Efecto de la disponibilidad de recursos sobre la eficacia reproductiva de *Juniperus thurifera*

Resource availability and reproductive efficacy of the dioecious tree *Juniperus thurifera*

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“**Los árboles son una cosa maravillosa.**
Permanecen fijos en el paisaje, a menudo durante siglos, tomando carbono del aire y oxígeno e hidrógeno del agua del suelo, y combinan estos elementos para formar capas de madera superpuestas en sucesión anual.”

“**Trees are wonderful things.**
They stand fixed in the landscape, often for centuries, taking carbon from the air, and oxygen and hydrogen from soil water, and combine these components into layers of wood laid down in annual succession.”

Danny McCarroll and Neil J. Loader
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Introducción:

Juniperus thurifera, una especie dioica, vecera y relíctica

La especie *Juniperus thurifera*

Juniperus thurifera L. (Cupressaceae), comúnmente conocida como sabina albar, es una especie arbórea originada durante el Terciario. Contemporáneamente presenta una distribución relíctica en la cuenca mediterránea occidental, encontrándose poblaciones de distinto tamaño en los Alpes, Córcega, los Pirineos, a lo largo de la península Ibérica, en los Atlas Marroquíes y en Argelia. Junto con las dos especies vicariantes *J. excelsa* y *J. procera*, de similar biología, se distribuyen por toda la cuenca mediterránea, el oeste de África y el este de Asia. Habitualmente es la especie dominante en los lugares donde se encuentra, formando bosques de baja densidad en zonas semiáridas de alta montaña, aunque puede coexistir en masas mixtas de *Quercus ilex*, *Q. faginea* y *Pinus nigra*.

Habitualmente los árboles tienen una altura de entre cuatro y diez metros y suelen alcanzar la madurez sexual alrededor de los 30 años (Pavón-García 2005) alargando su vida durante más de 200 años (Bertaudière et al. 1999). *J. thurifera* es una especie dioica, esto es, con pies masculinos y femeninos independientes. Machos y hembras preforman sus flores desde el verano y florecen al final del periodo invernal y tras la polinización anemófila las hembras producen conos reproductivos carnosos (gálbulos) que crecen y maduran durante un periodo de 20 meses, hasta que la dispersión de semillas se produce (Amaral-Franco 1986). Por ello, las hembras suelen ser portadoras de dos cohortes de gálbulos: los inmaduros, que han estado creciendo durante 12 meses y proceden de la floración del año anterior, presentando una coloración verdosa (en este trabajo nos referiremos a ellos como Fr1); y los maduros, que provienen de la floración de dos años antes y que han estado creciendo y madurando durante al menos 22 meses, presentando una coloración azulada oscura (nos referiremos a ellos como Fr2) y conteniendo entre una y siete semillas (media=3.5). Estas semillas presentan una tasa de viabilidad y germinación extraordinariamente bajas, debido a altas tasas de partenocarpia, aborto y predación predispersiva de semillas (Ceballos & Ruiz de la Torre 1979; Melero & García-Fayos 2001). Las semillas son predadas por una gran variedad de insectos, siendo sus principales predadores el calcídido *Megastigmus bipunctatus* (en adelante *Megastigmus*) y el ácaro *Trisetacus quadrisetus* (en adelante *Trisetacus*). *Megastigmus* oviposita en semillas inmaduras durante el primer verano tras la polinización y sus larvas se desarrollan y crecen durante un año antes de emerger de la semilla a través de un orificio de salida característico (Roques et al. 1984). La oviposición de *Trisetacus* ocurre mucho antes, a menudo antes de la polinización. Estos ácaros viven en colonias y utilizan las semillas como cámara de crecimiento, lo que suele conllevar la destrucción de la totalidad de semillas del gálbulo. Las semillas afectadas por la actividad de colonias de *Trisetacus* presentan una morfología típica "fibrosa" y marrón (Roques et al. 1984).

J. thurifera es una especie vecera: su producción de gálbulos varía fuertemente entre años, presentando un alto grado de variabilidad, sincronía poblacional y periodicidad temporal.

El esfuerzo reproductivo medio por rama durante un año no vecero es similar para ambos sexos. Por el contrario, durante los años veceros las hembras tienen que invertir una cantidad de recursos en reproducción muy superior a la de los machos. Esta diferencia reproductiva podría tener un coste somático. Comparando el crecimiento radial de anillos de tres árboles macho y tres árboles hembra de sabina albar en Marruecos, Gauquelin *et al.* (2002) encontraron que los árboles macho crecían significativamente más que las hembras, aunque en esa misma población las hembras eran más altas que los machos.

Al menos el 55% de los gálbulos de sabina albar son dispersados por aves de talla media del género *Turdus* (Santos & Tellería 1994; Santos et al. 1999) que, cuando invernan en bosques de sabina albar, se alimentan casi exclusivamente de gálbulos de *J. thurifera* y su abundancia y actividad se suele incrementar paralelamente al tamaño de cosecha de las sabinas (Zamora 1990; Jordano 1993; Santos et al. 1999; García & Ortiz-Pulido 2004). Habitualmente sus semillas germinan tras ser ingeridas por tordos aunque también pueden germinar directamente desde gálbulos no dispersados por aves (com. pers. P. García-Fayos). Otros ungulados y mamíferos también se pueden alimentar de gálbulos de sabina, aunque sólo representan una pequeña parte de su dieta (Santos et al. 1999). Por ello, y aunque estos mamíferos pueden viajar y dispersar semillas a largas distancias, juegan un papel cuantitativamente menor comparados con los tordos (Santos et al. 1999).

Lugares de estudio de *Juniperus thurifera*

La mayoría de los trabajos se realizaron en La Puebla de San Miguel, Ademuz, en la provincia española de Valencia. Los trabajos y parcelas experimentales se localizaron en las cercanías del lugar donde un grupo de sabinas albares monumentales recibe el nombre de Las Blancas (ver capítulos I,II y IV). Este lugar se encuentra a una altitud de 1500 m y constituye un bosque bastante homogéneo de *J. thurifera* con una densidad de 423 individuos por hectárea. La altura media de los individuos masculinos de esta población es de 4.73±0.84 m y la de los femeninos de 4.63±0.83 m, no encontrándose diferencias significativas entre ellos (F=0.14; df=1; p=0.71; N=40). La vegetación natural cubre menos del 40% de la superficie, y se compone principalmente de sabina albar, con individuos aislados de *J. communis*, *Thymus vulgaris*. y *Genista scorpius*. Líquenes y vegetación herbácea estacional aparecen ocasionalmente. Los suelos son poco profundos, con menos de 30 cm de profundidad, aunque las grietas de las rocas permiten a las raíces de sabina penetrar a profundidades mayores (Verdú et al. 2004).

El clima es mediterráneo, con inviernos fríos (el periodo de heladas es de más de 120 días al año) y veranos cálidos y secos. La precipitación media anual, según la serie (20 años) de la estación más cercana (Sesga) es de 486 mm, siendo octubre el mes más húmedo (58.6 mm) y julio el más seco (26.9 mm). La temperatura media anual es de 13º C, siendo agosto el mes más cálido (media de 22.8º C) y enero el más frío (media 4.8º C). El área de estudio fue usada durante siglos para agricultura, extracción de madera y ganadería extensiva. En sus estrechos valles se cultivó cebada y centeno hasta el éxodo rural de los años sesenta (Rodrigo 1999). En las últimas décadas, los cambios económicos y sociales han propiciado la recuperación de las masas forestales debido al abandono de los usos tradicionales (Lasanta 1996), aunque todavía es posible observar el ramoneo ocasional de ovejas y cabras.

En esta misma localidad, a unos 2 km del lugar anterior y a 1600 m de altitud, en una zona cercana al área conocida como El Verdinal, se marcaron 40 sabinas albares de ambos sexos para el estudio de los ciclos de vecería a largo plazo (ver capítulo III). A unos 100 km de esta primera población se encuentra una segunda población, en El Portell de Morella (provincia de Castellón) en la que se marcaron otras 40 sabinas albares de ambos sexos con la intención de replicar los resultados del estudio de vecería a largo plazo. Esta población se encuentra a 1150 m de altitud y la vegetación se encuentra dominada por *J. thurifera* y *Quercus ilex*, acompañados por individuos aislados de *Juniperus communis*, *Thymus vulgaris* y *Genista scorpius*. La precipitación anual media según la serie (30 años) de la estación más cercana (Vilafranca del Cid) es de 665 mm siendo mayo el mes más húmedo (81 mm) y julio el más seco (35 mm). La temperatura media anual es de 11.2º C, siendo Julio el mes más cálido (media de 20.1º C) y enero el más frío (media de 3.8º C).

Por último, y para estudiar la tasa de viabilidad de semillas y el crecimiento foliar de la especie en un amplio rango geográfico y ambiental, se seleccionaron nueve poblaciones más a lo largo y ancho de la península Ibérica y en los pirineos franceses. Para una descripción ambiental y geográfica de dichas poblaciones ver la figura y tabla 5.1 en el capítulo V.

***Juniperus thurifera*, una especie dioica, vecera y relíctica**

Mucho se ha investigado descrito y escrito sobre la ecología poblacional de plantas mediterráneas. Tanto, que no es arriesgado decir que, a la hora de resumir todo el conocimiento que anualmente se genera, uno de los mayores retos con que se encuentra la ciencia es la síntesis de esa miríada de datos en generalizaciones manejables. Ante dicha necesidad, han aparecido una serie de conceptos y generalizaciones que tratan de hacer fácilmente utilizables los conceptos ecológicos a distintos tipos de plantas, siendo la aparición y auge del concepto de grupos funcionales es sólo un ejemplo de varios.

El sexo es claramente otra de las claras diferenciaciones genéricas que se pueden hacer fácilmente para predecir el comportamiento de un grupo de individuos respecto a otros (p. ej. machos respecto a hembras, pero también machos frente a hermafroditas, etc.). Mucho se ha trabajado y se está trabajando al respecto y sin embargo mucho queda por hacer aún en este campo. La dioecia es un elemental y claro punto de partida para estudiar el efecto del sexo y los costes reproductivos sobre la biología de las plantas. Estudio que se torna más difícil si abordamos especies de sexualidad más compleja (androdioicas, ginodioicas, etc.).

La sexualidad tiene implicaciones a lo largo de todo el ciclo vital de las plantas. El hecho de ser macho o hembra puede acarrear diferencias desde la dispersión de semillas y supervivencia de plántulas hasta, naturalmente, la morfología y fisiología, y por supuesto en los sutiles compromisos de eficacia (*trade-offs*) entre reproducción y crecimiento. En esta introducción resumimos el marco conceptual necesario para investigar el efecto de la interacción entre la dioecia y la variabilidad en la disponibilidad de recursos sobre la eficacia reproductiva de *Juniperus thurifera* a través de todos sus estadíos vitales: desde los mecanismos de dispersión y establecimiento de plántulas (capítulo I), hasta la reproducción (capítulos II y III), la fisiología (capítulo IV) y el efecto del cambio climático (capítulo V).

En ecosistemas mediterráneos la sequía estival constituye la principal limitación ambiental y la principal causa de mortalidad juvenil de plantas (p. ej. Herrera et al. 1994; García-Fayos & Verdú 1998; Traveset et al. 2003; Gulías et al. 2004). En ambientes estresantes, la facilitación es un fenómeno común (Callaway 1995; Callaway et al. 2002) y las plantas nodriza mejoran las condiciones físicas y la germinación y supervivencia de plántulas (Verdú & García-Fayos 1996; Castro et al. 2004; Gómez-Aparicio et al. 2004) protegiéndolas a menudo de los herbívoros (García & Obeso 2003).

La facilitación puede ser específica de especie (Callaway 1998). Sin embargo, raramente se ha estudiado diferencias entre sexos de especies dioicas en búsqueda de interacciones específicas de sexo (aunque ver Verdú & García-Fayos 2003; Verdú et al. 2004). Diferentes sexos generan diferentes condiciones ambientales, constituyendo diferentes microhábitats. La variación en las condiciones ambientales puede determinar la supervivencia de plántulas lo que frecuentemente genera una discordancia espacial entre microhábitats (Jordano & Herrera 1995; Schupp 1995). Los factores que propician las diferencias entre microhábitats son múltiples a lo largo del ciclo reproductivo y vital de las plantas. Las aves frugívoras se alimentan de los gálbulos que portan las hembras, lo que genera que una cantidad desproporcionadas de semillas se depositen inmediatamente bajo la copa de individuos hembra (Holthuijzen & Sharik 1985; Herrera 1988; Herrera et al. 1994; Nanami et al. 1999; Calviño-Cancela 2002). Además, la deposición de frutos no dispersados bajo copa de hembras reforzaría este patrón dado que las semillas son capaces de germinar a partir de frutos no dispersados por endozoocoria (P. García-Fayos com. pers.). La predación postdispersiva de semillas tambien puede diferir entre microhabitats (Hulme 1997; Tomita et al. 2002; Hollander & Vander Wall 2004; Gulías et al. 2004). Cuando las semillas que escapan a la predación terminan enterradas en el suelo, su longevidad y germinación pueden ser influenciadas por las propiedades ambientales de la superficie del suelo, que también varía entre microhábitats (Rotundo & Aguiar 2005). Tras la germinación, la sombra que proporcionan las plantas nodriza protege a las plántulas de la sequía reduciendo la temperatura del suelo durante el verano y mejorando el balance hídrico del suelo (Callaway 1995; Verdú & García-Fayos 1996; 2003). Bajo las plantas nodriza se encuentra mayor proporción de materia orgánica y mayor contenido en nitrógeno que en espacios abiertos (Callaway 1995; Joy & Young 2002; Verdú & García-

Fayos 2003; Verdú et al. 2004). Además, las hembras de plantas de fruto carnoso tienden a incrementar la disponibilidad de nutrientes bajo su copa, debido a la deposición de frutos y heces de aves que acuden a alimentarse (Verdú & García-Fayos 1996; Verdú & García-Fayos 2003).

