bellotas (200 por especie).

Toma de datos

En cada unidad experimental se midió con un distanciómetro (Leica Disto TM) la distancia al pino (*Pinus pinaster* Aiton.), encina y arbusto más próximo. En un círculo de 10 m de radio alrededor de cada réplica y se establecieron categorías de hábitat con relevancia para los posibles predadores (Pons y Pausas, 2007c): pasto, tronco árbol, hojarasca (> 1 cm profundidad), suelo desnudo, matorral, piedras y vivar. El porcentaje de cada categoría se determinó mediante estima visual. Con estos datos se calculó un índice de diversidad estructural en cada réplica, empleando la fórmula del índice de diversidad de Simpson ($1-\sum c_i^2$, siendo c_i el porcentaje de cada una de las categorías de hábitat).

Se realizó un primer censo a los dos días, otro semanal durante el primer mes, uno a los dos meses y un último censo a los 4 meses. En cada censo se anotaba la presencia/ausencia de cada bellota, y también si la bellota presentaba perforaciones por larvas, síntomas de depredación post-dispersiva (mordeduras) o si había germinado. Debido a que las bellotas con síntomas de depredación post-dispersiva *in situ* supusieron un porcentaje muy bajo del total (1%), éstas se consideraron en el análisis sin hacer distinción. En todos los censos se realizó una estima de la disponibilidad de alimento (bellotas) para cada una de las réplicas situadas bajo encina. Para ello se lanzaba al azar tres veces un cuadro de 25 cm de lado y se contaban las bellotas que caían dentro. Con ese dato se estimó un valor medio de cantidad de bellotas por m^2 en cada censo y réplica.

Análisis estadístico

Caracterización de los micrositios

Las diferencias entre micrositios (bajo encina y claro) en distancia al pino más próximo, índice de diversidad estructural, y en las variables de hábitat estructural más relevantes (% hojarasca y ramas, % suelo desnudo, % herbáceas, % matorral), se estudiaron mediante Anova de una vía.

Debido a que en los claros la disponibilidad de alimento fue muy pequeña (< 1 bellota por m^2) esta variable se estudió considerando sólo las unidades experimentales bajo encina. Las diferencias en disponibilidad de alimento entre las unidades experimentales situadas dentro y fuera del cercado se analizaron mediante anova de medidas repetidas. En este análisis no se incluyeron las medidas del último censo al ser todos los valores de cero.

Efecto del cercado sobre la depredación

En cada réplica y para cada especie se calculó un porcentaje de remoción temprana de bellotas (bellotas desaparecidas en la primera semana). Las diferencias entre la remoción dentro y fuera del cercado para cada especie se analizó mediante un test no paramétricos (U de Mann –Whitney).

Depredación post-dispersiva dentro del cercado de exclusión

Con el objetivo de estudiar la depredación por parte de pequeños depredadores (excluyendo a ungulados y jabalíes), se trabajó con la submuestra de unidades experimentales situadas dentro del cercado.

Para conocer si hubo selección de los depredadores a nivel de especie, tamaño de bellota y micrositio, se emplearon los porcentajes de remoción correspondientes a los primeros 64 días, ya que en el último censo prácticamente el 100% de las bellotas habían desaparecido (número de bellotas recuperadas *Q. ilex* = 2; *Q. suber* = 1; *Q. faginea* = 1; *Q. pyrenaica* = 0).

Para el estudio de la selección a nivel de especies, se estimó una curva de probabilidad de supervivencia para cada una de ellas (entendiendo como supervivencia la permanencia de la bellota) usando el método de Kaplan-Meier (Kaplan y Meier, 1958). También se calculó el índice de electividad (Pons y Pausas, 2007c) por especie y réplica como $Ei = (d – p) / (d + p)$, siendo d el número de bellotas que habían desaparecido en el censo del día 64 y p el número de bellotas que permanecieron. El índice de electividad varía entre -1 y 1, indicando los valores más bajos *evitación*, mientras que valores altos indican *preferencia*. La diferencia en índice de electividad entre especies se comparó mediante Anova de un factor, considerando como factor la especie y como variable dependiente el índice de electividad.

Ya que se detectaron diferencias de peso de bellota entre especies, siendo las bellotas de *Q. pyrenaica* más grandes que el resto (Kruskal-Wallis test, $P < 0,001$; Tabla 1), la selección por tamaño se evalúo de forma independiente para cada especie mediante anova de un factor. La variable “peso de bellota” fue transformada logarítmicamente para cumplir los requisitos de normalidad y homocedasticidad. La exploración gráfica de los residuos del modelo no indicó la necesidad de considerar la unidad experimental como factor aleatorio (Zuur et al., 2009).

Tabla 1. Media, desviación estándar, mínimo y máximo de pesos secos de las bellotas utilizadas en el experimento para cada una de las especies. (*Qi*: *Quercus ilex subsp ballota*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*).

	Peso bellota	
	media ± sd	rango
Qi	1,45 ± 0,45	0,61 – 2,99
Qs	2,26 ± 0,90	0,36 – 4,90
Qf	1,05 ± 0,29	0,47 – 1,80
Qp	3,68 ± 0,7	1,45 – 5,48

Las diferencias de selección entre micrositios (sombra de encina y claros) se analizaron mediante Anova de una vía (siendo el porcentaje de remoción la variable dependiente y micrositio el factor) para cada especie.

Depredación fuera del cercado

Con el objetivo de comprobar si las tendencias observadas dentro del cercado se mantenían fuera de él, se hicieron los mismos análisis con la submuestra de datos de fuera del cercado. Como variable indicativa del consumo se usó el porcentaje de remoción temprana (primera semana).

Todos los análisis estadísticos se hicieron con el programa Statistica 8.0. (Statsoft, Inc.).

RESULTADOS

Caracterización de los micrositios

La tabla 2 muestra las características de los micrositios considerados (bajo encina y claro). Las unidades experimentales bajo cobertura de encina presentaron mayores índices de diversidad estructural que los claros ($P < 0,05$), y porcentajes más altos de hojarasca ($P < 0,01$).

Se encontraron diferencias en disponibilidad de bellotas entre las encinas situadas dentro y fuera del cercado (*P* = 0,04). Fuera del cercado la media de disponibilidad por censo osciló entre 0 - 40,8 bellotas m^{-2} mientras que dentro las disponibilidad media osciló entre 56,7 - 72,8 bellotas m^{-2} (en ningún caso se tiene en cuenta el último censo en el que no se encontraron bellotas en ninguna unidad experimental).

Tabla 2. Caracterización de los micrositios estudiados para distintas variables de hábitat (media ± desviación estándar). Letras diferentes indican diferencias significativas (p < 0,05) entre grupos.

	Claro	**Bajo encina**
Distancia pino más próximo (m)	8,83 ± 3,74 a	9,03 ± 3,18 a
Indice diversidad estructural	0,47 ± 0,15 **a**	0,59 ± 0,13 **b**
Hojarasca y ramas (%)	13,33 ± 20,22 **a**	38,06 ± 19,48 **b**
Herbáceas (%)	28,33 ± 31,57 a	17,33 ± 25,00 a
Suelo desnudo (%)	35,33 ± 29,75 a	21,73 ± 18,95 a
Matorral (%)	17,40 ± 21,06 a	12,66 ± 18,09 a

Efecto del cercado sobre la remoción temprana

Después de una semana, en las unidades experimentales situadas fuera del cercado de exclusión la remoción de bellotas fue casi total (alrededor de un 80%), mientras que en las de dentro del cercado el porcentaje de remoción fue muy bajo (alrededor de 10%) (Fig. 1). Las diferencias entre fuera del cercado y dentro fueron significativas para todas las especies (*P* < 0,001 en todos los casos). Después de un mes, fuera del cercado habían desaparecido prácticamente el 100% de las bellotas.

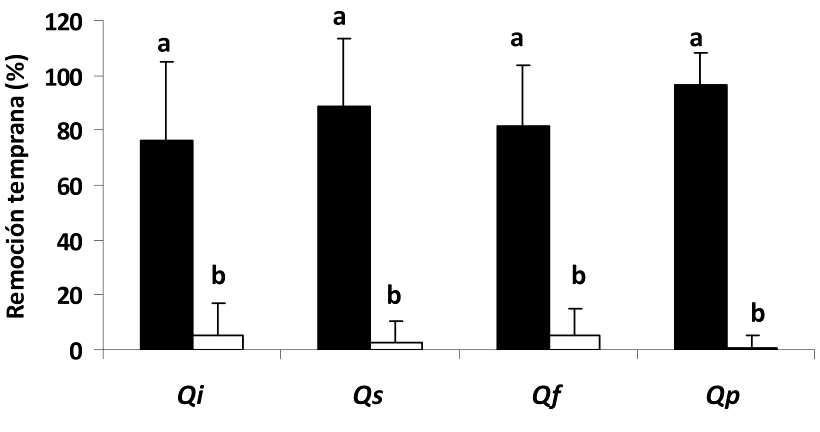

Figura 1. Porcentaje de remoción dentro (barras blancas) y fuera del cercado (barras negras) después de una semana para cada especie (*Qi*: *Quercus ilex subsp ballota*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*). Distintas letras indican diferencias significativas (*P* < 0.001) entre grupos para cada especie.

Depredación fuera del cercado

Fuera del cercado se encontraron diferencias significativas en el índice de electividad, siendo las bellotas de *Q. suber* y *Q. pyrenaica* las más consumidas (Tabla 3). No se encontró selección de tamaños de bellota para ninguna especie, si bien en general la tendencia fue a un consumo de bellotas mayores (*Q. ilex*: *P*=0,28; *Q. suber*: *P*=0,08; *Q. faginea*: *P*=0,06; *Q. pyrenaica*: *P*=0,58) (Fig 2). A nivel de micrositio no se encontraron diferencias significativas de depredación entre sombra y claros para ninguna especie (*Q. ilex*: *P*=0,15; *Q. suber*: *P*=0,57; *Q. faginea*: *P*=0,47; *Q. pyrenaica*: *P*=0,66).

Depredación post-dispersiva dentro del cercado de exclusión

Considerando los datos dentro del cercado de exclusión, hubo una selección de las bellotas de *Q. ilex* y *Q. faginea,* ya que tuvieron una menor probabilidad de permanencia durante los 64 días (Fig. 3). Esto también se comprobó con el índice de electividad, ya que se encontraron diferencias entre especies en los valores de este índice (*P* <0.001), prefiriendo los depredadores las bellotas de *Q. ilex* y *Q. faginea* frente a las de *Q. suber* y *Q. pyrenaica* (Tabla 3).

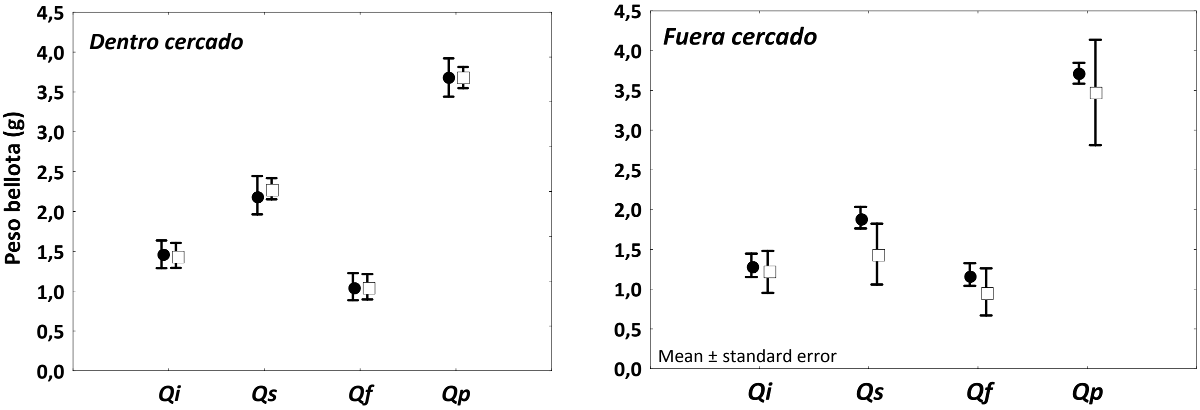

Figura 2. Peso (media ± error estándar) de las bellotas desaparecidas (círculos negros) y encontradas (cuadrados blancos) para cada especie dentro del cercado (64 días después de comenzar el experimento) y fuera del mismo (una semana después) (*Qi*: *Quercus ilex subsp ballota*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*)

En relación a la preferencia por tamaño de semilla, no se encontraron diferencias significativas en la selección en relación al peso de la bellota para ninguna especie (*Q. ilex*: *P* = 0,92; *Q. suber*: *P* = 0,53; *Q.faginea*: *P* = 0,8; *Q. pyrenaica*: *P* = 0,89) (Fig. 3).

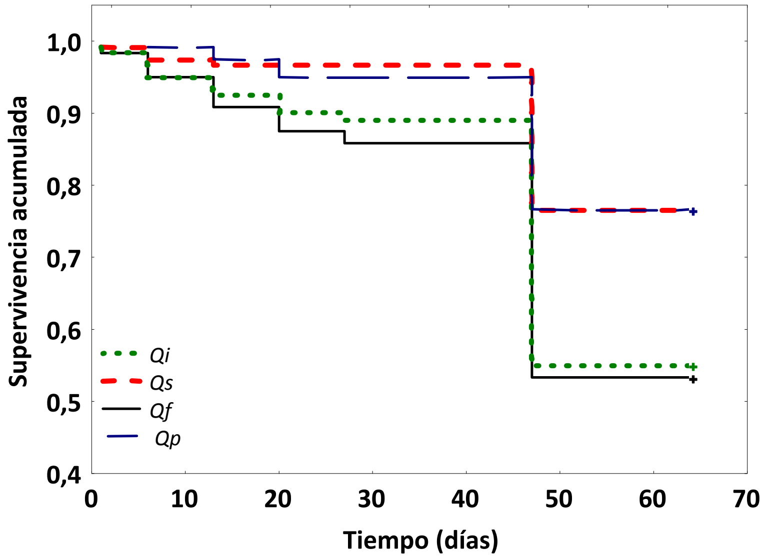

Figura 3. Curva de probabilidad (Kaplan Meier) de la supervivencia acumulada dentro del cercado (entendiendo por supervivencia la permanencia de cada bellota) para cada especie desde el inicio del experimento. (*Qi*: *Quercus ilex subsp ballota*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*)

Tabla 3. Media, desviación estándar, mínimo y máximo de índice de electividad para cada especie dentro del cercado (a los 64 días de experimento) y fuera del mismo (a la semana). Letras diferentes indican diferencias significativas (*P* < 0,05) entre especies (Qi: Quercus ilex subsp ballota; Qs: Q. suber; Qf: Q. faginea; Qp: Q. pyrenaica).

	Dentro cercado		**Fuera cercado**	
	media ± sd	rango	media ± sd	rango
Qi	-0,1 ± 0,56 **a**	-1 , 1	0,52 ± 0,57 **a**	-1 , 1
Qs	-0,55 ± 0,51 **b**	-1 , 0,5	0,77 ± 0,49 **a,b**	-1 , 1
Qf	-0,06 ± 0,62 **a**	-1 , 1	0,62 ± 0,45 **a,b**	-0,5 , 1
Qp	-0,53 ± 0,54 **b**	-1 , 1	0,92 ± 0,24 **b**	0 , 1

Se encontraron diferencias significativas entre micrositios para los porcentajes de remoción de bellota de encina y roble en enero (*Q.ilex: P*=0,02; *Q. pyrenaica: P*=0,04), y en alcornoque y quejigo la depredación bajo cobertura de encina fue mayor aunque no significativa (Fig. 4).

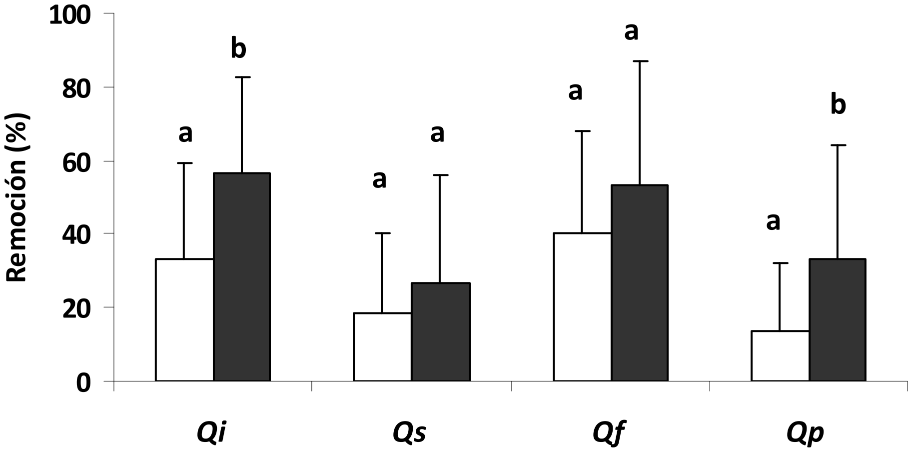

Figura 4. Porcentaje de remoción a los 64 días (censo de enero) para las unidades experimentales en claro (columnas blancas) y bajo encina (columnas negras). Letras diferentes indican diferencias significativas ($P < 0.05$) entre grupos para cada especie. (*Qi*: *Quercus ilex subsp ballota*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*).

DISCUSIÓN

La remoción de semillas fue muy alta para todas las especies y micrositios estudiados, tanto dentro como fuera del cercado de exclusión, ya que a los 4 meses de comenzar el experimento habían desaparecido prácticamente el 100% de las bellotas. La fase post-dispersiva supone por tanto una fuerte limitación en la regeneración natural de las especies de *Quercus* (Gómez, 2004b; Pulido y Díaz, 2005; Pérez-Ramos et al., 2010). Las mayores tasas de remoción, que llegaron al 80% en tan sólo una semana, se dieron en las bellotas situadas fuera del cercado de exclusión. Además en estas zonas la disponibilidad de bellotas fue menor, demostrando la importancia de los jabalíes y ciervos como grandes consumidores en esta fase del establecimiento (Gómez, 2004b; Pulido y Díaz, 2005).

Fuera del cercado, la posición de las bellotas no determinó su probabilidad de escapar a la depredación, indicando que este tipo de depredadores no manifiesta preferencia por ningún tipo de hábitat en concreto. Se encontró sin embargo una selección a nivel de especie, siendo *Q. suber* y *Q. pyrenaica* las consumidas en primer lugar. Este resultado es sorprendente y contrasta con otros estudios ya que generalmente las bellotas de *Q. ilex* son más apetecibles debido a sus características nutricionales (Pons y Pausas, 2007b). El hecho de que *Q. suber* y *Q. pyrenaica* presentaran un mayor tamaño de semilla hace pensar que ésta fue la causa de una mayor preferencia (Pérez-Ramos et al., 2008). Dicha selección de bellotas mayores no se detecta a nivel intraespecífico, seguramente por el menor rango de variabilidad en pesos de semilla existente dentro de cada especie.

Dentro del cercado, sin embargo, las tendencias cambian y la selección fue por especies. Los consumidores prefirieron las bellotas de *Q. ilex* y *Q. faginea*, que a su vez fueron las especies con semillas más pequeñas. La preferencia por estas dos especies probablemente se deba a las diferencias en contenido nutricional (León-Camacho et al., 2004), la dureza de la cáscara (Zhang et a., 2004) o la presencia de compuestos secundarios tóxicos como los taninos (Shimada y Saitoh, 2003). En este sentido, las bellotas de *Q. suber* presentan más proporción de compuestos fenólicos en la cáscara, lo que les confiere un sabor amargo que puede ser poco atractivo para los depredadores (Cantos et al., 2003). Otros autores han encontrado que los roedores son capaces de detectar el grado de dormancia de la semilla, de forma que ésta es rechazada si se encuentra próxima a la germinación (Smallwood et al., 2001). Debido a ello, especies con una germinación más temprana como las de *Q. pyrenaica* podrían ser evitadas por los depredadores. Por otro lado, aunque las 4 especies están representadas en el parque, en la parcela de estudio son precisamente *Q. ilex* y *Q. faginea* las únicas presentes. Este resultado podría sugerir una preferencia por parte de los depredadores (Janzen, 1971). Las selección específica por parte de los depredadores podría modificar el reclutamiento y influir en la abundancia de las especies que coexisten en un área (Crawley, 2000).

Por otro lado, no se encontró selección en función del tamaño de la semilla en ninguna especie. Aunque en general se ha observado una preferencia hacia bellotas más grandes (Gómez, 2004a; Cansen et al., 2004; Xiao et al., 2006), este resultado no es general (Xiao et al., 2004; Pons y Pausas, 2007c), pudiendo deberse a un rango pequeño de pesos de semilla dentro de cada especie o a la interacción de otros factores no considerados.

Con respecto al hábitat, la selección (en *Q. ilex* y *Q. pyrenaica)* fue mayor en los sitios bajo encina, que pueden representar sitios más seguros para los micromamíferos debido a su mayor diversidad estructural y hojarasca. Se ha observado que el riesgo de consumo de bellotas aumenta en las áreas de matorral donde los roedores concentran su actividad (Herrera, 1995; Alcántara et al., 2000; Pulido, 2002). Sin embargo, Gómez et al. (2003) no encontraron efecto del hábitat sobre la depredación de bellotas de *Q. pyrenaica*. En este caso, al tratarse de un área sin ningún tipo de exclusión, la selección por parte del jabalí esconde las preferencias de hábitat de los ratones. En nuestro estudio, para dos de las especies (*Q. suber* y *Q. faginea)*, las diferencias en porcentajes de remoción entre zonas bajo encina y claros no fueron significativas. Los arrendajos suelen consumir bellotas en zonas abiertas (Pons y Pausas, 2007a), teniendo por tanto una preferencia de hábitat opuesta a los micromamíferos y pudiendo enmascarar sus preferencias.

Por otro lado, los pinares parecen sitios menos visitados, con mayores zonas de suelo desnudo, donde los roedores sufren mayor riesgo de ser depredados (Pons y Pausas, 2007c). Este efecto no se observó en nuestra área de estudio donde los pinos son ejemplares dispersos y tanto los micrositios en claros como aquellos bajo encina se encontraban a distancias parecidas de los pinos.

Hay que tener en cuenta, sin embargo, que no todas las semillas desaparecidas tienen porqué haber sido consumidas. Muchos trabajos muestran cómo una parte de las semillas son comidas por roedores *in situ*, y la otra parte enterrada (Kollmann y Schill, 1996; Santos y Tellería, 1997). Aunque la mayoría de bellotas enterradas son después redescubiertas y consumidas (Kollmann y Schill, 1996; Seiwa et al., 2002) un pequeño porcentaje puede permanecer sin descubrir y escapar a la depredación. De esta forma los roedores pueden actuar como importantes agentes dispersores (Siscarta et al., 1999; Pulido and Díaz, 2005).

Algunos estudios han encontrado diferencias en las tasas de depredación entre años, estando este hecho relacionado con las fluctuaciones poblacionales de los consumidores y de la producción de bellotas (Silvertown, 1980; Wolf, 1996; Pérez-

Ramos et al., 2008). En el año de estudio se dio un pico de producción de bellotas (datos no publicados, área de Cardeña), por lo que sería de esperar que muchas de las bellotas enterradas no fueran después relocalizadas por un efecto de saciación de los depredadores (Janzen, 1971; Silvertown, 1980; Espelta et al., 2009). Aunque en nuestro experimento no evaluamos los movimientos de las bellotas, el hecho de encontrar un porcentaje bajo de restos de bellotas comidas *in situ* (< 2% en todos los censos) hace pensar en un mayor porcentaje de enterramiento. Por otro lado, Leiva y Fernández-Alés (2003) tampoco encontraron evidencias de saciación a lo largo de dos años de estudio. Es posible que el cercado haya tenido un efecto de aumento de la abundancia de roedores al excluir a sus depredadores (Pérez-Ramos y Marañón, 2008), de forma que su impacto sobre el reclutamiento siga siendo igualmente grande incluso en los años de mayor producción.

Fuera de los cercados, el reclutamiento puede verse fuertemente limitado, ya que los ciervos y jabalíes, presuntamente responsables de las mayores tasas de desaparición, no actúan como dispersores al consumir las bellotas *in situ*. Además, la abundancia de ungulados silvestres puede provocar una reducción en las poblaciones de depredadores-dispersores, especialmente en zonas con poco matorral como es el caso de la zona de estudio (Muñoz et al., 2009)

CONCLUSIONES

La depredación post-dispersiva supone un importante limitante a la regeneración natural de las especies de *Quercus* presentes en el P.N. Sierra de Cardeña y Montoro. Fuera de los cercados las bellotas desaparecieron rápidamente (una semana), y los depredadores parecen preferir las especies con bellotas de mayor tamaño (*Q. suber* y *Q. pyrenaica* en este caso). La presencia de cercados de exclusión proporciona lugares donde los jabalíes, responsables de esta alta tasa de remoción, no pueden acceder. Es interesante el hecho de que dentro del cercado la selección fuera distinta, consumiéndose preferentemente las bellotas de *Q. ilex* y *Q. faginea*, posiblemente debido a su mayor valor nutricional. Dentro del cercado no hubo selección en función del tamaño de la semilla, y la remoción fue significativamente mayor para dos especies en los sitio bajo encina. En estas zonas, y en años de alta producción de bellotas, podría haber una mayor probabilidad de escape a la depredación, debido a una menor tasa de desenterramiento de las bellotas por parte de sus depredadores. Para comprobar esta hipótesis y llegar a una mejor comprensión de las interacciones animal-semilla y sus consecuencias sobre el reclutamiento en esta zona será necesario realizar estudios de seguimiento de la depredación de bellotas y de las poblaciones de sus depredadores naturales.

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CAPÍTULO 2

Maternal influences on seed-mass effect and initial seedling growth in four *Quercus* species

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- *En segunda revisión en Acta Oecologica*

ABSTRACT

Seed mass represents the reserves available for growth in the first stages of plant establishment. Variation in seed mass is an important trait which may have consequences for growth and survival of seedlings. Three mechanisms have been proposed to explain how seed mass influences seedling development: the reserve use effect, the metabolic effect and the seedling-size effect. Few studies have evaluated at the same time the three hypotheses within species and none have evaluated the effect of the mother trees. We studied four *Quercus* species by selecting five mother trees per species. Seeds were sown in a glasshouse and use of seed reserves, seedling growth and morphology were measured. Considering all mothers of the same species together, we did not find the reserve effect for any species, the metabolic effect was observed in all species except for *Q. suber*, and the seedling-size effect was matched for all the species. Within species, maternal origin modified the studied mechanisms as we did not observe seed-mass effects on all mothers from each species. Moreover, the metabolic effect was not found in any mother of *Q. ilex* and *Q. faginea*. We concluded that a maternal effect can change seed-mass relationships with traits related to seedling establishment. The conservation of this high intra-specific variability must be considered to guarantee species performance in heterogeneous environments and in particular in the current context of climate change.

Keywords: *biomass allocation; intra-specific variability, oak; RGR; seedling establishment; SLA*

RESUMEN

El peso de una semilla representa las reservas disponibles para el crecimiento durante las primeras etapas de la vida de una plántula, siendo por tanto un rasgo que puede tener consecuencias importantes sobre su establecimiento. Se han propuesto tres mecanismos para explicar cómo el peso de la semilla influye sobre el desarrollo de la plántula: el efecto de la reserva, el efecto metabólico y el efecto plántula. Pocos estudios han evaluado las tres hipótesis sobre varias especies a la vez, y ninguno ha considerado el efecto del árbol madre. En este trabajo se estudiaron cuatro especies de *Quercus*, seleccionando cinco árboles madre por especie. Se sembraron las semillas en invernadero y se midió el uso de las reservas y otros parámetros morfológicos y de crecimiento. Considerando de forma conjunta a todas las madres de cada especie, no se encontró el efecto de la reserva en ninguna especie, el efecto metabólico se dio en todas las especies excepto en *Q. suber*, y el efecto plántula se observó en todas las especies. El árbol madre modificó los mecanismos estudiados ya que los efectos del peso de la semilla no se observaron para todas las madres dentro de cada especie. Además, el efecto metabólico no se encontró en ninguna madre de *Q. ilex* y *Q. faginea*. En conclusión, el árbol madre puede modificar los efectos del peso de la semilla sobre características de las plántulas relacionadas con el establecimiento. La conservación de esta variabilidad intra-específica debería ser considerada con el fin de garantizar la persistencia de las especies en ambientes heterogéneos, particularmente en el actual contexto de cambio climático.

Palabras clave: *distribución de biomasa; establecimiento; RGR; SLA; variabilidad intraespecífica*

INTRODUCCIÓN

Natural oak regeneration is constrained by different factors such as acorn predation, summer drought, soil impoverishment or herbivores (Pulido and Díaz, 2005; Pérez-Ramos and Marañón, 2008; Gómez-Aparicio et al., 2008). Many studies have focused on the factors driving seedling recruitment of *Quercus* oaks at different levels (Castro et al., 2006; Pausas et al., 2006). One important trait is seed mass as it represents the amount of reserves available for the growth of an embryo in the first stages of its life, thus having important consequences on plant early establishment (Navarro et al., 2006; Pérez-Ramos et al., 2010). Larger seeded plants could have advantages such as higher germination or emergence rates (Vázquez, 1998; Gómez, 2004a; Urbieta et al., 2008a) and a greater probability of survival (Gómez, 2004a; Moles and Westoby, 2004; Baraloto et al., 2005), especially in adverse environments like deep shady areas (Leishman and Westoby, 1994; Saverimuttu and Westoby, 1996) or in nutrient-poor soils (Milberg and Lamont, 1997). Moreover, seed mass seems to be a good predictor of other morphological traits describing fitness of species in their environment such as specific leaf area (*SLA*) or leaf mass fraction (*LMF*) in different habitats (Poorter and Rose 2005, Quero et al., 2008a).

Westoby et al. (1996) proposed three hypotheses to explain the mechanisms leading to a more successful establishment of larger-seeded species. Firstly, the ***“reserve effect*** postulates that the larger-sized seeds retain a larger amount of reserves, thus leaving a higher proportion of the reserves available to stand up to subsequent stress episodes (Westoby et al., 1996; Bonfil, 1998), and would increase survival possibilities under adverse environment conditions. The second hypothesis is the so-called ***“metabolic effect”***, in which it is predicted that species with larger seeds have a slower relative growth rate (*RGR*) (Marañón and Grubb, 1993; Baraloto et al. 2005). This effect has been observed specially during the early growth of species from different ecosystems (Poorter and Rose, 2005; Pérez-Ramos et al., 2010).

Finally, the ***“seedling-size effect”*** suggests that larger seeds produce larger seedlings (Hendrix et al*.,* 1991; Chacón and Bustamante, 2001; Green and Juniper, 2004; Castro et al,. 2008). These seedlings can develop longer roots thus reaching deeper layers of the soil, in which there are more water resources available (Milberg and Lamont, 1997). In addition, they have a greater aboveground growth, which permits them to develop a larger photosynthetic surface.

Quero et al. (2007) have suggested that the three hypotheses are connected. Therefore, the relationship between the seed and the seedling biomass is mediated by two effects: the use of the reserves and the metabolic effect. Increasing the use of reserves has a positive effect on seedling biomass, but a strong metabolic effect (a negative relationship between RGR and seed mass) could lead to a lack of any relationship between seed mass and seedling biomass. Most studies on the implications of the variation in seed size have been carried out by comparing different species. Differences in mean seed-size between species have been interpreted as being differential adaptations to a wide spectrum of ecological niches (Westoby et al., 1996). However, studies at an intra-specific level are also of interest, since they can provide a better understanding of the effect of variation in seed size on seedling establishment (Bonfil, 1998). Many studies have detected intra-specific variations in mean seed size within and between populations (Michaels et al., 1988; Ågren and Gren, 1989; Susko and Lovett-Doust, 2000; Castro et al., 2008; Rámirez-Valiente et al., 2009). This variability is also reflected in other characters like germination or aerial biomass (Sills and Nienhuis, 1995; Castro et al., 2008). This between–plant variability seems to be due to genetic differences between mother plants as well as environment conditions present at the moment of the seed’s development (Wulff, 1986; Ågren and Gren, 1989; Baskin and Baskin, 2001; Castro et al., 2008; Souza et al., 2010).

Quercus genus has high genetic diversity (Michaud et al., 1992), and phenotypic plasticity (Quero et al., 2008b). The existence of inter-individual variation in acorn yields has been studied for different species (Ramírez and Gómez, 1982; Ducousso et al,. 1993; Gómez 2004a). In fact, acorn size is a factor traditionally used for the selection of trees in open forest areas (*dehesas*) (Vazquez, 1998). Tilki and Alptekin (2005) found differences in germination rates among *Quercus aucheri* seeds from different provenances. Fernández-Rebollo et al. (2008) have found differences in acorn moisture percentage and chemical composition within populations of *Q. ilex*. This high variability among and within populations is also reflected in leaf traits (Bruschi et al., 2003; González-Rodríguez and Oyama, 2005; López de Heredía and Gil, 2006) and ecophysiological characteristics (Leiva and Fernández-Alés, 1998; Himrane et al., 2004; Sánchez-Vilas and Retuerto, 2007). Therefore, the mother provenance may determine seed mass, and also other traits associated with the seedling establishment (Castro et al., 2008). Since Leiva and Fernández-Alés (1998), no specific studies in order to assess maternal effects in *Quercus* species on seed-seedling relationships have been developed.

The aims of this study were: (1) to test the three seed-mass effect hypotheses (Westoby et al., 1996) at the same time for seeds collected from different mother trees, and made comparisons within and across four Mediterranean oak species and (2) to study the effect of the mother tree on seedling morphological attributes. *Quercus* acorns, as mentioned above, have a great inter-individual variability in seed mass. They also have two cotyledons, which are not photosynthetic but serve as resource storage (Bonner, 2003), making them suited for study of seed reserves. Furthermore, the study of traits related to oak establishment is of great interest due to their limited regeneration in the Mediterranean Basin (Marañón et. al, 2004). To our knowledge, this is the first work that combines an inter-specific and intra-specific approach in the study of seed mass effects on establishment.

MATERIAL AND METHODS

Selection of species and study area

Study area and species are described in general methods (p. 21-29)

Seed collection

Acorn collection was carried out in autumn 2006. Mother trees selection, acorn collection and seed dry mass predictions are described in general methods section (pag 29).

Plant cultivation

In December 2006, 10 acorns from each mother tree were selected from the stored ones, giving a total of 200 seeds (10 acorns × 5 mothers × 4 species). These acorns did not have any fungi or signs of predation, and were selected by flotation in order to eliminate any damaged ones. After weighing each fresh acorn, they were individually sown in pots in a greenhouse at Córdoba University, Spain (37º 51´N, 4º 48´W, at 100 m above sea level). Seed mass ranged from 1.87 ± 1.05 g (mean ± SD) in *Q. faginea* to 3.13 ± 1.47 g (*Q. ilex*). *Q. suber* and *Q. pyrenaica* acorns had intermediate mass values (2.5 ± 0.89 g and 2.51 ± 1.08 g, respectively) (Appendix S2).

The arrangement of the replicates in the greenhouse was completely random. Pots were made with PVC tubes of 50 cm in height and 10.5 cm in diameter to allow the development of a good root system. The substrate was a mixture of peat, sand and perlite at a 2:1:1 volume. Plants were watered until saturation by a daily drip irrigation system to ensure that water was not a limiting factor in their growth. The mean temperature was 16.4 ± 7.4°C during the three months of the experiment. The mean ± SD of the photosynthetic active radiation measured (with EMS7, canopy transmission meter, PPsystem, UK) during a clear day (February 2th, 2007) was 574 ± 191 µmol photon m^{-2} s^{-1}, and the total daily mean radiation throughout the experiment was 11.9 mol m^{-2} day^{-1}. Previous studies have not shown any effect of greenhouse structure on light quality (red: far red ratio = 1) (Quero et al., 2007). Every two days, individual emergence and number of leaves was recorded.

Seedling harvest

During February and March 2007 (67 ± 12 days after sowing), ten seedlings for each mother tree were randomly selected and harvested when its first group of leaves was completely unfurled (following Green and Juniper, 2004). At that moment, seedlings were considered independent from the seed reserves. After carefully taking the roots out of the substrate, each plant was divided up into leaves, stem, roots and remaining cotyledons and the fresh mass of each part was obtained. Leaves of each seedling were placed in individual bags with moistened filter paper and kept in a portable icebox until they were taken to the laboratory. The rest of the samples (stems and roots) were placed in paper envelopes. Once in the laboratory, all the leaves were scanned (HP Scan-jet 6300c), placed in envelopes and oven-dried at 70°C for a minimum of 48 hours, and subsequently weighed to obtain their dry mass. From the leaf images, the total leaf area of each harvested seedling was calculated using image analysis software (Image Pro-plus 4.5; Media Cybernetics, Inc).

The used seed reserves (*USR*) were calculated as $USR = Mi - Mr$, where Mi is the dry mass of the initial seed mass (determined from the regressions in Appendix S1, see Supplemental Data with the online version of this article) and Mr the dry mass of the remaining seed (cotyledon mass was only considered because this is where the seed reserves are localized). The relative growth rate (*RGR*) was calculated according to Steege et al. (1994) and Quero et al. (2007) as RGR (mg $g^{-1}day^{-1}$) = (log S – log USR)/ time, where S is the dry mass of the seedling with no cotyledons. The efficiency in the use of the reserves was calculated as Efficiency of reserve use (%) = $S \times 100/ USR$. Leaf area ratio (*LAR*) was calculated as the total area of leaves divided by the total seedling dry mass. Seedling biomass allocation —root mass fraction (*RMF*), stem mass fraction (*SMF*), and leaf mass fraction (*LMF*)— was calculated as the dry mass of root, stem, and leaves, respectively, divided by the total seedling dry mass (Hunt, 1990). Time of emergence was the time between sowing and stem emergence.

Thurnbull et al. (2008) have found that because *RGR* declines as individual plants grow, it could be heavily biased by initial size. In our study, we harvested all plants at the same time (about 67 days after sowing) when the development stage was similar (when its first group of leaves was completely unfurled) to avoid this confounding factor.

Statistical analysis

We studied the difference in seed mass between the different species by an analysis of variance where seed mass was the dependent variable and species the independent variable. We also did an analysis of variance for each species where seed mass was the dependent variable and mother tree the random factor.

To evaluate the three hypotheses of seed-mass effect, bivariate trait relationships were analysed by fitting Standardised Major Axis (SMA) lines to log scaled variables. SMA techniques provide a better estimate of the line summarizing the relationship between two variables (i.e. the main axis along which two variables are correlated) to that of ordinary linear regression, because the residual variance is minimized in both “x” and “y” dimensions, rather than the y dimension only (McArdle, 1988; Sokal and Rohlf, 1995). The analysis also determined the differences between the slopes obtained for each species or for each mother tree, so that a significant *P* indicated differences between the slopes of the groups studied. The free software statistics package SMART (Warton et al., 2006) was used.

For the *reserve effect* hypotheses to be supported two conditions must be fulfilled: 1) the slope of the relationship between the seed mass and the used seed reserve should be lower than 1, and, 2) the slope (*S*) of the seed mass–seedling biomass relationship should be significantly greater than the slope of the seed mass–reserves used relationship (Green and Juniper, 2004). If the slopes of these scaling relationships were the same, the ratio of reserve mass to seedling biomass would be the same across species or mothers differing in seed mass. This would be inconsistent with the idea that seedlings from larger seeded species are better provisioned to deal with hazards than those of smaller seeded species (Green and Juniper 2004). For the *metabolic effect* to be supported, the relationship between the seed mass and *RGR* should be significant and negative. For the *seedling size effect* to be supported there should be a positive relationship between the seed mass and the seedling biomass. Firstly, these relationships were evaluated for each of the four species, and secondly within the species for each of the mother trees.

We set up a mixed model ANCOVA to study the effects of mother tree (random factor) and seed mass (covariable) on different variables (time of emergence, total leaf area, efficiency of use of reserves, *RMF*, *SMF*, *LMF*, *LAR*). When necessary, a logarithmic transformation of the data was made to fulfill the requirements of normality and variance homogeneity (Zar, 1984). The statistical analyses were done using STATISTICA (version 7.1, Statsoft Inc.).

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).

RESULTS

Inter-specific level

The results of the SMA analysis for testing the three hypotheses are shown in Fig. 1 (analysis per species, considering together the different mother trees). For the **reserve effect hypothesis**, a significant and positive relation between seed mass and the reserves used was observed for all the species (with very high correlation coefficients ranging from 0.83 to 0.95, $P < 0.001$; Fig. 1A). The SMA slopes of *Q. ilex* ($S = 0.87$) and *Q. faginea* ($S = 0.91$) were significantly lower than 1 ($P < 0.05$) and for *Q. pyrenaica* the slope ($S = 0.87$) was near significant ($0.1 > P > 0.05$). This indicates that, in these species, an increase in the seed mass produces a proportionally lower increase in the consumption of the reserves.

For the **metabolic effect hypothesis**, there were different trends depending on the species. In *Q. ilex, Q. faginea* and *Q. pyrenaica*, the larger seeds had slower RGR (with correlation coefficients ranging from 0.38 to 0.89, $P < 0.01$, Fig. 1B). However, for *Q. suber* no relationship between the seed mass and RGR was observed ($P > 0.01$). Therefore, for the three species (*Q. ilex*, *Q. faginea* and *Q. pyrenaica*) the metabolic effect was fulfilled.

All the species showed an increase in seedling biomass related to an increase in seed mass (with correlation coefficients of between 0.68 to 0.89, $P < 0.001$) and, therefore, the **seedling size effect** hypothesis was fulfilled (Fig. 1C).

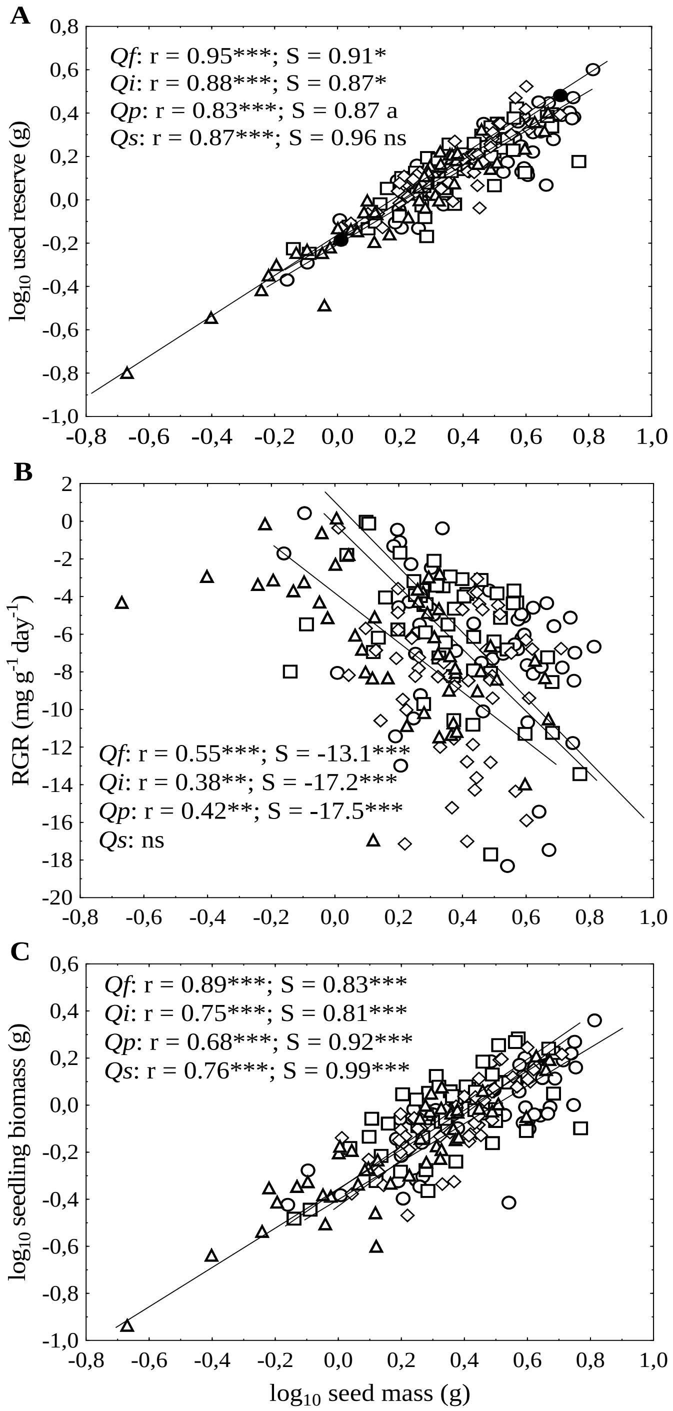


Figure 1. Relationships of initial seed dry mass versus (A) used seed reserve, (B) relative growth rate (RGR), and (C) seedling biomass after ca. 67 days of growth in four Quercus species. Pearson correlation (r) and significance are indicated as: ns, not significant; a, 0.05 < P < 0.1; *, P < 0.05; **, P < 0.01; ***, P < 0.001. The standardized major axis regression (SMA) lines are given when they are significant or marginally significant (0.1 > *P* > 0.05). All values remained significant after controlling the false discovery rate. The slope of each SMA regression (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) is given. Δ: *Quercus faginea* (*Qf*); ○: *Quercus ilex* (*Qi*); □: *Quercus pyrenaica* (*Qp*); ◊: *Quercus suber* (*Qs*).

Intra-specific level

Seed mass variability

As expected, seed-mass varied across maternal trees (Fig. 2). However, some mother trees had seeds with similar sizes; for instance, no differences were found between the seeds of mothers 4 and 5 in *Q. ilex* (Tuckey HSD post hoc test, Fig. 2). A similar situation was found for the rest of the species, since differences were found between some mother trees but not between others. All the mother trees had coefficients of variation in seed mass between 16% and 42% (Fig. 2), so there was also a variation within the seeds of one mother tree.

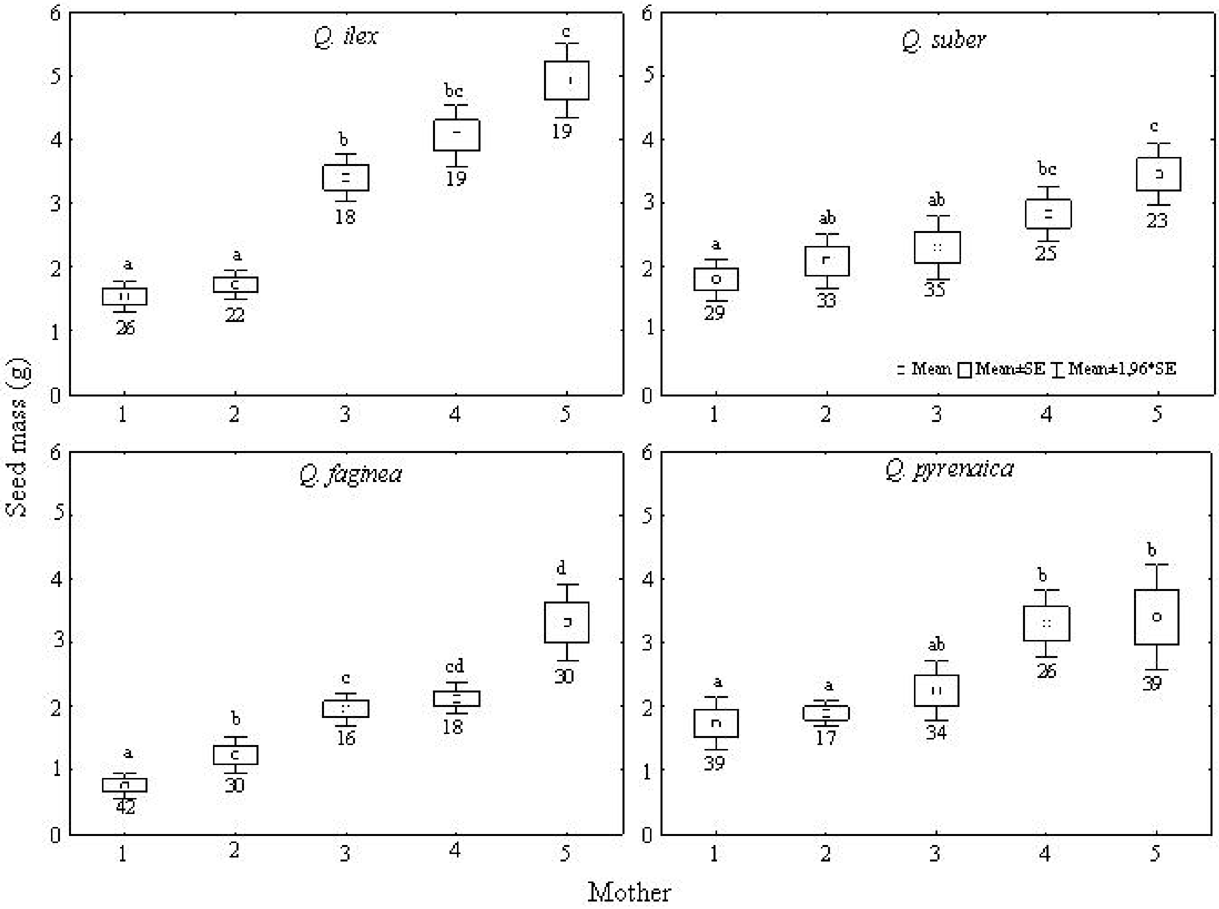


Figure 2. Mean seed mass of the different mothers for each species. Different letters represent statistically different groups according to post-hoc Tukey test ($P < 0.05$). The coefficient of variation is also shown below each box.

Testing the three hypotheses

For the **reserve effect hypothesis**, the relationship between the seed mass and the use of the reserves was positive and significant for all the mother trees of *Q. faginea* (correlations coefficients ranging from 0.74 to 0.99, $P < 0.05$, Fig. 3). Mother trees M_1, M_2, M_3 and M_5 had a slope (*S*) non-significantly different from 1 (slopes from 0.95 to 1.15, $P > 0.05$, Fig. 3), but mother tree M_4 showed a slope steeper than 1 ($S = 1.84$, $P < 0.001$, Fig. 3). This indicates that an increase in seed mass produced a proportionally higher consumption of seed reserves; the opposite to the reserve effect. Seeds produced by mother tree M_4 were different in size from those of mother trees M_1 and M_2, but no significant differences were found with mother trees M_3 and M_5 (Fig. 2). Thus, the different response was not caused by differences in seed mass. *Q. ilex* showed a significant and positive relationship between seed mass and the reserves used for mother trees M_1, M_2 and M_5 (correlation coefficients ranging from 0.78 to 0.88, $P < 0.05$, Fig. 3). The SMA slope for mother M_1 and M_2 was not different from 1 but that of mother tree M_5 ($S = 1.81$) was higher than 1 ($P < 0.01$) (Fig. 3), which was contrary to the reserve effect hypothesis. Two mother trees, M_1 and M_3 of *Q. pyrenaica,* showed a significant increase in the use of seed reserves with the increase in seed mass. Finally, all the mother trees of *Q. suber* showed a significant and positive relationship between seed mass and the use of its reserves (correlation coefficients from 0.69 to 0.88; $P < 0.05$, Fig. 3). In all the mother trees, the slope was close to 1, although for mother M_3 there was a pattern ($0.1 > P > 0.05$) of a slope lower than 1 ($S = 0.72$).

Concerning the **metabolic effect hypothesis**, the relationship between seed mass and *RGR* showed differences among mother trees. Mother trees of *Q. ilex* and *Q. faginea* did not show a significant relationship between seed mass and *RGR* (Fig. 3). One mother tree (M_5) of *Q. pyrenaica* showed a significant and negative relationship between seed mass and *RGR* and for mother tree M_4 there was also a negative pattern ($0.1 > P > 0.05$), where *RGR* decreased as the seed mass increased. In the case of *Q. suber*, only M_1 showed a significant and negative relationship between seed mass and

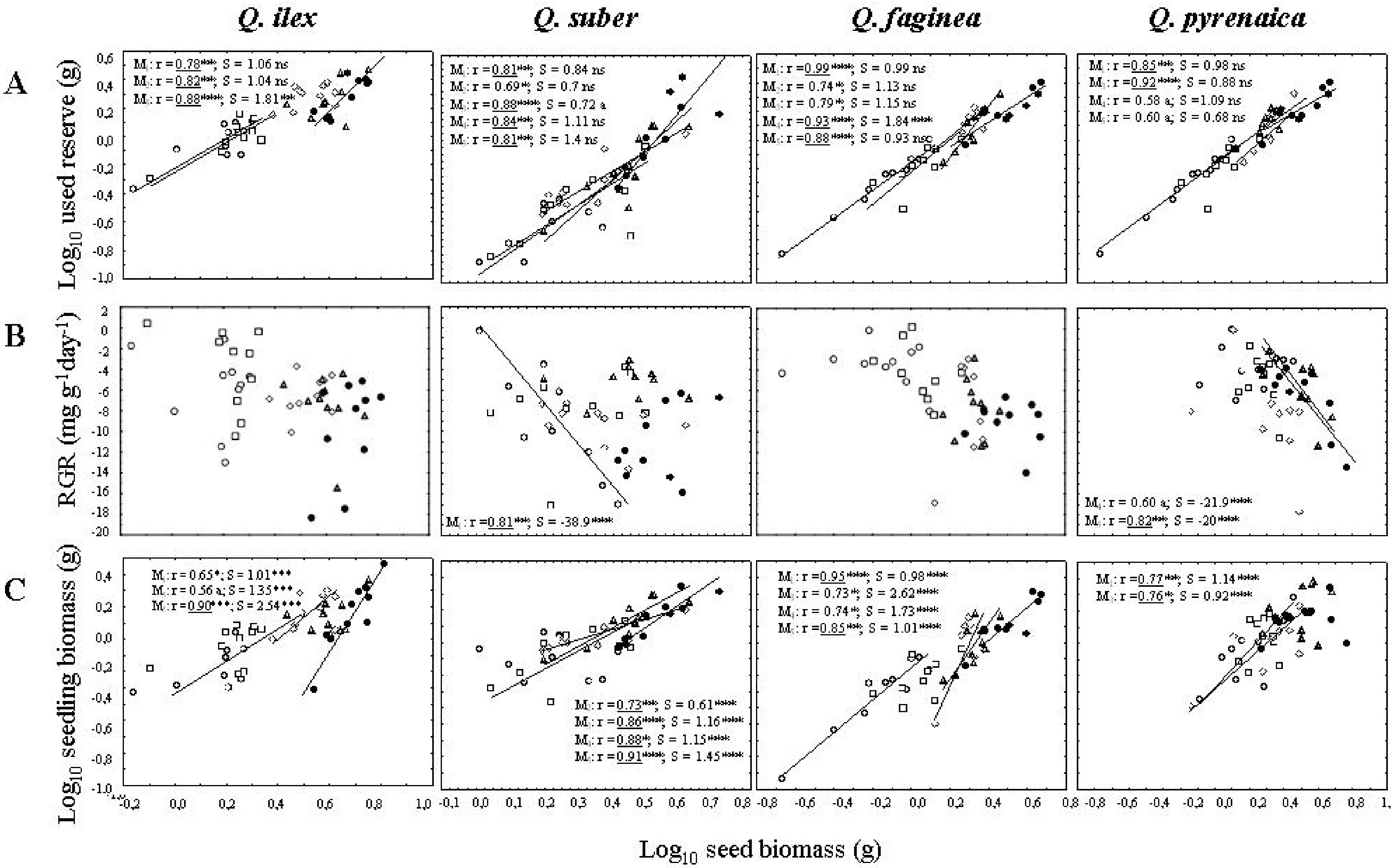
RGR (r = 0.81, $P < 0.01$). No metabolic effect was observed in the rest of the mother trees.

For the **seedling-size effect** within each species, differences between mother trees were also found (Fig. 3). For *Q. ilex,* mother trees M_1 and M_5 showed a significant and positive relationship between seed mass and seedling biomass; for mother tree M_3, the relationship was nearly significant (r = 0.56, 0.1> P >0.05). *Q. suber* presented a positive and significant relationship between seed mass and seedling biomass for all mothers (except M_1), thus confirming the seedling-size effect (Fig. 3). All mother trees of *Q. faginea* (except mother tree M_2), showed a significant and positive relationship between seed mass and seedling biomass (correlation coefficients ranging from 0.73 to 0.95; $P < 0.05$, Fig. 3). The slopes were significantly different from zero for all the mother trees, with mother M_3 and M_4 being the highest (M_3: S = 2.62, M_4: S = 1.73). Thus, confirming the seedling-size effect for all mothers (except M_2). Finally, for *Q. pyrenaica,* only two mother trees (M_1 and M_3) displayed a significant and positive relationship between seed mass and seedling biomass.

Maternal influences on morphological variables

The effect of the mother tree was significant for different variables depending on the species (Table 1). The mother effect was observed in the total leaf area of *Q. ilex* seedlings. For *Q. suber,* seeds of different mother trees differed in time of emergence and efficiency use of seed reserves. For *Q. faginea* no mother effects were observed for any of the variables studied. Seedlings of *Q. pyrenaica* differed among mother trees in the root and stem ratios (*RMF*, *SMF*) and the leaf area ratio (*LAR*).

Figure 3 (next page). Relationships of initial seed dry mass versus (A) used seed reserve, (B) relative growth rate (RGR), and (C) seedling biomass after ca. 67 days of growth within five progenitors of the four *Quercus* species. Pearson correlation (r) and significance are indicated as: ns, not significant; a, 0.05 < P < 0.1; *, P < 0.05; **, P < 0.01; ***, P < 0.001. r values remaining significant after controlling the false discovery rate are underlined. The standardized major axis regression (SMA) lines are given when they are significant or marginally significant (0.1 > P > 0.05). The slope of each SMA regression (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) is given. Mother trees: ○ M1; □ M2; ◊ M3; Δ M4; ● M5.


Q. ilex
Q. suber
Q. faginea
Q. pyrenaica
A
B
C
Log10 used reserve (g)
RGR (mg g-1day-1)
Log10 seedling biomass (g)
Log10 seed biomass (g)
M_1: r = 0.78***; S = 1.06 ns
M_2: r = 0.82***; S = 1.04 ns
M_3: r = 0.88****; S = 1.81***
M_1: r = 0.81***; S = 0.84 ns
M_2: r = 0.69**; S = 0.7 ns
M_3: r = 0.88****; S = 0.72 a
M_4: r = 0.84***; S = 1.11 ns
M_5: r = 0.81***; S = 1.4 ns
M_1: r = 0.99****; S = 0.99 ns
M_2: r = 0.74**; S = 1.13 ns
M_3: r = 0.79**; S = 1.15 ns
M_4: r = 0.93****; S = 1.84****
M_5: r = 0.88****; S = 0.93 nsc
M_1: r = 0.85***; S = 0.98 ns
M_2: r = 0.92****; S = 0.88 ns
M_4: r = 0.58 a; S = 1.09 ns
M_5: r = 0.60 a; S = 0.68 ns
M_1: r = 0.81***; S = -38.9****
M_4: r = 0.60 a; S = -21.9****
M_5: r = 0.82***; S = -20****
M_1: r = 0.65*; S = 1.01***
M_2: r = 0.56 a; S = 1.35***
M_3: r = 0.90***; S = 2.54***
M_2: r = 0.73***; S = 0.61****
M_3: r = 0.86****; S = 1.16****
M_4: r = 0.88**; S = 1.15****
M_5: r = 0.91****; S = 1.45****
M_1: r = 0.95****; S = 0.98****
M_3: r = 0.73**; S = 2.62****
M_4: r = 0.74**; S = 1.73****
M_5: r = 0.85***; S = 1.01****
M_1: r = 0.77***; S = 1.14****
M_5: r = 0.76**; S = 0.92****
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
-0,1
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
-0,8
-0,6
-0,4
-0,2
0,0
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0,6
0,8
-0,8
-0,6
-0,4
-0,2
0,0
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0,6
0,8
1,

Table 1. Results of mixed model ANCOVA to study the effects of mother tree (M, random factor), seed mass (covariable) and the interaction between both factors (M x Seed) on different variables (time of emergence, total leaf area, efficiency of use of reserves, RMF, SMF, LMF and LAR). Numbers indicate the % of variance explained by each factor calculated as SS_x / SS_{total}, where x is the factor. R^2 (x 100) is the variance explained by the model. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; Values of the variance explained by each factor remaining significant after controlling the false discovery rate are underlined. Efficiency use is the calculated as: seedling biomass with no cotyledons × 100/ reserves used; RMF (root mass fraction; root/ plant biomass), SMF (stem mass fraction; stem/plant biomass), LMF (leaf mass fraction; leaf/plant biomass), LAR (leaf area ratio; total leaf area/plant biomass). All biomass was expressed on dry mass basis.

Species	**Effect**	**Time Emergence**	**Total leaf area**	**Efficiency Use**	**RMF**	**SMF**	**LMF**	**LAR**
Q. ilex	Mother (M)	8.39	**15.83****	12.35	7.37	3.53	11.83	12.32
	Seed	13.76	**28.35****	0.18	0.90	3.97	0.01	1.24
	M x Seed	**17.20***	11.52	7.89	6.22	5.21	8.12	6.60
	R^2	**45.61****	**74.30****	28.01	15.50	15.51	22.21	**34.60***
Q. suber	Mother (M)	**17.20***	14.77	**23.23***	5.22	7.51	4.26	13.37
	Seed	1.51	5.45	1.37	**13.04***	0.51	**17.0****	4.09
	M x Seed	**17.39***	9.46	**22.74***	6.76	13.63	3.25	12.10
	R^2	**49.98*****	30.01	**39.07****	**53.6*****	**34.83***	**50.4*****	30.10
Q. faginea	Mother (M)	7.58	14.27	12.78	5.31	6.34	5.25	8.59
	Seed	0.86	6.67	1.33	12.71	0.70	**15.99***	4.13
	M x Seed	13.70	10.94	10.09	10.30	7.67	7.77	13.64
	R^2	**39.97***	**59.61*****	**35.21***	**36.72***	12.64	**40.34***	**38.76***
Q. pyrenaica	Mother (M)	17.16	8.11	2.47	**19.6****	**22.41****	13.72	**19.4****
	Seed	0.06	**19.84****	3.19	4.31	0.92	5.34	9.01
	M x Seed	10.36	10.35	3.64	**18.24***	15.12	14.33	**19.8****
	R^2	31.55	**43.80****	24.71	29.79	**45.22****	23.27	**36.99***

DISCUSSION

We analyzed the influence of the seed mass on the seedling size by means of the use of its reserves and of its growth rate, both within and across four oak Mediterranean species. In spite of low sample sizes, we found significant among-trees differences in these relationships, thus showing that maternal origin may modify seed mass effects.

Seed mass effect

As described above, the seed mass effect can be explained by three hypotheses: *reserve effect*, *metabolic effect* and *seedling-size effect*. Two conditions are necessary in order for the ***reserve effect*** to be accomplished (Green and Juniper, 2004). First, the slope of the relationship between the initial seed mass and the use of reserves should be less than one. Second, the slope of the seedling size effect should be greater than that of the reserve effect. In our experiment, the first condition was met for *Q. ilex* and *Q. faginea,* but the second condition was not fulfilled, so that the reserve effect was not supported, contradicting results found by Kidson and Westoby (2000). This effect has not been clearly shown for the genus *Quercus* (Quero et al*.,* 2007) and nor in other species (Green and Juniper, 2004) so it seems that the reserve effect is not generalized. It is known that *Quercus* acorns are recalcitrant so their viability is very short as they are desiccation-sensitive, and, moreover, they decompose soon because of their high moisture content (Roberts, 1973; Finch-Savage, 1992). Therefore, for this type of seed, there is no a clear advantage in retaining their reserves as they will not be useful later. Most of the studies on reserve effect are on total reserves (mainly carbohydrates), but other resources may also be important, as for example nutrients. In this way, a recently study found that *Q. ilex* seedlings depend on remobilization of acorn N reserves to roots during the first stages of life (Villar-Salvador et al., 2009).

Interestingly, different trends have been found when analyzing the seed mass hypothesis between mother trees within species. In the case of the reserve effect, differences between mother trees cannot be explained by mean seed mass differences, so it appears to be a factor associated with the mother tree, which has an influence on the use of the seed reserves. We have found a contrary effect to the reserve effect for some mothers (i.e. a higher increase of the reserves used with an increase in seed mass) (M_5 in *Q. ilex,* M_4 in *Q. faginea*). This result could be due to the recalcitrant characteristics of *Quercus* acorns as explained above, where a fast mobilization of the seed reserves could be another successful strategy.

The ***metabolic effect*** hyphoteses assumes that seedlings from larger seeds have a slower *RGR*. This hypothesis was confirmed for all the species except for *Q. suber*, corroborating results of Quero et al. (2007). This effect could be due either to lower respiration rates, a slower consumption of the seed resources or a lower efficiency in the conversion of seed reserves to seedling biomass. Thurnbull et al. (2008) found that calculation of *RGR* could be heavily biased by initial size. In our study, because we harvest all plants at the same time and with similar development stage we think this problem does not occur.

The metabolic effect changes dramatically within species considering the mother trees. For example, although there was a significant and negative relationship between seed mass and *RGR* for *Q. ilex* and *Q. faginea*, we did not found any metabolic effect in any mother of the two species. This could be due to each mother tree occupying a small range of seed mass (Fig. 2), so it is possible that there was not enough variability in the seed size within mother trees to show this effect. This would also indicate that *RGR* is not strongly associated with seed mass as the reserves used or seedling size are, whose effects appear even for small ranges of seed mass (Fig. 3). Similar results were found by Castro et al. (2008) in Scots pine seedlings from different maternal plants. In that study *RGR* was weakly correlated to seed mass, suggesting that the relationship between *RGR* and seed mass is not causal, but reflects an evolutionary covariation in these traits.

According to the ***seedling-size effect*** hypotheses***,*** a larger seed mass is related to a larger seedling biomass, which would confer a series of advantages on the seedling in its establishment (Hendrix et al., 1991; Eriksson, 1999; Chacón and Bustamante, 2001; Khan, 2004; Castro et al., 2008). In this study, this hypothesis has been supported by all species. Quero et al. (2007) only observed the seedling size effect in two out of four *Quercus* species under similar light availability. However, they studied seedlings in a more advanced ontogenetic phase, in which seed-mass effects might be masked with photosynthetic gains. Light conditions may influence the seedling-size effect, as this effect was mainly observed under moderate or dense shade conditions by Quero et al. (2007) and Leishman and Westoby (1994). However, Chacón and Bustamante (2001) and Quero et al. (2008a), found the *seedling size effect* under different environment conditions: both in drought and irrigation regimes, the latter being comparable to the conditions in our study.

Considering the mother tree, at least for one mother tree per species, the seedling-size effect was not found. It seems that, regardless of the seed size, there are other factors related to maternal influence. Different causes could be responsible for this, such as a different chemical composition of the seeds, efficiency of reserve use or biomass allocation (Leiva and Fernández-Alés, 1998; Rodríguez-Estévez et al., 2008).

To summarize, an intensive use of the reserves determined larger seedlings in the first development stages, as in the case of M_4 of *Q. faginea*, or M_5 of *Q. ilex* (Fig. 3). These results encourage the idea that the production of larger seedlings from larger seeds is more related to the amount of reserves stored in the cotyledons and their use rather than to the initial growth rates (Baskin and Baskin, 2001; Castro et al., 2008). However, for some mother trees, the metabolic effect showed up (M_4 and M_5 of *Q. pyrenaica* or M_1 of *Q. suber*) and this was the cause of the lack of relationship between seed mass and seedling biomass. This was predicted by the model of Quero et al. (2007). For other mother trees (M_2 of *Q. faginea*) neither the metabolic effect nor the seedling effect were found. For some reason, these plants were less efficient in the use of their reserves. The chemical composition and proportion of carbohydrates in the seeds or the respiration rates during germination could offer some explanation.

Assessing maternal influences

As described above, we found different trends between mother trees when evaluating seed mass effects, and maternal origin seems to determine other traits acting independently from the seed size, such as acorn genetic variability or physiological status (Merouani et al., 2001; Goodman et al., 2005). This idea is corroborated by other studies in which a mother effect in *Q. ilex* on characteristics associated with establishment and survival was found (Leiva and Fernández-Alés, 1998).

The mother effect could also introduce differences into certain morphological characteristics of the plants. For instance, in the case of *Q. pyrenaica*, the investment in their roots and stems was different depending on the mother, indicating diverse strategies. Some plants invest more in their roots so that they can capture more nutrients and reach deeper layers of the soil, whereas the strategy of others is to develop more photosynthetic tissue and support organs to grow faster (Villar et al., 2008).

It has been proposed that maintaining high phenotypic diversity may be crucial for species inhabiting in heterogeneous environments (Sánchez-Vilas and Retuerto, 2007). In this way, intra-species and interspecies variability needs to be considered for an applied perspective. One widely employed practice in seed companies and restoration programs is to select mother trees with big acorns, from which more robust plants could be obtained (Vázquez, 1998). Sometimes collection of seeds is done in a limited number of mother trees, which lead to plantations or restoration programs, where genetic diversity is significantly reduced (Rajora, 1999; Burgarella et al., 2007). Moreover, as Leiva and Fernández-Alés (1998) and this study have found, seedlings from different mothers showed a different biomass allocation pattern, which could confer a different drought resistance. Therefore, if we consider that retention of high variability and genetic resources is vital for species to survive in unpredictable and heterogeneous environments and face climate change, these practices should be revised.

CONCLUSIONS

This experiment was aimed to address the three hypotheses of seed-mass effect (Westoby et al., 1996) for four species of *Quercus* and among different mother trees of each species. At species level, the reserve effect was not observed for any species, probably because of the recalcitrant character of *Quercus* seeds. The metabolic effect was observed in three species, and the seedling-size effect was matched for all the species. Interestingly, within species, the mother trees introduced differences in the three hypotheses, as seed effects were found just for some mothers. In general, it seems that the production of larger seedlings from larger seeds is more related to the amount of reserves stored in the cotyledons and its use rather than to the initial growth rates. Further research is needed on the mechanisms regulating these relationships (such as the composition of the reserves, the respiration rates and the mobilization of carbohydrates). This study supports the idea that the high variability in mean seed mass within populations in *Quercus* species could affect other traits associated with establishment. Therefore, it is important to conserve this diversity as it provides the potential for surviving in heterogeneous environments and in the actual context of climate change.

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SUPPLEMENTARY INDEX

Appendix S1. Regression equations used for calculating the initial seed dry mass (S_{DM}) using the acorn fresh mass (A_{FM}) for each of the mother trees. All the weights are expressed in grams. For each species, the mothers have been numbered in the first column from 1 to 5. N and R^2 of the regressions is given. All the regressions are significant ($P < 0.001$).

	Quercus ilex	*Quercus faginea*	*Quercus suber*	*Quercus faginea*	*Quercus pyrenaica*
1	$S_{DM} = - 0.48 + 0.48 \times A_{FM}$ ($N = 9$; $R^2 = 0.88$)	$S_{DM} = - 0.12 + 0.50 \times A_{FM}$ ($N = 10$; $R^2 = 0.99$)	$S_{DM} = - 0.28 + 0.44 \times A_{FM}$ ($N = 9$; $R^2 = 0.93$)	$S_{DM} = - 0.12 + 0.50 \times A_{FM}$ ($N = 10$; $R^2 = 0.99$)	$S_{DM} = - 0.02 + 0.50 \times A_{FM}$ ($N = 10$; $R^2 = 0.99$)
2	$S_{DM} = - 0.47 + 0.52 \times A_{FM}$ ($N = 9$; $R^2 = 0.97$)	$S_{DM} = 0.02 + 0.36 \times A_{FM}$ ($N = 9$; $R^2 = 0.86$)	$S_{DM} = - 0.58 + 0.51 \times A_{FM}$ ($N = 8$; $R^2 = 0.95$)	$S_{DM} = 0.02 + 0.36 \times A_{FM}$ ($N = 9$; $R^2 = 0.86$)	$S_{DM} = - 0.01 + 0.49 \times A_{FM}$ ($N = 10$; $R^2 = 0.97$)
3	$S_{DM} = - 0.03 + 0.48 \times A_{FM}$ ($N = 10$; $R^2 = 0.98$)	$S_{DM} = - 0.34 + 0.57 \times A_{FM}$ ($N = 9$; $R^2 = 0.94$)	$S_{DM} = 0.01 + 0.48 \times A_{FM}$ ($N = 9$; $R^2 = 0.98$)	$S_{DM} = - 0.34 + 0.57 \times A_{FM}$ ($N = 9$; $R^2 = 0.94$)	$S_{DM} = - 0.63 + 0.54 \times A_{FM}$ ($N = 8$; $R^2 = 0.93$)
4	$S_{DM} = 0.37 + 0.43 \times A_{FM}$ ($N = 9$; $R^2 = 0.94$)	$S_{DM} = - 0.22 + 0.50 \times A_{FM}$ ($N = 9$; $R^2 = 0.93$)	$S_{DM} = - 0.36 + 0.52 \times A_{FM}$ ($N = 10$; $R^2 = 0.99$)	$S_{DM} = - 0.22 + 0.50 \times A_{FM}$ ($N = 9$; $R^2 = 0.93$)	$S_{DM} = - 0.50 + 0.51 \times A_{FM}$ ($N = 9$; $R^2 = 0.96$)
5	$S_{DM} = - 0.04 + 0.41 \times A_{FM}$ ($N = 8$; $R^2 = 0.96$)	$S_{DM} = - 0.23 + 0.51 \times A_{FM}$ ($N = 9$; $R^2 = 0.91$)	$S_{DM} = - 0.38 + 0.50 \times A_{FM}$ ($N = 9$; $R^2 = 0.96$)	$S_{DM} = - 0.23 + 0.51 \times A_{FM}$ ($N = 9$; $R^2 = 0.91$)	$S_{DM} = - 0.22 + 0.54 \times A_{FM}$ ($N = 9$; $R^2 = 0.97$)

Appendix S2. Mean values ± SD of different variables for the five mother trees of the four *Quercus* species studied (M = mother tree). Also the mean values for each species are presented.