Por lo tanto, machos y hembras de una misma especie pueden afectar significativamente a la estructura especial de sus poblaciones y favorecer la regeneración bajo uno de los morfos sexuales y/o sobre otros microhábitats (véase capítulo I).

Cuando las plantas dioicas alcanzan la madurez sexual es quizá cuando las diferencias entre sexos se maximizan. Dado que reproducirse es costoso, las plantas compensan estos costes reduciendo el crecimiento vegetativo (véase capítulo II), reduciendo la frecuencia de reproducción (véase capítulo III) o mejorando su fisiología (véase capítulo IV) (Delph 1999; Obeso 2002).

La reproducción es costosa y normalmente se correlaciona negativamente con el crecimiento vegetativo, indicando que ambas funciones biológicas compiten por los mismos recursos (Harper 1977; Koenig & Knops 1998). Las plantas pueden compensar estos esfuerzos reproductivos reduciendo los recursos asignados a crecimiento y mantenimiento vegetativo (Obeso 2002). Sin embargo, detectar esta reducción en el crecimiento vegetativo puede ser difícil en el corto plazo debido a la frecuente existencia de mecanismos de compensación fisiológica (Obeso & Retuerto 2002). Por lo tanto, el uso de variables acumulativas, como el los anillos de crecimiento anual, puede ser una buena aproximación para valorar la asignación de recursos a funciones vegetativas en plantas leñosas (e.g. Obeso 1997; Silvertown & Dodd 1999). Con todo, tradicionalmente se ha prestado poca atención al efecto de la reproducción en el crecimiento anual de anillos en especies dioicas. Los costes de reproducción son específicos de edad y el esfuerzo reproductivo varía a lo largo de la vida de las plantas (Silvertown & Dodd 1999). Además, plantas de diferente sexo pueden incurrir en costes reproductivos diferentes y, por lo tanto, presentar diferentes compromisos de eficacia (*trade-offs*) en el uso de recursos, entre reproducción y crecimiento y pueden desarrollar diferentes estrategias de uso de recursos (Obeso 2002). Sin embargo se ha prestado poca atención a la interacción entre edad y sexo de especies dioicas y la mayoría de las investigaciones no han estimado cómo el crecimiento de anillos varía con la edad o el tamaño de planta (Silvertown & Dodd 1999; aunque, ver Bañuelos & Obeso 2004).

La escala estacional de inversión de recursos también puede jugar un papel relevante en la asignación de recursos específica de sexo. En especies dioicas los machos incurren en el máximo gasto en el periodo de floración. Una vez el polen es liberado, sin embargo, los machos no precisan invertir más recursos en reproducción. Por el contrario, las hembras incurren en el mayor gasto reproductivo inmediatamente tras la polinización dado que el crecimiento y maduración de frutos es muy costoso en comparación con la producción de flores (Verdu & Garcia-Fayos 1998; Obeso 2002). Por lo tanto, se esperaría que en el corto plazo machos y hembras utilicen sus recursos de forma diferente. La relación entre crecimiento de anillos y precipitación de meses anteriores puede ayudarnos también a discernir como diferentes sexos responden ante la variación de recursos disponibles dado que la precipitación se correlaciona positivamente con el crecimiento de anillos en la mayoría de las especies y es una aproximación fiable de la disponibilidad de recursos en ambientes semiáridos (Cherubini et al. 2003; Austin et al. 2004).

Además de ser una especie dioica y mediterránea, *J. thurifera* es una especie vecera; es decir, que sus poblaciones se reproducen de forma variable, periódica y sincrónica. La vecería es un fenómeno resultante de la interacción de varios factores funcionales y evolutivos. En ausencia de selección por mayor o menor variabilidad la reproducción de una población debería variar en paralelo a los recursos disponibles (como lo es la lluvia en ambientes semiáridos). Este comportamiento ha sido llamado "de concordancia de recursos" (*resource matching*) (Kelly & Sork 2002). Sin embargo, las especies veceras a menudo presentan una variabilidad reproductiva mayor que la variabilidad ambiental, por lo tanto, debe haber causas adicionales que la expliquen. Se han propuesto dos explicaciones evolutivas de la vecería: (i) el saciado de predadores, que sugiere que la producción intermitente de grandes cosechas reduce la pérdida de semillas por saciado de los predadores; y (ii) eficiencia de la polinización, que propone que concentrar la polinización en algunos años incrementa la eficiencia en la polinización en especies anemófilas (Kelly & Sork 2002; Rees et al. 2002; Piovesan & Adams 2005). Kelly (1994) basándose en un estudio de Sork & Bramble (1993) sugirió que diferentes fuerzas selectivas podrían estar actuando sobre la floración vecera (p. ej. la eficiencia en la polinización anemófila) y sobre la fructificación vecera (p. ej. la saciación de predadores). Por lo tanto, las fuerzas evolutivas que favorecen el comportamiento vecero pueden afectar diferencialmente a machos y hembras de especies dioicas. Pese a todo, las ventajas selectivas de la vecería se obtienen de economías de escala (Norton & Kelly 1988) y los pulsos de vecería dependen en mayor o menor grado de las reservas de recursos disponibles y acumulados (Sork et al. 1993; Yamauchi 1996; Isagi et al. 1997). Por lo tanto, cualquier explicación de la vecería debería considerar la importancia de la disponibilidad de recursos (Yamauchi 1996).

Dado que los costes de reproducción son diferentes para machos y hembras de especies dioicas, la reproducción puede llegar a presentar patrones de variación diferentes entre sexos, y en ocasiones los machos se reproducen con más frecuencias que las hembras (Antos & Allen 1999). En su revisión, Delph (1999) encontró que los machos florecen más a menudo que las hembras en el 63% de los casos estudiados, y no encontró ningún caso en el que lo contrario fuera cierto. No obstante, esta revisión pretendía esclarecer el efecto de los costes reproductivos en cada sexo, lo que finalmente resultó en reproducción variable. Sin embargo esa revisión no consideró la periodicidad reproductiva o la sincronía poblacional, que junto con la reproducción variable son los tres elementos característicos de la vecería (Janzen 1976; Kelly 1994; Kelly & Sork 2002; Rees et al. 2002). Aunque por el momento no se ha estudiado, los costes diferenciales de las especies veceras dioicas probablemente generan diferencias en la periodicidad y tamaño del ciclo reproductivo similares a los de especies dioicas no veceras.

Sin embargo, no está claro hasta que punto las diferencias fisiológicas entre sexos se mantienen durante los momentos en que la inversión reproductiva es similar en ambos sexos, como ocurre a menudo en especies veceras durante años no veceros. En una reciente revisión, (Obeso 2002) se describe una serie de mecanismos fisiológicos y funcionales de compensación de costes de reproducción diferenciales: desarrollo de estructuras reproductivas fotosintéticamente activas, retraso en la edad de madurez sexual, reabsorción de nutrientes de estructuras senescentes, especialización de módulos y aumento de la tasa fotosintética de hembras. Gracias a estos mecanismos de compensación, algunas plantas pueden llegar a neutralizar sus costes de reproducción (Delph 1990). Dada la plasticidad de las variables fisiológicas, a menudo se asume que la respuesta fisiológica responde inmediatamente a las variaciones ambientales y la demanda de recursos. Encontrar diferencias fisiológicas entre plantas de distinto sexo durante periodos en que ambas invierten cantidades similares de recursos a reproducción confirmaría la existencia de verdaderas estrategias fisiológicas específicas de sexo, que estarían prefijadas para cada sexo. Además, los compromisos de eficacia (trade-offs) pueden ser detectados bajo unas condiciones ambientales determinadas, pero no en otras, debido a la plasticidad fenotípica (Stearns 1989; Correia & Diaz Barradas 2000; Verdú et al. 2004). Por ello, el incremento experimental de la disponibilidad de recursos permitiría el estudio de la variación de las estrategias fisiológicas entre distintas condiciones ambientales.

Si la variación en las condiciones ambientales es capaz de influenciar significativamente la reproducción de las plantas, el cambio climático actual puede sin duda alterar el equilibrio fisiológico y reproductivo de especies de alta montaña. *Juniperus thurifera* es una especie originaria del terciario y habita ambientes semiáridos fríos en la región Holártica (Suárez Cardona et al. 1991). El progresivo calentamiento desde la última glaciación ha reducido su distribución a una serie de poblaciones dispersas en las altas montañas de la cuenca mediterránea occidental. La migración altitudinal de la flora de alta montaña es una tendencia generalizada en respuesta al cambio climático. (Grabherr et al. 1994; Klanderud & Birks 2003; Walther 2003). El incremento altitudinal de los límites inferiores de distribución de las plantas de montaña normalmente no es debido a estrés térmico e hídrico, sino más bien al incremento del límite altitudinal superior de especies competidoras con tasas de crecimiento mayores (Loehle 1998; Walther 2003; Alward et al. 2006). Para aumentar sus límites de distribución las plantas tienen que hacer frente a un compendio de condiciones ambientales, progresivamente peores, y características de alta montaña, como un incremento en la radiación (IR, UV y visible); menor presión parcial de CO_2 y O_2; vientos fuertes que pueden destruir mecánicamente tejidos y eventos de sequía más frecuentes (Barceló et al. 1992; De Lillis et al. 2004). Estas condiciones ambientales pueden llegar a inhibir la actividad fotosintética (Kofidis et al. 2003) y reducir la productividad primaria neta (Luo et al. 2004). Así, la asignación de recursos tiende a disminuir con la altitud (Hemborg & Karlsson 1998; Obeso 2002; Sakai et al. 2006) y las plantas pueden mitigar los costes reproductivos produciendo semillas de baja calidad (Houle & Barbeux 1994). Como muchas sabinas, *J. thurifera* presenta bajas tasas de viabilidad (p. ej. Roques et al. 1984; García 1998). Como hemos visto, la fertilidad de las plantas se relaciona claramente con las condiciones ambientales pero los factores geográficos también pueden afectar directamente a la viabilidad de semillas (García et al. 2000). La fertilidad de sabinas y enebros puede verse limitada por la dureza ambiental (García et al. 2000; Wesche et al. 2005), por la disponibilidad de nutrientes y agua (Stephenson 1981; García et al. 1999; Drenovsky & Richards 2005) y por fallos en la polinización (Ortiz et al. 2002). Las especies del género *Juniperus* típicamente presentan altas tasas de partenocarpia (Fuentes 2000) y tasas de aborto y prelación predispersiva de semillas moderadas (Rappaport et al. 1993; García 1998; El Alaoui El Fels et al. 1999). La partenocarpia puede estar causada por daños al óvulo por heladas, a carencia de nutrientes y también puede ser una defensa contra los predadores de semillas (Traveset 1993; Verdú & García-Fayos 1998). Las especies predadoras de semillas también migran altitudinalmente en respuesta al calentamiento global, elevando su límite de distribución a mayor velocidad que las plantas, incrementando las tasas de predación en poblaciones y especies previamente intactas (Hódar & Zamora 2004). Las plantas de alta montaña también presentan menores tasas de crecimiento de hojas y menores "áreas foliares específicas" (specific leaf area, SLA) a mayores altitudes (De Lillis et al. 2004; Angert 2006). Dado que el SLA se correlaciona positivamente con la longevidad foliar (Westoby et al. 2006), se espera que las plantas de altitud muestren menor longevidad foliar. Además, la mayor radiación UV presente a mayor altitud puede ser un factor dañino para las hojas (Robberecht & Caldwell 1980) y podría reducir aun más la longevidad foliar de plantas de montaña.

El hábitat actual de las poblaciones de *J. thurifera*, relegado a manchas relícticas aisladas geográficamente, podría ser consecuencia de un compromiso entre elevado estrés ambiental y baja presencia de especies competidoras. (Gómez-Manzaneque 1997). Un incremento en las temperaturas y/o reducción en la precipitación como los previstos en la cuenca mediterránea (IPCC 2001; Schröter et al. 2005) podría incrementar la presión de especies competidoras en el límite de distribución inferior de las sabinas, mientras que las condiciones ambientales propias de la alta montaña podrían limitar la migración altitudinal y expondría a esta especie a una extinción en el medio plazo. Para prever la certeza de estas hipótesis es necesario estudiar el efecto de la variabilidad ambiental y geográfica de sus poblaciones actuales, para tratar de encontrar gradientes ambientales indicadores de la reacción de *J. thurifera* a los escenarios de cambio global predichos.

A lo largo de este trabajo describiremos la estructura espacial de las poblaciones de *J. thurifera*, combinaremos métodos descriptivos y experimentales para describir la ecología de la especie a través de todos sus estadios vitales y desde los enfoques fisiológico y funcional. Específicamente, trataremos de contestar a las siguientes preguntas:

Capítulo I: Las mamás son mejores nodrizas que los papás: autofacilitación sexualmente sesgada en un árbol dioico.

- ¿Se distribuyen aleatoriamente en el espacio los adultos y plántulas en las poblaciones naturales de *J. thurifera*?
- ¿Muestran todos los estadios vitales la misma tendencia de regeneración entre microhábitats (*stage coupling*)?
- ¿Está el establecimiento de plántulas limitado por el estrés hídrico en todos los microhábitats?
- ¿Existe interacción entre el estrés hídrico y el microhábitat?

Capítulo II: Cuando, cómo y cuanto: estrategias sexualmente sesgadas de uso de recursos en el árbol dioico *Juniperus thurifera*

- ¿Afecta la madurez sexual al crecimiento radial de anillos?
- ¿Difieren las hembras reproductivas de los machos en su inversión vegetativa?
- ¿Muestran las correlaciones entre precipitación y crecimiento radial de anillos la existencia de estrategias de inversión de recursos diferenciales para machos y hembras?

Capítulo III: Desacoplamiento de la vecería: no todos los episodios de floración vecera resultan en fructificación vecera.