Species	**Mothers**	**Acorn fresh mass (g)**	**Acorn dry mass (g)**	**Seed dry mass (g)**	**Use of reserves (g)**	**RGR (mg g^{-1}day^{-1})**	**Seedling biomass (g)**
Q. ilex	**M_1**	4.15 ± 0.81	2.28 ± 0.46	1.54 ± 0.39	0.95 ± 0.28	-6.02 ± 3.83	0.58 ± 0.19
	M_2	4.18 ± 0.73	2.49 ± 0.46	1.72 ± 0.38	1.02 ± 0.26	-3,82 ± 3.9	0.76 ± 0.19
	M_3	7.11 ± 1.27	4.42 ± 0.71	3.40 ± 0.61	1.96 ± 0.34	-6.50 ± 1.90	1.20 ± 0.29
	M_4	8.46 ± 1.78	5.13 ± 0.84	4.06 ± 0.77	1.82 ± 0.61	-7.38 ± 3.11	1.13 ± 0.30
	M_5	11.99 ± 2.26	6.21 ± 1.16	4.93 ± 0.93	2.25 ± 0.80	-9.65 ± 4.83	1.22 ± 0.54
	All mothers	7.18 ± 3.29	4.11 ± 1.70	3.13 ± 1.47	1.60 ± 0.72	-6.67 ± 3.98	0.98 ± 0.41
Q. suber	**M_1**	4.70 ± 1.19	2.62 ± 0.69	1.81 ± 0.52	1.09 ± 0.27	-8.94 ± 5.14	0.68 ± 0.19
	M_2	5.24 ± 1.36	2.67 ± 0.75	2.1 ± 0.69	1.22 ± 0.33	-7.81 ± 3.66	0.76 ± 0.26
	M_3	4.71 ± 1.64	2.89 ± 0.99	2.31 ± 0.79	1.46 ± 0.35	-8.96 ± 2.21	0.88 ± 0.18
	M_4	6.13 ± 1.34	3.83 ± 0.85	2.83 ± 0.69	1.69 ± 0.47	-5.23 ± 1.57	1.14 ± 0.32
	M_5	7.59 ± 1.54	4.37 ± 0.88	3.46 ± 0.77	2.16 ± 0.65	-11.14± 3.5	1.15 ± 0.35
	All mothers	5.67 ± 1.75	3.28 ± 1.07	2.5 ± 0.89	1.52 ± 0.56	-8.42 ± 3.83	0.92 ± 0.32
Q. faginea	**M_1**	1.75 ± 0.64	1.03 ± 0.38	0.76 ± 0.32	0.55 ± 0.24	-3.46 ± 2.10	0.42 ± 0.16
	M_2	3.04 ± 0.92	1.58 ± 0.45	1.14 ± 0.33	0.68 ± 0.20	-4.24 ± 2.76	0.49 ± 0.14
	M_3	4.08 ±0.57	2.52 ± 0.35	1.98 ± 0.32	1.33 ± 0.26	-7.85 ± 4.98	0.81 ± 0.27
	M_4	4.69 ±0.78	2.75 ± 0.47	2.12 ± 0.39	1.33 ± 0.43	-7.74 ± 2.80	0.80 ± 0.25
	M_5	6.84 ± 1.90	4.13 ± 1.26	3.32 ± 0.98	1.70 ± 0.47	-9.00 ± 2.1	1.09 ± 0.33
	All mothers	4.10 ± 2.06	2.41 ± 1.29	1.87 ± 1.05	1.12 ± 0.55	-6.44 ± 3.65	0.72 ± 0.34
Q. pyrenaica	**M_1**	3.45 ± 1.31	2.12 ± 0.80	1.73 ± 0.66	1.04 ± 0.43	-3.72 ± 2.03	0.80 ± 0.35
	M_2	3.84 ± 0.65	2.30 ± 0.36	1.89 ± 0.32	1.18 ± 0.24	-4.87 ± 2.5	0.87 ± 0.24
	M_3	5.33 ± 1.42	3.23 ± 0.95	2.25 ± 0.76	1.36 ± 0.47	-8.17 ± 4.51	0.88 ± 0.30
	M_4	7.43 ± 1.67	4.39 ± 1.06	3.30 ± 0.85	1.86 ± 0.55	-5.8 ± 0.85	1.33 ± 0.44
	M_5	6.64 ± 2.41	4.15 ± 1.51	3.40 ± 1.31	1.70 ± 0.40	-6.55 ± 3.25	1.14 ± 0.27
	All mothers	5.38 ± 2.18	3.24 ± 1.34	2.51 ± 1.08	1.43 ± 0.52	-5.82 ± 3.35	1.00 ± 0.37

CAPÍTULO 3

Spatial and temporal heterogeneity effects on seedling growth and establishment in four *Quercus* species

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ABSTRACT

The high spatial and temporal heterogeneity of Mediterranean ecosystems can influence establishment success in woody species, natural regeneration of which occurs to a very small extent in such ecosystems. In this work, the effect of the spatial pattern of environmental variables on growth and establishment success was examined by using a spatially explicit design. Seeds of four *Quercus* species differing in leaf longevity (*Q. ilex*, *Q. suber*, *Q. faginea* and *Q. pyrenaica*) were sowed at nodes 4 m apart in two 40 × 40 m plots located in a holm oak forest in the "Sierra de Cardeña y Montoro" Natural Park (Córdoba, S Spain). Light availability, soil moisture and herb production were measured, and seedling emergence, growth, survival and establishment success monitored over a period of one year. The spatial pattern of the studied variables was examined via Spatial Analysis by Distance Indices (SADIE). Multiple regression analysis was developed in order to find the factors which best predicted growth and plant morphology. All environmental variables exhibited an aggregated spatial pattern. There was, however, no spatial covariance between them; rather, a number of abiotic factor combinations were observed, suggestive of the presence of a wide variety of microsites. This is consistent with the absence of association between the spatial pattern for environmental variables and those for emergence and survival. Only soil moisture during the dry season was associated with all species establishment success. The distribution pattern for establishment success persisted throughout the dry period and only disappeared in those cases where a high mortality essentially derived from low soil water availability was observed. No aggregated spatial pattern for morphological or growth traits was apparent and multiple regression analysis showed that these traits were more dependent on seed mass than on environmental factors.

Keywords: *oak, regeneration, seedling performance, spatial analysis by distance indices, survival, variability*

RESUMEN

La gran heterogeneidad espacial y temporal en los ecosistemas mediterráneos puede influir en el establecimiento de las plantas leñosas, cuya regeneración suele estar muy limitada. En este trabajo se examinó el efecto del patrón espacial de las variables ambientales sobre el crecimiento y establecimiento mediante un diseño experimental espacialmente explícito. Se diseñaron 2 mallas de 40 x 40 m, con una resolución de 4 m en un encinar del Parque Natural Sierra de Cardeña y Montoro (SE Córdoba, S España). En cada nodo se sembraron semillas de 4 especies de *Quercus* que difieren en la longevidad foliar (*Q. ilex, Q. suber, Q. faginea y Q. pyrenaica*) y se tomaron medidas de luz, humedad del suelo y producción de pasto. Durante un año se estudió la emergencia, la supervivencia, el éxito en el establecimiento y el crecimiento de las plántulas de *Quercus*. El patrón espacial de las variables estudiadas se analizaron mediante índices de distancias (SADIE). Todas las variables ambientales (luz, humedad del suelo y producción de pasto) presentaron una distribución espacial agregada. Sin embargo, no se encontró apenas covariación espacial entre ellas, sugiriendo una gran variedad en las combinaciones de los factores estudiados. Esto es consistente con la ausencia de asociación entre el patrón espacial de las variables ambientales con la emergencia y supervivencia, debido seguramente a la coincidencia en el espacio de factores con efectos opuestos. Sólo la humedad del suelo durante la estación seca se asoció con el establecimiento al considerar a todas las especies en conjunto. El patrón de agregación espacial del éxito en el establecimiento persistió a lo largo del periodo seco y sólo desapareció en los casos en los que, debido a la falta de agua, la mortalidad fue muy elevada. No se encontró distribución agregada en las variables morfológicas y de crecimiento, siendo estos rasgos más dependientes del peso de la semilla que de los factores externos.

Palabras clave: *análisis espacial mediante índices de distancias (SADIE), regeneración, supervivencia, variabilidad*

INTRODUCTION

Natural regeneration in Mediterranean vegetation is strongly limited at all stages (Jordano et al., 2008), but particularly in seedlings, which are highly sensitive to their microenvironmental conditions (Grubb, 1977; González-Rodríguez et al., 2008a; Urbieta et al., 2008a). The availability of major resources including light, water and nutrients for plants can change within a few metres (Gallardo, 2003; Gómez et al., 2004; Quero, 2006). Also, these environmental factors exhibit complex mutual relationships (Sack and Grubb, 2001; Gallardo, 2003; Marañón et al., 2004) and can vary widely in space and time, affecting ecosystem structure and composition (Terradas, 2001; Maestre, 2006). Thus, open forest areas possess a light availability that influences not only abiotic resources such as nutrients or soil moisture (Denslow et *al.,* 1998), but also the structure and composition of the herbaceous layer (Milton, 1995). Herb abundance can either diminish seedling survival through competition for water (Rey Benayas et al., 2005) or increase it by reducing evaporation and alleviating the effects of high temperatures (Thomas and Davis, 1989; Gómez-Aparicio et al., 2005). In addition, the spatial structure of seedling recruitment may be governed not only by resources availability, but also by factors involved in the earliest regeneration stages such as seed rain and dispersal (Gómez et al., 2004; García and Houle, 2005).

Therefore, the distribution of environmental variables, which usually take the form of gradients or patches, are usually of a non-random nature and promote the formation of heterogeneous microsites to which plants respond differently (Jurena and Archer, 2003), thereby facilitating coexistence between species (Beckage and Clark, 2003).

The spatial structure of environmental variables is also time-dependent. The significance of temporal variability in the resources for establishment success has been the subject of many studies (Paynter et al., 1998; Castro et al., 2004; Gómez-Aparicio et al., 2005), especially under Mediterranean and semi-arid climates, where water is a highly restrictive resource (Milton, 1995; Veenendaal et al., 1996). Thus, Herrero et al. (2008) found the spatial structure of plant survival to vary between years with different precipitation. In very wet years, survival can be high enough to prevent aggregation; on the other hand, in especially dry years survival is usually too low for any aggregation to be observed. Therefore, experimental designs and statistical analyses should consider the above-described spatial and temporal heterogeneity (Legendre, 1993; Zas, 2006) in order to accurately assess the influence of some key factors influencing early recruitment stages. Although an increasing number of spatially explicit experimental designs is being used to examine aggregation patterns for recruitment-related variables (Maestre et al., 2003; García and Houle, 2005; Quero, 2007a), few authors have applied them simultaneously to several philogenetically related species.

Moreover, no study appears to have so far been conducted to establish the aggregation patterns for morphological and growth related variables in seedlings (but see Laliberté et al., 2008), which might have an impact on plant establishment. Seedling growth rates are very often influenced by environmental factors (Poorter, 2001; Villar et al., 2008); therefore, changes in such factors along spatial and temporal gradients might lead to the presence of areas with bigger or faster growing seedlings. In addition to environmental factors, some intrinsec traits as seed mass determine early seedling performance (ver capítulo 5). Experiments that evaluate seedling growth have been typically carried out under controlled conditions, and field experiments use to limit growth measures just to seedling height (Beckage and Clarck, 2003; Laliberté et al., 2008). However, it is necessary to contrast the results and conclusions of this kind of works with more complete field studies (Pérez–Ramos et al., 2010) in which more morphological traits were involved.

The genus *Quercus* is widely represented in the Mediterranean basin. Evergreen species are abundant in the semi-humid Mediterranean mountains of southern Spain; by contrast, semi-deciduous and deciduous species are scantier and occupy microsites with higher water availability owing to their lower drought tolerance (Acheral and Rambal, 1992; Carrión et al., 2000, Urbieta et al., 2008b). Moreover, deciduous oaks usually have higher leaf areas and growth rates than evergreens (Ruiz Robleto and Villar, 2005; Quero et al., 2006).

The present work was undertaken with four primary purposes: (*a*) to explore small-scale spatial heterogeneity in three environmental factors which may have a high impact in the Mediterranean region (*viz.* light, soil moisture and herb production); (*b*) to assess the influence of such factors on the spatial patterns for seedling emergence, growth and survival in four *Quercus* species differing in leaf longevity; (*c*) to study changes in the aggregation patterns for plant survival and establishment success during the dry period and (d) to explore the influence of seed mass and environmental factors in growth and morphology in these species developing in natural field conditions.

MATERIAL AND METHODS

Study area and species

The study area and species are described in general methods section (pag 21-29).

Experimental design

Acorn collection was carried out in autumn 2006. In order to eliminate mother effect, acorns of each species were selected from one mother tree (*Q. ilex* M_1; *Q. suber* M_3; *Q. faginea* M_5; *Q. pyrenaica* M_2) Collection and dry mass estimations are described in general methods section (pag. 29).

The study was conducted in two 40 × 40 m plots where nodes 4 m apart were used to establish a total of 121 sampling points per plot . The two plots were 50 m apart. In January 2007, one acorn of each species was weighed and sown at each sampling point. Table 1 shows the estimated seed dry mass for each species and plot.

Table 1. Mean ± standard deviation and range (in brackets) of estimated seed dry mass (g) at each species and plot.

	Plot 1	**Plot 2**
Q. ilex	1.94 ± 0.38	1.89 ± 0.27
	[0.93 - 3.79]	[1.14 - 2.68]
Q. suber	1.55 ± 0.62	1.58 ± 0.64
	[0.44 ± 3.66]	[0.22 - 3.52]
Q. faginea	3.03 ± 0.58	3.01 ± 0.58
	[1.56 - 4.63]	[1.24 - 4.57]
Q. pyrenaica	1.48 ± 0.27	1.40 ± 0.25
	[0.85 - 2.31]	[0.81 - 2.09]

Q. faginea exhibited the bigger acorns. At each species no differences in seed dry mass were found between plots except for *Q. pyrenaica*, which exhibited bigger acorns in plot one ($P = 0.02$). The distance between acorns of each species at each node was about 20 cm. This minimized competition between individuals since *Quercus* plants produce a pivot root.

Data collection

Seedlings were monitored for emergence and survival twice a month between January and September 2007. Emergence rate was calculated as percentage of seeds emerged from the sown seeds. Survival rate was calculated as percentage of seedlings surviving from the emerged seeds and the seedling establishment success as percentage of seedlings surviving from the sown seeds. Seedlings were subjected to the following measurements: basal and apical length, and diameter of the main stem and all secondary branches. The data were used to calculate the total stem volume for each plant, using the truncated cone formula. Total leaf area was estimated according to Poorter et al. (2004), using a point grid printed on a transparent sheet. The number of grid intersections found within a certain area provides a good estimate of such an area. Three sheets differing in grid size (3, 5 or 7 mm) were used depending on the size of the leaves.

Stem and leaf biomass were estimated from the previous measurements from the aerial fraction of a sample of seedlings consisting of 15–25 plants per species sown in the experimental site but outside the two plots. Previously, the non-destructive measurements (leaf area and stem volume) were taken. All leaves were scanned with a HP Scanjet 6300c scanner, and both stems and leaves dried in a stove at 70°C for at least 48 h prior to obtaining the dry weight of each plant organ. Linear regressions between stem volume and stem dry weight for each species and sampling date ($r^2 > 0.75$) were used to estimate stem mass from the previously calculated volume (Pérez-Ramos et al., 2010). The scanned leaf images were used to calculate the total leaf area for each harvested seedling by using the software Image Pro-plus 4.5 (Media Cybernetics, Inc.). Leaf mass per unit area (LMA) was calculated as the ratio of leaf dry mass to leaf area. Because LMA depends on both the particular species and incident light (Aranda et al., 2004; Poorter et al., 2009), linear regressions between LMA and light availability (Global Site Factor, GSF) were calculated for each species in order to obtain accurate estimates of LMA in relation to GSF ($r^2 > 0.40$). LMA and the leaf area estimates were used to calculate leaf dry mass per seedling.

At the end of the growth season (July 2007), dead seedlings were harvested for calculation of stem and leaf dry weight. No significant differences in spring aerial seedling biomass were found between surviving seedlings after summer and dead seedlings. Therefore, seedling biomass of dead seedlings could be assumed as representative of the population. In those seedlings measured on both sampling dates (beginning and end of the growth season) stems relative growth rate (RGR_t) and aboveground relative growth rate (RGR_a) was calculated as:

(1) $RGR = (\ln M_2 - \ln M_1) / t$,

were M_1 and M_2 were biomass (aboveground or stems) at the beginning and at the end of the growing season, respectively, and t is the time interval between the two measurements. Aerial leaf mass fraction (LMF_a) was calculated at the end of the growth season (t_2) as:

(2) $LMF_a = M_l / M_t$,

were M_l was leaf mass and M_t was total aboveground biomass, both estimated at t_2.

Environmental variables (light availability, soil moisture and herb production) were measured at each node. For simplicity, herb prodution was named as "environmental variable" because it was studied as an external factor that may influence seedling growth and establishment. Light availability was estimated via the Global Site Factor (GSF) (Rich, 198), using hemispherical photographs taken with a Coolpix camera fitted with an FC E8 fisheye lens, both from Nikon (Tokyo, Japan), in the spring of 2007. Photograhps were processed with the software Hemiview Canopy Analysis v. 2.1 (Delta-T, Cambridge, UK). Soil moisture was measured as the volumetric content of water at each sampling point on a monthly basis from February to September 2007. Measurements were made with a TDR (Model 100, Spectrum Technologies, Inc., Plainfield, IL, USA) fitted with 20 cm long rods –by exception, the

July measurements were made with 7 cm rods since the soil was too dry for the longer ones. Herbaceous aboveground biomass was harvested in July 2007 within a 25 × 25 cm square centred at each nodal point and oven-dried in a stove at 70°C for at least 48 h prior to determine dry weight.

Statistical analysis

The spatial pattern for the studied variables was examined by Spatial Analysis by Distance Indices (SADIE) (Perry 1998) as implemented in SadieShell v 1.3 (www.rothamsted.ac.uk/pie/sadie). The spatial pattern for each factor was assessed in terms of the aggregation index (I_a) and clustering index (υ). I_a is a measure of global aggregation in the variable concerned; depending on whether I_a is unity, greater than unity or less than unity, the pattern is of the random, aggregate or regular type, respectively (Maestre, 2003). Clustering index (υ) quantifies the contribution of each sampling unit to the overall spatial pattern; because is a continuous variable, it can be used for linear interpolation (Legendre and Legendre, 1998) in order to obtain maps clearly showing patches (zones with $\upsilon > 1.5$) and gaps ($\upsilon < 1.5$).

A spatial covariance analysis was performed in order to identify any coincidences in space between aggregated zones for the different variables (Perry and Dixon 2002). SADIE measurements provided an overall clustering index X (degree of spatial coincidence between two variables) ranging from –1 (dissociation) to 1 (association). In addition, they provided a local clustering index χ which estimates the contribution of each sampling unit to the overall clustering pattern. Like the clustering index (υ), these two indices are continuous and afford mapping in order to better envisage zones of association and dissociation between pairs of variables. In this work, maps were produced by linear interpolation with the software SURFER v. 8 (Golden Software, Inc.).

Data were not subjected to spatial analysis in those cases where the number of living plants was less than 10. The spatial distribution of survival and establishment success for each species was studied at three points in time during the dry period (July–September). Establishment success was also assessed at each sampling point for all species in combination; this variable therefore ranged from 0 when all seedlings had died or failed to emerge at the time of measurement to 4 when all four seedlings were alive. The aggregation pattern was examined separately on the three sampling dates.

As no spatial aggregation was detected in all growth and morphology variables (except from four cases from 72, see results), these data were assumed to be space-independent. Thus, morphology variables (Stem height and leaf area at the beginning of the growing season, aboveground biomass and LMF_a at the end of the growing season, RGR_t and RGR_a) were compared across species and between the two plots by a two way ANOVA (plot and species as categorical factors). When necessary, a logarithmic transformation of the data was made to fulfill the requirements of normality and variance homogeneity. A non-parametric Kruskal–Wallis test was applied in those cases where the transformed data failed to fulfill the ANOVA assumptions (leaf area and RGR_t, first for each species separately to compare variables between plots and second for each plot separately to make comparisons across species.

A multiple regression analysis was applied in order to identify the factors influencing growth and morphology at each species and plot. Seed mass, herb production, mean soil moisture of the growing season and light availability were used as independent variables. As emergence time was not correlated to any of the dependent variables and did not improve any model adjustment, and in order to not over-parameterize the models, this factor was not included in the analysis., Morphological variables were used as dependent variables.

Q. ilex and *Q. suber* leaf area was log transformed in order to fulfil regression requests. For RGR_a and $RGR_{t,}$ (x + 1) values were used in the analysis in order to avoid negative values. For each regression a subset of predictors was selected using a forward stepwise method. Model fit was evaluated using the adjusted R^2, which accounts for different degrees of freedom and, hence, the extra regression parameters. On each model, error terms and y-values were normality distributed and had constant variance. Absence of collinearity across dependent variables was checked as the variance inflation factor (FIV) that was in all cases below two (Graham, 2003). Although models for those dependent variables that presented aggregates spatial distribution were developed, residuals of all models (including both regression and ANOVA ones) were checked for autocorrelation by building spatial correlograms but no significant patterns were found, and thus independence could be safely assumed (Keitt et al., 2002; Lichstein et al., 2002). Non spatial statistical analyses were done with the software STATISTICA 8.0. (Statsoft, Inc.) and R version 2.9.1 (R Development Core Team 2009).

RESULTS

Spatial aggregation in environmental factors

Most of environmental variables studied (light availability, soil moisture at different times and herb production) changed according to an aggregated spatial pattern (9 of the 12 possible cases presented spatial aggregation, Table 2). Herb production in plot 1 exhibited the highest aggregation index (I_a, Table 2). The clustering index maps produced (Fig. 1) showed patches and gaps (zones with υ greater and less than 1.5, respectively) for some selected variables. Although the overall pattern was of the random type in some cases (*e.g.* maximum spring moisture in plot 1), there were zones of significant local aggregation.

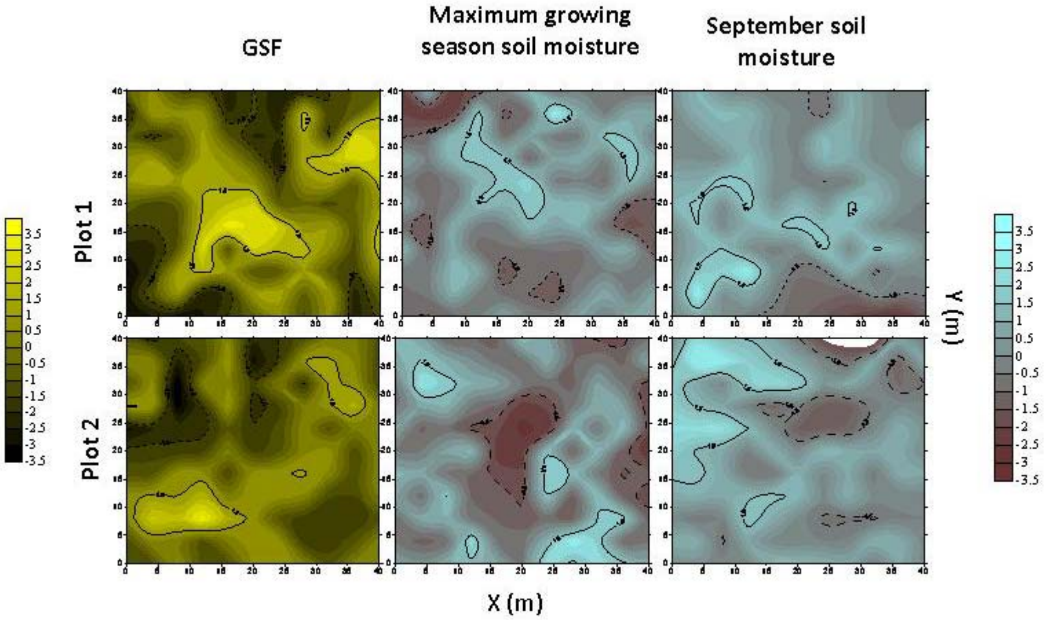

Figure 1. Maps of clustering indices (υ) for selected variables in each sampled plot. The zones bounded by solid lines are patches with high values (υ > 1.5) in the variable concerned and those bound by dashed lines gaps of low values in the variable (υ > 1.5).

Table 2. Means, standard deviations, ranges and aggregation indices (I_a) for the measured variables in the two studied plots. The spatial pattern is aggregated when I_a is significant and greater than unity (*a* $P < 0.07$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *ns* not significant). I_a values correspond to a regular pattern and near-unity values to a random pattern (Perry *et al.* 1999). The growing season spanned from February to May.

		PLOT 1	PLOT 2
Light (GSF)	**Mean ± ds**	0.43 ± 0.12	0.60 ± 0.13
	Min- Max	0.18 – 0.68	0.23 – 0.83
	I_a	**1.6 ****	**1.4 ****
Mean soil moisture growing season (%)	**Mean ± ds**	10.84 ± 1.99	11.0 ± 3.2
	Min- Max	6.6 – 21.9	5.6 – 31.4
	I_a	1.2 ns	**1.4 ****
Maximum soil moisture growing season (%)	**Mean ± ds**	15.7± 3.7	17.2 ± 5.3
	Min- Max	8 – 41	5 – 45
	I_a	1.1 ns	**1.4 ***
July soil moisture (%)	**Mean ± ds**	1 ± 0.9	0.3 ± 0.5
	Min- Max	0 – 3	0 – 2
	I_a	**1.9 *****	**1.7 ****
September soil moisture (%)	**Mean ± ds**	6.0 ± 1	5.5 ± 1.2
	Min- Max	4 – 10	4 – 11
	I_a	**1.3 ***	**1.4 ****
Herb prodution (g/0.25m^2)	**Mean ± ds**	5.02 ± 4.87	6.6 ± 3.8
	Min- Max	0 – 39.7	0.10 – 20.41
	I_a	**2.4 *****	1.3 a

Spatial pattern in emergence, survival, establishment success and growth

Emergence ranged from 36% for *Q. faginea* in plot 1 to 68% for *Q. suber* in plot 2 (Table 3). Only *Q. ilex* in plot 1 and *Q. pyrenaica* in plot 2 exhibited a spatial aggregation pattern for emergence.

Survival at the end of the summer (September) ranged from 1% for *Q. pyrenaica* in both plots to 36% for *Q. ilex* in plot 1 (Table 3). As a rule, all species exhibited lower survival rates in plot 2. Survival at the end of the summer exhibited an aggregation pattern for *Q. ilex* and *Q. suber* in plot 1; however, it could not be assessed in plot 2 owing to the low number of living plants remaining. The aggregation pattern for survival persisted over time (July to September); thus, if survival was aggregated at the beginning, it remained aggregated at the end of summer.

Table 3. Emergence and temporal variation of plant survival and establishment success for the four species in the two studied plots. 1/0 denotes the studied cases, where (1) is the number of emerged (emergence), dead (survival) and dead or non-emerged (establishment success) plants, respectively. The number of cases in the entry "All species" has been omitted because points exhibited values from 0 to 4 (see Methods). An aggregation index (I_a) less than 1 corresponds to a regular pattern and a near-unity one to a random pattern. The spatial pattern is aggregated when I_a is significant and greater than unity (a $P < 0.07$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns not significant). The dashes in some entries indicate that spatial aggregation could not be assessed owing to the scarcity of cases in some category.

PLOT 1		**Emergence**	**July survival**	**August survival**	**September survival**	**July success**	**August success**	**September success**
Q. ilex	**%**	54.5	78.6	44.9	36.2	45.5	25.6	22.3
	1/0	66 / 55	55 / 15	31 / 38	25 / 44	55 / 66	31 / 90	25 / 97
	I_a	**1.36 ****	**1.22 a**	**1.71 *****	**1.54 ****	**1.62 *****	**1.91 *****	**1.69 *****
Q. suber	**%**	60.3	74.0	31.5	24.7	44.6	19.0	14.9
	1/0	73 / 48	54 / 19	23 / 50	18 / 55	54 / 67	23 / 98	18 / 103
	I_a	0.92 ns	**1.27 ***	**1.3 a**	**1.28 ***	**1.32 ***	**1.31 ***	**1.31 ***
Q. faginea	**%**	36.4	60.9	21.3	14.9	23.1	8.3	5.8
	1/0	44 / 77	28 / 18	10 / 37	7 / 40	28 / 93	10 / 111	7 / 113
	I_a	1.11 ns	1.14 ns	**1.63 ****	-	1.02 ns	**1.35 ***	1.05 ns
Q. pyrenaica	**%**	57.0	50.7	5.6	1.4	29.8	3.3	2.5
	1/0	69 / 52	36 / 35	4 / 67	1 / 70	36 / 85	4 / 117	1 / 120
	I_a	0.95 ns	**1.38 ***	-	-	1.14 ns	-	-
All species	**%**	52.1				35.7	14.0	10.5
	I_a	1.03 ns				**1.56 ****	**1.94 *****	**1.78 *****
PLOT 2								
Q. ilex	**%**	48.8	58.6	19.3	15.8	28.1	9.1	8.3
	1/0	59 / 62	34 / 24	11 / 46	9 / 48	34 / 87	11 / 110	9 /112
	I_a	0.86 ns	0.86 ns	1.15 ns	-	0.88 ns	-	-
Q. suber	**%**	67.8	52.5	6.3	2.5	34.7	4.1	3.3
	1/0	82 / 39	42 / 38	5 / 74	2 / 77	42 / 79	5 / 116	2 / 119
	I_a	1.24 a	1.17 ns	-	-	0.92 ns	-	-
Q. faginea	**%**	41.3	37.3	2.0	2.0	15.7	0.8	0.8
	1/0	50 / 71	19 / 32	1 / 49	7 / 40	19 / 102	1 / 120	7 / 113
	I_a	1.02 ns	0.93 ns	-	-	0.97 ns	-	-
Q. pyrenaica	**%**	62.8	25.0	1.4	1.4	14.9	0.8	0.8
	1/0	76 / 45	18 / 54	1 / 72	1 / 70	18 / 103	1 / 120	1 / 120
	I_a	**1.31 ***	**1.40 ****	-	-	**1.57 ****	-	-
All species	**%**	55.2				23.3	3.7	3.9
	I_a	0.92 ns				1.11 ns	**1.39 ***	**1.33 ***

Establishment success (a combination of emergence and survival) at the end of the summer was generally higher for *Q. ilex* (22%) and *Q. suber* (15%) in plot 1 (Table 3). Establishment success in July, where an adequate number of plants for assessment of each species remained, exhibited an aggregation pattern in *Q. ilex* and *Q. suber* in plot 1 (Table 3, Fig. 2). The pattern for *Q. ilex* reflected aggregation in both emergence and survival, whereas that for *Q. suber* was aggregated in survival only. On the other hand, only *Q. pyrenaica* in plot 2 exhibited aggregation in establishment success — reflecting aggregation in both emergence and survival — in July (Table 3, Fig. 2). Establishment success for the four species as a whole was aggregated in the three sampling months except in plot 2 in July —where small patches of local aggregation were apparent, however (Fig. 2). As with survival, the aggregation patterns for establishment success persisted throughout the dry period. Also, low-success gaps increased during the dry season (July to September, Appendix 1). Success for all species as a whole at the end of the summer also exhibited an aggregation pattern (Fig. 3A).

No spatial aggregation in plant growth was detected in growth-related parameters (leaf mass, aerial and total stem mass, RGR_{stem} and RGR_{aerial}) in the four species. Only *Q. ilex* in plot 1 exhibited an aggregated spatial pattern in stem biomass, leaf area and RGR_{stem}, and in stem height in plot 2 (Appendix S2).

Spatial covariance between variables

Some variables were found to exhibit significant positive association (Table 4). Thus, plot 1 exhibited covariance between light availability (GSF) and moisture in September. Also, establishment success for *Q. ilex* in July was spatially associated to moisture in the same month. There was no covariance, however, between other environmental factors and emergence or survival of *Quercus* in this plot. Stem biomass for *Q. ilex* in plot 1 was associated to light availability.

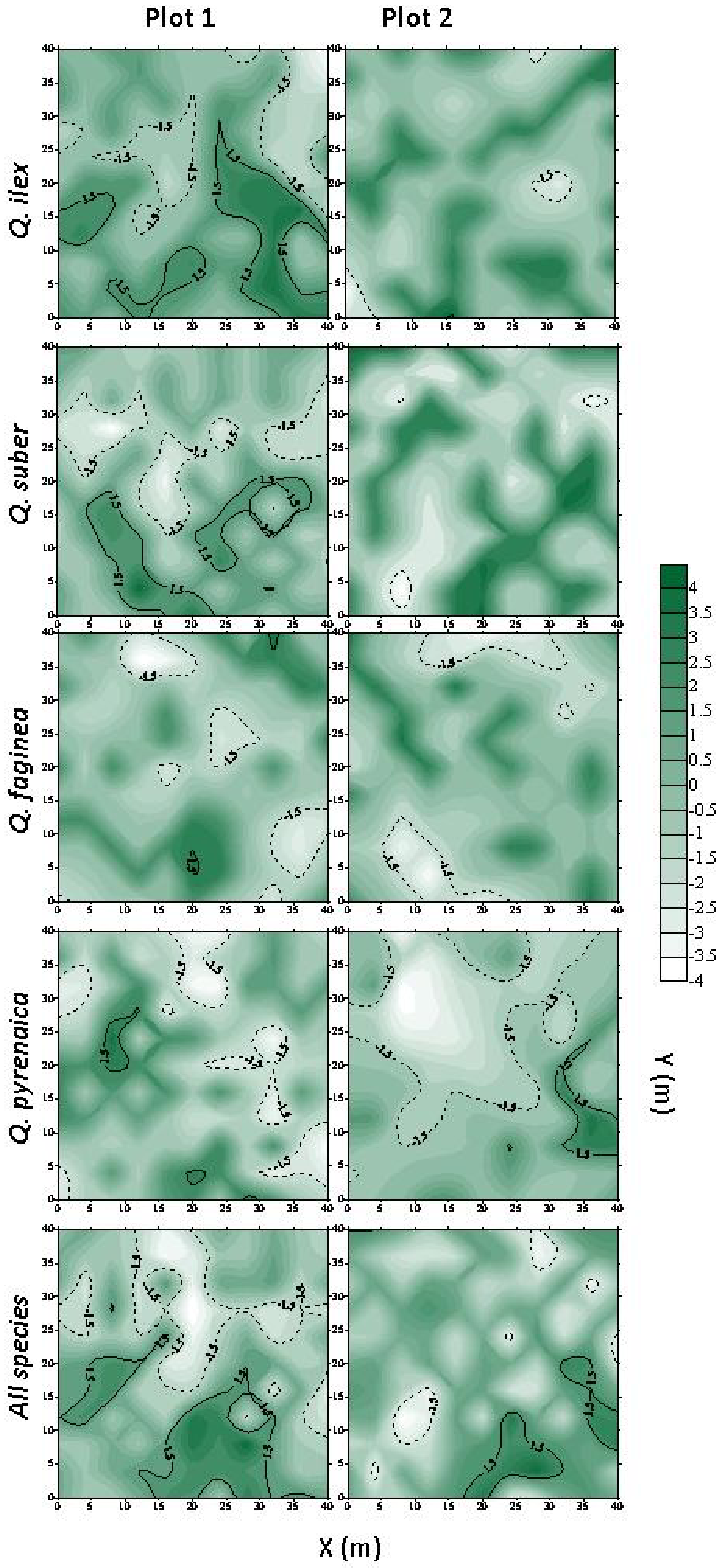

Figure 2. Maps of clustering indices (υ) for establishment success (living seedlings with respect to dead or non-emerged plants) in the four species and both studied plots in July. For details, see Fig. 1.

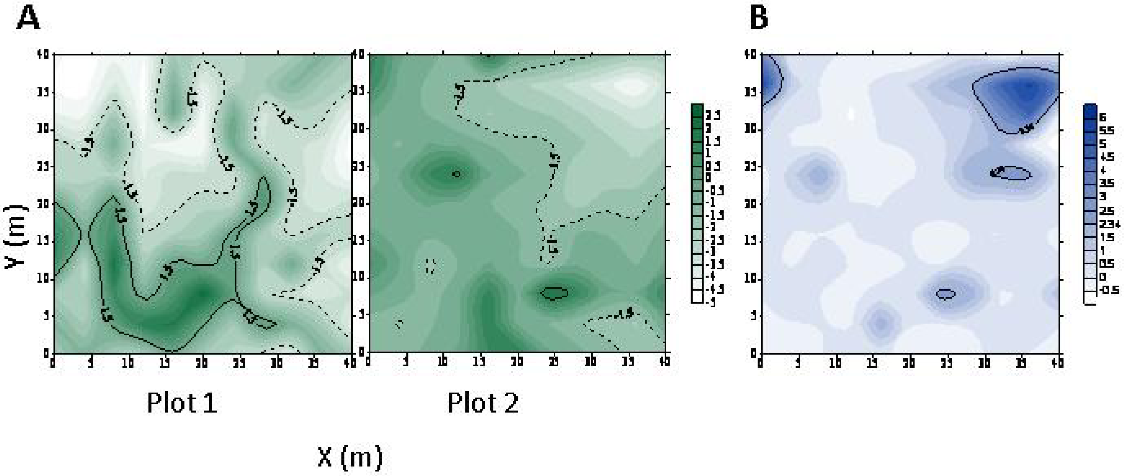

Figure 3. (A) Maps of clustering indices (υ) for establishment success (living seedlings seedlings with respect to dead or non-emerged plants) in the four species and both studied plots in September. For a description of patches and gaps, see Fig. 1. (B) Map of covariance between moisture in July and sowing success on all species in plot 2. The patches represent association zones and the gaps dissociation zones for the variables, and are bounded by lines if significant at the $P < 0.05$ level.

There was association between maximum moisture in spring and moisture in September in plot 2. Regarding *Quercus* sowing-related variables, there was association between emergence of *Q. pyrenaica* and maximum moisture in spring, especially in a zone where low moisture levels led to low oak emergence rates. Plot 2 also exhibited association between sowing success in all species at the end of the summer and moisture in July. In this case, there was an association gap where low moisture levels corresponded to zones of little or no sowing success (Fig. 3B).