- ¿Muestran machos y hembras el mismo patrón de sincronía, variabilidad y periodicidad reproductiva?
- ¿Son similares para ambos sexos los compromisos de asignación de recursos (*trade-offs*) entre los ciclos reproductivo y vegetativo?
- ¿Existe algún tipo de coste fisiológico de la reproducción para ambos sexos?

Capítulo IV: Los machos gastan mientras las hembras ahorran: la mitigación del estrés revela estrategias funcionales específicas de género en un árbol dioico.

- ¿Existen diferencias fisiológicas y/o funcionales entre machos y hembras?
- Si las hay, ¿son esas diferencias fisiológicas y funcionales el resultado de una demanda de recursos inmediata, o están presentes también durante periodos con inversiones reproductivas similares para ambos sexos?
- Si las hay, ¿cómo afecta la disponibilidad de recursos a la fisiología de machos y hembras de *J. thurifera*?

Capítulo V: El coste de ser relíctico: la viabilidad de semillas se encuentra limitada por la altitud en *Juniperus thurifera*

- ¿Cuáles son los determinantes ambientales y geográficos más limitantes para la viabilidad de semillas de sabina albar?
- ¿Cómo afecta la disponibilidad de recursos a la fertilidad de hembras?
- ¿Cuál podría ser el resultado de un incremento en la temperatura media y una reducción en la precipitación para la fertilidad de las poblaciones de *J. thurifera*?

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I – Las mamás son mejores nodrizas que los papás: autofacilitación sexualmente sesgada en un árbol dioico.

I - Moms are better nurses than dads: sex biased self-facilitation in a dioecious juniper tree

I – Las mamás son mejores nodrizas que los papás: autofacilitación sexualmente sesgada en un árbol dioico.

Introducción La facilitación es frecuente durante los procesos de regeneración en comunidades con fuerte estrés ambiental. Sin embargo, los conflictos semilla-plántula (*seed-seedling conflicts*) y los conflictos entre estadíos vitales son comunes, y la facilitación en un estadío vital puede convertirse fácilmente en competencia en el estadío siguiente. Además, las especies dioicas pueden mostrar patrones de facilitación diferentes dependiendo del sexo de las plantas nodriza. En este trabajo se estudia el árbol dioico *Juniperus thurifera* (la sabina albar) para tratar de contestar las siguientes preguntas: ¿Afecta el sexo de las plantas nodriza a los patrones de regeneración y la estructura poblacional? ¿Hay conflicto entre semillas y plántulas en este proceso de regeneración? ¿Qué factores son responsables de la agregación de estructura espacial de adultos?

Material y Métodos El patrón espacial de adultos de *J. thurifera* se estudió por medio de la K de Ripley. Se usó el análisis de Chi-cuadrado para testar la frecuencia de plántulas en cada uno de los tres microhábitats: bajo hembra, bajo macho, y en espacios abiertos. El patrón observado en poblaciones naturales se explicó por medio de la plantación y estudio experimental de plántulas en cada microhábitat durante dos años. Para cada microhábitat se calculó la probabilidad de supervivencia de cada estadío vital desde la dispersión de semillas hasta la supervivencia al segundo verano.

Resultados El análisis del patrón espacial de adultos indicó que los árboles se distribuían de forma agregada sobre el espacio y que la mayoría de las plántulas se encontraban bajo sabinas hembra. Los estudios experimentales mostraron que desde la dispersión de semillas hasta la supervivencia de plántulas, todos los estadíos vitales muestran la misma tendencia positiva o negativa dentro de un microhábitat dado, indicando acoplamiento entre estadíos (*stage coupling*) y ausencia de conflicto entre semillas y plántulas (*seed-seedling conflict*). Las hembras portadoras de conos maduros ("*frutos*") atraen aves dispersoras que a su vez defecan bajo sus copas haciendo que una cantidad desproporcionada de semillas se dispersen en este microhábitat. Por su parte esta deposición, junto con la deposición de frutos y hojas hace que se encuentre más materia orgánica y nutrientes bajo copa de árboles, y particularmente bajo hembras (N), lo que facilita el establecimiento de plántulas bajo hembras más que bajo machos y ahí más que en espacios abiertos.

Conclusiones En especies dioicas el género de las plantas nodriza puede determinar significativamente la estructura espacial de las poblaciones. En bosques de *J. thurifera* la facilitación bajo hembra ocurre a lo largo de todo el ciclo vital sin signo de conflicto entre estadíos vitales. Los factores más críticos determinando dicha estructura son la dispersión de semillas dirigida a hembras y una mejora ambiental bajo la copa de árboles este sexo.

I - Moms are better nurses than dads: sex biased self-facilitation in a dioecious juniper tree

Introduction Facilitation occurs often during plant establishment processes in communities under strong environmental stress. However, seed-seedling and life stage conflicts are common and facilitation in one life stage often becomes competition in another. Furthermore, dioecious species could show different facilitation patterns depending on sex of nurse plants. We studied the dioecious tree *Juniperus thurifera* in order to answer the following questions: Can gender of nurse plants affect regeneration patterns and spatial population structure? Is there a seed-seedling conflict in the regeneration process? What factors are responsible for the clumped spatial population structure observed for adult *J. thurifera* trees?

Materials and Methods The spatial pattern of adult *J. thurifera* trees was studied by means of Ripley's K analysis. Chi-square analyses were used to test for natural seedling frequency in each of three main microhabitats: under female and male tree canopies and in open interspaces. The observed pattern was explained experimentally by studying seed and seedling survival for two years. Survival probabilities were calculated across life stages for each of three main microhabitats.

Results Adult *J. thurifera* trees were aggregated in space. Most seedlings were found underneath female *J. thurifera* trees. Experimental studies showed that from seed dispersal to seedling survival all life stages showed the same positive or negative trend within a given microhabitat, indicating stage coupling and no seed-seedling conflicts. Attraction of frugivorous birds by reproductive female junipers and improvement of environmental conditions beneath tree canopies were the main factors responsible for the variation in seedling density among microhabitats; highest underneath female trees and lowest in open interspaces.

Conclusions In dioecious species, the gender of nurse plants can significantly determine the spatial population structure. In *J. thurifera* forests, facilitation beneath female trees occurs among all life stages without any sign of seed-seedling conflict. The most critical factors shaping the spatial population structure were directed seed dispersal and environmental amelioration beneath conspecific female trees.

Introduction

Facilitation is common a phenomenon under stressful environmental conditions (Callaway 1995; Callaway et al. 2002). In Mediterranean ecosystems summer drought is the primary environmental stressor and the main cause of juvenile plant mortality (e.g., Herrera et al. 1994; García-Fayos & Verdú 1998; Traveset et al. 2003; Gulías et al. 2004). In these arid environments, nurse plants improve physical conditions and enhance seed germination and seedling survival (Verdú & García-Fayos 1996a; Castro et al. 2004; Gómez-Aparicio et al. 2004) and offer protection from herbivores (García & Obeso 2003).

Facilitation can be species-specific (Callaway 1998), varying in intensity within a species because of phenologically plastic morphology (Callaway et al. 1991; Callaway et al. 2003). However, plant ecologists have rarely compared genders within one plant species in search for gender-specific facilitative interactions (but see Verdú & García-Fayos 1996a; Verdú & García-Fayos 2003; Verdú et al. 2004). Variation in survival between different microhabitats may yield different survival outcomes, resulting in spatial discordance (Jordano & Herrera 1995; Schupp 1995).

Frugivorous birds feed on fleshy fruits of female plants, resulting in disproportionate amounts of seeds being dispersed just below the canopies (Holthuijzen & Sharik 1985; Herrera 1988; Herrera et al. 1994; Nanami et al. 1999; Calviño-Cancela 2002). Post-dispersal seed predators may also attack hosts with different intensities depending on microhabitats (Hulme 1997; Tomita et al. 2002; Hollander & Vander Wall 2004; Gulías et al. 2004). When seeds escape predation by being buried in the soil, their longevity and germination are likely to be influenced by the environmental properties of the soil surface, which vary among microhabitats (Rotundo & Aguiar 2005 and references therein). After seed germination, shading provided by nurse plants protects the seedlings from desiccation by decreasing soil temperatures during summer and enhancing the soil water balance (Callaway 1995; Verdú & García-Fayos 1996a; 2003). Organic matter and total nitrogen content are frequently higher beneath shrubs than in open spaces (Callaway 1995; Joy & Young 2002; Verdú & García-Fayos 2003; Verdú et al. 2004). Furthermore, females of fleshy-fruited species tend to increase nutrient availability beneath their canopies due to fruit deposition and accumulation of disperser’s faeces (Verdú & García-Fayos 1996a; Verdú & García-Fayos 2003).

Previous studies have demonstrated that the spatial population structure is affected by many factors, which may differ among life stages. For each microhabitat the sign and intensity of selection may be coupled or uncoupled across life stages. When coupled, all life stages respond similarly, either positively or negatively, to the microhabitat matrix. Uncoupling occurs when the survival probability from a particular demographic stage deviates from that from previous stages (Jordano & Herrera 1995). These relationships have also been referred to as concordant and discordant, respectively (Schupp 1995).

In this work we describe the heterogeneous spatial population structure of the dioecious *Juniperus thurifera* and use experimental methods to study the processes that affect early life stages in three different microhabitats: beneath male trees, beneath female trees and in open interspaces. Furthermore, we calculate survival probabilities across life stages for each of the three main microhabitats and compare these to the observed spatial pattern to test the robustness of our estimations. Specific questions addressed in this work are: (a) Are adults and seedlings of *J. thurifera* distributed randomly in space? (b) Are regeneration rates higher in some microhabitats than in others? (c) Do all life stages show the same trend among microhabitats (stage coupling or concordance)? (d) Is water stress an equally strong limiting factor for seedling establishment in all microhabitats?

Methods

Study species

Juniperus thurifera is a dioecious long-lived tree with Tertiary relictual distribution throughout the western Mediterranean Basin, usually dominating in high-mountain low-density forests. Trees are typically 5–10 m high and often live for centuries (Bertaudière et al. 1999). Female trees bear fleshy cones with 1–7 seeds (mean=3.5, N=2000). Seed viability and germination are extremely low (Ceballos & Ruiz de la Torre 1979; Melero & García-Fayos 2001). Seeds germinate from cones without bird gut-passage (García-Fayos unpublished data). At least 55% of cones are dispersed by medium-sized passerines of the genus *Turdus* (Santos & Tellería 1994; Santos et al. 1999) which, when overwintering in *J. thurifera* forests, are almost monophagous on *J. thurifera* cones and their abundance and activity increases with increasing juniper crop size (Zamora 1990; Jordano 1993; Santos et al. 1999; García & Ortiz-Pulido 2004). Juniper cones represent only a minor part of the diet of carnivores and ungulates (Santos et al. 1999); although these mammals may travel and disperse seeds long distances, they play a quantitatively inferior role compared to thrushes (Santos et al. 1999).

Study site

The study was performed at La Puebla de San Miguel, Ademuz, in the province of Valencia, southeastern Spain. The study site is situated at 1500 m a.s.l. and is dominated by *J. thurifera*. Natural vegetation covers less than 40% of the rocky soil surface while the rest of the open spaces had herbaceous ephemeral vegetation, lichens and scattered *Thymus* and *Genista* plant individuals. The soils are limestones <30 cm deep, although cracks in the rocks allow roots to reach greater depths (Verdú et al. 2004). The climate is Mediterranean, with cold winters (freezing period of more than 120 days/year) and warm and dry summers. Mean annual total precipitation is 486 mm with October the wettest month (58.6 mm) and July the driest

(26.86 mm). Annual mean temperature is 13º C, with August the warmest month (mean 22.8º C) and January the coldest (mean 4.8º C). The study area was used for agriculture, timber, and extensive livestock grazing for centuries. Narrow valleys were tilled for rye and barley until land abandonment in 1960 (Rodrigo 1999). In the last decades, social and economic changes have motivated a return to forest by abandonment of traditional land uses (Lasanta 1996), although livestock grazing is still noticeable (D. Montesinos, pers. obs.).

Natural population structure

All adult individuals (> 2 m high) in one randomly chosen hectare square were located on a grid and coordinates (to the nearest m of trunk centers) were recorded. Spatial population structure was assessed by Ripley's *K(r)* test. Any individual more than 2 m high was considered an adult tree and classified as male, female or non-reproductive. The analysis was done with the freeware SPPA 2.0 designed by Dr. P. Haase (http://haasep.homepage.t-online.de/). Ripley's *K(r)* compares the observed number of plants within a distance *r* of an arbitrary plant with the expected number of plants within that distance for randomly distributed plants. Observed densities above the critical interval for a random distribution indicate significant clumping while the converse indicates significant overdispersion. To facilitate interpretation, the derived Ripley's *K* sampling statistic [$\sqrt{(K(r)/\pi)}-r$] was used, which has zero expectation for all *r* when the pattern is random.

In the spring of 2004 we intensively sampled seedlings and saplings in a square with total surface area of 2 ha which included the 1 ha sampled for the study of adult population spatial structure. For practical reasons the sampled area was subdivided into subplots 30 m wide and 10 m long. In this 2-ha square we recorded the proportion occupied by of each three microhabitats along 66 linear transects, each 30 m long and 10 m apart. Microhabitats were: beneath male trees (18% of the soil surface), beneath female trees (10% of the soil surface) and open interspaces (71% of the soil surface), for simplicity referred to as male tree, female tree and open, respectively. All seedlings (plants with needle-like leaves only, up to about 3 years old) and saplings (non-reproductive plants less than 2 m in height, with cypress-like leaves) were counted and the microhabitat in which they were located was typified by inspection. Microhabitat proportions were used for the calculation of the expected probabilities of seedling occurrence in Chi-square tests. Analyses were made with the statistical software R, version 2.3.0 (Ihaka & Gentleman 1996 www.r-project.org). This type of Chi-square test compares and calculates the expected probabilities according to an a priori given proportion (i.e. relative proportion of microhabitats). We tested if seedlings and saplings were found more often under adults than in open spaces and under females more often than under male adults. Adults without signs of recent reproduction, even without remains of fallen cones under their canopies, were classified as non-reproductive. Cones are resinous and seeds have a thick seed coat. Hence, cone decomposition is slow and females that had reproduced recently were characterised by presence of cone remains and/or seeds around their stems (D. Montesinos, pers. obs.). We assumed that individuals classified as non-reproductive had not been a seed source for years, and that these were influencing their immediate surroundings the same way males do. Consequently, they were grouped with males for this analysis.