Table 4. Summary of spatial covariance between studied variables. The clustering index and its significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) for each case are shown. All statistically significant covariance were positive (i.e. associations).

Variables	PLOT 1
Light – Sept. soil moisture	0.28 **
Q. ilex success july - July soil moisture	0.17 *
Q. ilex stem mass - light	0.28 *
	PLOT 2
Max. growing season soil moisture – Sept. soil moisture	0.16 *
Light - Herb prodution	0.22 *
Q. pyrenaica emergence – max. growing season soil moisture	0.16 *
Q. pyrenaica July success - herb prodution	0.17 *
All species Sept. success - July soil moisture	0.51 ***

Herb production in plot 2 was associated to light availability and to establishment success for *Q. pyrenaica* in July (Fig. 4), *i.e.* this species was especially successful in zones with an abundant herbaceous cover.

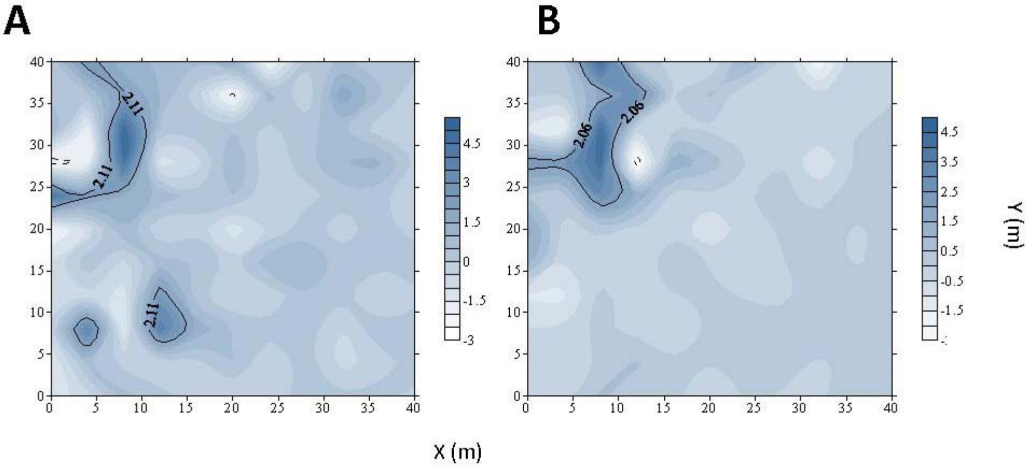

Figure 4. (A) Map of local covariance between light availability (GSF) and grass biomass in plot 2. (B) Map of local association between grass biomass in plot 2 and establishment success of *Q. pyrenaica* in July. For a description of covariance patches and gaps, see Fig. 3B.

Non spatial analysis of growth

Interspecific and site differences

Stem height varied across species and plot, being in general higher in plot 2 (P = 0.01) (Appendix S2). *Q. suber* seedlings were the tallest. Abovegroung biomass and RGR_a showed significant differences across species. *Q. faginea* had the highest aboveground biomass and *Q. pyrenaica* the lower. RGR_a was higher in *Q. faginea* and *Q. suber*. Leaf area, LMF_a and RGR_t were similar in different species and plots (Appendix S2).

Factors affecting growth and morphology

Seed mass was one of the best predictors for growth and morphology as it appears in 39 % of the 48 models fitted (six variables per species and plot), being practically the only factor selected in models of plot 1 (Table 5) (see also Appendix 3). Figure 5 shows seed mass – aboveground biomass relations in plot 1.

Table 5. Significant predictors in the selected models analysing the response of growth and morphology variables to seed mass and environmental factors (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). The sign of the effect of the independent variable (positive or negative) and the adjusted R^2 of the model are also indicated. LMR_a, leaf mass fraction; RGR_t, stem relative growth rate; RGR_a, aboveground relative growth rate. Herb refers to herb prodution. Soil moisture reflects mean soil moisture during the growing season.

		Plot 1			Plot 2		
Species	**Growth variable**	**Predictors**	**Relation**	**adj. R^2**	**Predictors**	**Relation**	**adj. R^2**
Q. ilex	Stem height t_1	-			light * herb *	- +	0.14
	Leaf area t_1	-			seed mass *	+	0.07
	Aboveground biomass t_2	seed mass **	+	0.23	seed mass *	+	0.12
	LMR_a t_2	-			-		
	RGR_t	-			seed mass **	+	0.14
	RGR_a	-			-		
Q. suber	Stem height t_1	-			seed mass * soil moisture *	+ +	0.09
	Leaf area t_1	seed mass *	+	0.08	-		
	Aboveground biomass t_2	seed mass *	+	0.10	seed mass *** soil moisture *	+ +	0.31
	LMR_a t_2	-			soil moisture *	+	0.07
	RGR_t	seed mass **	+	0.16	seed mass ***	+	0.24
	RGR_a	-			seed mass***	+	0.16
Q. faginea	Stem height t_1	-			-		
	Leaf area t_1	-			-		
	Aboveground biomass t_2	seed mass *	+	0.14	soil moisture *** herb **	+ -	0.25
	LMR_a t_2	-			-		
	RGR_t	seed mass *	+	0.17	-		
	RGR_a	-			soil moisture * herb *	+ -	0.14
Q. pyrenaica	Stem height t_1	-			seed mass ***	+	0.14
	Leaf area t_1	seed mass *	+	0.06	seed mass **	+	0.11
	Aboveground biomass t_2	seed mass * light *	+ +	0.18	-		
	LMR_a t_2	-			light ** seed mass *	+ -	0.17
	RGR_t	-			seed mass *	+	0.07
	RGR_a	-			herb **	-	0.10

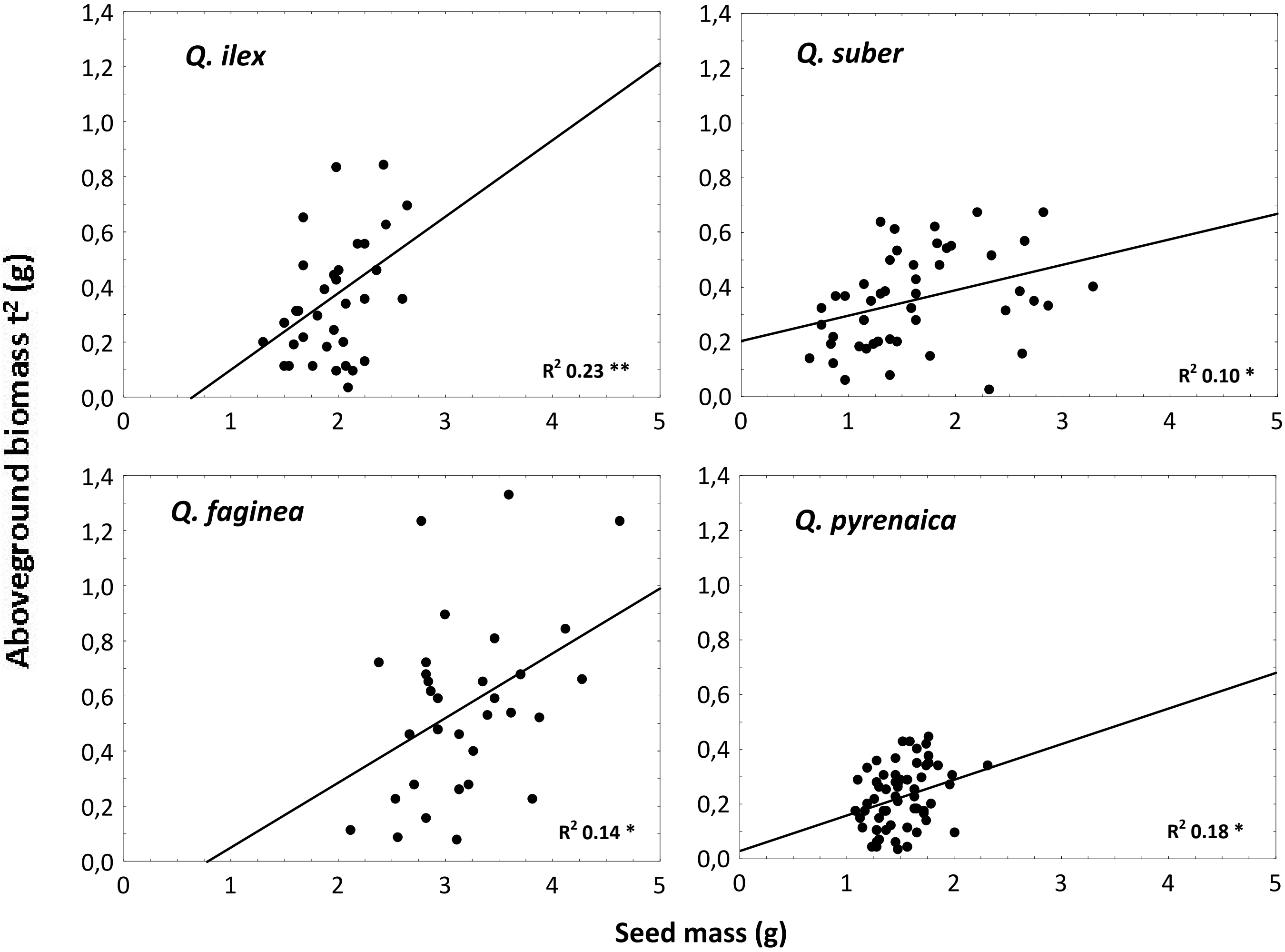

Figure 5. Relationship of seedling aboveground biomass at the end of the growing season in plot 1 as a function of seed dry mass. Lines are the regression lines

Environmental variables were more explanatory on plot 2, appearing as best predictors either with or without seed mass effect. Soil moisture effects were always positive on morphology and growth variables. Seed mass effect was positive in all cases except on LMF_a of *Q. pyrenaica* in plot 2. Light effect was positively related with aboveground biomass of *Q. pyrenaica* in plot 1, and negatively correlated with stem height of *Q. ilex* in plot 2. Herbaceous production effect was negative in all cases apart of stem height of *Q. ilex* in plot 2 (Table 5 and Fig. 6).

Adjusted R^2 varied from 0.06 to 0.31. In general model explaining aboveground biomass and RGR fitted better that those related to leaf traits (Table 5).

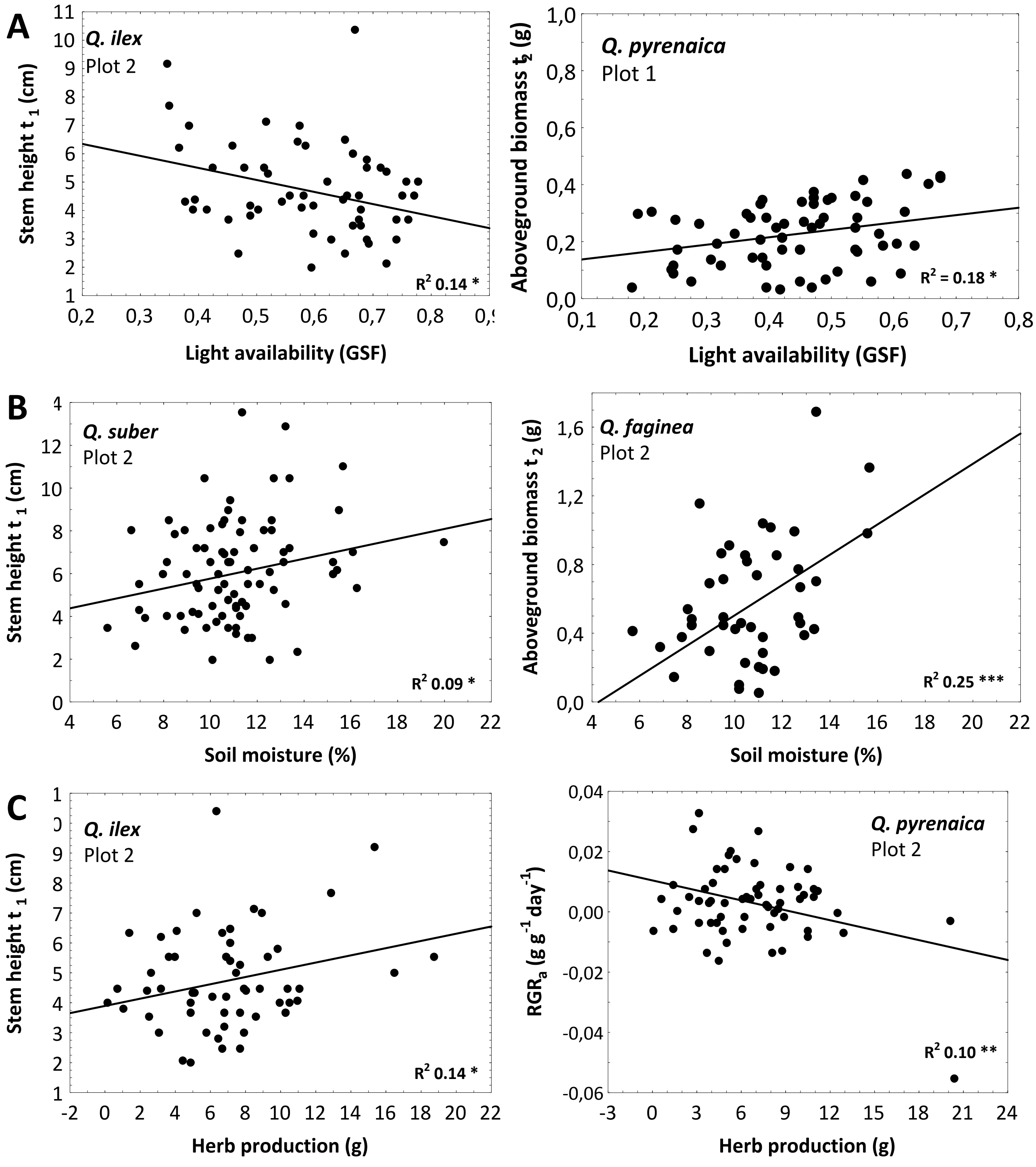

Figure 6. Relationship of different morphology and growth variables with (A) light availability, (B) soil moisture and (C) herb prodution in different plots. Lines are the regression lines.

DISCUSSION

Environmental factors

The primary aim of this work was to characterize the spatial distribution and potential association between environmental variables influencing establishment success and growth in wood seedlings. Most environmental variables exhibited an aggregation pattern, showing the small-scale heterogeneity in the environment that is consistent with previous results of spatial variability in Mediterranean areas (Gallardo et al., 2000; Valladares and Guzmán, 2006; Herrero et al., 2008). There was, however, virtually no spatial covariance between the measured environmental variables (light, soil moisture and herb production). This suggests, for example, that the study area encompasses high light zones of widely variable soil moisture and *vice versa*. As a result, the area spans a wide spectrum of microsites, which is consistent with the high complexity of the relationships between these factors (Sack and Grubb, 2001; Valladares and Pearcy, 2002). Only plot 1 contained a zone where high light levels were associated to high moisture levels in September (after the earliest autumn rains). This may have resulted from the tree canopy intercepting rainwater and the more open zones having increased moisture levels as a consequence of less marked evaporation in autumn (Tiëlborger and Kadmon, 2000). Also, there were association patches between maximum moisture in spring and moisture in September (after the earliest autumn rains) in plot 2, which is suggestive of spatial and temporal consistency in soil water availability.

Emergence, survival and establishment

As the studied samples were seedlings from artificially sown seeds and the absence of post-dispersal predation was verified (seeds were buried and no holes or other predation signals were found), we confirm that the spatial pattern observed was independent of earlier regeneration stages such as dispersal or post-dispersal predation. García and Houle (2005) found the effect of environmental gradients did not suppress the recruitment patterns generated by seed rain. Although the effect of the pattern resulting from seed dispersal was suppressed in our case, the survival patterns observed may also be influenced by the spatial structure of the emergence.

No spatial aggregation patterns for emergence were virtually found. This suggests that emergence is largely independent of environmental factors or that, as noted above, the differential distribution of environmental variables in space conceals their effect on plant performance (Gallardo, 2003; García et al., 2006; Pérez-Ramos et al., 2010). The two exceptional emergence aggregation patterns corresponded to *Q. ilex* in plot 1 and *Q. pyrenaica* in plot 2. The aggregation pattern for *Q. ilex* was associated to none of the variables studied. On the other hand, that for *Q. pyrenaica* was positively associated to maximum moisture in spring. Therefore, the least moist zone in winter has a strong limiting effect on emergence of *Q. pyrenaica* seedlings, consistent with its increased moisture requirements (Costa et al., 1997). Success in this species at the beginning of the dry period was associated to herbaceous biomass, which facilitated establishment in those microsites with especially abundant herb production. Some studies have shown herb species to use large amounts of water for grow, thereby limiting survival of woody plants at an early growth stage (Davis et al., 1999; Rey Benayas et al., 2005). However, *Q. pyrenaica* is one of the *Quercus* species with the highest root investments (Quero et al., 2006), which allows it to evade water competition. In addition, an abundance of herbaceous cover may indicate increased light and moisture levels in spring and hence easier establishment of oaks at the emergence stage. Although this emergence pattern influenced the survival pattern at the beginning of the dry period, plant mortality increased as drought progressed and the spatial pattern vanished.

Spatial aggregation in survival and establishment success persisted throughout the studied period. The zones with the lowest survival rates expanded as the dry period progressed and the patterns disappeared only when plant mortality was virtually 100% (deciduous species, *Q. faginea* and *Q. pyrenaica*, in plot 2 at the end of the summer). Some studies (Quero, 2007a; Herrero et al., 2008) have found spatial patterns in survival to differ depending on whether the climatic conditions were intermediate (aggregation) or extreme (random mortality). However, these conclusions have been reached by comparing plants sown in rather different habitats or years. Probably, the differences in soil moisture between the beginning and end of the summer in our study were not large enough to alter aggregation patterns so markedly. Also, Maestre (2006) previously found that survival spatial patterns persisted despite mortality episodes in subsequent years, which suggests that the earliest months of growth are very important for successful establishment. Although precipitation during the study was close to the average for the area (709 mm between September 2006 and August 2007), the amount of rainfall collected during the summer (June-August) was only 3 mm. Such a dry summer may cause both the low survival observed and the disappearance of some spatial patterns. The four species are therefore expected to retain the above-described patterns in rainier years.

Establishment success for the four species as a whole exhibited aggregation in both plots, with zones especially favouring performance of *Quercus* seedlings. Identifying the zones bound to facilitate regeneration and examining their persistence may provide useful hints with a view to focusing restoration endeavours on microenvironments with high survival rates (Maestre et al., 2006). What are the specific factors governing establishment success? In plot 1, the spatial distribution of light, soil moisture and herb production was not directly associated to success. Therefore, other factors such as topography, soil stoniness or nutrient distribution (Maestre et al., 2006) may determine the presence of zones of low or high establishment success. Plot 2 exhibited some zones where a decrease in moisture during July was associated to a low establishment success. Water is known to be a major limiting factor in Mediterranean ecosystems (Aschman, 1973), especially during the summer drought. Site factors such as microtopography, vegetation patches or soil type determine spatially and temporally heterogeneity in soil moisture (Aguiar and Sala, 1999; Maestre and Cortina, 2002; Quero, 2007a) influencing species performance. As a result, seedlings in especially dry microsites may have inadequate water resources to survive. Overall, survival and establishment success were lower in plot 2 than in plot 1, possibly as a result of increasing water stress during the summer in the former. Therefore, water was seemingly a more limiting factor in plot 2, with especially dry zones where all species were bound to exhibit a high mortality.

Growth and morphology

Unexpectedly, growth-related variables exhibited virtually no aggregation patterns. Laliberté et al. (2008) found the spatial patterns for stem diameter and height to account for a very low fraction of the overall variance. Pérez-Ramos et al., (2010) found growth of *Quercus* seedlings during the first year to depend more markedly on the seed reserves than on the environmental conditions, as seedlings have a limited photosynthesis capacity during these first stages. In our study, seed mass was the main factor explaining morphology and growth variance, and it was especially important on plot 1. Seed-mass represents the amount of reserves available for growth development on the first stages of seedling development. Larger seed-mass is related to a larger seedling biomass, which confer some advantages on seedling stablishment (Hendrix et al., 1991; Chacón and Bustamante, 2001).

In general, variance explained in all models was not very high. Pérez–Ramos et al. (2010) found similar values for first year growth in *Quercus* species, and models fit increased in the second year, in which environmental variables were more explicative. As noted above, it seems that during the first year seedlings are more dependent of intrinsic characteristics as seed-mass or other factors such as seed chemical composition no contemplated in this study but which could be more critical (Villar-Salvador et al., 2009).

Although environmental variables explained less variance than seed mass, they were selected as predictors in many models in plot 2 whereas they were just included in one model of plot 1. This confirms the high variability in Mediterranean ecosystems, where, as we have seen, resources availability may change in few meters, determining two areas very closed each other but in which seedling performance was different.

Light availability is an important resource which determines species distribution in Mediterranean environments (Beckage and Clark, 2003; Zavala and Zea, 2003). However, it doesn´t appear as a key factor explaining *Quercus* growth variability. This may be caused by the fact that growth of large seeded species is less responsive to environmental conditions (Beckage and Clark, 2003). Moreover, natural shading in the experimental site was mainly produced by *Q. ilex*, *Pinus pinaster* and *Cistus ladanifer*. The last two species alter physico-chemical properties of the top-soil by emiting allelochemical compounds or modifying the amount of nutrients (Gallardo, 2003; Gómez–Aparicio et al., 2005; Puerta-Piñero, 2006). These plant-plant interactions take place in different signs depending on the nurse plant and could hide the specific responses to light availability *per se*. In fact, Perez-Ramos et al. (2010) found that light and nutrient availability are negatively related, therefore the effect of each factor isolated may be masked.

According to a similar experiment carried out in Sierra Nevada (Spain) (Gómez-Aparicio, 2008), light increased aboveground biomass and leaf mass fraction of *Q. pyrenaica*. As canopy intercepts rainfall during the growing season (Aussenac, 2000), shaded sites could be occassionaly drier, so oak seedlings growing in open and wet areas could be favorished, even although this species is shade tolerant in early life stages (Quero et al., 2006). On the other hand, stem height of *Q. ilex* increased on the shade. This effect have been previously observed in plants growing in areas with a reduced R:FR ratio (Ziegenhagen and Kausch, 1995; Ammer, 2003). In this experiment no deep shaded microsites were represented as they are scant in the study area so other strong negative relations between light and growth are rarely to appear. Moreover, the studied species are in general, intermediate or good shade tolerant (Cardillo and Bernal, 2006, Quero et al., 2006, Pérez-Ramos et al, 2010).

As observed on survival and establishment, soil moisture during the growing season affected positively *Quercus* growth and biomass. Holm oak was not influenced by soil moisture, maybe due to its higher drought tolerance (Mediavilla and Escudero, 2003; David et al., 2007).

Competition between herbs and woody seedlings is mainly for water (Davis et al., 1999; Rey Benayas et al., 2007). In the present study the herbaceous layer was related to a decrease in RGR_a and aerial biomass and to an increase of stem height in *Q. ilex* at the beginning of the growing season. Holmgren et al. (2000) found similar herb-effect on Chilean woody seedlings. Competition could be, in this case, for light as well as for water, diminishing aboveground growth in biomass but enhancing height growth in the same way that shade conditions did.

Differences across species

Species differed in survival and establishment success. *Q. ilex* was the most successful species in both plots. This species develops an effective water stress avoiding strategy (Valladares et al., 2008) and possesses the highest drought resistance (Costa et al., 1997). By contrast, the deciduous species (*Q. faginea and Q. pyrenaica*) exhibited lower survival, possibly as a result of their lower drought tolerance (Quero et al., 2006; Villar et al., 2008). *Q. pyrenaica* exhibited virtually no survival or establishment success. This species in scantily represented in Sierra de Cardeña y Montoro, where they only occurs in especially moist areas (Castillo and Castillo, 2004; Quero and Villar, 2009).

Species growth differed just slightly in their response to environmental gradients. Other studies have shown how interspecific differences in response to drought and shade disappeared in the first stages of life, when seedlings from different *Quercus* species showed a common strategy (Mediavilla and Escudero, 2003; Quero et al., 2006). A striking outcome of this study is that *Q. faginea* had the highest aboveground biomass at the end of growing season but it could be an experimental design effect. *Q. faginea* seed mass average was the highest and this trait was positively correlated with seedling mass (see above). On the other hand, the lower aboveground inversion of *Q. pyrenaica* seedlings could be due to the higher root allocation of this species (Quero et al., 2006).

CONCLUSIONS

In this work, we characterized the spatial distribution and potential association between environmental variables influencing establishment success in woody seedlings. Heterogeneity at the microsite level was found to govern the small-scale availability of some resources including light and water, determining that in the two studied plots seedling performance was different, although the plots were very close each other. The seedling emergence process appears to be scarcely influenced by the environmental conditions. The same happens for plant growth during the first year, which is more markedly dependent on seed reserves than on the particular environmental conditions. On the other hand, survival and establishment success depend, at least partly, on water availability. A scarcity of this resource can lead to strongly limited survival in some zones. The spatial aggregation patterns for seedling survival and establishment success persist during the dry season and only vanish when plant mortality is very high (*e.g.* under extremely dry conditions such as that of summer in the studied year or in species with a high sensitivity to this limitation).

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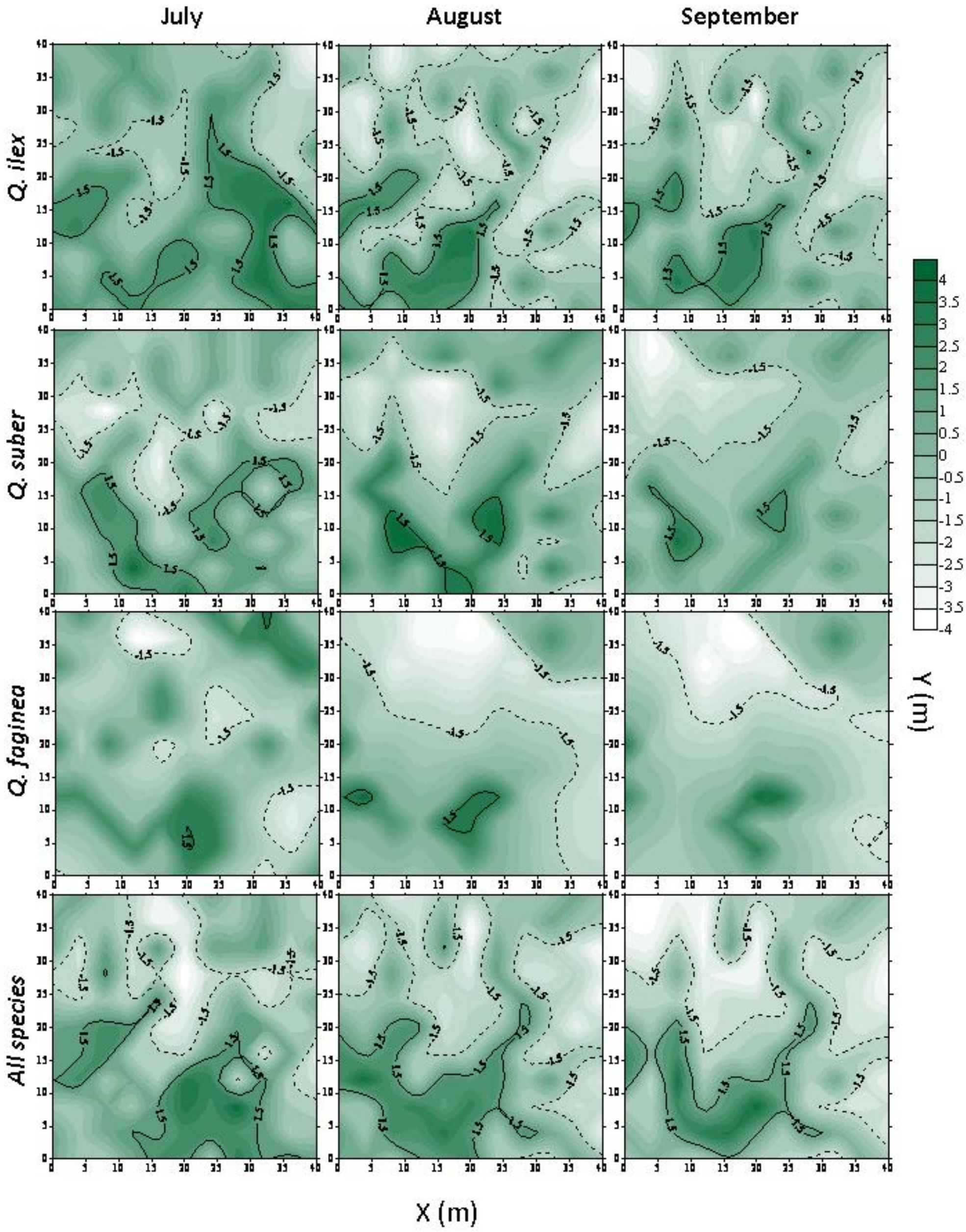
SUPPLEMENTARY INDEX

Appendix S1. Maps of clustering indices (υ) during the dry season for establishment success (living seedlings versus dead or non-emerged plants) in plot 1 for each species and for all as a whole. No data for plot 2 and for Q. pyrenaica in plot 1 are shown since the number of living plants present in the August and September censuses were inadequate to examine their aggregation patterns. The zones bounded by a solid line correspond to patches with high values of the variable (υ > 1.5) and those bounded by a dashed line gaps with low values of the variable (υ < 1.5).



Appendix S2. Means, standard deviations, ranges and aggregation indices (I_a) for the different variables related to growth and morphology for the four species in the two plots. An aggregation index (I_a) less than 1 corresponds to a regular pattern and a near-unity one to a random pattern. The spatial pattern is aggregated when I_a is significant and greater than unity (a $P < 0.07$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns not significant). LMFa (leaf mass fraction aerial is the ratio of leaf mass to aboveground biomass, RGR_s (relative growth rate of stems), RGR_a (relative growth rate of aboveground biomass), subscript t_1 and t_2 refers to spring and summer, respectively. Different letters indicate significant differences ($P < 0.05$) across species at plot 1 (capital letters) and at plot 2 (lower case letters). No difference were found between plots, except from stem height that was higher in plot 2 ($P = 0.01$). No letters indicate no significant differences.

		Q. ilex		*Q. suber*		*Q. faginea*		*Q. pyrenaica*	
		PLOT 1	**PLOT 2**	**PLOT 1**	**PLOT 2**	**PLOT 1**	**PLOT 2**	**PLOT 1**	**PLOT 2**
Stem height t_1 (cm)	**Mean ± ds**	4.28 ± 1.51 **A**	4.78 ± 1.64 **a,b**	5.74 ± 2.21 **B**	6.15 ± 2.38 **c**	5.05 ± 2.20 **A,B**	5.60 ± 2.28 **a,c**	4.00 ± 1.56 **A**	3.95 ± 1.17 **b**
	Min - Max	1.5 – 7.5	2 – 13.5	1.7 – 12.3	2 – 10.4	1.28 – 12.4	2.5 - 12	1.5 – 7.5	1.7 - 7
	I_a	1.13 n.s	**1.35** *	1.09 n.s	1.18 n.s	1.03 n.s	1.01 n.s	1.08 n.s	1.10 n.s
	N	61	56	72	82	42	48	68	71
Leaf area t_1 (cm^2)	**Mean ± ds**	13.9 ± 8.33	17.1 ± 8.6	18.1 ± 12.3	17.9 ± 12.6	26.8 ± 23.2	26.5 ± 18.9	21.5 ± 13.3	20.3 ± 10.6
	Min - Max	1.8 – 35.0	1.5 – 45.0	1.8 – 50.9	0.4 – 68.5	1.3 – 121	3.75 – 74.5	3.5 – 50.0	2.7 – 52.9
	I_a	**1.63** **	0.76 n.s	0.97 n.s	1.29 n.s	1.02 n.s	1.02 n.s	0.91 n.s	0.96 n.s
	N	60	57	71	82	40	48	66	71
LMFa t_2	**Mean ± ds**	0.73 ± 0.18	0.77 ± 0.12	0.73 ± 0.07	0.74 ± 0.06	0.74 ± 0.10	0.72 ± 0.12	0.65 ± 0.17	0.68 ± 0.18
	Min - Max	0.08 – 0.90	0.21 – 0.89	0.58 – 0.87	0.47 – 0.87	0.33 – 0.86	0.39 – 0.86	0.20 – 0.84	0.08 – 0.86
	I_a	1.28 ns	1.11 n.s	1.05 n.s	0.85 n.s	0.88 n.s	1.21 n.s	0.89 n.s	1.71 n.s
	N	33	38	46	68	30	42	63	64
Stem mass t_2 (g)	**Mean ± ds**	0.08 ± 0.05	0.08 ± 0.07	0.09 ± 0.05	0.10 ± 0.05	0.14 ± 0.08	0.14 ± 0.08	0.07 ± 0.04	0.08 ± 0.11
	Min - Max	0.02 - 0.25	0.01 - 0.40	0.01 - 0.22	0.01 - 0.26	0.02 - 0.36	0.03 - 0.35	0.02 - 0.23	0.01 - 0.71
	I_a	**1.46** *	1.20 n.s	1.18 n.s	0.95 n.s	0.95 n.s	0.82 n.s	0.98 n.s	0.87 n.s
	N	33	38	46	68	30	42	63	64

(Appendix S2 continuation)

		Q. ilex		***Q. suber***		***Q. faginea***		***Q. pyrenaica***	
		PLOT 1	**PLOT 2**	**PLOT 1**	**PLOT 2**	**PLOT 1**	**PLOT 2**	**PLOT 1**	**PLOT 2**
Leaf mass t_2 (g)	**Mean ± ds**	0.27 ± 0.18	0.27 ± 0.15	0.26 ± 0.13	0.28 ± 0.17	0.43 ± 0.25	0.44 ± 0.30	0.16 ± 0.10	0.16 ± 0.11
	Min - Max	0.02 - 0.70	0.03 - 0.61	0.02 - 0.54	0.01 - 0.75	0.03 - 0.99	0.03 - 1.34	0.01 - 0.36	0.02 - 0.82
	I_a	0.78 n.s	1.04 n.s	1.05 n.s	0.92 n.s	0.96 n.s	1.03 n.s	0.85 n.s	0.90 n.s
	N	33	38	46	68	30	42	63	64
Aboveground mass t_2 (g)	**Mean ± ds**	0.34 ± 0.21 **A,B**	0.35 ± 0.18 **a,b**	0.35 ± 0.17 **A**	0.38 ± 0.22 **a**	0.56 ± 0.32 **A**	0.59 ± 0.36 **c**	0.23 ± 0.11 **B**	0.24 ± 0.17 **b**
	Min - Max	0.04 - 0.85	0.03 - 0.73	0.03 - 0.67	0.01 - 0.92	0.08 - 1.33	0.06 - 1.69	0.03 - 0.44	0.05 - 1.14
	I_a	0.89 n.s	0.96 n.s	1.09 n.s	0.91 n.s	0.98 n.s	1.01 n.s	0.88 n.s	0.72 n.s
	N	33	38	46	68	30	42	63	64
RGR_s (mg gr^{-1} day^{-1})	**Mean ± ds**	12.7 ± 9.5	14.9 ± 13.4	13.3 ± 6.3	11.5 ± 5.4	15.1 ± 6.5	15.5 ± 15.5	15.2 ± 8.9	13.6 ± 12.2
	Min - Max	1.1 - 40.5	1.3 - 74.4	3.1 - 36.8	1.1 - 25.3	1.5 - 26.6	1.52 - 85.92	0.8 - 51.6	1.1 - 64.3
	I_a	**1.37 ***	0.80 n.s	1 n.s	0.84 n.s	0.87 n.s	0.81 n.s	1.30 n.s	0.91 n.s
	N	26	38	42	61	30	37	58	55
RGR_a (mg gr^{-1} day^{-1})	**Mean ± ds**	8.3 ± 7.1 **A**	11.4 ± 11.7	14.5 ± 8.6 **B**	13.1 ± 7.8	13.8 ± 8.8 **B**	17.6 ± 8.0	11.1 ± 8.7 **A**	16.2 ± 7.6
	Min - Max	1.9 - 25.1	3.8 - 57.1	2.1 - 37.8	3.4 - 46.9	1.6 - 32.2	9.6 - 39.5	0.1 - 39.2	7.5 - 33.0
	I_a	0.98 n.s	0.84 n.s	0.89 n.s	0.85 n.s	1.21 n.s	0.94 n.s	0.95 n.s	1.01 n.s
	N	18	27	35	54	21	30	39	37

CAPÍTULO 4

Whithin population variability influences early seedling establishment on four Mediterranean Oaks

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- *Enviado a Journal of Vegetation Science*

ABSTRACT

Regeneration of Mediterranean forests is severely limited. Multiple abiotic factors, such as summer drought or light availability, are known to constrain the establishment of woody seedlings at its first phases, but less attention has been paid to the role of intraspecific variation in seedling performance. In this study we collected acorns from different mother trees (as determinants of within population variability) of four Mediterranean oaks (*Q. ilex, Q. suber, Q. faginea* and *Q. pyrenaica*). Acorns were sowed in field to study their early establishment, from emergence and early growth to second-year survival across a gradient of environmental factors. Maternal origin had an effect on most of the phases studied (time of emergence, percentage of emergence, establishment success and morphological traits) but main drivers for each phase were slightly different for each species. In addition to a direct effect, in many cases intrinsic traits due to maternal origin and seed mass modulated the effects of environmental conditions on seedling performance. The role of intrinsic factors was masked under ameliorated conditions (i.e. summer irrigation), indicating the relevant role of within population variability to cope with the highly heterogeneous and unpredictable Mediterranean environments. This high variability should be taken into account in restoration programs.