Microhabitat characterization

In the summer of 2004 we randomly selected across the 2-ha square 15 points in each of the three microhabitats to record: photosynthetically active radiation (PAR) at ground level, soil bulk density, soil compaction and three chemical soil properties (organic matter, total nitrogen and available phosphorous). PAR was measured on a totally clear day at noon with a quantum sensor (Skye Instruments, Llandrindod-Wells, UK) as the mean of three measurements. Soil compaction was measured with a penetrometer (Eijkelkamp type IB, Giesbeek, NL) with conic head (measurement range 5 to 400 Newtons cm^{-2}) as the mean of 10 measurements. Data were log-transformed to achieve a homogeneous variance distribution and approximate normal distribution of errors. Soil bulk density was calculated as the dry weight of the soil contained in cylindrical cores 5 cm deep by 5 cm of diameter, divided by the volume of that cylinder. The samples used for soil bulk density determination were also used for soil chemical properties, using the Walkley, Kjeldahl and Olsen procedures for quantification of organic matter, total N and available P, respectively (Page 1982). Generalized Linear Models with Gaussian distribution of errors were fit to the data to test differences among microhabitats, when necessary with Tamhane post-hoc analyses (Zar 1996). The SPSS statistical software Version 11.0 (Norusis 2002) was used.

Seed dispersal

During the peak of the dispersal period (December 16, 2003; January 14, 2004 and February 4, 2004), we searched for fresh bird faeces and regurgitations containing *J. thurifera* seeds on a random linear transects one m wide with total area of 1196m^2, distributed across the 2-ha square. Only fresh excrements (i.e. from the same day) were considered. For each m^2 along each transect microhabitat type was visually classified, and faeces were collected and visually classified by dispersal agent. In the laboratory, seeds were hand extracted and counted. According to our hypothesis, birds direct dispersal towards trees as a result of regurgitation and defecation under the perches where they rest, and towards females, as birds are attracted to trees that bear fleshy fruits (Herrera 1988; Herrera et al. 1994; Verdú & García-Fayos 1996a; Verdú & García-Fayos 2003). Accordingly, the spatial distribution of dispersed seeds was expected to be consistent among years (Nathan & Muller-Landau H.C. 2000; Hampe 2004). Chi-square tests for observed vs expected numbers of observations (the latter calculated from proportion of area occupied by each microhabitat) were performed to assess differences in seed dispersal between open spaces and under tree canopies, and between female and male (including non-reproductive) tree canopies.

Post-dispersal seed predation

Sets of five seeds were offered to ants and mice. Seeds offered to ants (N=400) were placed randomly across the studied population inside Petri dishes with a 5-mm wide hole and a small stone over the lid in order to protect them from removal by rain or wind. Seeds offered to mice (N=400) were glued with Loctite® to a plastic mesh and nailed to the soil. Twenty dishes and 20 meshes were randomly placed under different male and female trees, 40 dishes and 40 meshes in open space which occupies more than 60% of the area. Seed removal was recorded monthly from 12 December 2002 to 30 September 2003. Because many units were destroyed by livestock, we also placed sets of 5 dishes and 5 meshes under each of male and female trees and 10 dishes and 10 meshes in open spaces inside fenced areas. Recorded was made from 28 April 2003 to 30 September 2003. There were no statistically significant differences between fenced and non-fenced treatments and all data were pooled for statistical analysis. Generalized Linear Models with Poisson distributions of errors (McCullagh & Nelder 1989) were fit to data for each experiment (each predator) to test if seed removal and predation differed among microhabitats.

Soil seed bank

Four mesh bags, each with 250 seeds, were buried in the soil at 5 cm depth in October 2002. Mean seed viability at that time, visually assessed by judgement if seeds were filled or not, was determined from a sub-sample of 100 seeds to be 5%. Seeds were extracted from the soil in April 2005 and viability was again checked. Our visual assessment of viability was compared with the standard tetrazolium test for viability and no difference between the two procedures was found (D. Montesinos, unpublished results). Seed germination normally occurs in the field 16–18 months after seed dispersal. We therefore assumed viability after 30 months to provide a reliable estimate for the relative size of the seed bank of *J. thurifera* at the second germination period after seed dispersal.

Seedling emergence

Sets (female and male trees: 10 sets each; open: 19 sets) of 30 viable seeds (non-viable seeds, i.e. that floated in water, were discarded), collected in the field at the study site in October 2002, were sown at random locations in the field in November 2002 at 1 cm depth under 50 x 50 cm wire mesh cages to prevent seed predation by mice. Mean seed viability after seed selection by flotation was 35% (N=100). In each microhabitat half of the cages (nine in open microsites) received a watering treatment consisting of 5 litres of water every month from March to October, except in July and August, the months with the greatest water deficit, in which they received 5 litres twice per month. This treatment increased the annual water supply by 40%. Final seedling emergence was recorded in July 2004. GLMs with Poisson distributions of errors were used to test if microhabitat and/or watering treatment had an effect on seedling emergence.

Seedling survival

In early April 2003, 15-month old seedlings were manually planted at a density of 10 seedlings per 10 m^2 plot. Each seedling was planted 1 m from the next nearest seedling. Plots were fenced to exclude herbivory. Sample sizes were: open (20 plots, N=200 seedlings), male trees (10 plots, N=100) and female trees (10 plots, N=100). Half the plots received a watering treatment consisting of 1 l of water per seedling per month until October, except in July and August when they received 1 l twice per month. Water was poured immediately below the seedlings. If we consider a seedling's root influence area to be 25 x 25 cm, each watering event corresponded to addition of 16 mm of precipitation. This watering treatment, repeated 10 times per year, was equivalent to an increase in annual precipitation of approximately 30%.

An additional set of 20 plots (10 plots in the open, five under males and five under females; N=200 seedlings) were exposed to herbivory by sheep and goats. None of these seedlings received a watering treatment. On 6 November 2003 and on 22 September 2004 survival was recorded. During the experiment four plots from which herbivores were excluded, were lost. Data were analyzed with GLM with binomial distributions of errors. First we analyzed the effect of herbivory on seedling survival. As no significant effect was found, data from seedlings exposed to herbivores and from exclusion areas were pooled and re-analyzed with GLM for the survival of seedlings over the first and the second summer, depending on the watering treatment and the microhabitat. Microhabitat was considered as an ordered factor following the expected pattern (open<male trees<female trees), as seedling survival was expected to be higher beneath trees where environmental conditions were expected to be more favorable, especially beneath female trees where soil nutrient concentrations were expected to be higher than in the open due to enrichment by cone deposition (Verdú & García-Fayos 1996a; Verdú & García-Fayos 2003 and references therein).

Transition probabilities

Transition probabilities (indicating survival probabilities) were calculated for each microhabitat for the following life-stage transitions: (1) proportion of seed rain in each microhabitat; (2) survival to post-dispersal seed predation; (3) seedling emergence (4) seedling survival to the first year and (5) seedling survival to the second year. Seedling emergence was extremely low, zero in some treatments. To avoid null values in some columns of the transition matrix, we assigned a single mean seedling emergence value to all columns since no statistical differences were detected among microhabitats. This mean value was similar to other germination studies with *J. thurifera* seeds in natural conditions (P. García-Fayos, unpublished data). The product of all of individual transition probabilities is the probability with which dispersed seeds arrived at a specific microhabitat and developed into established saplings two years later.

To assess the reliability of the cumulative transition values, we set up simple simulations for each microhabitat, each rerun 1000 times, using values for each transition randomly generated from a normal distribution, with means and standard deviations derived from the mean transition values for the microhabitat. Negative values on the left tail of distributions were meaningless and hence truncated to zero. A confidence interval of 95% was then established for each of the six sets of 1000 values each, and significant differences between microhabitats were established to the 0.05 significance level.

Independent validation of the precision of results was made by comparing expected values obtained from transition probabilities with available data of crop size from three years in the study site. To do so, we assumed that all factors were equal among years, and applied transition probabilities to crop sizes for years 2000 to 2002 (2000=37,500 cones/ha; 2001=163,900 cones/ha; 2002= 141,900 cones/ha). In year 2003 crop size was 9,740 cones per ha. In that year, we directly measured an average of 0.78 dispersed seeds/m^2. Projecting that proportion to crop size from previous years we estimated dispersed seed densities of 3.18 seeds/m^2, 15.35 seeds/m^2, and 13.29 seeds/m^2 for years 2000, 2001 and 2002, respectively. We applied those values to the transition rates with the following considerations: for the year 2000, we assumed that seedling survival from the third year was the same as for the second year; for the year 2002, transition probabilities were calculated only up to first year seedling survival. Since seeds germinate 16 months after dispersal and seedlings remain in the needle-leaved state up to 3 years we could estimate the expected density of seedlings up to 3 years old. We summed final estimations of seedling densities from the three cohorts and obtained the expected seedling densities. Then, we compared these densities with the results of observed seedling densities.

Results

Natural population structure

Adult *J. thurifera* density in the study area was 269 trees per ha. Adult population structure showed significant aggregation, notably for spatial distances below 4 m (Fig. 1.1).

Seedling and sapling occurrence (N=161 and N=587, respectively) were strongly dependent on the microhabitat (Fig. 1.2). Seedlings were found beneath trees more than twice as often as in open interspaces (X^2_1= 31.3, $p<0.001$) and threefold more often beneath female trees than beneath male trees (X^2_1= 20.9, $p<0.001$). A similar trend was found for saplings both under tree canopies *vs.* open (X^2_1= 22.2, $p<0.001$) and beneath female *vs.* male trees (X^2_1=12.7, $p<0.001$).

Microhabitat characterization

Open interspaces had significantly greater PAR than microhabitats underneath trees and differed significantly also in all other variables, presenting less favorable soil conditions for plant growth (Table 1.1). The only measured variable for which significant differences between male and female trees was observed was soil nitrogen concentration (63% higher under female than under male trees).

Seed dispersal

We extracted a total of 532 seeds from 283 units of faeces. A mean of 0.06 seeds/m^2 were dispersed to open habitats; 0.55 seeds/m^2 to underneath male trees and 1.74 seeds/m^2 to underneath female trees. Significantly more seeds were dispersed under trees than into open (X^2_1=712.2, $p<0.001$), and seed dispersal to female trees was significantly greater than dispersal to male trees (X^2_1=137.4, $p<0.001$).

Post-dispersal seed predation

Final seed predation differed among microhabitats (Table 1.2; X^2_{77}=10.8, $p<0.001$). Significantly more seeds were harvested by mice in open spaces than under trees ($p<0.001$). Seed coat remnants eaten by mice were often found around the experimental sets, evidencing effective seed predation. Seed predation by mice did not differ between male and female trees ($p=0.815$). Seed removal by ants did not differ among the studied microhabitats (X^2_{63}=21.41, $p=0.351$).

Soil seed bank

Initial seed viability just after seed collection was 5%. Only three out of 1000 buried seeds (0.3%) were still viable 30 months after collection.

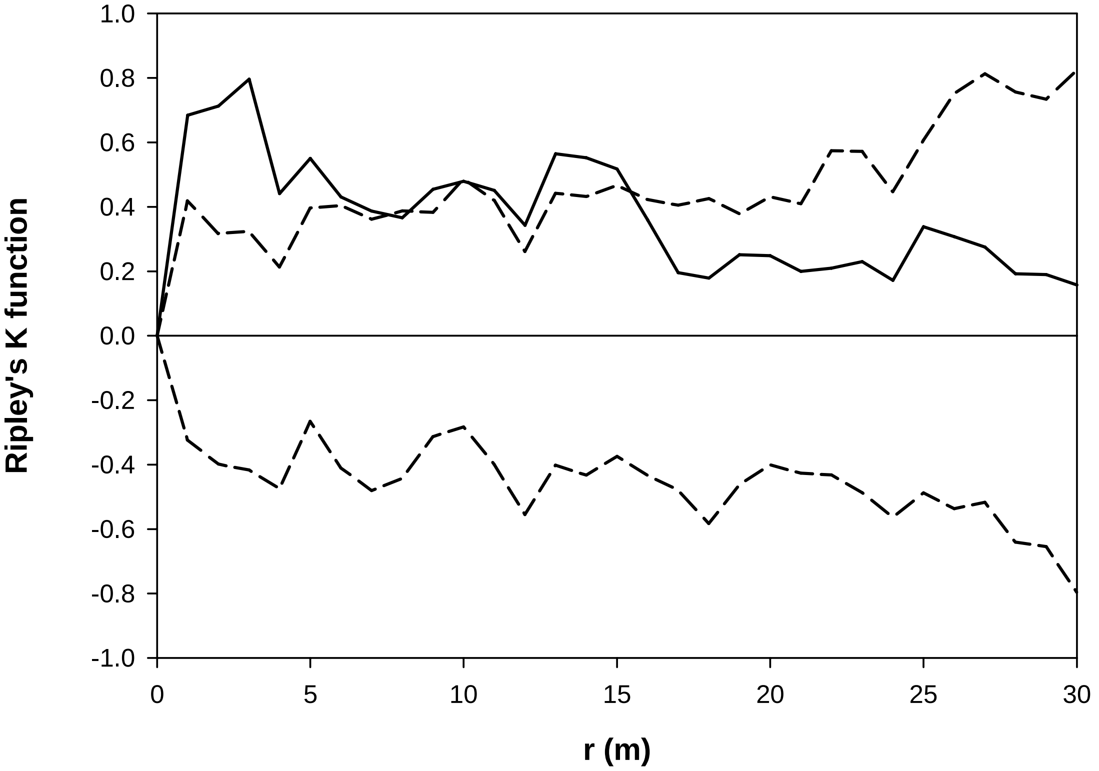

Fig. 1.1. Tree density is higher than expected at low distances and especially below 5 m. Ripley's *K* compares the observed number of plants within a distance of an arbitrary plant with the expected number of plants within that distance in a random distribution. To facilitate interpretation, Ripley's *K* function [√(K(r)/π)-r] is plotted for which the zero line represents the expected density of plants for each distance *r* (in meters) in a random spatial distribution. Dashed lines represent the 95% critical interval for a random distribution. The solid line shows the observed distribution of the studied population. Positive values indicate plant aggregation and negative values overdispersion.

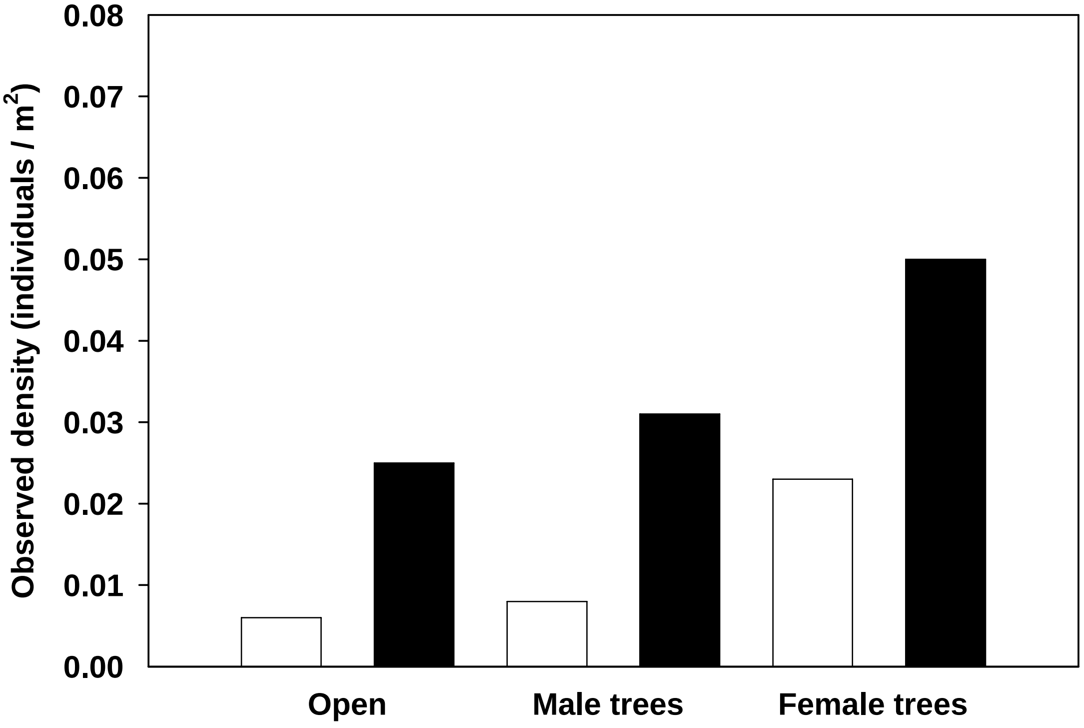

Fig. 1.2. White bars represent observed seedling densities and black bars observed sapling densities. Both are significantly higher below trees than in open and higher below female trees than anywhere else.

Table 1.1. Open spaces had greater PAR but less favorable soil properties than sites under tree canopies. Mean values ± standard deviations are given for each environment. F values for the GLM are given (df=2); different letters indicate significant differences among microhabitats according to the post-hoc analyses ($p \leq 0.05$).

	Open	**Male trees**	**Female trees**	**F**
PAR (μMol/m^2/sec)	1481.6±404.9^a	56.2±17.5^b	190.4±385.1^b	89.2***
Soil compaction (N/cm^2)	27.30±20.36^a	0.65±1.29^b	1.53±3.06^b	24.2***
Soil bulk density (g/cm^3)	0.62±0.09^a	0.34±0.17^b	0.34±0.16^b	16.0***
Organic matter (%)	13.41±5.04^a	37.90±14.29^b	35.54±16.08^b	16.7***
P_2O_5 (mg/100g soil)	3.11±1.36^a	9.22±3.82^b	7.72±3.28^b	16.8***
N_T (%)	0.81±0.27^a	1.47±0.47^b	2.34±0.87^c	25.3***

*** $p \leq 0.001$

Table 1.2. Percent of mean seed removal (± SD) by mice and ants for each microhabitat. Different letters indicate significant differences ($p<0.05$) among microhabitats.

	Open	**Male trees**	**Female trees**	***Total***
Mice	83.00±18.42%^{a}	50.00±37.10%^{b}	48.18±26.66%^{b}	66.00±30.75%
Ants	17.58±24.87%^{a}	6.67±9.76%^{a}	26.67±31.44%^{a}	17.58±25.18%

Seedling emergence

Seedlings emerged 16 months after sowing (spring 2004). Only eight seedlings emerged out of the 1170 sown seeds: five under non watered females; one under a watered female and two in non watered open spaces. GLM with Poisson distribution of errors did not show any effect of microhabitats (t_{35}=1.7, p=0.10) or watering (t_{35}=-0.007, p=0.99) on seedling emergence.

Seedling survival

Survival of planted seedlings (N=600) varied among watering treatments, microhabitats and years (Fig. 1.3). Herbivore exclusion neither affected seedling survival the first year (42±23% vs. 29±8% in excluded vs. non-excluded plots respectively; estimate= -0.29±0.55, t=-0.53, p=0.599) nor the second year (94±12 vs. 84±35 in excluded vs. non-excluded plots respectively; estimate= -0.35±0.98, t=-0.36, p=0.724). Watering had a significantly positive effect on seedling survival the first year (estimate= 2.04±0.41, t=4.99, p<0.001), increasing survival almost four fold. In contrast, differences were not significant the second year (estimate= 6.75±1065.51, t=0.01, p=0.995). Seedling survival was significantly higher under female than under male trees, and higher under male trees than in the open (linear contrast 1.80±0.57, t=3.14, p<0.005). Differences were not significant in the second year (linear contrast 0.91±0.77 t=1.81, p=0.248). No significant interaction between microhabitat and watering treatments was observed (linear contrasts: first year: 0.03±0.72, t=0.04, p=0.966; second year: 0.51±1.03, t=0.49, p=0.628).

Transition probabilities

Transition (survival) probabilities between subsequent life stages revealed that seeds were dispersed more often, and that seeds and seedlings survived more often, under female than under male trees, and under male trees more often than in open spaces (Table 1.3). Cumulative survival probabilities were enhanced by watering and were higher underneath female trees than in other microhabitats. Confidence intervals (95%) showed two independent subgroups: open microhabitats (open-no watering: $1.6{\cdot}10^{-8}$ to $7.7{\cdot}10^{-6}$; open-watering: $4.4{\cdot}10^{-7}$ to $5.8{\cdot}10^{-5}$); males overlapping between the two subgroups (under male-no watering: $9{\cdot}10^{-6}$ to $8{\cdot}10^{-4}$; under male-watering: $4.3{\cdot}10^{-5}$ to $1.6{\cdot}10^{-3}$) and females on a second subgroup (under female-no watering: $4.3{\cdot}10^{-5}$ to $3.3{\cdot}10^{-3}$; under female-watering: $3.4{\cdot}10^{-4}$ to $5.4{\cdot}10^{-3}$).

When compared, predicted and observed seedling densities were closely similar (Fig. 1.2 and Fig. 1.4), although observed seedling density in open was higher than expected. A one-sample t-test was made between the observed and expected (not watered) values of seedling density. There were no statistically significant differences between observed and expected seedling densities under male (t_{64}=1.16, p=0.250) and female microhabitats (t=-0.04, df=64, p=0.964). Expected and observed values for open areas were significantly different (t_{64}=5.28, p<0.001).

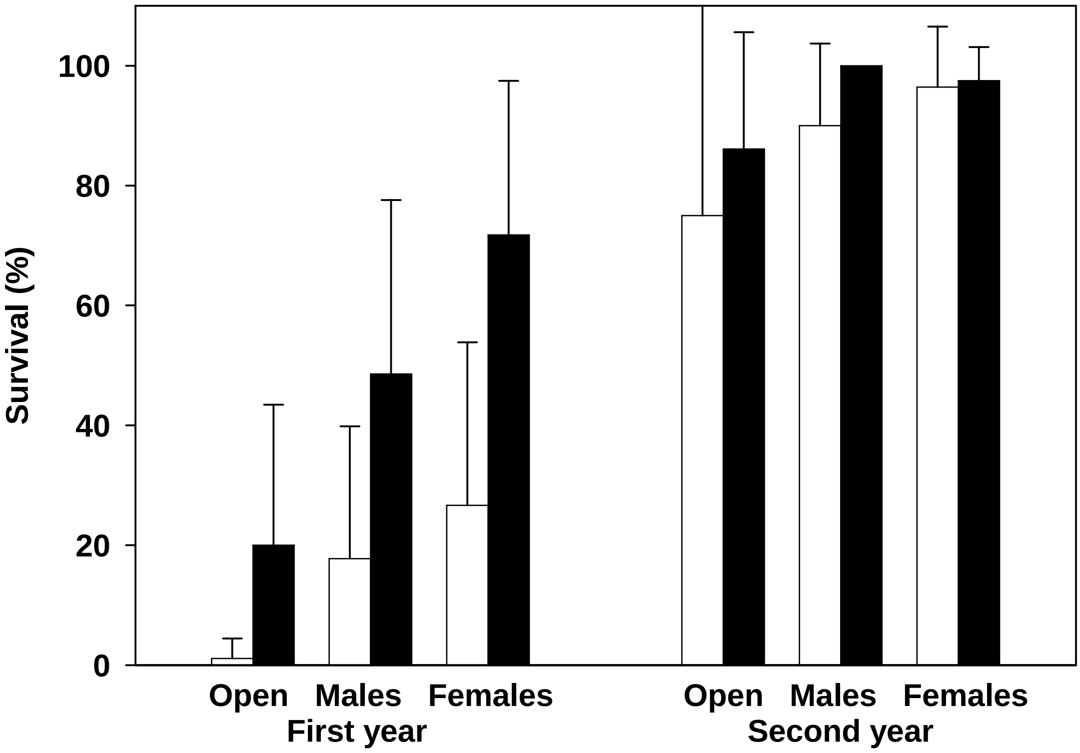

Fig. 1.3. Mean percentages of seedling survival ± SD are given. White bars represent controls and black bars watered seedlings. Watering regime and environment significantly affected seedling survival during the first year, but not during the second.

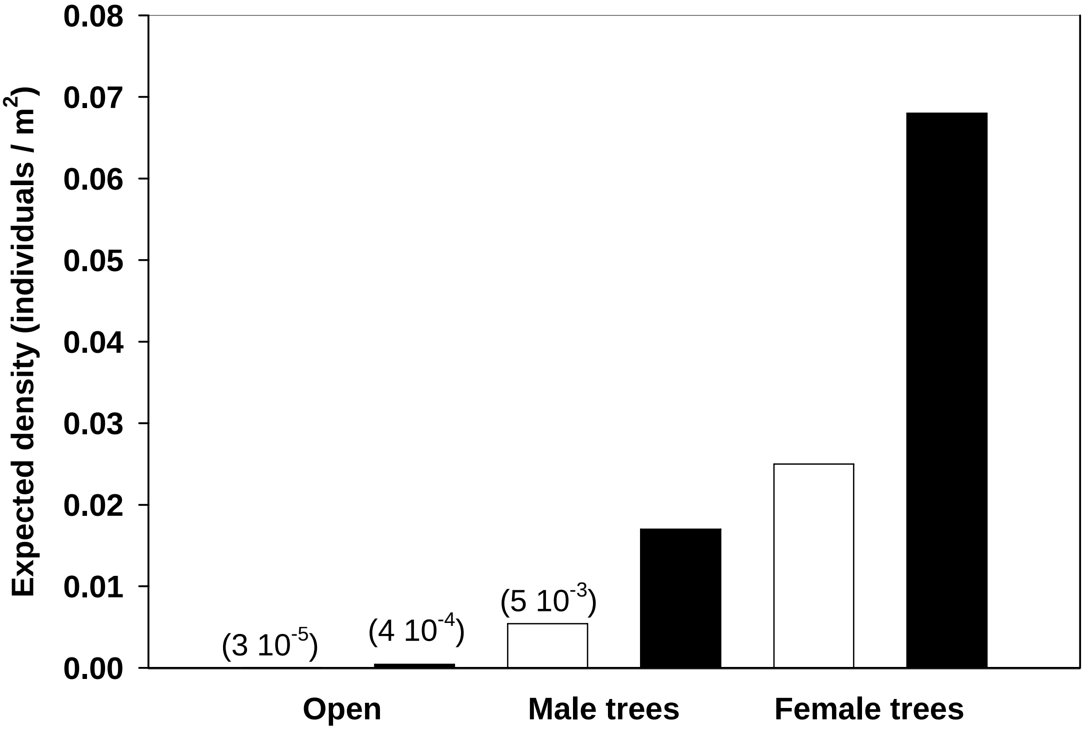

Fig 1.4. Expected seedling density (seedlings/m^2) in each environment and watering treatment based on probabilities from Table 3 and considering crop size from years 2000, 2001 and 2002. White bars represent expected seedling density under natural conditions and black bars when watered.

Table 1.3. Transition probabilities between subsequent life stages of *J. thurifera* (and cumulative values in brackets), showing a clear pattern of spatial discordance and a strong degree of stage coupling.