Keywords: *aboveground biomass, emergence, growth, mother plant, seed, SLA, survival*

RESUMEN

La regeneración de los bosques mediterráneos está fuertemente limitada. Las primeras fases del establecimiento están condicionadas por muchos factores abióticos como la luz y la sequía estival, pero se ha prestado menos atención al papel de la variabilidad intraespecífica en la regeneración. En este estudio se recolectaron bellotas en diferentes árboles madre de tres *Quercus* mediterráneos (*Q. ilex, Q. suber, Q. faginea* y *Q. pyrenaica*). Las bellotas se sembraron en campo para estudiar su establecimiento y crecimiento a lo largo de un gradiente de factores ambientales. El árbol madre tuvo efecto en casi todas las variables estudiadas (tiempo de emergencia, porcentaje de emergencia, éxito en el establecimiento, crecimiento y caracteres morfológicos), pero los factores con más influencia fueron diferentes según la especie. Además del efecto directo, los efectos de los factores ambientales fueron en muchos casos diferentes según la madre. El papel de los factores intrínsecos desapareció bajo condiciones favorables (riego suplementario en verano). Esto puede indicar la importancia de la variabilidad intra poblacional en ambientes tan impredecibles y heterogéneos como los mediterráneos, variabilidad que debe ser tenida en cuenta en los programas de restauración.

Palabras clave*: árbol madre, biomasa aérea, crecimiento, emergencia, semilla, SLA, supervivencia*

INTRODUCTION

A vast proportion of the forested areas in temperate and subtropical regions of the Northern Hemisphere are dominated by species belonging to *Quercus* genus (Pulido, 2002). Traditional land uses (i.e. agriculture and cattle rising) have dramatically reduced or deeply altered forested areas. Coupled with these management practices, climatic and environmental factors determine a very limited regeneration with very low seedling establishment and fast ageing populations. In Mediterranean areas, oak forests are one of the main ecosystem types. The Mediterranean climate is characterized by large intra and inter-annual variations in environmental conditions (Blondel and Aronson, 1995). In addition, resource availability may change spatially, and it is also distributed across gradients that determine a great variability of combinations of different factors (Sack and Grubb, 2002; Gallardo, 2003). Plant species have to cope with this spatial and temporal heterogeneity, which is especially important in the first stages of establishment, when seedlings are especially sensitive to environmental conditions (Pulido, 2002). Multiple factors constrain the establishment of woody seedlings at its first phases in Mediterranean areas. Summer drought is known as a major cause of mortality (Herrera et al., 1994) and its impact is likely to increase in the Mediterranean area under a climate change scenario (Peñuelas et al., 2004). Rainy summers may thus represent a recruitment opportunity, especially in species less tolerant to hydric stress (Mendoza et al., 2009). Another limiting factor for growth and survival is light availability, whose spatial gradient determines niche differentiation along successional stages (Beckage and Clark, 2003). Many studies have demonstrated that moderate shade benefits seedling survival by protecting against excessive evapotranspiration and photo damage caused by high radiation (Puerta-Piñero et al., 2007; Gómez-Aparicio et al., 2008).

It has been proposed that maintaining high phenotypic diversity may be crucial for species inhabiting in such unpredictable and heterogeneous environments (Sánchez-Vilas and Retuerto, 2007). In this way, the study of adaptive variation within and among populations is necessary to understand how species deal with local environmental constraints (McKay et al., 2005). Many studies focusing on the analysis of the variability among populations, have found significant population-associated variations in physiological, structural and growth parameters (Sánchez-Vilas and Retuerto, 2007; Villar-Salvador et al. 2008; Ramírez-Valiente et al. 2009).

In the same sense, within-population variability driven by maternal traits might play a key role in the adaptation to local conditions. Seed mass is one of the traits influenced by the maternal plant (Byers et al., 1997; Castro 1999; Merouani et al., 2001) with a well-recognised importance on regeneration, especially on large-seeded species growing in unfavourable environments (Leishman and Westoby, 1994; Saverimuttu and Westoby, 1996). Seed mass is determined by maternal genotype, maternal environment or both (Schmitt et al., 1992; Ramírez-Valiente et al., 2009). Plants from larger seeds could have some advantages, such as higher germination or emergence rates higher biomass or a greater probability of survival (Gómez, 2004a; Moles and Westoby, 2004; Quero et al. 2008b). The existence of inter-individual variation in acorn yield size has been studied for different oak species (Rice et al, 1993; Gómez, 2004a) and seed mass has shown to be highly correlated to mother tree. Therefore, the effect of seed mass can be confounded with other traits determined by maternal source when experiments are conducted with seeds from different plants (Castro, 1999).

Maternal source may also determine other traits that enhance seedling establishment. The within-population variability in leaf traits is well-recognised (González-Rodríguez and Oyama, 2005; Ramírez-Valiente et al., 2009). Rice et al. (1993) suggested the possibility of genetic variation for plasticity in traits such as the specific leaf area that can be especially important in heterogeneous environments such as Mediterranean-type areas. Seedling growth and other physiological traits related to drought resistance seem as well to have a heritable component (Leiva and Fernández-Alés, 1998; Castro et al., 2008).

The preservation of adaptive genetic variation within and among populations ensures that evolutionary potential is maintained and inbreeding avoided, determining species adaptability to the environment and their conservation (McKay and Latta, 2002). Genetic diversity among and within populations varies greatly among taxa (Byers and Waller, 1999), so the study of these factors on species used in restoration is necessary to adapt seeding collects on forest management actions (Vander Mijnsbrugge et al., 2010). Many experiments in both controlled and field conditions have evaluated multiple environmental constrains in Mediterranean oaks (Sánchez-Gómez et al., 2006; Gómez-Aparicio et al., 2008, Urbieta et al., 2008a). However, it is also necessary to consider the role of intra specific variability in an ecological context in order to a better understanding of the mechanisms underlying seedling establishment. To our knowledge, this is the first field experiment that combines maternal and environmental effects in seedling establishment of various species at the same time.

The aim of our study was to investigate the effect of mother plants as determinants of within-population variability on the establishment of four Mediterranean oaks, from emergence and early growth to survival in the second year, across a gradient of environmental factors. Specifically, we designed an experiment to meet four objectives: 1) tease apart pure seed-mass effects from mother influence, 2) identify which factors are more critical (environmental vs. intrinsic) at each establishment phase, 3) evaluate the benefits of artificial irrigation that simulated a rainy summer in the first year, and its delayed effects on the second year, and 4) compare these effects on four late-successional *Quercus* species that differ in leaf longevity.

METHODS

Study area and species

The study area and species are described in general methods section (pag 21-29).

Experimental design

Acorn collection was carried out in autumn 2006. Mother trees selection, acorn collection and seed dry mass estimations are described in general methods section (pag 29). In order to characterize better the seed characteristics, seed moisture content was determined in seeds used to estimate seed dry mass (Appendix S1).

Twenty five experimental plots were set on each of 3 light categories: open woodlands, beneath cover of *Q. ilex*, and beneath cover of *Q. ilex* and simulated shrub cover. To simulate shrub cover, a green mesh that allowed through 27 % of incident radiation was placed over the corresponding experimental plots on a rectangular structure of 2 x 1 m horizontally held on four iron sticks 40 cm over the soil. These light categories were established to cover a wide range of light availability and to estimate leaf traits based on light conditions (see below). Each experimental plot was divided in two replicates (separated 50 cm), so there were 150 replicates in total. Sixteen acorns (4 per species) were sown at each replicate, 4 cm deep and 5 cm apart in a square grid of 4 x 4 acorns. Each replicate was protected by a wire square (50 x 50 cm, 1 cm mesh width), put up and half-buried to avoid predation by rodents (J. Pausas personal communication). Weeds were removed by hand during the first spring to avoid competition.

In each experimental plot, replicates were randomly assigned to an irrigation treatment (irrigated vs. non-irrigated). Water was applied during the summer months of the first year of the experiment (70 days, June – August 2007) at 15-30 days intervals. In total, 51 L m^{-2} was added, simulating a rainy summer in this area (data from Consejería de Agricultura, Junta de Andalucía).

Data collection

The main environmental variables measured that likely influenced seedling establishment were light availability and soil moisture. The spatial variability of light was explored with hemispherical digital photography (Rich, 1989). At each plot, a photograph was taken at the seedling level (about 30 cm over the soil) using a horizontally-levelled digital camera aimed at the zenith, using a 180° field of view fish-eye lens. The images were analysed using Hemiview canopy analysis software v 2.1, which estimates a global site factor (GSF) by combining the proportion of direct and diffuse radiation. In further analyses this measurement was used as a proxy for light availability, rather than the three light categories described above, unless specified otherwise.

To estimate soil moisture, the volumetric water content of the topsoil (20 cm depth) was monthly measured using a time-domain reflectometer (TDR model 100; Spectrum Technologies, Inc., Plainfield, IL, USA Spectrum). Three measurements were taken at each experimental plot from December to May, when soil in the upper layers was almost dry. Measurements were pooled from December to February to account for soil moisture in the pre-emergence period, and from December to May to reflect spring soil water availability.

Experimental plots were visited every two weeks for the first year and once every three months for the second year. Seedling performance was evaluated through seedling emergence (i.e. percentage of seeds emerged from the sown seeds), time to emergence (number of days after sowing when seedlings emerged), survival in two successive growing seasons (percentage of seedlings surviving from the emerged seeds in the first and second year of the experiment) and an overall quantification of seedling establishment success at the end of the experiment (percentage of seedlings surviving from the sown seeds).

To assess seedling morphological traits a subsample of the emerged seedlings was randomly selected (7-18 seedlings per mother tree, except for one mother tree for which only 3 seedlings could be used) and non-destructive measurements in stems and leaves were taken in the field at end of the growing season (May-July 2007). Stem volume was estimated measuring length and diameter of stems and branches at two positions (at the base and at the top) and applying the formula for conical frustums. Total leaf area was estimated in the field following Poorter et al. (2004) using a point grid printed on a transparent sheet. The number of grid intersections found within a certain area is a good estimator of that area. Three sheets with different grid sizes were used (3, 5 and 7 mm) depending on leaf size.

To estimate stem biomass from the non-destructive measurements, 10-15 additional seedlings per species were harvested at the end of the growing season and all non-destructive stem volume measurements were taken as described above. Seedlings were placed in individual bags with moistened filter paper and kept in a portable icebox until taken to the laboratory. Once in the laboratory, harvested seedlings were oven-dried at 70° C for a minimum of 48 hours, and subsequently weighed to obtain dry mass of the stem fraction. Linear regressions between stem volume and stem dry mass were calculated per species and sampling date ($R^2 > 0.80$), and were used to estimate stem mass from the non-destructive estimate of stem volume (Pérez-Ramos et al., 2010). A linear regression per species may suffice, since in a previous nursery-experiment using acorns from the same provenance we found no differences in wood density across seedlings from different mothers within each species (VGR unpublished data).

To estimate leaf mass from the non-destructive measurements taken in the field, leaves were harvested from an additional fifteen randomly-selected seedlings per mother tree at the end of the growing season (June 2007), since leaf traits are known to be related to both species and incident light (Aranda et al., 2004; Poorter et al., 2009), but might be as well influenced by mother tree (VGR unpublished data). Measurements were taken from one expanded leaf at medium height on each seedling. All leaves were scanned (HP Scan-jet 6300c), oven-dried at 70º C for a minimum of 48 hours, and subsequently weighed to obtain dry mass. Leaf area for each harvested leaf was calculated using image analysis software (Image Pro-plus 4.5; Media Cybernetics, Inc), and specific leaf area (SLA) was calculated dividing leaf area by leaf dry mass. Average SLA values per mother tree and light category combination were calculated from the harvested leaves ($R^2 > 0.40$). Leaf mass was calculated as estimated leaf area divided by SLA.

Aboveground biomass at the end of the first growing season was calculated as the sum of the estimated stem and leaf masses. Aerial leaf mass fraction (LMF_a) was also calculated as the ratio between leaf mass and estimated aboveground biomass.

Data analyses

To investigate the effect of different variables on seedling emergence we used generalized mixed models (GLMM) with a binomial distribution of errors and a logit link function, and constructed a model for each species. Seedling emergence was taken as 1 if plants emerged and 0 otherwise. Experimental plot identity was included as a random factor to account for the nestedness of the data, and light availability, pre-emergence soil moisture, seed mass, mother plant and all two-way interactions were taken as the fixed factors. In all cases, seed mass was heavily dependent upon the mother plant (ANOVA; *Q. faginea*: R^2=0.71, *P*=0.000; *Q. ilex*: R^2=0.77, *P*=0.000; *Q. pyrenaica*: R^2=0.74, *P*=0.000; *Q. suber*: R^2=0.28, *P*=0.000). To avoid multicollinearity problems we regressed seed mass against mother tree, and replaced the former variable with the residuals from the regression (Graham 2003); therefore, the residuals for seed mass would represent the unique contribution of seed mass, independent from mother-induced effects (Castro, 1999).

To detect potential factors influencing time to emergence in each *Quercus* species we used Linear Mixed Models (LMM), in which experimental plot identity was included as a random factor, to account for the structure of the data. As fixed factors we considered the same variables as above, and all two-way interactions.

To analyse seedling survival in two successive years after plantation and establishment success at the end of the experiment we built binomial generalized mixed models (GLMM) for each species. Seedling survival was taken as 1 if emerged seedlings were alive and 0 otherwise, and establishment success was coded as 1 for those seedlings found alive at the end of the experiment and 0 for those plants dead or not even emerged. In all cases, experimental plot identity was included as a random factor to account for the structure of the data. Light availability as measured through GSF, residuals of seed mass (see above), mother plant, irrigation treatment, and all two-way interactions were taken as the fixed factors. For models in year 1, soil moisture in spring was also included to reflect early growth conditions.

In addition, to explore the main factors that determine survival of seedlings of the four *Quercus* species in our study area in year 1 without ameliorating conditions through summer irrigation, we built binomial generalized mixed models (GLMM) for each species. Survival of non-irrigated seedlings was taken as the response variable, and experimental plot was taken as the random factor. As fixed components we included the independent effects of light availability, spring soil moisture, residuals of seed mass and maternal plants.

To investigate the influence of mother tree on different growth and morphological traits, i.e. the aboveground biomass, aerial leaf mass fraction (LMF_a), and the specific leaf area (SLA), we built Linear Models (LM) or Linear Mixed Models (LMM) for each species. The random effect of experimental plot identity was only included if it significantly improved model fit. Aboveground biomass measured at the end of the growing season was square root-transformed to achieve normality. Different environmental and plant-related factors likely to have an impact on morphologic and growth traits (i.e. soil moisture in spring, light availability and residuals of seed mass) were included in the fixed part of the model. In addition, for SLA models we tested the effect of the interaction between light availability and maternal plant that has been described for other tree species (Rice et al., 1993).

Model selection was based on AIC and Log-likelihood Ratio Tests (LRT) using Maximum Likelihood, and for the binomial GLMMs it followed a backward procedure based on a 'Laplace' approximation of likelihood, implemented in the package *lme4* (Bates and Maechler 2009). This GLMM approach allows for a comparison of models with different fixed effects, but does not allow F-tests for fixed effects. Therefore we report here the χ^2 statistics of the likelihood ratio tests between models with and without a certain fixed term. The direction of the effect of a variable is based on evaluation of its estimated coefficient. Modelling assumptions were checked (Zuur et al., 2009) and all analyses were performed using the statistical software R 2.10.1 (R Development Core Team 2009).

RESULTS

Seedling emergence

Time to emergence differed between the four *Quercus* species studied (*P*=0.000; Table 1), with the shortest time of emergence for *Q. faginea* and the longest for *Q. ilex*. Average values ranged between 154 and 170 days, but it is noteworthy that in all species a percentage of seedlings did not emerge until the second year (*Q. ilex* 4.7%; *Q. suber* 2.7%; *Q. faginea* 5.5%; *Q. pyrenaica* 3.7%).

Table1. Mean ± standard deviation and range (in brackets) of time to emergence, aboveground biomass, aerial leaf mass fraction (LMF_a), specific leaf area (SLA), and percentages of emergence, survival and establishment success in years 1 and 2 for the four *Quercus* species studied. Survival relates to the number of emerged seedlings that are found alive in the following year, while success refers to the number of planted acorns that were recruited at the end of the experiment.

	Evergreens		*Deciduous*	
	Quercus ilex	*Quercus suber*	*Quercus faginea*	*Quercus pyrenaica*
Mean time to emergence (days)	170.2 ± 27.0 [97 - 665]	160.0 ± 28.6 [97 - 567]	153.8 ± 31.2 [97 - 791]	155.6 ± 27.5 [83 - 567]
Emergence (%)	33.6	73.5	54.2	71.0
Survival Y1 (%)	37.9	22.8	19.5	12.4
Survival Y1 non irrigated (%)	31.1	17.8	14.2	7.21
Survival Y2 (%)	24.0	10.4	10.9	7.7
Establishment Success (%)	8.5	7.8	6.2	5.71
Aboveground biomass (g)	0.54 ± 0.29 [0.10 – 1.11]	0.56 ± 0.27 [0.05 – 1.29]	0.67 ± 0.44 [0.11 – 2.10]	0.67 ± 0.39 [0.16 – 2.37]
LMF_a	0.84 ± 0.06 [0.69 – 0.93]	0.85 ± 0.06 [0.55 – 0.96]	0.83 ± 0.06 [0.61 – 0.93]	0.81 ± 0.06 [0.60 – 0.91]
SLA (m^2 kg^{-1})	7.3 ± 1.9 [5.0 – 16.9]	11.5 ± 2.7 [7.4 – 17.7]	11.3 ± 2.3 [7.2 – 17.6]	13.7 ± 2.8 [9.6 – 21.7]

Time to emergence was determined by different variables in each species (Table 2), but in most cases (3 species) it was related to light availability and to intrinsic traits related to maternal origin (Fig. 1). *Q. ilex* was the species with the largest mean time to emergence, which was only negatively determined by light availability. In *Q. suber* however, time to emergence was negatively affected by seed mass, and also depended on mother tree. No differences were found in mean time to emergence between deciduous species (Tukey contrasts; z=0.892, *P*=0.807), which was on average lower than evergreens. For *Q. faginea*, the species with the lowest time to emergence, a complex set of parameters was involved: the interaction term between light availability and mother tree, the interaction between soil moisture and mother tree, and a negative effect of seed mass. An interaction between light availability and mother tree was the only term retained in the model for *Q. pyrenaica*, the species with the second lowest average time to emergence. In the case of *Q. faginea* the interaction between light and mother tree determined neutral or negative effects of light availability on time to emergence depending on the mother tree, while for *Q. pyrenaica* these effects were positive or negative depending on the mother tree (Fig.1).

Seedling emergence was highest for *Q. suber* and *Q. pyrenaica* (73.5 % and 71.0% respectively; Table 1), and for these species it was determined by the independent effects of seed mass and mother tree (Table 2). In both cases, seed mass had a positive influence on seedling emergence (Fig. 2). On the contrary, seed mass had a different effect on emergence of seedlings from different mother trees in *Q. faginea* and *Q. ilex*, as revealed by a significant interaction between mother and seed mass (Table 2). In the case of *Q. faginea* seed mass had a positive influence on emergence but with different slopes for each maternal plant. On the contrary, for *Q. ilex* this relationship was either positive or negative depending on the mother tree (Fig. 2). In addition these two species showed lower rates of emergence (54.2 % and 33.6 % respectively; Table 1).

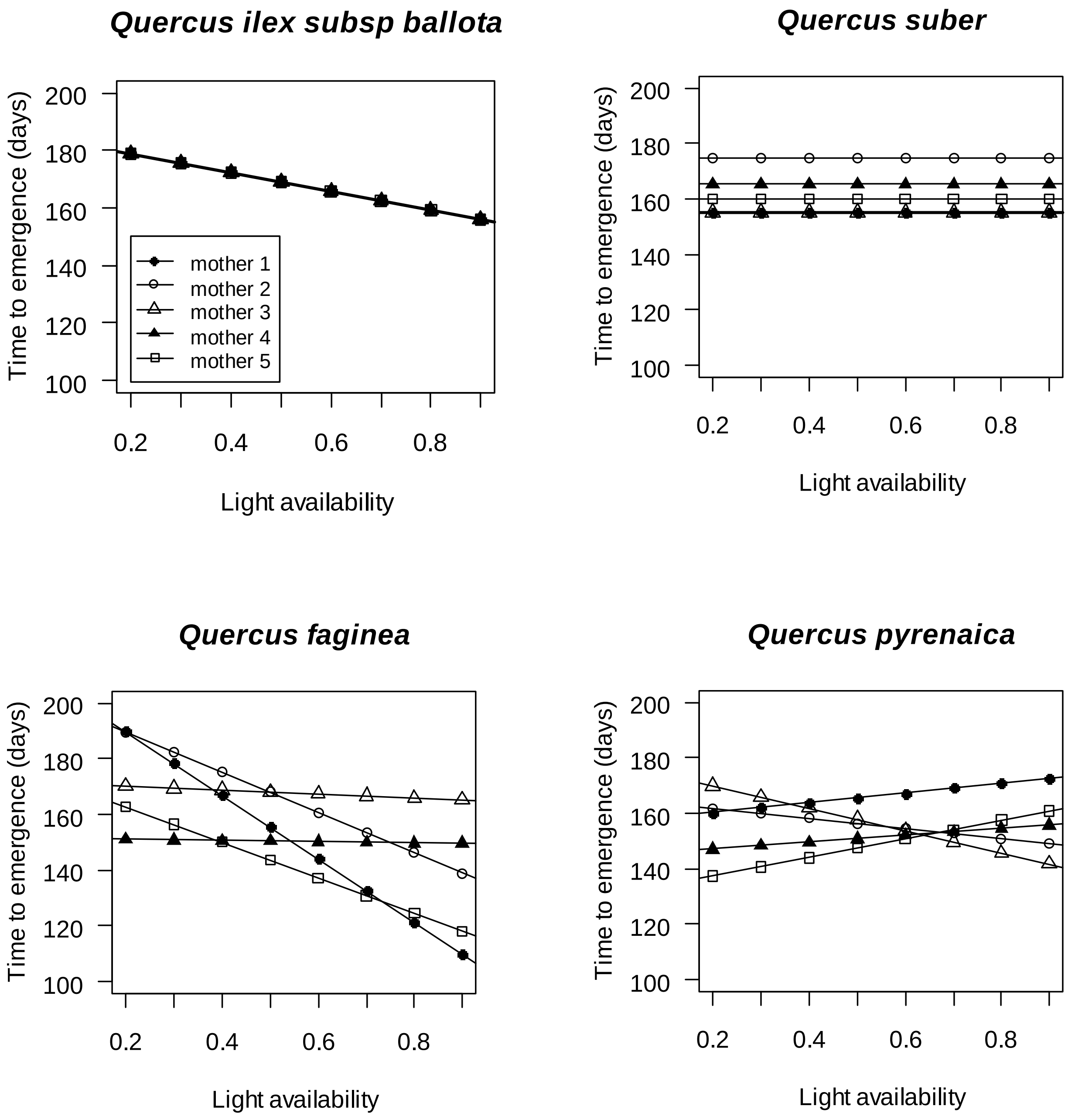

Figure 1. Predicted values for time to emergence in relation to light availability measured through GSF (Global Site Factor) for the four Quercus species studied. Lines indicate predicted values of time to emergence (days) for different mother trees, which are depicted by different symbols. Parallel or overlapping lines (thick lines) indicate non-significant interactions between light availability and mother tree.

Seedling survival and establishment success

Summer irrigation had a positive effect on seedling survival in year 1 for all four *Quercus* species (Table 2). The greatest effect was detected on *Q. pyrenaica*, for which seedlings with summer irrigation increased their probabilities of survival in 60.9% relative to those without irrigation, according to model predictions. These percentages were 45.4, 37.7 and 35.5 for *Q. faginea*, *Q. suber* and *Q. ilex*, respectively. In addition, for *Q. suber* other factors were involved, such as seed mass and the interaction terms between light availability and mother tree, and light availability and soil moisture. Thus, cork oaks responded differently to light conditions depending on both mother tree and water availability in the soil. The response to light was either positive or negative depending on mother tree.

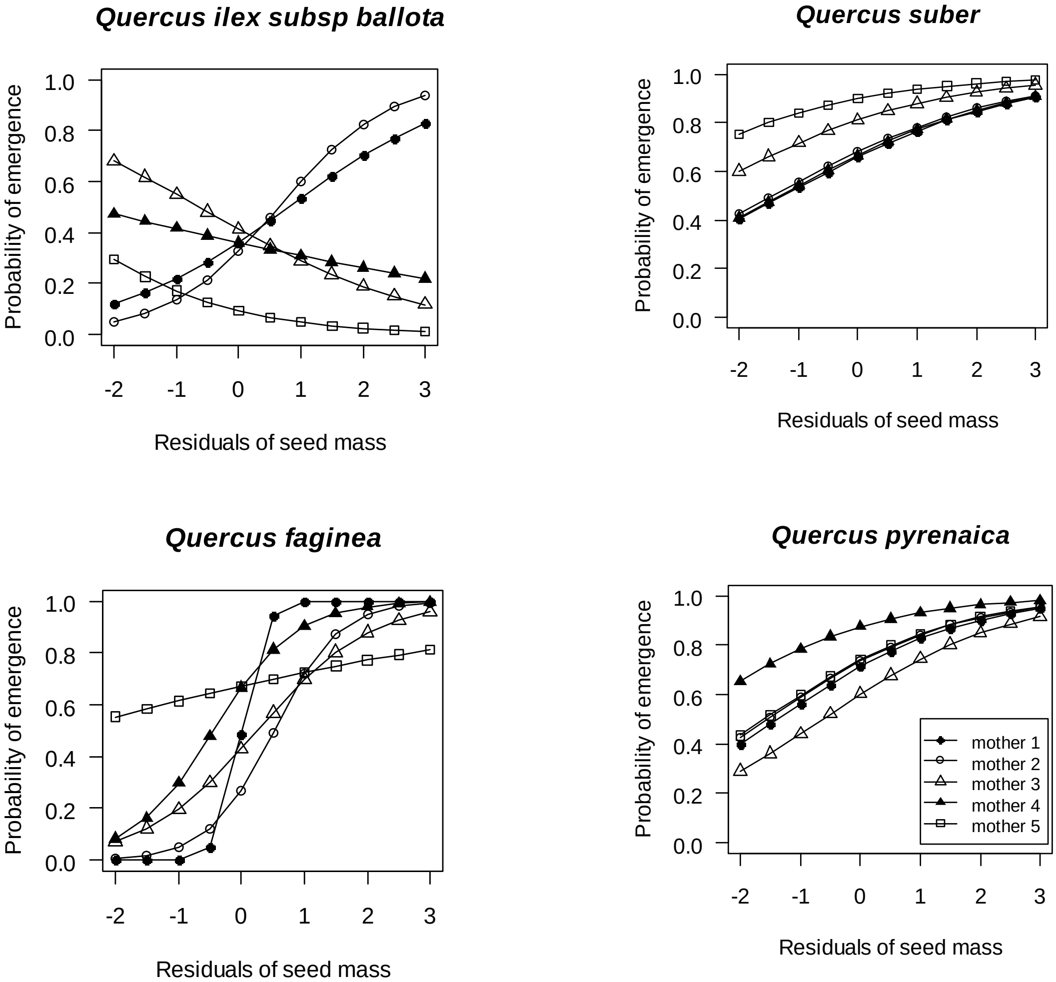

Figure 2. Predicted probabilities of emergence from a binomial generalized linear model (GLMM) across values of seed mass. The residuals from regressing mother tree against seed mass were taken to avoid collinearity problems and to identify the unique contribution of seed mass, independent of mother tree on the probability of emergence. Different maternal plants are depicted by different symbols. Intersecting curves indicate a significant interaction between maternal plant and the residuals of seed mass, and only one line indicates a significant independent effect of residuals of seed mass.

Table 2. Linear Mixed Models (LMM) for time to emergence and Generalized Linear Mixed Models (GLMM) for seedling emergence, survival in years 1 and 2, survival in year 1 for non-irrigated seedlings and establishment success of the four *Quercus* species studied. For LMM results of likelihood ratio tests (LRT) are given, while for GLMMs significance is assessed by comparing nested models with a Chi-square statistic. Signs in brackets indicate the direction of the effect of continuous predictors on the response variable. Irrigation effects were always positive. 'GSF': light availability; 'seed mass': residuals of seed mass; 'mother': maternal plant; 'irrigation': presence of summer watering in year 1; 'moisture': soil moisture.

	Quercus ilex				***Quercus suber***				***Quercus faginea***				***Quercus pyrenaica***			
		LRT		**Sig.**		**LRT**		**Sig.**		**LRT**		**Sig.**		**LRT**		**Sig.**
Time to emergence	GSF (-)	4.33		0.038	Seed mass (-)	20.60		0.000	GSF*mother	13.38		0.010	GSF*mother	10.71		0.030
					Mother	28.50		0.000	Moisture*mother	17.34		0.002				
									Seed mass (-)	9.78		0.002				
		Chisq	**df**	**Sig.**		**Chisq**	**df**	**Sig.**		**Chisq**	**df**	**Sig.**		**Chisq**	**df**	**Sig.**
Emergence	Seed mass*mother	13.34	4	0.009	Seed mass (+)	11.54	1	0.001	Seed mass*mother	29.25	4	0.000	Seed mass (+)	16.48	1	0.000
					mother	29.62	4	0.000					Mother	18.50	4	0.001
Survival Y1	Irrigation	3.47	1	0.062	Irrigation	6.33	1	0.012	Irrigation	4.68	1	0.031	Irrigation	9.93	1	0.002
					GSF*moisture	4.58	1	0.032								
					GSF*mother	11.46	4	0.022								
					Seed mass (+)	5.67	1	0.018								
Survival Y1 for non-irrigated seedlings	Mother	12.64	4	0.013	Mother	10.63	4	0.031					Mother	16.91	4	0.002
					Seed mass (+)	5.01	1	0.025								
					Moisture (+)	7.07	1	0.008								
Survival Y2	Irrigation*mother	10.11	4	0.039	Seed mass (+)	3.64	1	0.056	Irrigation*mother	10.20	1	0.037	GSF*seed mass	7.20	1	0.007
Establishment success	Mother	9.54	4	0.049	Seed mass (+)	6.52	1	0.011	Irrigation	5.96	1	0.015	GSF*seed mass	5.18	1	0.023
													Irrigation*mother	10.65	4	0.031

For non-irrigated seedlings, survival after the first summer was mainly determined by the mother tree, except in the case of *Q. faginea*, for which none of the studied variables had a significant effect (Table 2). In addition, for *Q. suber*, survival of non-irrigated plants depended on seed mass and spring soil moisture, both of them having a positive effect.

Second-year survival was much lower than first year-survival for all species (Table 1). Summer irrigation in the previous year influenced second-year seedling survival for both *Q. ilex* and *Q. faginea,* but its effect differed between mother trees (Table 2). In the case of *Q. suber*, survival in year 2 was related to seed mass although this effect was only marginally significant. For *Q. pyrenaica* seed mass also played a role, being its effect on second-year survival positive but with differing slopes between light conditions (Table 2).

Establishment success relates to the partial contribution of consecutive processes, from seedling emergence to subsequent survival. Therefore, much lower values were found, ranging between 5.7 and 8.5% (Table 1). For *Q. ilex* it was determined by maternal factors, for *Q. faginea* by summer irrigation in the first year, for *Q. suber* by seed mass (positive) and for *Q. pyrenaica* by the interactions between seed mass and light conditions and mother tree and irrigation (Table 2), which determined different responses to irrigation in year 1 depending on maternal origin, and different responses to light availability depending on seed mass.

Morphological traits and growth parameters

Aboveground biomass for *Q. ilex, Q. suber* and *Q.faginea* was only determined by maternal traits (Table 3, Fig. 3). For *Q. pyrenaica* none of the explored factors had a significant effect.

For *Q. ilex* none of the studied variables had any influence on aerial leaf mass fraction (LMF_a), but for the other species maternal plant had a significant effect. Additionally for *Q. faginea* light availability had a positive effect on LMF_a (Table 3).

Table 3. Model results for growth parameters in the four *Quercus* species studied: Linear Models (LM) for estimated aboveground biomass and Linear Mixed Models (LMM) for aerial leaf mass fraction (**LMF**$_a$), and specific leaf area. For LM results of F tests are given, while for LMMs log-likelihood ratio tests (LRT) and the corresponding significance levels (Sig.) are shown. Signs in brackets indicate the direction of the effect of continuous predictors on the response variable. 'GSF': light availability; 'seed mass': residuals of seed mass; 'mother': maternal plant.

	Quercus ilex				***Quercus suber***				***Quercus faginea***				***Quercus pyrenaica***			
		F	**df**	**Sig.**		**F**	**df**	**Sig.**		**F**	**df**	**Sig.**		**F**	**df**	**Sig.**
Aboveground biomass	Mother	12.75	36.40	0.000	Mother	4.59	60.65	0.001	Mother	13.22	57.62	0.000	-			
		LRT		**Sig.**		**LRT**		**Sig.**		**LRT**		**Sig.**		**LRT**		**Sig.**
LMF$_a$	Mother	8.95		0.062					Mother	9.99		0.041	Mother	16.57		0.023
									GSF (+)	10.29		0.001				
Specific leaf area	GSF (-)	15.73		0.000	GSF (-)	12.09		0.000	GSF*mother	10.21		0.037	GSF (-)	10.17		0.001
					Mother	11.19		0.0245								

For all four oak species light availability had a negative impact on specific leaf area. In the case of *Q. ilex* and *Q. pyrenaica* it was the only variable retained in the final model. For *Q. suber*, an independent effect of mother tree was also detected, while for *Q. faginea* the response to light availability depended on the mother tree, as sugested by a significant interaction term (Table 3).

Appendix S2 and S3 show mean values (± sd) of the different variables measured in the five mother trees at each species.

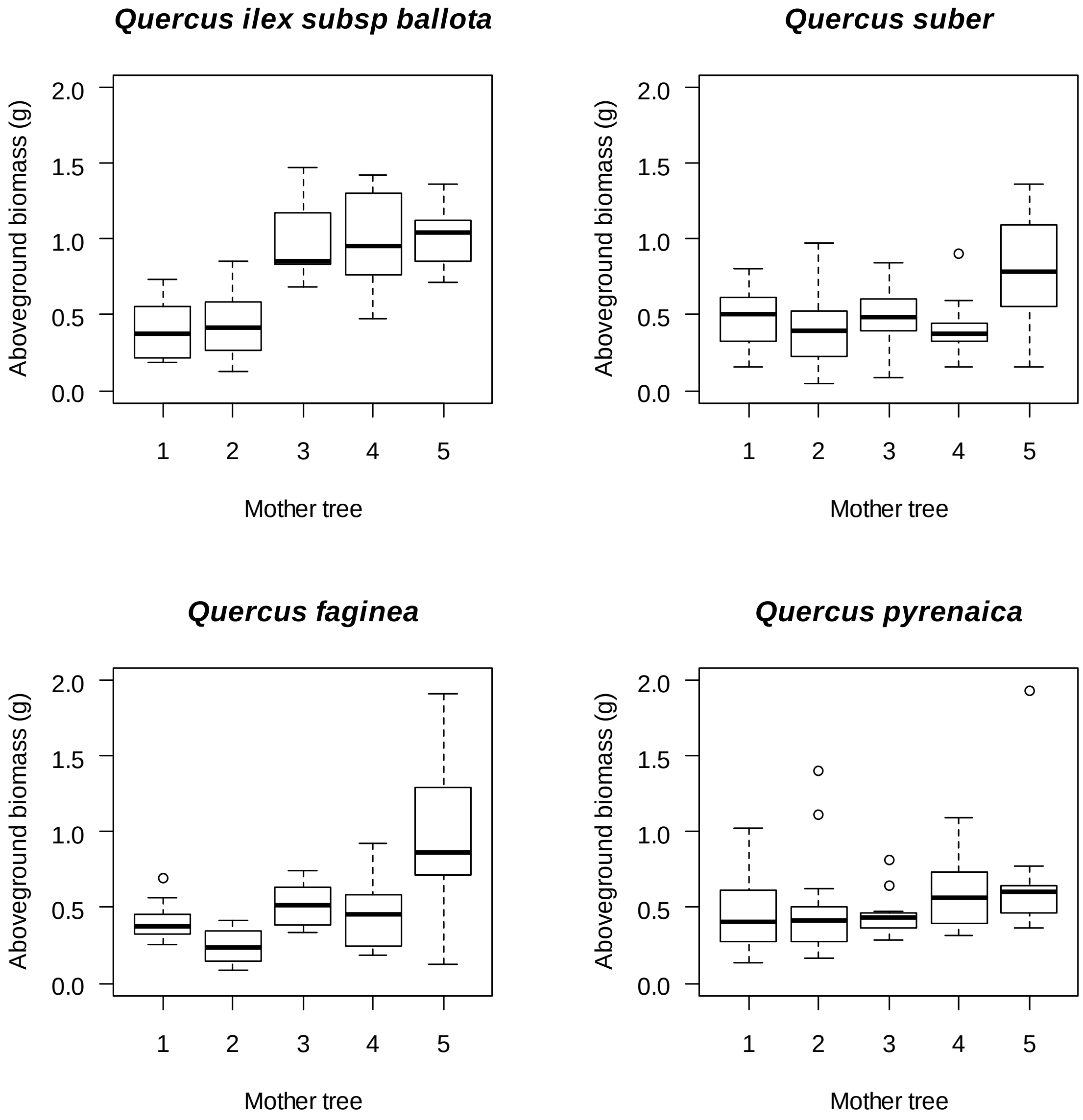

Figure 3. Box plot of aboveground biomass of one year old seedlings of the four different species studied with respect to mother tree.