	Open		**Male trees**		**Female trees**	
	No watering	**Watering**	**No watering**	**Watering**	**No watering**	**Watering**
Seed rain	0.02		0.24		0.74	
Post-disp. seed pred.	0.47 (0.01)		0.70 (0.16)		0.62 (0.45)	
Seedling emergence	$6.8\cdot10^{-3}$ ($8.0\cdot10^{-5}$)		$6.8\cdot10^{-3}$ ($1.1\cdot10^{-3}$)		$6.8\cdot10^{-3}$ ($3.1\cdot10^{-3}$)	
Seedling surv. 1st yr.	0.02 ($1.3\cdot10^{-6}$)	0.20 ($1.6\cdot10^{-5}$)	0.18 ($2.0\cdot10^{-4}$)	0.49 ($5.4\cdot10^{-4}$)	0.27 ($8.2\cdot10^{-4}$)	0.72 ($2.2\cdot10^{-3}$)
Seedling surv. 2nd yr.	0.75 ($0.9\cdot10^{-6}$)	0.86 ($1.4\cdot10^{-5}$)	0.90 ($1.8\cdot10^{-4}$)	1.00 ($5.4\cdot10^{-4}$)	0.96 ($7.9\cdot10^{-4}$)	0.97 ($2.2\cdot10^{-3}$)

Discussion

All life stages from seed dispersal to seedling establishment are favored underneath *J. thurifera* female conspecifics, resulting in a clumped adult population spatial structure.

As expected, and in agreement with previous studies with *J. sabina* (Verdú & García-Fayos 2003), birds dispersed significantly more seeds beneath females than beneath males and there more than in open spaces.

Seed removal by ants was similar among microhabitats (18%), in agreement with other studies (Rey et al. 2002 and references therein). Predation by the omnivorous and highly efficient mice (Hansson 1985; Santos & Tellería 1991) did, on the other hand, result in removal of of 66% of the available *J. thurifera* seeds, proportionally more in open spaces than under tree canopies. This contrasts the results of a majority of studies (e.g. Alcántara et al. 2000; Rey et al. 2002 and references therein) in which seed predation is often more intense under parent plants than elsewhere. The behavior of granivores is affected by many factors, among other interactions between altitude, temperature, microhabitat, predation risk, dependence of stable refuges and food accessibility (Diaz 1994; Verdú & García-Fayos 1996b; Rey et al. 2002). A full understanding of factors affecting post-dispersal seed predation of *J. thurifera* seeds requires further research.

Seed viability was negligible after the first germination period (0.3%), in accordance with other studies of *J. thurifera* (Orozco Bayo 1999). Accordingly only current seed rain appears to play a role in *J. thurifera* regeneration.

Because of extremely low seedling emergence in our experiments, no significant differences among treatments were found. On the other hand, experimentally planted seedlings survived more often under female than under male trees, and more often under male trees than in the open. Statistical analysis of the spatial distribution pattern of naturally occurring seedlings supported these experimental results. Seedling mortality was highest during the hot and dry summer months, as in other Mediterranean plants (Herrera et al. 1994; García-Fayos & Verdú 1998; Traveset et al. 2003) and other *Juniperus* species (Jackson & Van Auken 1997; Joy & Young 2002). Watering experiments greatly improved seedling survival and differences in seedling survival among microhabitats were reduced when seedlings were regularly watered. Furthermore, watering increased seedling survival proportionally more in the most unfavorable microhabitat. These results support the hypothesis that improvement of environmental conditions will attenuate facilitation (Bertness & Callaway 1994; Bertness & Yeh 1994; Greenlee & Callaway 1996; Ibáñez & Schupp 2001), and the general view that improvement of environmental conditions, notably reduction of water stress under nurse plants, are important aspects of facilitation in Mediterranean environments (Verdú & García-Fayos 1996a; García et al. 2000; García-Fayos & Gasque 2002;

Castro et al. 2004; Gómez-Aparicio et al. 2004). However, even with watering, the observed seedling density was higher than expected in open spaces. Coefficients of variation for seedling survival in open spaces were two-fold higher than those from male or female trees. This indicates greater unpredictability of performance in open interspaces, and may explain the difference between expected and observed seedling density in this microhabitat. Nonetheless, expected and observed data for the rest of the microhabitats are very similar.

Herbivory is known to affect plant spatial distributions (McAuliffe 1986; Ostfeld & Canham 1993; García et al. 2000) but no significant effect of herbivory on seedling survival is found in our study. Even though livestock densities are high in the study area, naturally occurring seedlings are often seen. Other authors have found similar results for another *Juniperus* species (Jackson & Van Auken 1997; although see Joy & Young 2002) and suggest that unpalatability of junipers may restrict herbivory.

Adult population structure was aggregated at fine spatial scales, with peak aggregation at 4 m. This distance coincides with tree canopy dimensions, under which seedling survival is enhanced. However, in the long term individuals growing under parental trees may experience reduction of illumination and competition for water and other resources as growth limitations (Callaway 1995; Jackson & Van Auken 1997; Verdú et al. 2004). Adult *J. thurifera* trees have been reported to be affected by competition (Pavón-García 2005). Thus, a turning point may occur in the life cycle of *J. thurifera,* at which life-stage conflicts weakens the strongly biased spatial pattern observed for seedlings, finally resulting in the slight aggregation observed for adult trees.

Conclusions

Both observational and experimental data show that the population structure of *J. thurifera* forests is shaped substantially by female trees, which attract dispersers, ameliorate environmental conditions and improve survival of young conspecifics. This accords with previous studies in which spatial discordance related to particular microhabitats has been attributed to effects of dispersal agents or improved environmental conditions (Jordano & Herrera 1995; Schupp 1995; Clark et al. 1999; Rey & Alcántara 2000; Traveset et al. 2003). However, life stages may or may not be coupled and most studies have found, to some degree, stage uncoupling or seed-seedling conflicts (Harper 1977; Herrera 1991; Schupp 1995; Rey & Alcántara 2000; Traveset et al. 2003). Our study is, to our knowledge, the first study in which absence of seed-seedling conflicts have been reported from Mediterranean habitats.

Facilitation seems to play a major role in regeneration of other *Juniperus* species in environments with limited water availability (see for instance Jackson & Van Auken 1997). A model in which survival probability decreases away from parent plants was proposed by

McCanny (1985) as a variation of the basic Janzen-Connell model for parent-offspring relationships. Patterns that accord with the McCanny model have rarely been observed, but our data conform to this model. Such a pattern may be quite common in semi-arid environments in which survival is strongly determined by water availability, and notably for species narrowly adapted to particular microhabitats (Turkington 1979; Montesinos et al. 2006).

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II - Cuando, cómo y cuanto: estrategias sexualmente sesgadas de uso de recursos en el árbol dioico *Juniperus thurifera*

II - When, how and how much: gender specific resource use strategies in the dioecious tree *Juniperus thurifera*

II - Cuando, cómo y cuanto: estrategias sexualmente sesgadas de uso de recursos en el árbol dioico *Juniperus thurifera*

Introducción Machos y hembras de especies dioicas experimentan diferentes fuerzas selectivas y a menudo incurren en costos reproductivos diferentes. Un incremento del gasto reproductivo habitualmente conduce a una reducción de los recursos disponibles para otras demandas biológicas, como el crecimiento vegetativo. Los anillos de crecimiento de árboles constituyen una medida integradora que permite resumir la inversión vegetativa a lo largo de la vida de la planta. Por ello, en especies dioicas, la medida de anillos de crecimiento radial permite el estudio de estrategias de asignación de recursos específicas de género.

Material y Métodos Se utilizaron metodologías standard de medida de crecimiento de anillos. Los análisis estadísticos de las series temporales se realizaron con Modelos Generales Mixtos con corrección de valores autocorrelacionados por medio de una estructura de covarianza autoregresiva de primer orden. Se utilizaron funciones de correlación *Bootstrap* para estudiar la relación entre el clima y el crecimiento de anillos.

Resultados Machos y hembras invirtieron una cantidad similar de recursos al crecimiento de anillos durante los primeros estadíos vitales de las plantas. Sin embargo, tras alcanzar la madurez sexual, el crecimiento de anillos se redujo en ambos sexos. Las hembras experimentaron una reducción significativamente mayor que los machos, lo que indica una asignación de recursos menor para el crecimiento vegetativo de hembras. Además, el crecimiento de anillos de árboles macho se correlacionó positivamente a la precipitación de primavera e invierno del año en curso, mientras que las hembras correlacionaron su crecimiento únicamente con las precipitaciones de la primavera del año en curso.

Conclusiones Una vez se alcanza la madurez reproductiva, los anillos crecen proporcionalmente más en machos que en hembras. Estas diferencias entre sexos podrían constituir una estrategia de respuesta a los diferentes costos reproductivos de machos y hembras. Por lo tanto, y contestando a las preguntas de cuando, como y cuanto enunciadas en el título, encontramos que los árboles macho invierten más recursos a crecimiento que las hembras pero únicamente tras alcanzar la madurez reproductiva, y utilizan esos recursos con un patrón temporal diferente al de hembras.

II - When, how and how much: gender specific resource use strategies in the dioecious tree *Juniperus thurifera*

Introduction In dioecious species male and female plants experience different selective pressures and often incur in different reproductive costs. An increase in reproductive investment habitually results in a reduction of the resources available to other demands, like vegetative growth. Tree ring growth is an integrative measure which tracks vegetative investment among the plant's entire life-span. This allows the study of gender-specific vegetative allocation strategies in dioecious tree species among life stages.

Material and Methods Standard dendrochronological procedures are used to measure tree ring-width. Analyses of time-series are made by means of General Mixed Models with correction of autocorrelated values by using an autoregressive covariance structure of order one. Bootstrapped correlation functions are used to study the relation between climate and tree-ring width.

Results Male and female trees invest a similar amount of resources to ring growth during the early life stages of *Juniperus thurifera*. However, after reaching sexual maturity, tree-ring growth is reduced for both sexes. Furthermore, females experience a significant stronger reduction in growth than males which indicates a lower vegetative allocation in females. In addition growth was positively correlated to precipitation from the current winter and spring in male trees but only to current spring in females.

Conclusions Once sexual maturity is achieved, tree-rings grow proportionally more in males than in females. Differences in tree-ring growth between genders could be a strategy to respond to different reproductive demands. Therefore, and responding to the questions of when, how and how much asked in the title, we show that male trees invest more resources to growth than female trees only after reaching sexual maturity, and they use these resources in a different temporal way.

Introduction

Reproduction is expensive to plants and often is inversely correlated to vegetative growth, indicating that both biological functions compete for the same resources (Harper 1977; Koenig & Knops 1998). Plants may compensate for reproductive efforts by improving physiological performance, reducing the number of reproductive events or reducing resources allocated to growth and maintenance (Obeso 2002). Nevertheless, detection of reproductive costs in the short term might be difficult due to physiological compensation mechanisms (Obeso & Retuerto 2002). Therefore, long term cumulative variables like tree ring width are widely used as a proxy of vegetative investment in woody plants (e.g. Obeso 1997; Silvertown & Dodd 1999).

However, little attention has been given to the effect of reproduction on tree-ring growth in dioecious species. Costs of reproduction are age specific and reproductive investment is variable during plants' lives (Silvertown & Dodd 1999). Furthermore, plants of different sexes might incur different reproductive costs and thus present different trade-offs between reproduction and growth and develop different resource-use strategies (Obeso 2002). Though, little attention has been drawn to the interaction between age and gender in dioecious species and most investigations have not estimated how tree-ring growth varies with size or age at all (Silvertown & Dodd 1999; but see Bañuelos & Obeso 2004).

The seasonal scale of resource investment can play a significant role in gender-specific resource allocation. In dioecious species males incur the maximum reproductive effort at the time of flower production. Once pollen is shed, males do not need to invest more resources into current reproduction. In contrast, the main reproductive effort for females starts once ovules are pollinated because fruit maturation and ripening is a costly task compared to flower production (Verdu & Garcia-Fayos 1998; Obeso 2002). Thus, in the short-term, males and females are expected to manage available resources differently. The relationship between tree-ring growth and precipitation from previous months can help us to discern how different sexes manage resource availability because precipitation is a proxy of resource availability in semi-arid environments that is positively correlated to tree-ring growth in most species (Cherubini et al. 2003; Austin et al. 2004).

J. thurifera is a dioecious tree inhabiting high mountain semi-arid areas. Flowering occurs during February and female cone growth and ripening takes 20 months. Tree-ring growth in this species has been reported to be positively correlated with precipitation from the previous year (Bertaudière et al. 1999). Pavón-García (2005) sampled cores from *J. thurifera* individuals of different reproductive stages and determined that reproductive maturity in this species started at the age of 31±3.6 (N=223). These characteristics make *J. thurifera* particularly appropriate to study age and gender specific reproductive trade-offs and climatic relations to stem growth.

The objectives of our research are to determine the effect of reaching sexual maturity in tree-ring growth of each gender of the dioecious tree *Juniperus thurifera* on stem growth, and to detect the resource-use strategies developed to deal with reproductive demands by each gender. More specifically we want to answer the following questions regarding resource investment: (a) *When*: Does sexual maturity affect tree ring growth? (b) *How much*: Do reproductive females differ from males in vegetative investment? and (c) *How:* Do climatic relations between precipitation and ring growth reveal gender specific resource allocation strategies?

Materials and Methods

Study species

Juniperus thurifera L. (Cupressaceae) is a dioecious long-lived tree with relict distribution from the Tertiary throughout the western Mediterranean basin. Together with two closely related vicariant species with similar biology (*J. excelsa* and *J. procera*) they distribute along the Mediterranean basin, western Asia and eastern Africa. It is usually the dominant species in high elevation low-density forests with semi-arid climate. Trees are 5-10 m high and often live for centuries (Bertaudière et al. 1999). Trees reach sexual maturity at the age of 31±3.6 (N=223) (Pavón-García 2005). Reproductive output varies strongly from year to year; male and female trees flower at the end of the winter period and after winter pollination females produce reproductive cones over a period of 20 months when seed dispersal occurs (Amaral-Franco 1986 and P. García-Fayos pers. obs.). Gauquelin *et al.* (2002) found that males grew significantly more than females comparing the ring-widths of 3 males and 3 females from Morocco (t-test, $p<0.01$); however in that population females were taller ($p=0.056$) than male trees.