DISCUSSION

In this study we identified environmental and intrinsic factors that may affect the early establishment of four Mediterranean oak species. Our more interesting results was that within population variability as determined by maternal origin affects different phases of establishment and can modify the responses of seedlings along resource gradients.

Emergence

For three of the studied species, light availability influenced time to emergence, being in general a negative factor (the more incident radiation, the lesser time to emergence). In areas with more incident radiation the soil temperature could be higher, and this could acelerate the germination and then shortens the time to emergence (Pugnaire at al., 2006). However, maternal traits modified the effects of light availability on emergence time of the two deciduous species. Light effects were negative, neutral or even positive depending on the mother tree. These differences between mothers can be related to seed moisture content, which happened to differ among maternal plants. Delayed emergence of seedlings has been related to hypocotyl dormancy, which is associated to higher seed moisture content (Merouani et al., 2001). On the other hand, we found that larger seeds of *Q. suber* and *Q. faginea* emerged earlier. This effect of seed mass on time to emergence has not been observed in other species (Castro, 1999; Jones et al, 1997), but it may be related to species-specific seed size and the ability of seeds to have a storage function. In our case, early emergence of larger seeds could also be a consequence of a less time of germination that has been found for other oak species (Gómez, 2004a).

Another interesting result was the delayed emergence that was observed for a proportion of seedlings in all species (between 2.7 to 5.5). This phenomenon has been reported for *Q. ilex* by Gómez (2004b), who found that some acorns germinated but remained shootless during the summer and emerged when environmental conditions became favourable. This strategy may thus represent a resistance mechanism against environmental extremes. This can be related to an opportunistic exploitation of resources that allows germination when minimal favourable conditions are met.

Across species, *Q. faginea* and *Q. pyrenaica* emerged before the evergreen species. Urbieta et al. (2008a) found the same effect for deciduous oaks in a similar field experiment, and it was also observed in a green-house experiment using acorns from the same sources (VGR, unpublished data). Thus, emergence date appears to be a species-specific character (Laliberté et al., 2008) that can be modified by maternal source.

Surprisingly, the probability of acorn emergence was not explained by environmental factors but only by intrinsic characteristics: maternal origin and seed mass. Although other studies have described the effects of environmental factors on emergence, most of them overlooked intrinsic factors related to maternal origin (Tyler et al., 2008; Mendoza et al, 2009). Urbieta and co-workers (2008a) accounted for seed mass in explaining probability of emergence in oaks and found no influence of seed mass on emergence rates, but their results might be unduly affected by extreme local conditions such as winter flooding that prevented seedling emergence. In the same sense, planting year has also found to be a crucial agent impacting emergence rates due to rainfall variability (Tyler et al., 2008). In our case, suitable light and temperature conditions in spring 2007 may have not limited seedling emergence that was thereby more dependent on seed characteristics. Seed mass benefits on seedling emergence have been previously reported (Winn, 1985; Seiwa, 2000). On the other hand, the relationship between seed mass and emergence may only hold for some maternal plants according to a similar essay with Scot pine (Castro, 1999). We found this effect for *Q. ilex*, for which mother tree determined a positive or negative effect of seed mass on emergence probability. On the other hand, *Q. ilex* had the lowest emergence rates. Since there were no predation signs, this limitation could be caused by a higher susceptibility to desiccation (Kollmann and Schill, 1996; Smit et al., 2009) or other uncontrolled factors. Acorn genetic variability and physiological status may also have an impact on germination and emergence rates (Merouani et al., 2001; Goodman et al., 2005).

Survival

Mediterranean climate is characterised by large water deficits in which belowground resources are more critical than light or other environmental factors (Retana et al., 1999; Coomes and Grubb, 2000; Maltez-Mouro et al., 2007). In fact, irrigation was the main factor that explained survival after the first summer for all species. The benefit of an extra amount of water during the dry period has been largely demonstrated on Mediterranean environments (Castro et al., 2005; Gómez-Aparicio et al., 2008; Mendoza et al., 2009). Indeed, rainy summers represent a recruitment opportunity for species growing in semi-arid ecosystems. Interestingly, when irrigation was not taken into account, maternal origin had a prime role in the survival of all species except *Q. faginea.* This result suggests that the effects of maternal source can be masked under the ameliorated conditions simulated by summer irrigation. In this sense, under extreme environmental conditions, such as the severe drought that occurred during the experiment (just 3 mm of precipitation during the 3 summer months), maternal traits are the main factors determining survival.

Other factors were implied in first-year survival of *Q. suber*, maybe showing the weaker effect of irrigation for this species that has been previously reported (Gómez-Aparicio et al., 2008). Depending on mother tree, radiation negatively affected *Q. suber* survival. Moderate shade provided by shrubs or trees creates a suitable microhabitat for recruitment that protects seedlings from excessive evapotraspiration (Puerta-Piñero et al., 2007; Gómez-Aparicio et al., 2008). This beneficial effect of shade was probably overwhelmed for the other species in our study by soil water availability because the summer of the experiment was exceptionally dry. Indeed, under non-irrigation conditions, survival of *Q.suber* depended on spring soil moisture, as well as on intrinsic parameters (seed mass and maternal traits).

Different effects explained survival after second summer for each *Quercus* species. Irrigation had a delayed effect only in two of the four species studied and interestingly, this effect varied across mothers. For these species, seedlings that survived the first year due to an increased water supply were able to survive in the second year, maybe because during the second growing season they could develop longer roots and thus reach deeper soil layers. For *Q. pyrenaica* and *Q. suber* there was a positive effect of seed mass on survival in the second year, which in the case of *Q. pyrenaica* was modulated by light availability. This effect can be a delayed effect of seed mass since *Quercus* acorns are recalcitrant (Finch-Savage, 1992) and also they decompose soon (less than one year, personal observation). Seed mas has been reported to have a positive effect on root biomass (Quero et al., 2008a) and an increase in root proportion has been positively related to summer survival (Lloret et al., 1999). Therefore, the effect of seed mass effect on second-year survival might also be caused by a higher investment in belowground resources.

Establishment success

In Mediterranean conditions, survival of recruited seedlings stabilizes after two years (Jordano et al., 2008). Thus, “establishment success” indicates the percentage of seedlings that have overcome successfully post-dispersal limitations and have a high probability of surviving and becoming adults. In general terms, the studied factors which were more relevant to the previous phases of establishment had significant effects on establishment success. Therefore, different factors limiting the different phases constrained recruitment across species. For example, low emergence percentage of *Q. ilex* was offset by higher survival relative to the other oak species. As a result, the percentage of established seedlings after two years was similar for all species (~ 7 %), although it was slightly higher in evergreens than in deciduous. This fact might be linked to their differential susceptibility to summer drought and their higher relative abundance in the study area and in general, in Mediterranean forests in the South of the Iberian Peninsula (Acheral y Rambal, 1992; Costa et al., 2005). Indeed, irrigation had a significant effect for *Q. pyrenaica* and *Q. faginea* which are the two most drought-sensitive species (Acheral y Rambal, 1992; Quero et al., 2006). In fact, the studied area is in the distribution limit of *Q. pyrenaica*, where this species is scantly represented and only found in the most humid sites (Castillo y Castillo 2004, Quero y Villar 2009).

Intrinsic factors explained establishment success in the two evergreen species, and also in *Q. pyrenaica*. Maternal origin was the only significant effect for *Q. ilex,* and seed mass was the only variable determining establishment success in *Q. suber.* In the case of *Q. pyrenaica*, intrinsic factors were modulating the response to environmental factors.

Growth and morphological traits

In this study we found that intrinsic factors explained most variation in growth and morphological traits in the first growing season (i.e. mother tree). Residual seed mass had not effect on aboveground biomass probably because of the narrow seed mass variation within mothers. Thus, mother tree may influence seedling biomass through seed mass. Heavy-seeded species are more dependent on seed resources during first stages of life, in which they have limited photosynthetic capacity (Beckage and Clark, 2003; Pérez-Ramos et al., 2010). A larger seedling biomass has been generally related to larger seed mass (Hendrix et al*.,* 1991; Green and Juniper, 2004). However, mother tree also may influence seedling biomass through other intrinsic traits, such as physiological status or nutrient content (Castro et al., 1999; Merouani et al., 2001). The ausence of mother tree effect on *Q. pyrenaica* can be due to the fact that this species invest a higher proportion of root in its first stage of development (Quero et al., 2008a), so in all mothers aboveground fraction could be similar.

As expected, light availability negatively influenced SLA. Higher SLA reflects a greater capacity to tolerate shade (Mooney and Dunn, 1970; King, 2003; Quero et al., 2006; Schumacher et al., 2009). For *Q. faginea* SLA depended on the interaction of light and mother source. Rice et al (1993) found the same effect in *Q. douglasii* seedlings, suggesting the possibility of heritable genetic variation for plasticity in leaf morphology.

On the other hand, *Q. faginea* seedlings invested more resources in leaf fraction in more iluminated sites. Similar results have been found in *Q. canariensis*, also a deciduous species (Perez-Ramos et al., 2009) and also in tropical seedlings, probably due to faster leaf production rates (Poorter et al., 2001)

Within-population variability: the role of maternal tree

It is known that maternal origin affects seedling performance by influencing seed size (Leiva and Fernandez-Alés, 1998; Castro, 1999; Merouani et al., 2001). To tease apart these effects, maternal trees that produce seeds in the same range of size variation might be chosen, in detriment of exploring the whole range of seed mass present in the population. In this study we chose to cover the whole range of seed mass and thus explore all maternal effects together in a complete ecological context and not only in a subsample of it. Moreover, our analytical approach let us study seed-mass factor *per se* at each mother source, separating this effect from other maternal influences. Our results also showed how maternal origin modulates environmental and seed mass effects on different establishment phases. This within population variability on responses across the resource gradients could be an adaptation to highly heterogeneous and unpredictable environments (Castro, 1999; Sánchez-Vilas and Retuerto, 2007). In order to evaluate this hypothesis, it would be interesting to study these effects on oak populations from less heterogeneous environments like temperate or tropical forests.

Maternal effects could be caused either by environmental conditions during seed development or by genetic variability (Wulff, 1986; Schmitt et al., 1992; Castro et al., 2008). Woody perennials have more than 90% of their total genetic diversity within populations rather than among them, thus being adapted to environmental variation at small microhabitat scales (Campbell, 1979; Hamrick, 2004). In the context of rapid climatic changes predicted for the Mediterranean region (IPCC, 2007), it is crucial to understand whether sufficient genetic variation resides within populations to allow a successfully recruitment and guarantee their persistence (Hamrick, 2004). In this experiment we found that maternal source modifies seedling performance at different stages of establishment, providing a first approach to the understanding of within population variability patterns in relation to other environmental constraints. Combining this kind of ecological studies of local adaptation with genetic approaches using molecular techniques would provide useful knowledge that completes conservation and restoration efforts (McKay et al., 2005).

CONCLUSIONS

This field study evaluated maternal influences and their interactions with various factors relevant to Mediterranean oaks establishment. Maternal origin had a crucial role on many of the phases studied. Intrinsic traits influenced emergence, survival, growth and morphological traits, especially under adverse growing conditions (i.e. extreme summer drought). Ameliorating conditions can favour oak survival rendering it less dependent on maternal traits. Environmental effects on seedling performance were also modified by the mother, and this within population variability could be an adaptation to high heterogeneous an unpredictable environments.

Acknowledgements

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SUPPLEMENTARY INDEX

Appendix S1. Moisture content (mean ± ds) of seeds used to estimate fresh mass/dry mass relation by mother tree. Different letters show significant differences (*P* < 0.05) across mothers (Kruskal-Wallis no parametric test)

Mother	***Q.ilex***	***Q.suber***	***Q.faginea***	***Q.pyrenaica***
1	0,464 ± 0,051 **a**	0,449 ± 0,033 **a**	0,411 ± 0,017 **a**	0,387 ± 0,010 **a,b**
2	0,431 ± 0,045 **a,b**	0,522 ± 0,075 **a**	0,484 ± 0,035 **b**	0,396 ± 0,012 **a,b**
3	0,379 ± 0,018 **b**	0,391 ± 0,023 **b,c**	0,374 ± 0,036 **a**	0,410 ± 0,025 **b**
4	0,390 ± 0,037 **b**	0,377 ± 0,014 **b**	0,431 ± 0,052 **a,b**	0,409 ± 0,014 **b**
5	0,477 ± 0,028 **a**	0,443 ± 0,062 **a,c**	0,417 ± 0,038 **a**	0,381 ± 0,022 **a**

Appendix S2. Percentages of emergence, survival and establishment success in years 1 and 2 (Y1 or Y2, respectively), for all the mother trees studied. Survival relates to the number of emerged seedlings that are found alive in the following year, while establishment success refers to percentage of seedlings surviving from the sown seeds.

Species	Mother	Emergence (%)	Survival Y1 (%)	Survival Y1 non irrigated (%)	Survival Y2 (%)	Establishment Success (%)
Q. ilex	1	37.82	34.09	31.03	22.44	9.40
	2	35.00	40.00	32.00	23.68	9.09
	3	41.96	43.86	2.94	28.81	12.05
	4	38.26	37.78	15.78	22.22	8.69
	5	12.61	21.43	40.00	17.60	2.52
Q.suber	1	65.55	11.39	5.71	6.32	2.79
	2	66.22	20.00	13.60	5.88	4.05
	3	79.27	28.68	25.75	13.07	10.42
	4	65.00	27.16	17.50	11.90	8.33
	5	86.55	22.33	1.85	10.67	9.24
Q.faginea	1	50.00	20.00	20.00	5.12	2.85
	2	29.36	15.15	6.89	8.57	2.75
	3	44.62	25.00	18.18	15.60	7.81
	4	64.50	17.76	11.32	10.60	6.58
	5	65.52	20.18	14.28	12.82	8.62
Q. pyrenaica	1	69.57	15.58	8.16	8.53	6.19
	2	73.76	6.60	4.00	5.60	4.16
	3	58.87	14.12	16.21	5.55	3.54
	4	86.57	17.24	4.16	11.66	10.44
	5	15.58	11.49	2.85	8.98	6.72

Appendix S3. Mean ± standard deviation and range (in brackets) of seed dry mass, time to emergence, aboveground biomass, LMFa (ratio between leaf mass and aboveground biomass), specific leaf area (SLA), for all the mother trees studied.

Species	Mother	Seed dry mass (g)	Mean time to emergence (days)	Aboveground biomass (g)	LMFa	SLA (m^2 kg^{-1})
Q. ilex	1	1.60 ± 0.37	209 ± 112.8	0.40 ± 0.19	0.88 ± 0.03	7.23 ± 1.32
		[0.58 - 2.57]	[112 - 665]	[0.18 - 0.73]	[0.82 - 0.92]	[5.22 - 9.52]
	2	1.61 ± 0.53	197.4 ± 103.4	0.44 ± 0.23	0.87 ± 0.06	7.60 ± 1.48
		[0.27 - 3.19]	[97 - 567]	[0.12 - 0.85]	[0.71 - 0.93]	[5.48 - 10.48]
	3	3.53 ± 0.57	178 ± 58.2	1.00 ± 0.27	0.82 ± 0.03	7.12 ± 2.00
		[2.21 - 5.89]	[97 - 567]	[0.68 - 1.47]	[0.79 - 0.86]	[5.38 - 11.48]
	4	3.88 ± 0.66	166.2 ± 29.2	0.97 ± 0.30	0.82 ± 0.07	7.69 ± 3.04
		[2.43 - 5.92]	[112 - 279]	[0.47 - 1.41]	0.69 - 0.93	[5.48 - 16.9]
	5	4.44 ± 0.79	198.2 ± 96.1	1.02 ± 0.24	0.81 ± 0.06	6.63 ± 1.15
		[2.78 - 6.46]	[97 - 483]	[0.71 - 1.35]	[0.77 - 0.89]	[5.00 - 8.62]
Q.suber	1	1.68 ± 0.48	156.5 ± 37.7	0.48 ± 0.21	0.86 ± 0.05	11.55 ± 2.86
		[0.64 - 3.20]	[97 - 302]	[0.15 - 0.89]	[0.75 - 0.96]	[7.97 - 17.73]
	2	2.16 ± 0.65	187.3 ± 67.2	0.40 ± 0.24	0.82 ± 0.11	11.8 ± 2.87
		[0.82 - 3.52]	[112 - 567]	[0.04 - 0.97]	[0.55 - 0.96]	[7.95 - 17.5]
	3	1.93 ± 0.53	160.8 ± 50.5	0.47 ± 0.18	0.86 ± 0.04	12.23 ± 2.58
		[0.84 - 3.51]	97 - 567	[0.08 - 0.84]	[0.78 - 0.93]	[9.25 - 16.16]
	4	2.51 ± 0.63	183.3 ± 82.6	0.43 ± 0.21	0.85 ± 0.03	11.7 ± 2.98
		[1.19 - 4.13]	[112 - 567]	[0.15 - 0.90]	[0.80 - 0.91]	[7.38 - 16.12]
	5	2.86 ± 1.23	160.3 ± 25.8	0.79 ± 0.33	0.84 ± 0.04	10.34 ± 2.18
		[0.59 - 6.05]	97 - 223	[0.15 - 1.36]	[0.78 - 0.91]	[8.06 - 14.8]
Q.faginea	1	0.87 ± 0.34	186.7 ± 104.16	0.44 ± 0.17	0.86 ± 0.04	11.94 ± 2.73
		[0.18 - 1.63]	[97 - 567]	[0.25 - 0.81]	[0.79 - 0.93]	[8.20 - 17.6]
	2	1.02 ± 0.24	209.2 ± 127.9	0.26 ± 0.12	0.82 ± 0.07	12.48 ± 2.19
		[0.40 - 1.69]	[125 - 791]	[0.08 - 0.41]	[0.69 - 0.92]	[8.46 - 16.06]
	3	1.98 ± 0.40	216.4 ± 135.7	0.51 ± 0.15	0.85 ± 0.04	10.14 ± 1.37
		[0.49 - 2.62]	[97 - 567]	[0.33 - 0.74]	[0.80 - 0.93]	[8.82 - 12.75]
	4	1.74 ± 0.36	165.6 ± 79.9	0.46 ± 0.24	0.81 ± 0.08	10.65 ± 2.38
		[0.92 - 3.07	[97 - 567]	[0.18 - 0.91]	[0.61 - 0.90]	[7.21 - 15.58]
	5	3.08 ± 0.77	157.3 ± 72.79	0.98 ± 0.43	0.81 ± 0.07	11.24 ± 2.24
		[1.02 - 5.46]	97 - 567	[0.12 - 1.90]	[0.64 - 0.93]	[8.22 - 15.3]
Q. pyrenaica	1	1.79 ± 0.48	177.8 ± 75.3	0.46 ± 0.26	0.81 ± 0.07	13.81 ± 3.27
		[0.88 - 3.27]	[112 - 567]	[0.13 - 1.02]	0.67 - 0.89	[9.62 - 21.71]
	2	1.59 ± 0.29	160.1 ± 47.62	0.49 ± 0.32	0.80 ± 0.05	13.6 ± 2.82
		[0.79 - 2.88]	[112 - 567]	[0.16 - 1.40]	[0.69 - 0.87]	[10.17 - 18.76]
	3	2.05 ± 0.65	182 ± 87.01	0.45 ± 0.14	0.84 ± 0.03	14.87 ± 2.48
		[0.29 - 3.78]	[97 - 567]	[0.28 - 0.81]	[0.79 - 0.91]	9.91 - 18.96
	4	3.35 ± 0.73	161.4 ± 67.6	0.59 ± 0.23	0.84 ± 0.03	12.5 ± 2.43
		[1.97 - 5.36]	97 - 567	[0.31 - 1.09]	[0.79 - 0.90]	[9.83 - 17.73]
	5	4.08 ± 0.80	153.4 ± 57.5	0.74 ± 0.47	0.76 ± 0.09	13.6 ± 2.64
		[2.08 - 6.19]	[83 - 567]	[0.36 - 1.93]	[0.60 - 0.89]	[10.44 - 17.56]

CAPÍTULO 5

Reforestation with *Quercus ilex* L. and *Q. suber L*. by direct seeding and planting in southern Spain

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ABSTRACT

The limited ability of *Quercus* species to regenerate naturally in Mediterranean forests has led to the development of various reforestation techniques; however, there is no general consensus as to what specific technique is the best for this purpose. In this work, we assessed growth and survival in two *Quercus* species (*Q. ilex* ssp *ballota* and *Q. suber*) used for reforestation in two different afforestation techniques (*viz.* direct seeding and planting) and two seedling quality (1-year-old seedlings and 3-year-old seedlings) in southern Spain. One-year-old seedlings of both species were found to exhibit the highest survival rates and direct-seeded plants intermediate survival values. Also, seed mass was found to have a significantly positive effect on establishment success in both species. No clear-cut trend in survival, however, was detected in 3-year-old seedlings. Survival in 3-year-old *Q. suber* seedlings and direct-seeded plants was similar, but not in *Q. ilex*, where 3-year-old seedlings survived less. The latter result may have been a consequence of cultivation in smaller containers leading to root deformation and limiting plant access to water. Differences in survival could not be ascribed to growth variables or stomatal conductance. Based on the results, all three afforestation methods can be similarly effective provided appropriate nursery cultivation conditions are used and seeds are protected against predators, the best choice in each case being dictated by the particular restoration goals.

Keywords: direct seeding, planting, stomatal conductance, survival

RESUMEN

La limitada capacidad de regeneración natural de las especies de *Quercus* ha llevado al desarrollo de varias técnicas de reforestación, sin embargo no hay un consenso general sobre cuál es la mejor técnica. En este trabajo se estudia el crecimiento y supervivencia de dos especies de *Quercus* (*Q. ilex* ssp *ballota* and *Q. suber*) con dos técnicas de repoblación (siembra directa y plantación) y dos edades de plántula (uno y tres años). En ambas especies, las plantas de un año tuvieron la supervivencia más alta, presentando las plantas procedentes de siembra directa un valor intermedio. En *Q. suber* la supervivencia fue similar para plantas de tres años y plantas de semilla, mientras que en *Q. ilex* las plántulas de tres años sobrevivieron menos. Este último resultado puede ser una consecuencia del cultivo en contenedores pequeños que lleva a deformaciones en la raíz y limita a la planta el acceso al agua. Las variables de crecimiento y la conductancia estomática no explicaron las diferencias en supervivencia. Basándose en los resultados, todos los métodos de repoblación podrían ser igual de efectivos, siempre que se den los adecuados cuidados culturales y las semillas empleadas se protejan frente a la depredación. La mejor elección en cada caso dependerá del objetivo particular de la restauración.

Palabras clave: conductancia estomática, plantación, siembra directa, supervivencia

INTRODUCTION

Holm oak [*Quercus ilex* L*. ssp ballota* (Desf.)] and cork oak (*Q. suber* L.) are two evergreen woody species widely represented in wild and managed forests of the Iberian Peninsula. Also, they constitute two essential elements of the agro-sylvopastoral system known as “dehesa”. Ensuring sustainable use of natural resources in savanna-like ecosystems (dehesas) is of a high economic importance for rural areas.

Establishment success in *Quercus* species is strongly limited and their regeneration quite difficult. The low natural regeneration ability of oaks has been ascribed to a number of factors including acorn predation, unsuitable germination conditions, soil impoverishment and the presence of herbivores (Watt, 1919; Leiva and Fernandez Ales, 2005; Pulido and Díaz, 2005; Pérez-Ramos and Marañón, 2008;). Oak tree mortality has increased considerably over the past 20 years by effect of the combined action of pathogens, xylophagous insects and adverse climatic conditions (Brasier, 1996; Sanchez et al., 2002). Poor regeneration and a high adult mortality in many areas have led to the implementation of reforestation programmes, mainly in areas where the tree population has declined or disappeared, or the land has been converted to agriculture, and transformation into forested areas is desirable (Costa, 2006).

Afforestation with *Quercus* trees is limited by their difficult natural regeneration, and low growth and survival rates (Villar et al., 2004; Quero et al., 2008b), especially when the first summer drought they are confronted with is very strong (Navarro-Cerrillo et al., 2005). In addition, *Quercus* seeds are recalcitrant, so they can only be stored for a few months (Roberts, 1973; Finch-Savage, 1992).

No consensus exists as to which is the best available reforestation method for *Quercus*. Oaks have heavy seeds, so they initially have large amounts of available resources (Quero et al., 2007; González-Rodríguez et al., 2008b) which can be used at an early growth stage until they can rely on photosynthesis. As a result, directly sown *Quercus* seeds have high emergence likelihood (Stanturf et al., 1998).

The greatest advantage of seeding is probably its low cost relative to planting; in fact, the latter requires nursery care and planting has higher associated costs than seeding (Bullard et al., 1992; Stanturf et al., 1998; Engel and Parrotta, 2001). Moreover, direct-seeded plants can develop natural root systems on site and longer roots can reach deeper soil layers and access water more readily during the first summer drought (Maestre et al., 2003). Lloret et al. (1999) found species with high root proportions to exhibited increased survival. Oak seedlings obtained by direct seeding must rely heavily on available acorn reserves. A relationship between seedling mass and seed mass in *Quercus* has been established (Chacón and Bustamante, 2001; Quero et al., 2007; González-Rodríguez et al., 2008b) based on which seeding large acorns can be expected to provide more vigorous seedlings with an increased survival likelihood.

However, direct-seeded plants remain in a more vulnerable condition over longer periods and are thus at an increased risk of failure (Stanturf et al., 1998). In fact, plants from direct-seeded plants of some species have been found to perform rather poorly (Engel and Parrotta, 2001). Also, long-term studies have shown direct-seeded oaks to grow to a lesser extent (Allen, 1990; Zaczek et al., 1996; Twedt and Wilson, 2002). One other disadvantage of acorns is vulnerability to predation by wild animals, especially in small openings or forested areas over the first few weeks after sowing (Madsen and Löf, 2005; Birkedal and Löf, 2007; Pérez-Ramos and Marañón, 2008). Therefore, direct seeding can only be a viable choice if the recommended procedures are strictly adhered to, particularly as regards acorn selection, storage and handling; species selection; size of opening and sowing depth; and site preparation before seeding (Allen, 1990; Stanturf et al., 1998; Dey et al., 2008; Löf and Birkedal, 2009).

Nursery production of oak seedlings is believed to be a more reliable regeneration method than direct seeding (King and Keeland, 1999). Thus, it considerably reduces initial predation, and results in increased growth and more efficient competition with grasses (Stanturf and Kennedy, 1996). This method is especially recommended when the seed supply is limited (King and Keeland, 1999). Spanish legislation has set various quality conditions for nursery seedlings including a maximum seedling age of 1–2 years for *Q. ilex* and 1 year for *Q. suber*. The growing use of plants to increase density and enrich existing dehesas has led nurseries to grow older *Q. ilex* and *Q. suber* seedlings (3–4 years) in larger containers (1000–3000 cm^3). This has allowed the initial growth period in the field to be shortened and more visible results to be obtained within a shorter time after planting. However, planting taller seedlings has increased associated costs (Stanturf et al., 2004). Also, older plants may exhibit abnormal root growth by effect of cultivation in small containers (Pemán et al., 2006) and develop inadequately after planting as a result (Dey et al., 2008), thereby being at an increased risk of dying during the first summer drought. However, Zaczek et al. (1996) found their oldest, tallest plating stock to exhibit the highest survival after 6 years. Moreover, although larger seedlings are more expensive to plant than are smaller seedlings, the former can be planted at lower densities in order to reduce costs (Dey et al., 2008).

Seedling quality and growing conditions can also be highly influential on plant establishment success (Villar-Salvador et al., 2004a; Zida et al., 2008). Nursery growth of container **s**eedlings is affected by the type of substrate, container size, and watering and fertilization regimes used (Aphalo and Rikala, 2003; Zida et al., 2008). Thus, seedlings grown in deep containers have root systems similar to those of naturally grown plants, and exhibit an increased root development capacity and hydraulic conductance than those growing in not so deeper containers (Pemán et al., 2006; Chirino et al., 2008). Plant morphology dictates growth and survival potential in the field (Navarro-Cerrillo et al., 2007). Various attributes including aerial biomass, height and the root/shoot biomass ratio have frequently been used to predict seedling performance in the field (Davis and Jacobs, 2005; Jacobs et al., 2005); there is, however, no general agreement as to what is the most suitable plant morphology for the Mediterranean environment. Some authors have found no relationship between plant survival and seedling morphology; also, plant early response is believed to be more markedly dependent on the particular local conditions (Dey et al., 2008; Zida et al., 2008; Palacios et al., 2009).

One of the main causes of failure in reforestation actions is summer drought. Water stress affects planting stock and seedling establishment (Grossnickle, 1988), and can reflect in changes in stomatal conductance (Stewart and Bernier, 1995;

Lamhamedi et al., 1998). In fact, stomatal conductance reflects the water use strategy of plants; thus, stomatal closure avoids excessive transpiration and xylem cavitation (Cochard et al., 1996), but can compromise photosynthesis and reduce the growth potential of plants as a result (Larcher, 1995). Because seedlings must develop long roots rapidly enough to compete with the herbaceous layer and access deeper water soil profiles during the dry season, adopting a spender strategy in order to facilitate growth may be more successful at an early development stage (Bragg et al., 1993).

In summary, the choice of a particular afforestation method is dictated by a number of factors including site conditions, seedling quality and water status, the particular species to be regenerated and the specific objectives of the restoration project (Dey et al., 2008). Identifying the best possible choice in each case usually entails assessing various methods under different conditions, and examining the morphological and physiological processes that underlie seedling outplanting performance. No previous simultaneous assessment of direct seeding and planting of 1- and 3-year-old seedling with *Q. ilex* and *Q. suber* oaks has to the authors' knowledge been done. The primary purposes of this work were thus as follows: (*a*) to compare direct seeding with planting of 1- and 3-year-old seedlings of the two most common oak species in the Iberian Peninsula; (*b*) to confirm whether morphological or physiological attributes can be used to explain seedling performance in the field; (*c*) and to identify the most suitable afforestation method for the two species.

MATERIAL AND METHODS

Site description

The study was carried out in an experimental plot at the University of Córdoba (Campus de Rabanales, Córdoba, Spain, 37°51'N, 4°48'W, 100 m a.s.l.) that was formerly used as agricultural land. The site has a small slope (< 2%) and the soil is a fluvisol consisting of heterometric silical gravels containing occasional calcareous boulders (Table 1).

Table 1. Selected physico-chemical properties of the soil in the study area (N = 15)

Property	**mean ± sd**
Clay (%)	10.43 ± 1.51
Sand (%)	53.37 ± 4.55
Silt (%)	36.20 ± 5.22
Penetration resistance (MPa)	1.64 ± 0,3
Depth of maximum penetration resistance (cm)	34.18 ± 10.57
Maximum measurement depth (cm)	49.40 ± 2.20
Soil organic matter (%)	1.66 ± 0.30
pH	6.79 ± 0.31
Organic N (%)	0.09 ± 0.01
Available P (ppm)	26.31 ± 7.87
Available K (ppm)	344.33 ± 109.80
Ca (meq/100g)	5.00 ± 0.69
Mg (meq/100g)	1.80 ± 0.53
Na (meq/100g)	0.41 ± 0.03
K (meq/100g)	0.79 ± 0.26
Cation-exchange capacity (meq/100 g)	9.88 ± 0.73

The climate is dry Mediterranean, with a mean annual temperature of 17.6 ºC, an average rainfall of 609 mm and dry summers. The average temperature and atmospheric humidity for the period from April to October 2008 were recorded with a data logger (HOBO Pro Series 8 Temp, RH) located in the area. The average temperature during the first eight months (February–September 2008) was 23.9 ± 5.1 ºC and accumulated precipitation 604 mm (Fig.1).

Species used (*Q. ilex* and *Q. suber*) are described in general methods (pp 26-29)

Seed collection and seedling assessment

Acorn collection was carried out in autumn 2007. Collection and seed dry mass estimations are described in general methods section (p. 29). Equation for *Q. ilex* was $S_{DM} = -0.2878 + 0.5412 \times A_{FM}$ ($r^2 = 0.95$, $P < 0.001$) and for *Q. suber* $S_{DM} = -0.1167 + 0.6019 \times A_{FM}$ ($r^2 = 0.98$, $P < 0.001$).

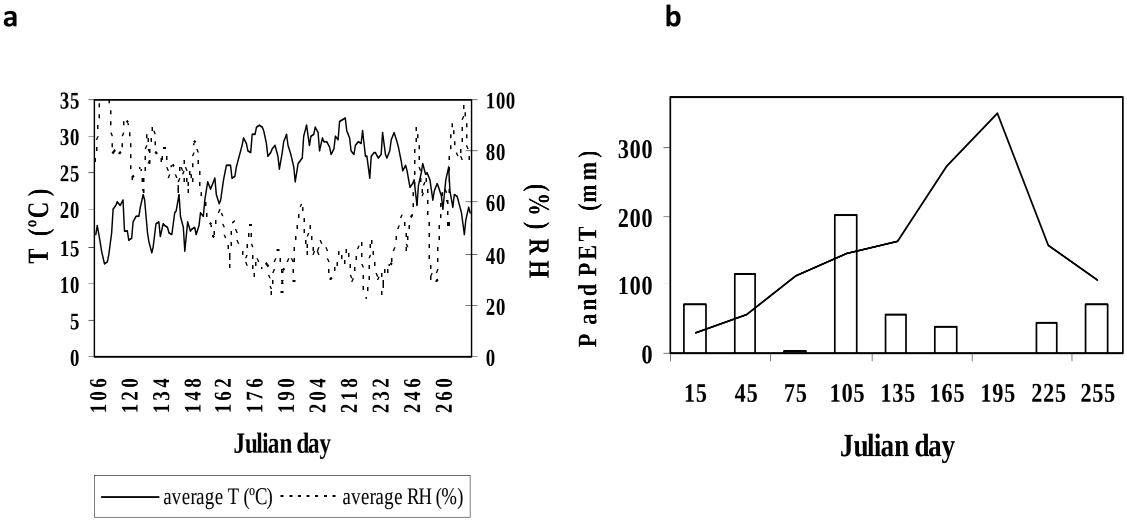

Figure 1. (a) Mean temperature and relative humidity, (b) precipitation (P, white bars) and potential evapotranspiration (PET, lines) during the experiment.

In January 2008, several sets of *Q. ilex* and *Q. suber* seedlings cultivated in a forest nursery (San Jerónimo, Consejería de Medio Ambiente, Junta de Andalucía, Seville, Spain) were selected. One-year-old seedlings of *Q. ilex* (height = 13.8 ± 2.2 cm; stem basal diameter = 0.35 ± 0.07 cm) and *Q. suber* (height = 34.7 ± 9.7 cm; stem basal diameter = 0.36 ± 0.07 cm) were grown in 400 cm^3 Forestpot® containers. Three-year-old seedlings of *Q. ilex* were grown in Forestpot® 3000 cm^3 containers (height = 61 ± 12.4 cm, stem basal diameter = 1.31 ± 0.29 cm) and *Q. suber* seedlings of the same age cultivated in 7200 cm^3 individual pots (no measurements available). A randomly selected sample of 15–20 seedlings of each species and age was harvested (first harvest planting) before planting for measurement of root, stem and leaf dry biomass after oven-drying at 70°C for at least 2 days. All leaves were scanned (HP Scan-jet 6300c) for calculation of total leaf area with the image analysis software Image Pro-plus 4 (Media Cybernetics, Inc.). Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass. Biomass allocation to leaves (LMF, leaf mass fraction), stems (SMF, stem mass fraction) and roots (RMF, root mass fraction) was calculated as the ratio of organ biomass to total biomass. Roots were manually classed as fine (less than 1 mm thick) or coarse (more than 1 mm thick).

Experimental design

The studied plot was tilled to a depth of 40 cm with an agricultural harrow and the soil removed with a single ripper. The experiment was arranged as a multi-factorial design involving two levels of species (*Quercus ilex* and *Quercus suber*) and three levels of age (seeds, 1-year-old seedlings and 3-year-old seedlings) in a completely randomized three-block design. Seeds were sown and seedlings planted in 2 m × 2 m spacing in each block. For direct seeding, 150 acorns of each species were distributed in each block. Three seeds per point were sown, at a depth of 5 cm, to ensure emergence. Seeds were placed 10 cm apart. The fresh mass of each acorn was recorded before seeding. In addition, 50 one-year-old seedlings and 50 three-year-old seedlings of each species were planted in each block. Therefore, a total of 750 samples from each species were used. Both direct seeding and planting were performed in late January 2008. Weed control during the experiment was done by manual removal and motocultor during the spring.

Survival assessment

Acorn emergence and seedling survival were assessed at 15-day intervals from February to September 2008, and once more in September 2009. Two survival rates were calculated as percentages, both for plants surviving after the first summer (September 2008) and for those still alive after the second (September 2009). Two performance-related variables (survival and success) were determined for directly seeded samples. Survival was calculated as the proportion of plants remaining alive after summer relative to emerged plants, and success as the number of plants surviving the summer divided by the total number of acorns sown. Therefore, the percent success was a combination of emergence and survival.

Morphological measurements

In April 2008, a sub-sample of 15 seedlings from directly sown seeds of each species was randomly selected from the three blocks for removal of their aerial fraction (first harvest seeding) and measurement of the dry mass of each part, leaf area and SLA as described above.

At the end of September of 2008, a sub-sample of 15 seedlings from each factorial treatment was randomly selected from the three blocks. Whole seedlings were removed from the soil, and their roots washed and cleaned (second harvest). The mean root depth was 28.4 ± 4.9cm. Total biomass was split into leaves, stems and roots, and morphological variables were calculated by following the above-described procedure. Absolute shoot and root mean increments were calculated as the mean differences between shoot or root dry mass of each afforestation method at the second and first harvest. Shoot and root mean increments for seedlings from directly sown seeds were taken to be the shoot or root dry mass values obtained at the second harvest as no shoots or roots existed at seeding time. The relative growth rate (RGR) for each method was calculated from the following expression (Hunt, 1990; Hoffman and Poorter, 2002):

$$\mathrm{RGR} = \ln(M_2) - \ln(M_1)/(t_2 - t_1)$$

were ln (M_2) and ln (M_1) denote the mean ln-transformed plant dry mass at time t_1 and t_2, respectively. M_2 and t_2 corresponded to the second harvest for all afforestation methods, whereas M_1 and t_1 differed between methods. For 1- and 3-year-old cultivated seedlings, M_1 and t_1 corresponded to the first harvest before planting; for seedlings from directly sown seeds, M_1 and t_1 corresponded to the first seeding harvest (after emergence of seedlings). The standard deviation for RGR was calculated according to Cornelissen et al. (1996).

Physiological measurements

Two types of measurements were done: stomatal conductance and leaf water content. A porometer (delta T porometer AP4) was used to measure leaf stomatal conductance. Measurements were made every 2 weeks at midday (10–12 am solar time) on four randomly selected replicates per species during the dry season (June to August 2008). Measurements were made on completely expanded young leaves receiving full sunlight.

From May to October 2008, 10 seedlings per afforestation method were selected monthly for measurement of leaf water content. A young leaf directly hit by sunlight was collected from each replicate. Leaves were rapidly transferred to individual plastic pots that were previously weighed and covered with parafilm. Samples were brought back to the laboratory for weighing (within one hour of collection) and then oven-dried at 70°C for at least 48 h to measure dry mass. Percentage water content was calculated as $WC = (FW - DW) \times 100/FW$, where FW and DW are fresh and dry weigh, respectively.

Data analyses

Differences in seedling survival for each species between afforestation methods were assessed by using log-rank survival curves constructed by following the Kaplan–Meier procedure (Kaplan and Meier, 1958). This analysis considers both seedling longevity and status (dead or alive) at the last survival assessment. Differences in morphological and physiological attributes were analysed by two-way ANOVA with species and type of afforestation method as factors, and a post-hoc Tukey test. When necessary, data were converted into logarithmic form in order to fulfil normality and variance homogeneity requirements. A non-parametric Kruskal–Wallis test was applied in those cases where the transformed data failed to fulfil the ANOVA assumptions. The potential effect of blocks was excluded from the statistical analysis since they introduced no changes in survival trends. All statistical analyses were done with the software STATISTICA 8.0. (Statsoft, Inc.).

RESULTS

Survival

Eight months after planting, 1-year-old *Q. suber* seedlings exhibited the highest survival rates (*ca.* 68 %), and 3-year-old *Q. ilex* seedlings the lowest (*ca.* 25 %)(Table 2). Twenty months after planting, all seedlings exhibited lower survival than after eight months and a high correlation between both survival rates ($r^2 = 0.90$, $P = 0.003$). Therefore, survival trends were similar in both periods. At species level, log-rank tests revealed significantly lower survival of 3-year-old *Q. ilex* seedlings relative to the other afforestation methods (Fig. 2). Also, 1-year-old *Q. suber* seedlings exhibited significantly higher survival rates than 3-year-old seedlings of the same species and directly seeded plants (Fig. 2).

Table 2. Percent **s**urvival and success 8 and 20 months after planting (after the first and second dry season, respectively). Mean and standard deviation for the three blocks.

		SURVIVAL (%)		**SUCCESS (%)**	
		First year	**Second year**	**First year**	**Second year**
Q. ilex	**ds**	51.5 ± 14.7	34.96 ± 9.2	17.2 ± 9.9	11.7 ± 7.2
	1ys	61.1 ± 19.8	45.28 ± 19.3		
	3ys	24.9 ± 18.7	17.74 ± 10.3		
Q. suber	**ds**	47.9 ± 9.4	33.6 ± 2.4	31.8 ± 13.9	22.3 ± 7.4
	1ys	67.7 ± 14.6	60.5 ± 11.1		
	3ys	45.1 ± 13.6	37.6 ± 25.1		

ds direct-seeded plants; 1ys 1-year-old seedlings; 3ys 3-year-old seedlings

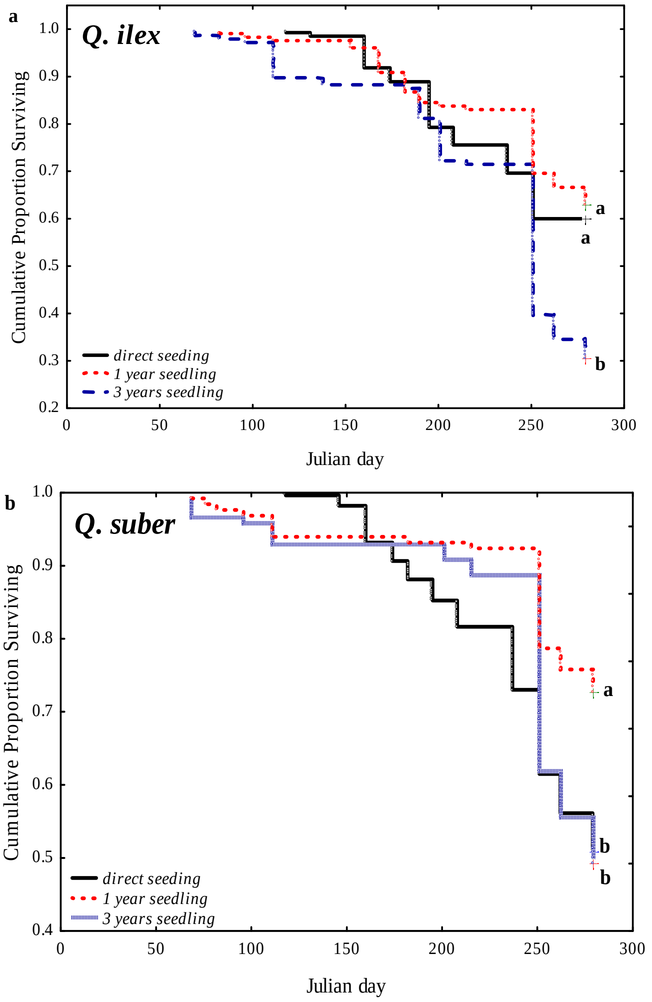

Figure 2. Percent survival of *Q. ilex* (*a*) and *Q. suber* (*b*) seedlings during the first dry season. Different letters represent statistically different groups according to Kaplan–Meier survival analysis (log-rank tests) ($P < 0.05$).

Seedling success (a combination of emergence and survival) in directly seeded plants was about 17 % for *Q. ilex* and 32 % for *Q. suber* the first year (Table 2). Second year seedling success decreased 31.9 % in *Q. ilex* and 29.8 % in *Q. suber* (Table 2). Success was significantly affected by seed mass (F = 14.3, P < 0.001). In both species, successful seedlings had larger acorns than the non successful, the effect being significant for *Q. ilex* (Fig. 3). Because seedling success was a combination of emergence and survival, seed mass may have influenced either variable. Interestingly, the favourable effect of seed mass was felt on survival in *Q. ilex* (F = 9.24, P = 0.002) but on emergence in *Q. suber* (F = 10.66, P = 0.001)

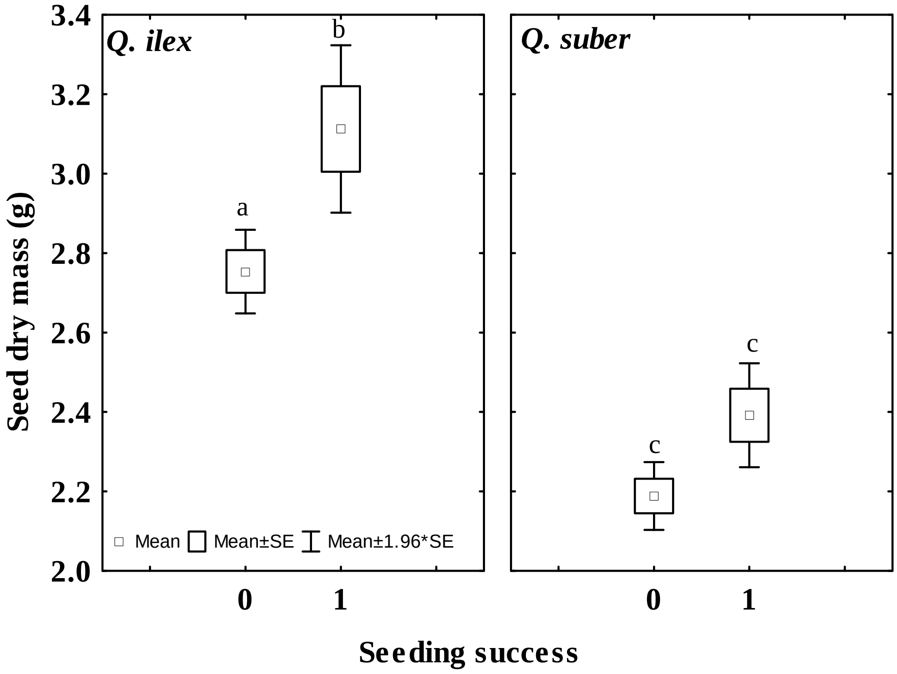

Figure 3. Differences in initial seed dry mass between emerged and alive seedlings (1) and non-emerged or dead seedlings after 8 months. Different letters represent statistically different groups according to the post-hoc Tukey test (P < 0.05).

Seedling growth and morphology

Morphological attributes differed between species and with seedling age, both on the planting date (Supplementary information, Appendix S1) and 8 months later (Table 3). The change in root biomass generally was lower in the older seeldings, and so did relative growth rate (RGR), specific leaf area (SLA) and leaf mass fraction (LMF) for both species (Table 3). The root/shoot ratio of the older seedlings was higher, except for the 3-year-old *Q. suber* seedlings, which exhibited the lowest ratios. The proportion of fine roots at the first harvest (*i.e.* planting time) differed significantly between the two species; thus, the proportion for 1-year-old *Q. suber* seedlings (16.7 ± 4.3%) was significantly higher (F = 11.15, $P < 0.001$) than those for 3-year-old *Q. ilex* seedlings (10.7 ± 4.7 %) and 1-year-old *Q. ilex* seedlings (10.2 ± 2.1) (see Supplementary information in Appendix S1).

Table 3. Summary of the biomass allocation and growth variables in *Q. ilex* and *Q. suber* at the end of the first dry season. Root, stem and leaf biomass, specific leaf area (SLA), leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), root/shoot ratio and proportion of fine roots as obtained from plants at second harvest (8 months after planting). See text for details about RGR (relative growth rate) calculation. Different letters in a row represent statistically different groups according to the post-hoc Tukey test ($P < 0.05$).

Variable	***Q. ilex***			***Q. suber***		
	ds	**1ys**	**3ys**	**ds**	**1ys**	**3ys**
Root biomass (g)	0.72 ± 0.45	3.97 ± 1.50	39.42 ± 10.20	0.83 ± 0.43	6.73 ± 3.86	47.13 ± 11.39
Stem biomass (g)	0.61 ± 0.28	2.30 ± 1.05	25.15 ± 15.82	0.51 ± 0.32	4.03 ± 2.17	60.48 ± 14.88
Leaf biomass (g)	0.60 ± 0.33	2.07 ± 0.59	9.26 ± 6.58	0.40 ± 0.21	1.79 ± 0.95	16.15 ± 5.40
Total biomass (g)	1.80 ± 0.87	8.15 ± 2.46	76.04 ± 31.38	1.82 ± 0.72	12.64 ± 5.51	124.26 ± 25.16
Δ root biomass (g)	0.72 ± 0.45	-0.43 ± 1.49	-1.64 ± 10.20	0.83 ± 0.43	0.53 ± 3.86	na
Δ shoot biomass (g)	1.21 ± 0.57	1.84 ± 1.44	8.56 ± 19.46	0.91 ± 0.46	1.64 ± 2.52	na
RGR (mg g^{-1} day^{-1})	4.1 ± 1.35	2.2 ± 0.88	1.2 ± 6.02	4.9 ± .2.45	1.3 ± 3.52	na
SLA (m^2 kg^{-1})	9.70 ± 5.22 **a,b**	7.53 ± 3.92 **a,c**	4.53 ± 0.30 **c**	12.73 ± 6.97 **d**	12.82 ± 7.70 **d**	10.79 ± 6.18 **b,d**
LMF (kg kg^{-1})	0.30 ± 0.10 **a**	0.25 ± 0.04 **a**	0.11 ± 0.05 **b**	0.21 ± 0.05 **a,c**	0.15 ± 0.04 **b,c**	0.13 ± 0.04 **a,b**
SMF (kg kg^{-1})	0.33 ± 0.07 **a**	0.26 ± 0.05 **a**	0.34 ± 0.10 **a**	0.32 ± 0.15 **a**	0.32 ± 0.20 **a**	0.49 ± 0.06 **a**
RMF (kg kg^{-1})	0.38 ± 0.12 **a**	0.48 ± 0.08 **a**	0.55 ± 0.11 **a**	0.47 ± 0.16 **a**	0.54 ± 0.18 **a**	0.38 ± 0.06 **a**
Fine roots (%)	9.02 ± 12.47 **a**	9.18 ± 6.07 **a**	15.44 ± 5.24 **a**	2.77 ± 4.42 **a**	17.12 ± 15.78 **a**	4.96 ± 3.41 **a**
Root/shoot ratio	0.66 ± 0.32 **a**	0.96 ± 0.28 **a,b**	1.38 ± 0.70 **b**	0.90 ± 0.50 **a,b**	1.40 ± 0.71 **b**	0.63 ± 0.18 **a**

ds direct-seeded plants; 1ys 1-year-old seedlings; 3ys 3-year-old seedlings

Ecophysiological measurements

Figure 4 shows the stomatal conductance evolution for the dry period. As can be seen, the 3-year-old seedlings of both species had lower mean conductance values than both their 1-year-old seedlings and the plants from directly sown seeds throughout the dry season (Fig. 4, Table 4). The mean maximum conductance at the start of the dry period ranged from 90 ± 67 mmol m^{-2} s^{-1} for 3-year-old *Q. ilex* seedlings to 226 ± 95 mmol m^{-2} s^{-1} for direct-seeded *Q. suber* plants (Fig. 4). Conductance decreased considerably throughout the dry season in all plant types. At the end of the dry period, 3-year-old *Q. suber* seedlings exhibited the lowest conductance values (6 ± 0.5 mmol m^{-2} s^{-1}) and 1-year-old *Q. suber* seedlings the highest (90 ± 29 mmol m^{-2} s^{-1}), the differences being significant —the conductance of 3-year-old *Q. suber* seedlings was significantly lower than those for the other treatments (*F* = 10.97, *P* = 0.003). There were also substantial differences between individual specimens of the same species (Fig. 4).

Leaf water content rate varied little with time (results not shown). The mean water content was highest for direct-seeded *Q. suber* plants (56.6 ± 9.5) and lowest for 3-year-old *Q. ilex* seedlings (47.6 ± 5.7); the differences, however, were not statistically significant.

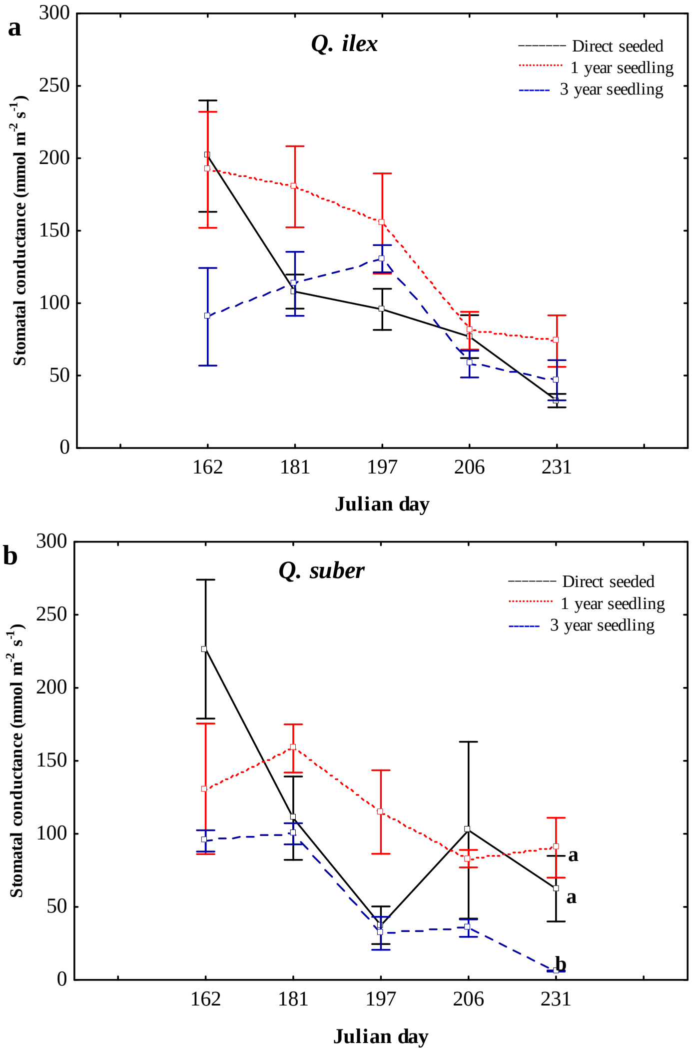

Figure 4. Stomatal conductance of *Q. ilex* (*a*) and *Q. suber* (*b*) at midday during the dry season. Bars represent standard errors and different letters statistically different groups according to the post-hoc Tukey test (*P* < 0.05) on a given date.

DISCUSSION

In this study, we simultaneously evaluated direct seeding and planting of 1- and 3-year-old seedlings of the two most common oak species in the Mediterranean region (*Q. ilex* and *Q. suber*). We found 1-year-old seedlings of both species to perform much better than the other afforestation methods. In fact, this age has been recommended as optimal for plantation in Mediterranean conditions (Villar-Salvador, 2003). Seedlings obtained by direct sowing had medium survival rates (about 50% the first year and 33% the second). Mendoza et al. (2009) found survival in *Q. ilex* and *Q. pyrenaica* in a Mediterranean environment to range from 30 to 50% depending on the particular habitat, and to be low in open sites. Gonzalez-Rodríguez et al. (2008b) also found poor survival of *Q. ilex* and *Q. suber* in open sites. Our study area was open and contained no trees or shrub cover, so our results should be comparable to theirs. On the other hand, two years after seeding Navarro et al. (2006) found high survival rates (80%) in directly sown seedlings and proposed the seeding method as a viable choice for forest restoration. This method, however, can fail by effect of acorn predation. Although no apparent signs of predation were detected in our study, this can be a substantial risk in areas with high density of wild and domestic animals (Pérez-Ramos and Marañón, 2008). Therefore, plant survival can be affected by the specific habitat and meteorological conditions of the site.

With direct seeding, plants from large acorns were more successful than those from small acorns as a result of the combined effect of emergence and survival. A number of studies have revealed that large seeds exhibit increased germination and emergence rates (Gómez, 2004a; Urbieta et al., 2008a), and also increased survival likelihood (Lloret et al., 1999; Gómez, 2004a; Moles and Westoby, 2004), especially under adverse environmental conditions. Although large seeds can increase sowing success, they are also under a greater risk of predation by animals such as wild boars and wood mice (Gómez, 2004a). Therefore, the direct seeding method can provide an effective, low-cost supplementary tool for afforestation (Bullard et al., 1992) provided an appropriate microhabitat is chosen and predation efficiently controlled (Johnson et al., 2002).

No clear conclusion can be drawn as regards survival in 3-year-old seedlings. Thus, *Q. suber* exhibited medium rates (about 50% the first year) that were comparable to those for direct-seeded plants, and *Q. ilex* had very low values (25 %) that departed significantly from those for the other methods.

These performance differences can seemingly not be explained in terms of the morphological variables studied. Previous studies aimed at identifying morphological attributes allowing seedling field performance to be predicted found either no definite relationships (Zida et al., 2008; Palacios et al., 2009) or relationships that changed with the meteorological conditions of the planting year (Del Campo et al., 2010). Palacios et al. (2009) claim that the effect of seedling quality must be studied jointly with those of other environmental factors —particularly, those with a high potential impact on reforestation. Thus, appropriate soil preparation and planting date selection, with provision for the local climate, might be the most influential factors for successful reforestation. Nursery cultivation variables may also have a prominent effect on post-planting establishment (Menzies et al., 2001; South et al, 2001; Villar-Salvador et al., 2004a). Container type and size are two such variables. Larger containers allow the development of deeper tap roots capable of colonizing increased volumes and absorbing more water as a result, thereby facilitating the establishment of seedlings —the greatest limitation for which is known to be imposed by summer droughts (Pemán et al., 2006; Chirino et al., 2008). The containers used to grow 3-year-old *Q. ilex* seedlings were considerably smaller than those for 3-year-old *Q. suber* seedlings. Moreover, removal of seedling roots from the soil after 8 months of growth in the field revealed that the nursery seedlings had developed few new roots outside of the nursery container root volumen; by contrast, the roots of direct-seeded plants had grown more vertically than horizontally. This effect may have been critical for 3-year-old *Q. ilex* oaks, which produced less deep roots than the others and may partly account for their poor survival by effect of their strengthening container constraints on root growth.

Although seedlings with an increased root/shoot ratio can be expected to survive quite well in dry environments (Lloret et al., 1999), we found no lower root/shoots ratios in 3-year-old *Q. ilex* seedlings by effect of their previous cultivation in small nursery containers or their lower survival. Similarly, previous studies in Mediterranean found no relationship between root/shoot ratio and seedling performance (Villar-Salvador et al., 2004a; Del Campo et al., 2010) or container size (Chirino et al., 2008). Our results are suggestive of slightly greater root mass allocation in the larger plants, but this requires confirmation by careful study of the morphology and structure of the roots. Possibly, there was a markedly thickened main root, so the actual surface area available for water and nutrient uptake was probably small. One-year-old *Q. suber* seedlings contained an increased proportion of fine roots at planting time and must therefore have had an increased physiological capacity for water and nutrient uptake (Eissenstat, 1992). The depth of the root system is one other crucial factor in this respect (Pemán et al., 2006).

All specimen types exhibited similar trends in stomatal conductance with time. Physiological parameters related to water use may change with plant age and developmental stage (Cavender-Bares and Bazzaz, 2000); also, differences between species may not be apparent at the seedling stage (Mediavilla and Escudero, 2004). Thus, although *Q. ilex* is known to develop an extremely conservative strategy for water use, it exhibits low stomatal sensitivity and high conductance at seedling stage (Nardini et al., 1999; Mediavilla and Escudero, 2004). The decrease in conductance in the dry season was a response to prevent xylem cavitation through stomatal control (Vilagrosa et al., 2003). Although all plant types exhibited a similar strategy, there were some differences between trends. Thus, directly seeded and 1-year-old seedlings seemingly had a better water status —reflecting in increased stomatal conductance— during the dry period. Direct seeded and 1-year-old seedlings may have had a more balanced root/shoot ratio than the 3-yr-old seedlings, which could explain some of the observations. The 3-yr-old seedlings had root/shoot ratios reflective of their growth in the nursery, hence may have had too much shoot, especially leaf material, for the outplanting conditions. Moreover, three-year-old seedlings (especially those of *Q. suber*) exhibited higher stomatal sensitivity, a strategy which may reduce its growth rate. Also, the older seedlings had lower leaf water contents (*Q. ilex*) and SLA values, both traits being closely related to RGR (Cornelissen et al., 1996; Ruiz-Robleto and Villar, 2005). In fact, SLA and RGR decreased with age, which was to be expected since younger seedlings need to develop more leaves and have higher photosynthetic rates in order to reach the growth potential they require for successful establishment. Increased SLA values are suggestive of rapid production of biomass, as are low values of efficient conservation of nutrients (Poorter and Garnier, 1999) and water (Poorter et al., 2009). On the other hand, the more conservative strategy of the older seedlings could be successful under extremely dry or stressing conditions. Thus, under conditions of high evaporative demand and low water availability, large seedlings may undergo more marked reductions in growth and conductance than small seedlings (Stewart and Bernier, 1995; Lamhamedi et al., 1998), as we indeed found here. Moreover, older oak seedlings had lower LMF values at both harvests. Finally, leaf dieback has been noted as a response to high water stress by reducing passive water losses (Vilagrosa et al., 2003) and may have been the specific strategy adopted by our 3-year-old oak seedlings.

CONCLUSIONS

Two different afforestation techniques (*viz.* direct seeding and planting) and two seedling ages (1-year-old seedlings and 3-year-old seedlings) were found to result in differential plant survival rates. Each method, however, has some advantages. Thus, seeding costs less than planting 1-year-old seedlings and results in higher survival rates; by contrast, planting 3-year-old seedlings shortens the vegetative period in the field. Seeding remains an effective alternative to planting, especially when planting nursery seedlings is subject to some limitation or support for afforestation activities is required. Success rates for direct-seeded plants can be raised by using large acorns. Regarding plant size, although 3-year-old seedlings of *Q. ilex* exhibited decreased survival rates, this method has the potential advantage that it shortens the plant juvenile period. However, it requires using large containers, which increases costs.

Large seedlings grown in small containers undergo root deformation and are exposed to increased water stress, which diminish stomatal conductance and RGR rates.

Acknowledgements

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SUPPLEMENTARY INDEX

Appendix S1. Summary of the biomass allocation and other morphological parameters in *Q. ilex* and *Q. suber* from seedlings before plantation and from direct seeded plants three months after seeding. Different letters in columns represent statistically different groups according to post-hoc Tukey test (*P*<0.05).

		Root biomass (g)	**Stem biomass (g)**	**Leaf biomass (g)**	**Shoot biomass (g)**	**SLA (m^2kg^{-1})**	**LMF ($kg\ kg^{-1}$)**	**SMF ($kg\ kg^{-1}$)**	**RMF ($kg\ kg^{-1}$)**	**Root/shoot**	**Fine roots (%)**
Q. ilex	**direct-seeded**		0.12 ± 0.05	0.56 ± 0.28	0.68 ± 0.31	7.94 ± 1.33					
	1 year-seedling	4.40 ± 2,09	1.23 ± 0.61	1.80 ± 0.74	2.53 ± 0.99	5.91 ± 2.26 **a**	0.25 ± 0.04 **a**	0.15 ± 0.04 **a**	0.60 ± 0.07	1.97 ± 1.55 **a**	10.21 ± 2.15 **a**
	3 year-seedling	41.06 ± 9.56	11.90 ± 7.93	7.30 ± 4.05	25.84 ± 4.97	4.77 ± 0.39 **b**	0.14 ± 0.03 **b**	0.24 ± 0.05 **b**	0.61 ± 0.05	1.61 ± 0.34 **.b**	10.73 ± 4.67 **a**
Q. suber	**direct-seeded**		0.09 ± 0.03	0,36 ± 0.18	0.46 ± 0.20	11.14 ± 1.46					
	1 year-seedling	6.20 ± 2.56	1.97 ± 0.80	2.19 ± 0.70	4.17 ± 1.39	6.95 ± 0.71 **c**	0.22 ± 0.03 **a**	0.20 ± 0.07**a,b**	0.58 ± 0.09	1.50 ± 0.47 **b**	16.67 ± 4.27 **b**
	3 year-seedling	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

DISCUSIÓN GENERAL

El objetivo general de esta tesis fue la identificación de algunos factores, tanto ambientales como inherentes a la plántula, con influencia en distintas fases del establecimiento y del crecimiento de cuatro especies de *Quercus* (dos perennifolias y dos caducifolias). En general, se ha observado que la importancia de cada factor depende de la fase considerada, y que en ocasiones estos factores presentan interacciones significativas, de forma que el efecto de un factor depende del otro. Desde un punto de vista aplicado, los resultados de cada experimento permiten extraer recomendaciones prácticas de utilidad para la restauración de los bosques de estas especies de regeneración lenta y muy limitada.

A continuación se presenta una discusión de los aspectos más importantes de la tesis.

Factores "intrínsecos"

El primer objetivo de la tesis fue evaluar la importancia del tamaño de la semilla y del árbol madre - denominados "factores intrínsecos"- en las distintas fases del establecimiento. La Tabla 1 muestra un resumen de los efectos del peso de la semilla encontrados en cada experimento, y el anexo 1 los pesos medios de semilla empleados para cada especie y experimento.

En general, el **peso de la semilla** tuvo un efecto positivo sobre las distintas fases de establecimiento (emergencia, supervivencia, crecimiento), confirmando las conclusiones de estudios previos (Moles y Westoby, 2004; Gómez, 2004; Urbieta et al., 2008). Sin embargo, su importancia fue distinta según la fase considerada. Por ejemplo, el efecto del peso de la semilla fue muy importante en el porcentaje de emergencia, mientras que en la supervivencia fueron más determinantes otros factores. Así, en el capítulo 3 se vio como en general la emergencia no se distribuía de forma agregada en el espacio, dando la impresión de ser un proceso más dependiente de las características intrínsecas de la planta que de las condiciones del medio y su variabilidad espacial. En el capítulo 4 se confirmó esta hipótesis, ya que las variables explicativas de la emergencia fueron siempre las relacionadas con el peso de la semilla y con las características del progenitor. Otros autores (Paz et al., 1999) encontraron que la emergencia venía mediada por factores externos más que por el peso de la semilla. Sin embargo, en este estudio se empleaban especies de la familia de las rubiáceas, por lo que los resultados no son comparables a nuestro trabajo ya que las características de las semillas son diferentes.

Por otro lado, las semillas más grandes emergieron antes, seguramente a consecuencia de un menor tiempo de germinación (Gómez, 2004b). De todas formas, el tamaños de la semilla explicó un porcentaje bajo de variación en el tiempo de emergencia (r^2 bajos), similar a lo que ocurre en otras fases. En cambio, el tiempo de emergencia parece estar más determinado por la especie (ver más abajo).

Como era de esperar, el efecto positivo del peso de la bellota sobre el peso de la plántula se observó en casi todos los experimentos, confirmando resultados de otros trabajos (Hendrix et al., 1991; Chacón y Bustamante, 2001; Green y Juniper, 2004; Quero et al., 2007). En el capítulo 2 se vio como el peso de la plántula venía más determinado por el uso de las reservas, y no se verificó en ningún caso la hipótesis del "efecto de reserva" de Westoby et al. (1996) que postula que una semilla más grande consume una proporción menor de reservas, teniendo así un reservorio "extra" para poder hacer frente a futuras perturbaciones (herbivoría, sequía, etc.). En este caso, parece que la estrategia de las especies de *Quercus* sea la de movilizar rápidamente los recursos hacia la raíz, ya que las bellotas son recalcitrantes y pierden viabilidad en poco tiempo (Finch-Savage, 1992). La semilla también influyó en otros rasgos morfológicos como en el área foliar, pero su efecto no fue tan marcado sobre la inversión en biomasa de los distintos órganos (hojas: LMF, raíz: RMF y tallo: SMF).

En general, no se encuentran presiones negativas del peso de la semilla sobre la morfología y las distintas fases de establecimiento estudiadas. Algunos estudios proponen la existencia de presiones selectivas opuestas relativas al tamaño de la semilla. Así, por ejemplo, las semillas más grandes son más apetecibles para los depredadores, teniendo por tanto más probabilidades de ser consumidas (Gómez et al., 2004; Xiao et al., 2006), pero en cambio una semilla más grande genera plántulas mayores (Quero et al. 2007, Quero et al. 2008, Perez-Ramos et al. 2010). En el estudio de depredación de esta tesis (capítulo 1), si bien los ungulados consumieron preferentemente las especies con bellotas mayores, no se encontró selección de tamaños dentro de cada especie. Por lo tanto, en este caso las presiones encontradas sobre el peso de la semilla no fueron negativas sino neutras. Resultados similares han sido ya constatados en otros trabajos (Xiao et al., 2004; Pons y Pausas, 2007).

Tabla 1. Variables medidas en relación al peso de la semilla en los cinco capítulos. Se muestra el signo de la relación existente (+,-) y las especies en las que se encuentra relación significativa (*Qi*: *Q. ilex*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*). En el capítulo 5 sólo se emplearon las especies *Qi* y *Qs*. Las casillas con "-"indican que la variable no se evaluó. Los "*" indican que los resultaros no se evaluaron en el capítulo correspondiente pero se muestran en el Anexo 2. RMF: proporción de raíz; SMF: proporción de tallo; LMF: proporción de hojas; ns: no significativo

	CAPÍTULO				
	1	**2**	**3**	**4**	**5**
DEPREDACIÓN POST DISPERSIVA	ns[1]	-	-	-	-
TIEMPO EMERGENCIA	-	**(-)** *Qi* *	-	**(-)** *Qs, Qf*	No significativo *
EMERGENCIA	-	-	-	**(+)** *Qs, Qp (Qi y Qf según madre)*	**(+)** *Qs* *
SUPERVIVENCIA	-	-	-	**+** *Qs*	**(+)** *Qi* *
SUPERVIVENCIA AÑO 2	-	-	-	**(+)** *Qs (Qp según madre)*	
ESTABLECIMIENTO	-	-	-	**(+)** *Qs (Qp según madre)*	**(+)** *Qi*
PESO AÉREO PLÁNTULA	-	**(+)** Todas	**(+)** Todas	ns	**(+)** *Qi* *
PESO RAÍCES	-	**(+)** Todas *	-	-	ns *
RGR reservas semilla	-	**(-)** *Qi, Qf, Qp*	-	-	
RGR parte aérea	-	**(-)**	**(+)** Todas	-	
ÁREA FOLIAR	-	**(+)** *Qi, Qp*	**(+)** *Qi, Qs, Qp*	-	**(+)** *Qi* *
RMF	-	**(+)** *Qs*	-	-	ns *
SMF	-	ns	-	-	ns *
LMF	-	**(-)** *Qs, Qf*	-	-	ns *

[1] No se encontró efecto del peso de la semilla dentro de cada especie, pero los ungulados prefirieron las especies con tamaño medio de semilla mayor (*Qs* y *Qp*)

En resumen, y al menos en las condiciones del área de estudio, las semillas más grandes son en general más exitosas, ya que tienen más probabilidad de emergencia, las plántulas son más vigorosas y con mayor probabilidad de supervivencia, no existiendo presiones negativas sobre el tamaño de la semilla en relación a la depredación. No obstante, hay que considerar otros factores que modifican estos efectos como pueden ser las influencias maternas. El estudio de la **variabilidad intrapoblacional** abordado en los capítulos 2 y 4 supone uno de los aspectos científicos más novedosos de esta tesis. En el capítulo 4 se vio cómo no necesariamente las madres que producían semillas mayores fueron las más exitosas. El ejemplo más claro es el de la M_5 de *Qi* que produjo las semillas mayores, y que fueron, sin embargo, las que presentaron tasas más bajas de emergencia y supervivencia en campo (capítulo 4). Las semillas de esa misma madre (recogidas en el mismo año), tuvieron también tasas de emergencia algo más bajas en el experimento en condiciones controladas (Anexo 3). En los dos experimentos en los que se ha considerado el factor madre, éste aparece modificando los efectos de otros factores estudiados, ya sea el efecto del peso de la semilla (capítulos 2 y 4), como el de otros factores ambientales (capítulo 4). Esta alta variabilidad intraespecífica puede venir mediada por diferencias genéticas, o bien por las condiciones ambientales que rodean a la madre en el momento de producir las semillas (Wulff 1986; Schmitt et al., 1992; Castro et al., 2008). La existencia de plagas o el distinto estado fisiológico de las semillas puede ser la causa de los comportamientos diferenciales de las semillas procedentes de distintos árboles madre. Por ejemplo, las madres que produjeron bellotas con mayor contenido hídrico (M_5 de *Qi*, M_2 de *Qf*, M_3 de *Qp*) tuvieron las probabilidades más bajas de emergencia en el experimento del capítulo 4. Sería interesante en el futuro estudiar los mecanismos causantes de las diferencias en emergencia considerando la repetición de las colectas de bellotas y experimentos en diferentes años, junto con un análisis detallado de la producción en cantidad y calidad de la semilla de cada árbol.

Factores ambientales

En los objetivos 2 y 3 de la tesis se planteó el conocimiento de la influencia de los factores ambientales sobre el establecimiento y el crecimiento, teniendo en cuenta que dichos factores no se distribuyen aleatoriamente en el tiempo y espacio. El clima mediterráneo está caracterizado por una enorme variabilidad a escala espacial y temporal (Blondel and Aronson, 1995; Terradas, 2001; Maestre, 2006) (Para un ejemplo ver Métodos Fig. 2 pág.23). Esto genera una enorme complejidad en los procesos ecológicos, y hace difícil el establecimiento de patrones o "reglas" fijas. La repetición de ensayos en diferentes años y zonas es necesaria para poder extraer conclusiones y realizar predicciones más fiables.