Study site

The study was performed at La Puebla de San Miguel, Valencia, Spain. The study site covers approximately 30 ha and is located at 1500m a.s.l. Vegetation is dominated by *J. thurifera* trees of different ages and tree density is 423 ind./ha. Vegetation covers less than 40% of the rocky soil surface while the rest of the open spaces have only herbaceous ephemeral vegetation, lichens and sparse *Thymus* and *Genista* plants. The mean tree height of male trees (n=40) was taller (4.73±0.84) than that of female trees (4.63±0.83). However differences between sexes were not significant (GLM; $F=0.14$; df=1; $p=0.71$). The climate is Mediterranean, with harsh winters (the duration of the freezing period is more than 120 days per year) and warm and dry summers. Mean annual precipitation is 486 mm with October the wettest month (58.6 mm) and July the driest (26.86 mm). Annual mean temperature is 13° C, with August the warmest month (mean 22.8º C) and January the coldest (mean 4.8º C). Winter precipitation is usually in the form of snow, and accounts for 21% of the total precipitation. The study area has been used for agriculture, timber, and extensive livestock grazing for centuries. Narrow valleys were tilled for rye and barley until land abandonment in 1960 (Rodrigo 1999). In recent times, reforestation is occurring due to human abandonment of traditional uses as a consequence of social and economic changes (Lasanta 1996), although livestock grazing pressure is still noticeable (D. Montesinos pers. obs.).

Tree-ring and statistical analysis

Logging of the study site in October 2001 gave us the opportunity to obtain discs of *J. thurifera* from 14 male and 14 female trees. Trees are representative of the variability in age and size of the studied population. Whole discs from the bottom of the trunk were collected and polished for tree-ring growth measurements. We selected two ring-series on each disk and ring-widths were measured to the nearest 0.01 mm using an incremental measuring table. The tree-ring series were visually cross-dated and synchronized with each other by calculating the 'Gleichläufigkeit' (GLK%) coefficient (Eckstein & Bauch 1969) and the t-value after Baillie and Pilcher (1973). Crossdating was verified statistically using COFECHA program (Holmes 1994). Despite difficulties while cross-dating due to the presence of double and missing rings, 11 males and 12 female trees were exactly dated and then used to build chronologies. We made two chronologies for both the male and female trees: a non-detrended, raw-data and a detrended residual chronology. The non-detrended, raw-data chronologies were used to test for differences in growth between male and female trees. The climatic influence on tree growth was studied using the residual version of the chronology, obtained by the program ARSTAN (Holmes 1994). Hereby, the individual raw tree-ring series were standardized in a two-step procedure to remove most of the low-frequency variability that is assumed to be unrelated to climate, such as that due to tree ageing and forest stand development (Cook & Peters 1981). First, the long-term trend was removed by fitting a negative exponential function or a regression line to each tree-ring series. Second, a more flexible detrending was made by a cubic smoothing spline with a 50% frequency response of 30 years to reduce further non-climatic variance. After that, autoregressive modelling of the residuals and bi-weight robust estimation of the mean were applied to construct residual chronologies (Cook & Peters 1981). Residual chronologies are commonly used in dendroclimatic studies because removal of serial autocorrelation is required to analyze the effects of year-to year climatic variability on tree growth.

Differences between non-detrended ring growth data from male and female trees were tested with SPSS 11.00 with a General Mixed Model with an autoregressive covariance structure of order one. This model permits the study of time-series accounting for temporal autocorrelations among continuous rings from the same tree. Two statistical analyses were made, one testing for differences between sexes among the first 30 years of life of each tree and another testing for differences among the following 120 years, once sexual maturity is achieved (Pavón-García 2005).

To test for climatic relations both for males and female trees, bootstrapped correlation functions (Guiot 1991) were computed using the software DENDROCLIM2002 (Biondi & Waikul 2004). In each case, the residual chronology was used as an independent variable. Since residual chronologies do not preserve the effects of previous years, the regressors were, separately, seasonal precipitation and temperature from summer and autumn of the previous growing season and winter, spring, summer and autumn of the year in which the ring was formed.

Results

Male and female trees grew increasingly more every year until the approximate age of 30. From that point, ring width decreased gradually in both sexes. Nonetheless, such reduction was significantly steeper for females resulting in greater overall ring widths for males compared to female trees (Figure 2.1 and Table 2.1). Mean age of sampled trees was 152±74 years (47-328).

Table 2.1. Male ring growth is higher than female's after reaching sexual maturity. Ring growth (Mean±SD; mm 10^{-2}) and Lineal Mixed Model values are given for male and female trees in each age class.

	Male trees	**Female trees**	**F**	**df**	**p**
Age 1-30	321.39±242.77	332.78±281.92	0.020	39.428	0.889
Age 31-150	293.76±205.61	249.44±174.42	4.797	111.664	0.031

On an annual basis, climate from current and previous year (precipitation and temperature) accounts for a high amount of variance in tree-ring widths for both sexes (r=0.72 and 0.73 for males and females respectively). Intra-annually however, males showed different growth response than females. Male growth was significantly correlated with current winter and spring precipitation (r=0.30 and 0.31 respectively) whereas female growth was only significantly correlated to current spring precipitation (r=0.38) (Figure 2.2). Temperature from the current year was also correlated with tree-ring width but there was no difference between the sexes. Current winter temperatures correlated positively with tree-ring width for both sexes (r=0.23 and 0.27 for males and females respectively) (Figure 2.3). In contrast, current summer temperatures correlated negatively with tree-ring width for both sexes (r=-0.31 and -0.25 for males and females respectively).

Fig. 2.1. Males and females grow similarly until the age of ca. 30, since then male trees grow significantly more than female trees. Cumulative ring growth is shown depending on tree age. Sample size among ages is shown for both sexes.

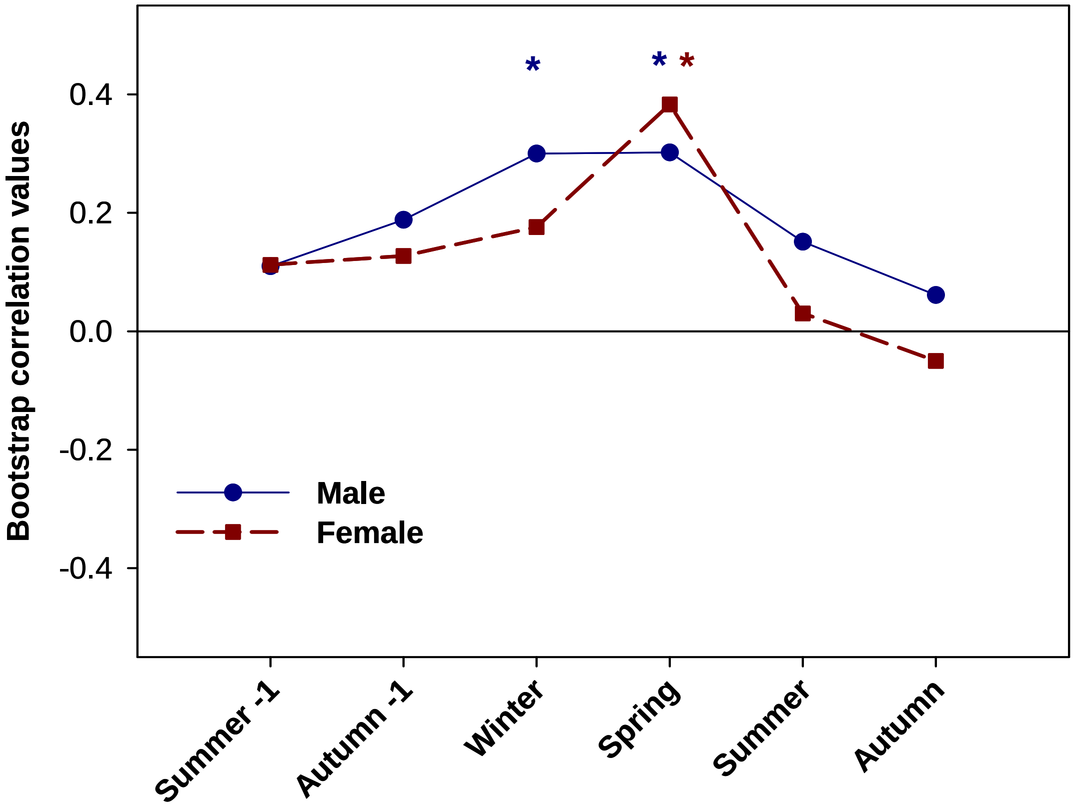

Fig. 2.2. Male tree ring-growth correlate significantly with winter and spring precipitation while female tree ring growth correlate only with spring precipitation. Bootstrap correlation values are shown for the previous six seasons. Asterisks represent 95% significant p-values ($p<0.05$).

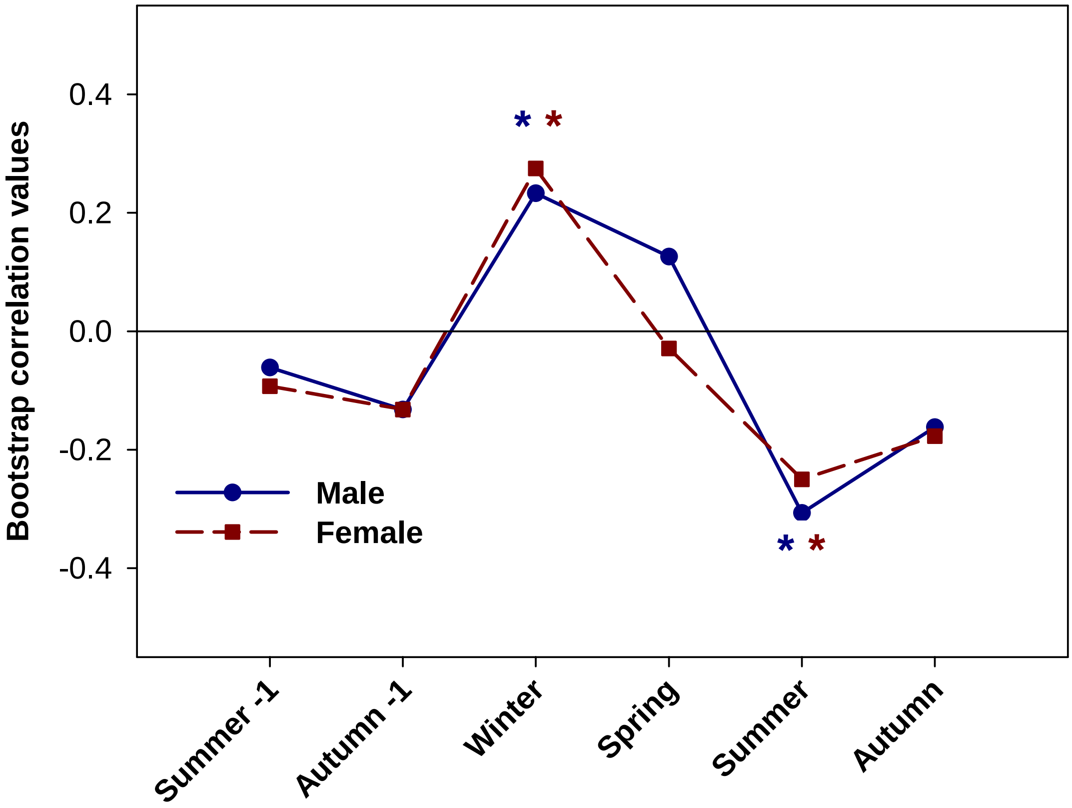

Fig. 3.3. Male and female tree ring-growth correlate positively with temperature from previous winter and negatively with temperature from previous summer. Bootstrap correlation values are shown for the previous six seasons. Asterisks represent 95% significant p-values ($p<0.05$).

Discussion

Sexual maturity marks a change-point in *J. thurifera* tree-ring growth pattern. When reproduction starts ring growth experiences a strong reduction for individuals of both sexes. Switching of resources between stem growth and reproduction is characteristic of Northern Hemisphere conifers (Koenig & Knops 1998) and *J. thurifera* is no exception. Furthermore, the trade-off between the reproductive and the vegetative function is sexually-biased, where females experience a stronger reduction in stem growth than males. The only other work known by us in which the interaction between gender, age of reproduction and stem growth was studied (Bañuelos & Obeso 2004) found a similar trade-off in the dioecious, insect-pollinated, *Rhamnus alpinus*.

In *J. thurifera*, stem diameter is positively correlated with tree height (data not shown) thus tree-ring growth is a good proxy to tree height. Males of anemophilous (wind-pollinated) species like *J. thurifera* experience stronger selective pressure than females to increase tree height, since taller males may produce more pollen (Obeso 2002) and may disperse pollen to longer distances thereby attaining greater pollination success (Burd & Allen 1988). In contrast, females do not benefit directly from increased height but do need to accumulate resources during periods of low reproductive investment to be able to grow and ripen reproductive cones during the following two years (Obeso 2002).

Water limitation can limit nutrient availability (Austin et al. 2004) particularly in Mediterranean environments where precipitation and temperature are the main determinants of tree-ring growth (Cherubini et al. 2003). Previous studies found a positive relationship between current year ring growth and previous year precipitation in *J. thurifera* (Bertaudière et al. 1999). However, in our study, both sexes were analyzed separately. Although yearly growth patterns in relation to climatic conditions are very similar for both sexes, growth patterns differ when seasonal (i.e. within-year) patterns are studied, and as far as we know this is the first work in which gender-specific climatic correlations have been found. The only other work known by us in which gender-specific climatic correlations were studied (Rovere et al. 2003) correlations between annual tree-ring growth and seasonal climatic variables did not differ between male and female trees of the conifer *Austrocedrus chilensis*.