En esta tesis la heterogeneidad espacial se ha considerado en los diferentes experimentos, ya sea mediante el establecimiento de bloques o unidades experimentales bien caracterizadas (capítulos 1, 4 y 5) o bien con diseños espacialmente explícitos que permitieran incorporar al estudio la estructura espacial de las variables consideradas (capítulo 3). De esta forma se ha podido constatar que, incluso en áreas pequeñas (2 Ha), existen micrositios más favorables para el reclutamiento que otros, debido a la distribución diferencial de los factores ambientales. Por ejemplo, en el capítulo 3 se vio que los dos cuadros de estudio presentaron tasas muy dispares de supervivencia tras el primer verano (siendo ésta prácticamente nula en uno de los cuadros). Mientras que la emergencia parece más determinada por las características de la semilla (peso y efecto materno), la supervivencia es más dependiente del medio, como así muestran los patrones de agregación de la supervivencia, que persistieron a lo largo de todo verano. La **sequía estival** es una de las mayores limitaciones al reclutamiento en zonas mediterráneas, especialmente en las primeras fases de vida de las plántulas (Herrera et al, 1994). La adición de un riego suplementario durante el verano aumentó significativamente la supervivencia (capítulo 4), reafirmando así los resultados de otros estudios similares (Castro et al., 2005; Gómez-Aparicio et al., 2008) (Fig.2). También en el capítulo 3 se encuentran indicios de la importancia del factor hídrico en el éxito de establecimiento de todas las especies, aunque estos efectos no son tan patentes debido seguramente a la distribución espacial de distintos factores ambientales con efectos opuestos sobre la supervivencia. Además, es necesario tener en cuenta la dificultad metodológica que supone la medida de humedad del suelo en campo, especialmente la de las capas más profundas que van a ser las más determinantes durante el verano, en el que se secan las capas superficiales. Debido a esto, las medidas de humedad realizadas en el verano no fueron muy diferentes de unos puntos de muestreo a otros, ya que se realizaron a poca profundidad (máximo 20 cm). Sería interesante desarrollar métodos de muestreo de humedad del suelo que fueran rápidos y con posibilidad de realizarse a distintas profundidades.

Con respecto a la competencia con las **herbáceas**, varios estudios han mostrado como éstas consumen muchos recursos hídricos durante su crecimiento, limitando así la supervivencia de las plántulas leñosas en sus primeras fases de desarrollo (Davis et al*.,* 1999, Rey Benayas et al., 2005). Este efecto no se encontró en nuestro estudio. Sin embargo, hay que tener en cuenta las condiciones meteorológicas del año, ya que durante el verano la precipitación fue prácticamente nula, y como ya se comentó en el capítulo 3, esto puede haber sido la causa de la desaparición de los patrones espaciales y de las asociaciones entre variables ambientales y establecimiento que se esperaba encontrar, pues todos los sitios estaban muy secos. En los años muy secos la supervivencia es muy baja y no se encuentran patrones de agregación espacial (Quero, 2007a) Por otro lado, la presencia de herbáceas disminuyó el crecimiento de *Q. faginea*, pudiendo ser ésta una consecuencia de la competencia, bien por luz, bien por agua (Davis *et al.,* 1999; Holmgren et al., 2000)

Otro de los factores considerados ha sido la **luz**, ya que este recurso es de gran importancia y puede determinar la distribución de las especies en los ecosistemas (Beckage y Clark, 2003). En esta tesis se ha comprobado como la luz influye en distintas fases del establecimiento, acelerando el tiempo de emergencia (capítulo 4) o modificando variables de tipo morfológico (capítulos 3 y 4), especialmente las relativas a las hojas, ya que éstas poseen una elevada plasticidad (Ramírez-Valiente et al., 2009). Asimismo, la respuesta de las plantas a la luz se vio modificada por la interacción con otros factores (madre y humedad del suelo, capítulo 4). La luz también determinó el crecimiento de una mayor cobertura de herbáceas (capítulo 3), que a su vez influyó en el establecimiento. Queda así de manifiesto la enorme heterogeneidad en las combinaciones entre los factores y procesos ecológicos, y la importancia de su evaluación conjunta, ya que la distribución de un factor puede condicionar la de otros, generándose así un amplio abanico de micrositios que influyen de forma diferencial en el establecimiento de las especies leñosas.

Como ya se ha visto en el apartado anterior, las variables relacionadas con el crecimiento y la morfología fueron en general más dependientes de los factores intrínsecos que de los ambientales (capítulos 3 y 4) (Fig.2). Pérez-Ramos et al. (2010) encontraron que durante el primer año de vida las plántulas eran más dependientes de la semilla, mientras que en el segundo año empezaban a entrar en juego otros factores (como la disponibilidad de luz y nutrientes). La dificultad de medir el crecimiento de plántulas en campo hace que los experimentos de este tipo sean escasos, especialmente en condiciones mediterráneas. La aproximación multi-específica al estudio de crecimiento de plántulas en campo realizada en esta tesis es por tanto de gran valía a la hora de confrontar los resultados con los de otros experimentos similares.

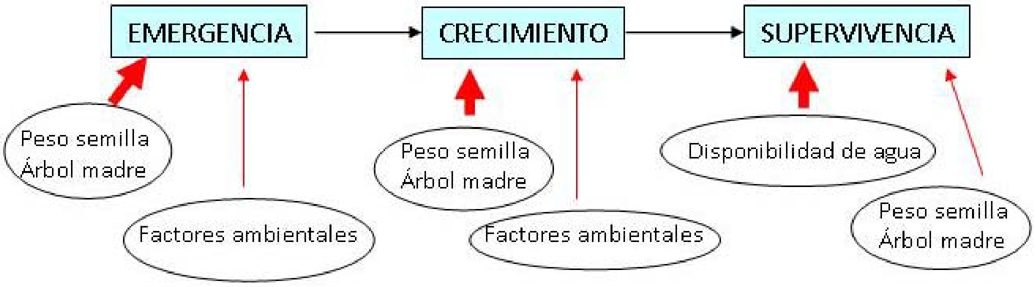


Figura 2. Síntesis de los factores que afectan las distintas fases del establecimiento estudiadas. Se muestran con flechas gruesas los factores de mayor importancia.

Diferencias entre especies

Respuesta a factores ambientales

Otro objetivo de la tesis fue el extender el estudio de los factores que afectan al establecimiento a varias especies del género *Quercus* muy abundantes en los bosques del sur de la Península Ibérica. El género *Quercus* presenta una alta variabilidad en su fisiología y ecología (Costa et al., 1997), y las especies estudiadas, aunque pueden coincidir en el espacio, presentan diferentes requerimientos. Así, las dos especies de carácter caducifolio o semi-cadudifolio (*Q.faginea y Q. pyrenaica*) presentan diferentes características morfológicas como un mayor SLA que implican una mayor susceptibilidad al déficit hídrico frente a las especies esclerófilas (Acheral y Rambal, 1992; Costa et al, 1997). Así se observó como en las zonas con el suelo más seco la emergencia de *Q. pyrenaica* se encontraba más limitada (capítulo 3). Además, ambas especies presentaron mucha más mortalidad tras el primer verano (capítulos 3 y 4) y el riego artificial favoreció especialmente su establecimiento (capítulo 4).

La importancia de los factores estudiados en el crecimiento y la morfología no fue muy diferente de unas especies a otras (capítulos 2, 3, 4, 5). Es posible que muchas de las diferencias interespecíficas desaparezcan en la fase de plántula (Mediavilla y Escudero, 2003) en la que las especies muestren estrategias comunes. Por otro lado, debido seguramente a la menor dureza de las hojas de las plantas caducifolias, especialmente las de *Q. pyrenaica*, éstas fueron más consumidas por los defoliadores durante la primavera (observaciones personales). Estas plántulas tendrían que hacer después un consumo “extra” de reservas de la bellota o de la raíz para poder rebrotar y regenerar la parte aérea. La estrategia del roble como rebrotadora ya ha sido documentada con anterioridad (Costa et al., 1997).

Por otro lado, los resultados del capítulo 1 muestran cómo en la zona de estudio los depredadores-dispersores (micromamíferos y aves) preferían, independientemente de su tamaño, las bellotas de *Q. ilex* y *Q. faginea*. Sin embargo, los ungulados mostraron preferencia por las semillas de las dos especies con mayor peso de semilla (*Q. suber* y *Q. pyrenaica*). Las diferencias interespecíficas en las probabilidades de reclutamiento se empiezan a manifestar, por tanto, ya en la fase de depredación post-dispersiva.

Tiempo de emergencia

Un resultado interesante y del que no hay muchos datos en la bibliografía es el referido a las diferencias interespecíficas en tiempo de emergencia. En el capítulo 4 se encontró que las especies perennifolias (*Q. ilex* y *Q. suber*) emergían más tarde, y este resultado fue consistente para *Q. ilex* en el capítulo 2 (Anexo 4A). Aunque la germinación no se evaluó directamente, en el capítulo 1 se puede ver como el porcentaje de bellotas germinadas sobre el total de las no desaparecidas siempre es menor para *Q. ilex* y *Q. suber* (Anexo 4B). El retraso en la emergencia (mediado por el retraso en la germinación) puede venir determinado por la dormancia del hipocotilo, que a su vez se relaciona con el contenido hídrico (Merouani et al., 2001). De este modo, las semillas con mayor contenido hídrico tardarían más en desecarse y por tanto en romper su dormancia. Al comparar el contenido hídrico de las bellotas empleadas como predictores del peso seco en los experimentos de los años 2007 y 2009 (capítulos 1, 2, 3 y 4), y se observó como, efectivamente, las bellotas de *Q. ilex* y *Q. suber* presentaban un mayor contenido hídrico (ver Anexo 5). Otra explicación podría venir por la fenología diferencial de cada especie, pero, al menos en la zona de estudio, si bien las bellotas de *Q. faginea* son las primeras en caer, las bellotas de *Q. pyrenaica* caen al mismo tiempo que las de *Q. ilex* (VGR observaciones personales y comunicación personal de Semillas Cantueso S.L.) por lo que esta explicación parece poco probable. Urbieta et al. (2008a) también encontraron que las especies caducifolias de *Quercus* emergían antes en un experimento de campo, por lo que estas diferencias parecen ser un rasgo característico de cada especie. Las caducifolias parecen entonces prolongar su primera estación de crecimiento, situación que puede suponer una ventaja para estas especies mas sensibles al déficit hídrico ya que así podrían tener tiempo para desarrollar raíces más profundas (Castro, 2006).

Aspectos aplicados

Aunque de todos los aspectos estudiados se pueden extraer recomendaciones de tipo práctico, al inicio de esta tesis se plantearon dos objetivos concretos (objetivos 5 y 6) para responder a problemas específicos en la gestión. Por un lado, se planteó la disyuntiva **siembra/plantación** que aún no ha sido resuelta, y los resultados dispares de las diferentes experiencias realizadas al respecto no han dado una respuesta clara. Muchos de estos ensayos se han llevado a cabo en Norteamérica, y no hay muchos estudios realizados en la cuenca mediterránea (pero ver Navarro et al., 2006). La comparativa del éxito de establecimiento para plántulas procedentes de siembra o plantación realizada en el capítulo 5 tampoco arroja un resultado concluyente, ya que tanto las plántulas procedentes de siembra directa como de siembra previa en vivero tuvieron resultados similares, y parece que las condiciones del cultivo en vivero tienen una importancia clave. Aunque no fue un factor directamente evaluado, las plántulas de 3 años de encina fueron cultivadas en contenedores de escaso volumen, y como ya se comentó en el capítulo 5, ésta podría ser una de las razones del menor éxito de este método de repoblación para esta especie. Por otro lado, a pesar de que las plántulas procedentes de siembra presentan un crecimiento más reducido, esta técnica supone una alternativa de bajo coste e impacto ambiental que además evita los problemas asociados a la deformación de raíces. Aunque en los capítulos 3 y 4 no se han mostrado los resultados relativos a la supervivencia de plántulas tres años después de la siembra, los censos realizados durante el mismo muestran que las plántulas establecidas en los dos primeros años tuvieron una probabilidad muy alta de supervivencia (ver Fig.2 Introducción, pág. 16), habiendo alcanzado posiblemente un tamaño que les permite hacer frente a las principales limitaciones del medio que son la sequía estival y la herbivoría. Estos datos de supervivencia del tercer año no se han analizado en los capítulos correspondientes ya que durante el tercer año se detectó presencia de conejos en el cercado experimental, y se consideró por tanto que este factor confundiría los resultados y los objetivos iniciales del estudio.

Otro factor que no se ha evaluado directamente pero que podría ser determinante en el éxito de una siembra artificial es el relativo a la **fecha de siembra.** Los experimentos de los capítulos 3 y 4 se realizaron en la misma parcela, con bellotas procedentes de la misma población y en el mismo año. La Tabla 2 muestra los datos de emergencia, supervivencia, éxito y biomasa aérea al inicio del verano para cada especie en ambos experimentos. Como se puede ver, los valores de las distintas variables correspondientes al capítulo 4 (siembra temprana) fueron, en muchos casos, mayores que los de la siembra tardía (capítulo 3). La siembra temprana aumentó el porcentaje de emergencia, supervivencia y establecimiento para todas las especies salvo *Q. ilex*. Las dos grandes diferencias de una siembra con respecto a la otra fueron: A) la protección frente a la depredación (protección con malla en capítulo 4 y sin protección en el capítulo 3); y B) la fecha de plantación (Noviembre en capítulo 4 y

Enero en capítulo 3). Con respecto a la depredación, ésta parece poco probable, ya que las bellotas fueron enterradas y en el caso de las bellotas no protegidas, no se observaron hoyos ni rastros de haber sido retiradas. Además, la encina parece una de las especies más preferidas por los consumidores (ver capítulo 1), y precisamente la emergencia de esta especie fue mayor en el experimento del capítulo 3 (sin protección).

Tabla 2. Porcentajes de emergencia, supervivencia en verano 1, éxito de establecimiento en verano 1 y peso aéreo de plántulas al final de la primera estación de crecimiento (media ± ds) de las plántulas de siembra temprana (Noviembre 2006, capítulo 4) y siembra tardía (Enero 2007, capítulo 3). Para las plántulas de siembra temprana se consideran sólo aquellas de los tratamientos control (sin riego suplementario en verano). *Qi*: *Q. ilex*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*. En las plántulas de siembra temprana se indica el valor para todas las madres, y entre corchetes el valor para la madre que se empleó en la siembra tardía (*Q. ilex* M_1; *Q. suber* M_3; *Q. faginea* M_5; *Q. pyrenaica* M_2)

	% emergencia		**% Supervivencia**		**% Establecimiento**		**Peso aéreo plántulas (g)**	
	Temprana	**Tardía**	**Temprana**	**Tardía**	**Temprana**	**Tardía**	**Temprana**	**Tardía**
Qi	33,39 [37,82]	51,65	30,76 [31,03]	26,01	8,00 [11,11]	15,29	0,60 ± 0,41 [0,36 ± 0,19]	0,35 ± 0,19
Qs	73,49 [79,27]	64,05	17,8 [37,00]	15,49	7,33 [13,25]	9,09	0,47 ± 0,36 [0,51 ±0,24]	0,36 ± 0,20
Qf	54,36 [65,52]	38,84	13,63 [14,28]	9,84	4,00 [5,63]	3,30	0,59 ± 0,25 [0,70 ± 0,28]	0,42 ± 0,26
Qp	70,67 [73,76]	59,91	6,96 [4,00]	3,44	4,33 [1,35]	1,65	0,58 ± 0,35 [0,47± 0,17]	0,23 ± 0,14

Es por ello que la diferencia de éxito parece debida más bien a la fecha de siembra, efecto que ya ha sido observado en plantaciones en condiciones mediterráneas (Radoglou et al., 2003; Palacios et al., 2009). Otro dato que apoya esta teoría es el mayor tamaño de planta aérea que alcanzaron las plántulas sembradas más pronto. Estas plántulas emergieron antes y por tanto dispondrían de más tiempo para el crecimiento (Jones et al, 1997; Seiwa, 1998; Castro, 2006). Aunque no se evaluó la inversión en biomasa subterránea, es previsible que las plántulas sembradas en noviembre tuvieran más tiempo para desarrollar un sistema radicular más profundo y de este modo alcanzar capas de agua más inaccesibles durante el verano, lo que les habría facilitado la supervivencia. No obstante, es necesario un diseño experimental específico y el análisis de la inversión en biomasa subterránea para comprobar esta hipótesis.

Actuaciones en Cardeña

De los diferentes experimentos realizados en el P.N. Sierra de Cardeña y Montoro se pueden extraer distintas recomendaciones de carácter práctico que puedan ser útiles para los gestores a la hora de llevar a cabo acciones de restauración y conservación. Éste era uno de los objetivos específicos de la tesis ya que son pocos los estudios de regeneración de *Quercus* llevados a cabo en el parque, y la mayoría se han centrado en la población de *Q. pyrenaica*. Como ya se indicó al inicio de la memoria, la normativa específica del parque contempla el mantenimiento y expansión de las quercíneas, y en ese sentido en los últimos años se han venido realizando aclareos selectivos de pinares y repoblaciones artificiales con *Quercus.*

Debido a las actuaciones del proyecto Life “Recuperación de las poblaciones de lince Ibérico en Andalucía”, a lo largo del parque se han establecido **cercados** para la cría de conejo. No todos estos cercados están poblados de conejos, y suponen por tanto zonas de exclusión de grandes herbívoros que se puede aprovechar para crear “islas” de vegetación a partir de las cuales fomentar la regeneración (Robinson y Andel, 2000; Rey Benayas et al., 2005), protegiendo a las plántulas en las primeras fases de vida es las que son muy susceptibles a la herbivoría. Estas islas supondrían fuentes de propágulos a partir de las cuales se favorecería la recolonización de nuevas áreas por efecto de los dispersores. Además, como ya se ha explicado más arriba, aún existiendo la posibilidad de que estos cercados sean posteriormente recolonizados por conejos, se ha observado que las plántulas establecidas ya no serían tan susceptibles a su presencia. Los conejos pueden causar daños a las leñosas por cortos periodos de tiempo, cuando las herbáceas escasean, pero en muchas especies estos daños no afectan a la regeneración a largo plazo (Tiver y Andrew, 1997).

El **enterramiento** de las bellotas también ofrece más garantías de éxito, y aunque no se ha comprobado directamente, parece que en esta zona no es necesaria una protección adicional al enterramiento. Como ya se ha explicado, en el experimento del capítulo 3 no se puso ningún tipo de malla, y no se encontraron indicios de depredación ya que el terreno, visitado con una frecuencia prácticamente semanal durante el primer año, no aparecía removido ni excavado. En el caso del capítulo 4, tampoco se encontraron hoyos ni galerías en los laterales de las mallas protectoras. Además, cuando después de un año de la siembra se procedió a desenterrar algunas de las bellotas no emergidas, se encontró prácticamente el 100 % de las mismas, algunas de ellas con radícula, por lo que el fracaso en la emergencia de estas plántulas no se debió a la depredación. Sin embargo, prácticamente el 100% de las bellotas depositadas en superficie (capítulo 1) desaparecieron. Como ya se comentó en este capítulo, muchas de las bellotas desaparecidas pueden ser enterradas por micromamíferos o aves en despensas y tener oportunidades de sobrevivir si no son relocalizadas más adelante. No obstante, sería necesario un estudio concreto de dispersión para comprobar esta hipótesis, teniendo en cuenta también la variabilidad interanual en la producción de bellotas y en las densidades poblacionales de los potenciales dispersores (Wolff, 1996). Lo que parece claro por tanto es que el enterramiento de las bellotas podría ser suficiente para escapar a la depredación en esta zona (García et al., 2002).

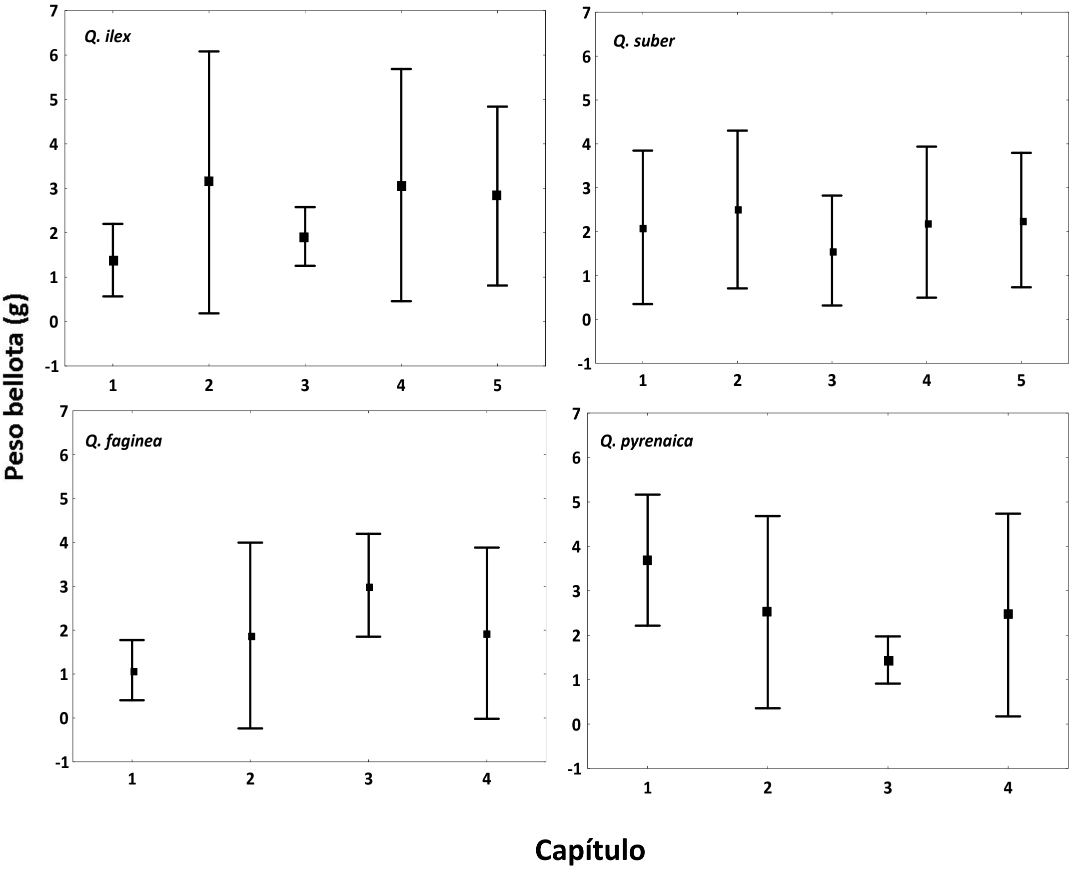
La elección de micrositios adecuados y la consideración de la variabilidad espacio-temporal en la distribución de los recursos es un factor clave para garantizar el éxito de las actividades de repoblación. La elección de sitios con condiciones microclimáticas de humedad mayores es importante, especialmente para las especies que son más susceptibles a la sequía, como son las caducifolias. En el capítulo 4 se mostraron los efectos beneficiosos de la aplicación de un riego suplementario durante el primer verano, pero estos resultados deben ser considerados con cautela, ya que el efecto del riego se manifestó de forma débil dos años después de la siembra. Parece necesario aplicar más cantidad de agua, o bien repetir el riego durante el segundo verano, al menos cuando las condiciones hayan sido especialmente secas, como fue el caso de nuestro primer verano de estudio. Es recomendable también el uso de arbustos como plantas nodriza, que si bien en esta memoria no se han evaluado, proporcionan protección a las plántulas durante las primeras etapas de vida (Gómez–

Aparicio et al., 2004), favoreciendo además la recuperación de las formaciones de leñosas autóctonas, que implican no sólo un estrato arbóreo, sino también un estrato arbustivo muy diverso y que proporciona el hábitat adecuado para la recuperación de las especies de fauna amenazada del parque (Quero y Villar, 2009)

Por último, la modulación en las respuestas a los factores ambientales mediada por el progenitor de origen es otro factor a tener en cuenta en la recolección de semillas para fines repobladores. La importancia de seleccionar árboles de poblaciones cercanas al lugar donde se realizará la siembra es un factor conocido y que se tiene en cuenta en la legislación y redacción de proyectos. Aunque las diferencias entre poblaciones son consideradas, no sucede lo mismo con la variabilidad intrapoblacional. Muchas veces, por razones económicas o por falta de tiempo, la recogida se limita a la selección de unos pocos árboles madre, generalmente aquellos que presentan semillas de mayor tamaño. Aunque se ha visto que las semillas de mayor tamaño suelen tener más probabilidad de éxito, no se debe olvidar el riesgo de avocar a las poblaciones a una depresión endogámica al reducir su diversidad. En esta memoria no se ha abordado el estudio genético de las semillas procedentes de cada árbol, pero los diferentes efectos maternos encontrados podrían deberse a diferencias tanto genéticas como ambientales (Wulff, 1986; Schmitt et al., 1992; Castro et al., 2008). Los efectos positivos del mantenimiento de la diversidad genética sobre el mantenimiento de las poblaciones han sido ampliamente estudiados ya que las hace más estables frente a las fluctuaciones del medio (Vander Mijnsbrugge et al., 2010). Este es un aspecto muy importante a tener en cuenta, especialmente en el caso de la población de *Q. pyrenaica* del parque, muy reducida y en la que muchos de los individuos sólo se reproducen de forma vegetativa.

APÉNDICE SUPLEMENTARIO

Anexo 1. Pesos secos medios de bellota de las cuatro especies empleados en cada capítulo. El método empleado para estimar el peso de la bellota se describe en el apartado de Métodos (pág. 29)



Anexo 2. Resultados del efecto del peso de la bellota no mostrados en los capítulos

A) Para los datos del capítulo 2 se evaluó la relación entre peso de bellota (variable independiente) y peso raíces o tiempo de emergencia (variables dependientes) mediante regresiones simples por especie. (*Qi*: *Q. ilex*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*) (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001)

	Tiempo emergencia (días)		**Peso raíces (g)**	
	Ecuación	**r^2**	**Ecuación**	**r^2**
Qi	y = 62,12 – 3,65x	0,06 *	y = 0,16 + 0,13x	0,60 ***
Qs	y = 41,17 – 0,07x	0,00	y = -0,04 + 0,26x	0,69 ***
Qf	y = 46,35 – 3,31x	0,03	y = 0,12 + 0,18x	0,69 ***
Qp	y = 44,16 – 1,48x	0,00	y = 0,40 + 0,15x	0,25 ***

B) Para los datos del capítulo 5 se evaluó la relación entre el peso de la bellota y la emergencia y supervivencia mediante anova de una vía (se muestra F, significación y signo del efecto), y el resto de variables mediante regresiones simples por especie (se muestra la ecuación, r^2 y significación) (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) (*Qi*: *Q. ilex*; *Qs*: *Q. suber*)

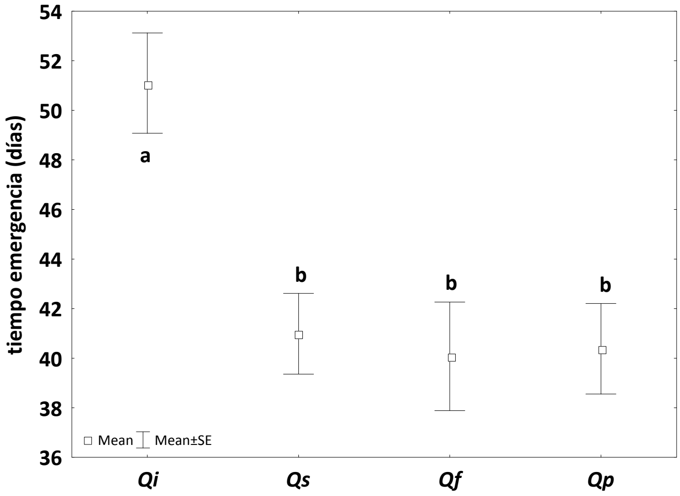
		Qi	***Qs***
Emergencia	F	1,38	12,00 *** (+)
Supervivencia	F	9,26 ** (+)	1.32
Tiempo emergencia (días)	Ecuación	y = 126,8 - 2,90x	120,12 - 3,40x
	r^2	0,01	0,009
Peso aéreo plántula (g)	Ecuación	y = -0,35 + 0,49x	y = 0,56 + 0,15x
	r^2	0,77 ***	0,006
Peso raíces (g)	Ecuación	y = -0,03 + 0,25x	y =0 ,30 + 0,22x
	r^2	0,28	0,19
Área foliar (cm^2)	Ecuación	y = -37,75 + 31,83x	y = 27.55 + 10,21x
	r^2	0,33 *	0,004
LMF (kg/kg)	Ecuación	y = 0,26 + 0,01x	y = 0,19 + 0,005x
	r^2	0,01	0,00
RMF (kg/kg)	Ecuación	y = 0,44 - 0,02x	y = 0,51 - 0,017x
	r^2	0,03	0,008
SMF (kg/kg)	Ecuación	y = 0,29 + 0,009x	y = 0,29 + 0,0115x
	r^2	0,016	0,004

Anexo 3: Porcentaje de bellotas emergidas por cada madre en el experimento del capítulo 2.

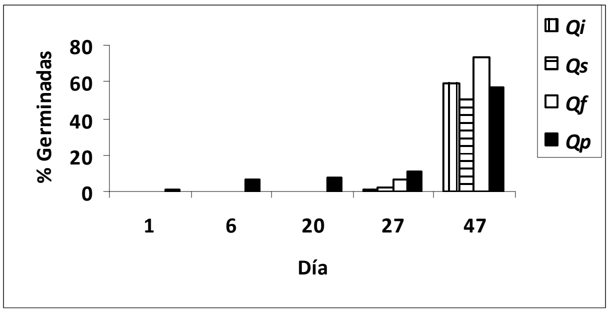
Madre	1	2	3	4	5
Q. ilex	66,6	63,3	63,3	80	43,3
Q. suber	76,6	86,6	90	96,6	93,3
Q. faginea	53,3	53,3	26,6	66,6	70
Q. pyrenaica	90	86,6	76,6	90	80

Anexo 4: Germinación y tiempo de emergencia entre especies

A) Diferencias en tiempo de emergencia entre especies en el experimento en condiciones controladas (Capítulo 2) según el test no paramétrico Kruskal-Wallis. Diferentes letras minúsculas indican diferencias significativas entre grupos (*P* < 0,05) (*Qi*: *Q. ilex*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*)

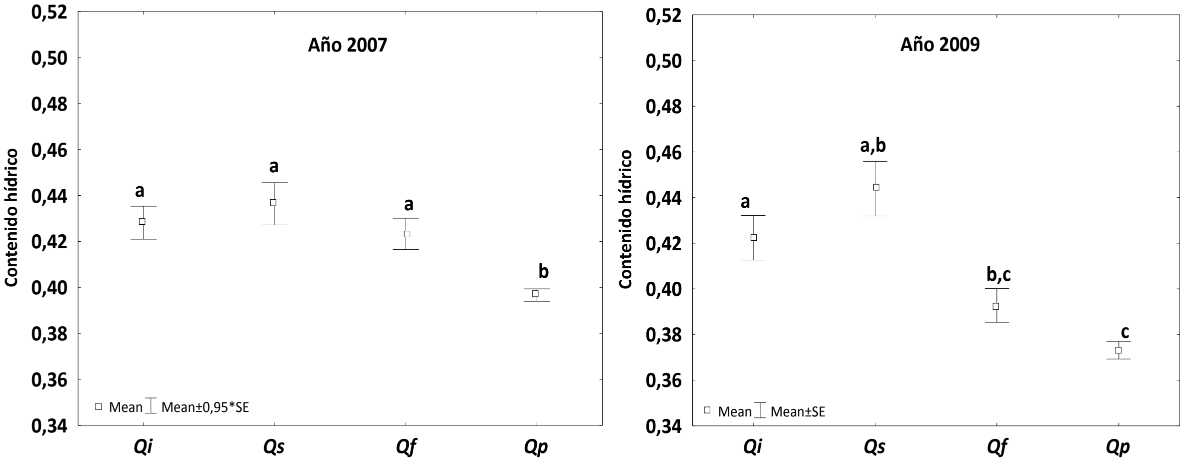


B) Porcentaje de bellotas germinadas sobre el total de las no desaparecidas para cada especie en el capítulo 1 (*Qi*: *Q. ilex*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*).



Anexo 5: Diferencias en contenido hídrico de la bellota

Diferencias en contenido hídrico de las bellotas empleadas para estimar la relación peso seco/peso fresco entre especies en los años 2007 y 2009 mediante test no paramétrico de Kruskal-Wallis. (Año 2007: H = 14,12, p = 0,002; Año 2009: H = 30,48; *P* = 0,000). Letras diferentes indican diferencias significativas entre especies (*P* < 0,05) (*Qi*: *Q. ilex*; *Qs*: *Q. suber*; *Qf*: *Q. faginea*; *Qp*: *Q. pyrenaica*).



CONCLUSIONES

1. En el área de estudio, los animales consumidores-dispersores (micromamíferos y aves) seleccionan las bellotas de *Q. ilex* y *Q. faginea*, independientemente del tamaño de la semilla. Sin embargo, los ungulados, estrictamente consumidores, seleccionan en primer lugar las especies con bellotas de mayor tamaño (*Q. suber* y *Q. pyrenaica*).

2. Las características intrínsecas como son el peso de la semilla y las características propias de la especie y progenitor de origen son más determinantes en la emergencia y crecimiento de las plántulas en los primeros meses de vida. Por el contrario, la supervivencia es más dependiente de factores externos, especialmente de la disponibilidad de agua.

3. Las plantas procedentes de semillas de mayor tamaño presentan mayores probabilidades de emergencia, supervivencia y establecimiento. Además, un aumento en el peso de la semilla está relacionado con una mayor biomasa y área foliar, no habiendo un efecto claro del peso de la semilla sobre la distribución de biomasa a hojas, tallos o raíces.

4. El progenitor de origen introduce diferencias en las fases de establecimiento y en el crecimiento que no siempre vienen mediadas por las diferencias en peso de semilla. Además, el factor materno modifica las respuestas a los factores ambientales o al propio peso de la semilla, siendo este efecto de importancia en ambientes heterogéneos como el mediterráneo y en un escenario de previsibles cambios climáticos.

5. La importancia del progenitor de origen y el peso de la semilla desaparece en condiciones favorables (como por ejemplo riego suplementario en verano). Esto puede indicar la importancia de la variabilidad intrapoblacional en ambientes con recursos limitantes.

6. La supervivencia de las plántulas no sigue patrones aleatorios en el espacio sino que se localiza en forma de manchas y claros, que vienen determinados en parte por la distribución diferencial de las condiciones y recursos ambientales. Las zonas con mayores recursos hídricos son lugares con alta probabilidad de supervivencia, aunque en condiciones de extremada sequía estos patrones de agregación desaparecen.

7. El tiempo de emergencia parece un rasgo característico de la especie. Las especies perennifolias (*Q. ilex* y *Q. suber*) presentan una emergencia retrasada con respecto a las caducifolias (*Q. faginea* y *Q. pyrenaica*). Por otro lado, el tiempo de emergencia se acortó en zonas con mayor disponibilidad de luz.

8. Los resultados apoyan la idea de que la fecha de siembra puede determinar el éxito de la misma, siendo recomendable una siembra temprana. De este modo las plántulas tienen más tiempo para crecer y desarrollar raíces profundas antes de la sequía estival.

9. Las técnicas de repoblación con semillas o plántulas no presentan grandes diferencias entre sí, aunque la probabilidad de supervivencia es mayor en las plántulas de un año. En el caso de las plántulas, las condiciones de cultivo en vivero son determinantes, ya que pueden modificar la estructura de la raíz. Las siembras suponen una alternativa de bajo coste económico e impacto ambiental y además evitan la deformación de la raíz.

10. El aprovechamiento de los cercados de exclusión de grandes herbívoros existentes en el P. N. Sierra de Cardeña y Montoro es recomendable para proteger a las semillas y plántulas de la depredación en las primeras fases de vida. En el interior de los cercados, las bellotas enterradas tienen gran probabilidad de escapar a la depredación post-dispersiva y no es necesario protegerlas.

11. El mantenimiento de la diversidad intrapoblacional supone una garantía de persistencia frente a las fluctuaciones del medio, siendo de especial importancia en poblaciones reducidas como la de *Q. pyrenaica* en el P.N. Sierra de Cardeña y Montoro. Para ello es importante contar con un amplio número de árboles madre a partir de los cuáles se efectúen las colectas de semilla.

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Vico
Córdoba, julio de 2010

ANEXO DE FOTOS

Foto 1. Bellotas de *Q. pyrenaica*

Foto 2. Madre 5 de *Q. faginea* en una dehesa del parque

Foto 3. Vista de la finca La Vegueta

Foto 4. Bellota de *Q. pyrenaica* en experimento de depredación (capítulo 1)

Foto 5. Experimento de invernadero (capítulo 2), poco después de la siembra

Foto 6. Plántula de *Q. faginea* en experimento capítulo 2

Foto 7. Cercado experimental. Vista de los nodos del experimento capítulo 3

Foto 8. Malla para simular la sombra de arbustos en experimento capítulo 4

Foto 10. Malla protectora frente a depredadores en capítulo 4

Foto 11. Técnicas de repoblación (capítulo 5). Vista parcial de un plot experimental

Foto 11. Raíces de plántulas de *Q. ilex* de tres años atrofiadas por el contenedor de escaso volumen en que fueron cultivadas. Se observa la raíz principal engrosada

Foto 12. Plántula de *Q. ilex* procedente de siembra directa en capítulo 5