In *J. thurifera*, at the seasonal temporal scale, male trees present a significantly positive correlation between tree-ring growth and both current winter and current spring precipitation. This indicates that males are investing a high proportion of their winter and spring available resources to growth and not to reproduction, since male cones are totally developed at the end of the winter. In contrast, current female growth correlates only with current spring precipitation. This would suggest that females do not use current winter precipitation for current vegetative growth but rather that they allocate resources for the subsequent cone maturation, and only surplus resources from current spring precipitation are used to current growth. Contrarily to our findings, correlations between annual growth and seasonal climatic variables did not differ between male and female trees of the conifer *Austrocedrus chilensis* in the only other work known by us in which gender-specific correlations were studied (Rovere et al. 2003). As far as we know this is the first work describing a differential ring growth response to climatic variation between male and female plants.

We suggest that male and female trees of *J. thurifera* have to deal with different selective pressures. Males would benefit from an increase in vegetative investment, since this could increase their reproductive success. On the other hand, females need to store resources to guarantee provisioning for cone maturation. Therefore, and responding to the questions asked in the title, we show that male trees invest more resources to growth than female trees only after reaching sexual maturity, and in a different temporal way.

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III - Desacoplamiento de la vecería: no todos los episodios de floración vecera resultan en fructificación vecera.

III - Masting uncoupling: mast seeding does not follow all mast flowering episodes in a dioecious tree.

III - Desacoplamiento de la vecería: no todos los episodios de floración vecera resultan en fructificación vecera.

Introducción En especies dioicas y veceras los costes reproductivos y las fuerzas selectivas a menudo afectan con diferente intensidad los procesos de floración y fructificación y, por lo tanto, a los machos y a las hembras. Consecuentemente, machos y hembras podrían llegar a diferir en sus ciclos reproductivos.

Material y Métodos El esfuerzo reproductivo (ER), medido en términos de la media de masa seca asignada a reproducción por rama, fue calculada para árboles macho y hembra de dos poblaciones durante seis años. El crecimiento de anillos y la composición isotópica de anillos fueron medidos en una de las poblaciones. Se corrieron Modelos Lineales Mixtos con estructura de covarianza autorregresiva de primer orden para testar las diferencias entre sexos y las correlaciones entre variables.

Resultados En la especie dioica y vecera *Juniperus thurifera* el esfuerzo reproductivo de las hembras es cuatro veces mayor que el de los machos. Durante seis años machos y hembras florecieron sincrónicamente en un ciclo bianual de *floración vecera*. Sin embargo, únicamente uno de los episodios de floración masiva resultó en una fructificación masiva o *fructificación vecera*, generando un *desacoplamiento de la vecería* entre la floración y la fructificación. Producir semillas tiene un coste enorme, sin embargo la floración femenina es muy barata. Por lo tanto, las hembras pueden beneficiarse de estrategias de *apuesta de perdidas limitadas* (*bet hedging*) y abscidir conos más tarde si los costes reproductivos se tornan excesivos. Por otra parte, el ER de machos se correlacionó negativamente con el crecimiento de anillos pero el de hembras no. Esto indica la existencia de un *compromiso de eficacia* (*trade-off*) entre reproducción y crecimiento en machos, mientras que otros estudios con esta misma especie (ver capítulo II) muestran el mismo compromiso de eficacia para hembras, pero a largo plazo.

Conclusiones Los episodios de floración masiva (floración vecera) no siempre resultaron en episodios de fructificación masiva (fructificación vecera), generando un *desacoplamiento de la vecería* de floración y fructificación. El desacoplamiento de la vecería es susceptible de estar modulado por fuerzas evolutivas, sin embargo, nuestro trabajo sugiere que los ciclos de vecería están influenciados también por costes reproductivos diferenciales para cada sexo y, por lo tanto, presentan patrones diferentes para machos y hembras de especies dioicas.

III - Masting uncoupling: mast seeding does not follow all mast flowering episodes in a dioecious tree

Introduction Dioecious masting species often experience reproductive costs and selective pressures which act with different intensity over flowering and fruiting and thus over males and females. As a result, males and females can present different reproductive cycles.

Materials and Methods Reproductive effort (RE), in terms of mean dry mass allocated to reproduction per branch, was calculated for male and female trees from two populations during six years. Tree-ring growth and tree-ring stable isotope composition was analyzed for one of the populations. Linear Mixed Models (GMM) with autoregressive covariance structure of order one were run to test for differences between sexes and years and for correlations between variables.

Results In the masting dioecious tree *Juniperus thurifera*, females' reproductive effort is four times larger than that of males. Along the six studied years male and female trees flowered synchronically in a biannual *mast flowering* cycle. However, only one out of three *mast flowering* episodes resulted in *mast seeding*, leading to *masting uncoupling* between flowering and seeding. Production of seeds had an enormous cost but females' flowering was inexpensive. Hence females could benefit from *bet hedging* strategies and abscise cones later in the reproductive cycle if costs became too high. On the other hand, males' RE correlated negatively with tree-ring growth while females' RE did not. This evidences a short term trade-off between reproduction and growth for male trees, whereas previous studies with this same species showed the same trade-off for females when longer time-frames were considered.

Conclussions *Mast flowering* episodes did not always result in *mast seeding*, leading to *masting uncoupling* between flowering and seeding. Masting uncoupling is likely to be affected by evolutionary forces. However, our study suggests that masting cycles are also influenced by reproductive costs and, therefore, present different patterns in male and female plants.

Introduction

Mast seeding (the variable, periodic and synchronic production of seeds in a population) is a phenomenon resulting from the interaction of many functional constraints and evolutionary forces. In the absence of selection for higher or lower variability reproductive output of a plant population would vary in parallel with available resources (i.e. with weather). This behavior has been referred to as resource matching (Kelly & Sork 2002). However, in masting species reproduction often presents higher variability than that of weather, therefore, there must be additional causes of mast seeding. Two main evolutionary explanations have been proposed to explain mast seeding: (i) predator satiation, suggesting that large intermittent seed crops reduce seed-loss due to predator satiation; and (ii) pollination efficiency, proposing that concentrating pollination in some years increases pollination efficiency in wind pollinated species (Kelly & Sork 2002; Rees et al. 2002; Piovesan & Adams 2005 and references therein). Kelly (1994) based in a study by Sork & Bramble (1993) suggested that different selective forces could be acting over mast flowering (e.g. wind pollination) and mast seeding (e.g. predator satiation). Therefore, evolutionary forces selecting for masting behavior could affect differently males and females of dioecious species.

Selective advantages of masting are achieved through economies of scale (Norton & Kelly 1988) and masting pulses depend on resource reserves and/or availability (Sork et al. 1993; Yamauchi 1996; Isagi et al. 1997). Consequently, any explanatory hypothesis of masting must take into account resource availability (Yamauchi 1996). Reproduction is expensive and plants can compensate for reproductive efforts by improving physiological performance, reducing resources allocated to vegetative growth or reducing the frequency of reproduction (Delph 1999; Obeso 2002). In dioecious plants different sexes might incur in different reproductive costs and thus present different trade-offs between reproduction and growth, developing different functional strategies (Obeso 2002). For instance, reproductive females often present lower leaf and tree-ring growth and lower water use efficiency (WUE) than males, specially in stressful conditions (Dawson & Bliss 1989; Obeso et al. 1998; Dawson et al. 2004). As a result, intensity of reproduction might present a different pattern of variability for each sex, with males flowering more frequently than females (Antos & Allen 1999). In a review, Delph (1999) found that males flowered more often than females in more than 63% of the cases studied, and there were no cases in which the contrary was found. Such review was intended to disentangle the factors affecting gender-associated costs of reproduction, which eventually resulted in variable reproduction. However, the review did not consider reproductive periodicity or population synchrony, which together with variable reproduction are the three characteristic elements of masting (Janzen 1976; Kelly 1994; Kelly

& Sork 2002; Rees et al. 2002). Dioecious masting species are likely to experience similar differences between sexes and, particularly, in reproductive cycle length and periodicity.

In this work we study the consequences of differential reproductive efforts and selective pressures on the reproductive cycles of male and female trees of the masting dioecious *Juniperus thurifera*. In a previous work we found that, in the long term, *J. thurifera* male trees invested more resources to tree-ring growth than female trees after reaching reproductive maturity (Montesinos et al. 2006). In this work we quantify the degree of variability, periodicity and synchrony in flowering within and between genders and also describe the degree of coupling between flowering and fruiting effort in females, clarifying if male and female trees present different reproductive cycles and if that generates different compensation mechanisms for each sex. More specifically, we want to answer the following questions: (a) Do male and female trees present the same pattern of reproductive synchrony, variability and periodicity? (b) Are trade-offs between reproductive and vegetative cycles similar for both sexes? (c) Is there a differential physiological cost of reproduction for both genders?

Methods

Study species

Juniperus thurifera L. (Cupressaceae) is a dioecious long-lived tree with relict distribution from the Tertiary throughout the western Mediterranean basin. Together with two closely related vicariant species with similar biology (*J. excelsa* and *J. procera*) they distribute along the Mediterranean basin, western Asia and eastern Africa. It is usually the dominant species in high elevation low-density forests with semi-arid climate. Trees are 4-10 m high and often live for centuries (Bertaudière et al. 1999). Trees reach sexual maturity at the approximate age of 31 (Pavón-García 2005). Reproductive output varies strongly from year to year; male and female trees flower at the end of the winter and after pollination females produce reproductive cones over a period of 20 months, when seed dispersal occurs (Amaral-Franco 1986 and P. García-Fayos pers. obs.).

Study sites

The study was performed in Eastern Spain at two different populations separated by 100 km: El Portell de Morella, in Castelló (hereafter population A), and La Puebla de San Miguel, in València (hereafter population B).

Population A is located at 1150 m a.s.l. and vegetation is dominated by *J. thurifera* and *Quercus ilex* trees, accompanied by sparse individuals of *Juniperus communis*, *Thymus* spp and *Genista* spp. Lichens and herbaceous ephemeral vegetation cover most of the rocky soil surface. The climate is Mediterranean, with harsh winters (the duration of the freezing period is more than 120 days per year), and warm dry summers. Mean annual precipitation is 665 mm with May being the wettest month (81 mm) and July being the driest (35 mm). Annual mean temperature is 11.2º C, with July being the warmest month (mean 20.1º C) and January the coldest (mean 3.8º C).

Population B is located at 1500 m a.s.l. Vegetation is dominated by *J. thurifera* trees with sparse *Thymus* spp. and *Genista* spp. plants. Lichens and herbaceous ephemeral vegetation cover most of the rocky soil surface. The climate is Mediterranean. Mean annual precipitation is 536 mm with May being the wettest month (66 mm) and July the driest (27 mm). Annual mean temperature is 12.6º C, with July being the warmest month (mean 20.9º C) and January the coldest (mean 4.2º C).

Both areas have been used for agriculture, timber, and extensive livestock grazing for centuries. Narrow valleys were tilled for rye and barley until land abandonment in 1960 (Rodrigo 1999). In recent times, reforestation is occurring due to human abandonment of traditional uses as a consequence of social and economic changes (Lasanta 1996), although livestock grazing pressure is still noticeable (Verdú & García-Fayos 2003).

Reproductive and vegetative cycles

A set of 20 male trees and 20 female trees of similar age were marked in 1999 throughout each of the two populations. Reproductive effort (RE) is difficult to evaluate in long-lived woody species (Antos & Allen 1999), therefore biomass is the most used currency to quantify RE and very few studies have considered any other resource (Delph 1999). Likewise, results from studies considering other currencies (N and P mainly) do not find different patterns that when considering biomass alone (Delph 1999 and references therein). Therefore RE is quantified in terms of dry biomass in this study.

To compare RE of male and females trees of *J. thurifera*, we counted the number of male and female flowers (Fw), and ripe feminine cones (Fr2, 22 months old) in 10 branches randomly sampled all around each tree (including non-reproductive branches) along six consecutively years (2000-2005). Since bigger trees will have more branches than smaller ones, therefore mean RE per branch gives a convenient, independent approximation to RE. In 2003 and 2004 we collected masculine and feminine reproductive cones from a different set of 10 male trees and 10 female trees from population B. From each male and female tree we weighted ten flowers (Fw), ten unripe feminine cones (Fr1, 12 months old) and ten ripe feminine cones (Fr2, 22 months old). Flowers and feminine cones were dried at 50 ºC for 48 h before weighting. For feminine cones, we calculated the proportion of weight produced during the first 12 months (Fr1) relative to the final feminine cone weight (Fr2). We determined that 64% of the weight of a ripe feminine cone was produced during the first year, and the remaining 36% is gained during the last year.

Yearly reproductive effort (RE) per branch was calculated as:

$$(W_{Fw(t)} \times N_{Fw(t)})+(0.36 \times W_{Fr2(t)} \times N_{Fr2(t)})+(0.64 \times W_{Fr2(t+1)} \times N_{Fr2(t+1)})$$

in which *W* refers to dry weight in grams; N, number of flowers or cones; *t*, current year and *t+1*, following year.

Although there is no clear boundary between masting and non-masting species (Herrera et al. 1998) the term *masting* is useful from an evolutionary point of view if a quantitative approach clarifies *how much masting* (i.e. variable, synchronic, periodic) a determinate species is (Koenig et al. 2003). To give a quantitative approach to our study we calculated the indexes proposed by Herrera (1998) to quantify masting (CVp, CVi and W; Herrera 1998b). Coefficients of variation (CV=SD/Mean) for male and female flowering RE and for total female RE were calculated independently for each population. CVp was calculated as the population coefficient of variation for RE among years. CVi was calculated as the mean of within-plant coefficients of variation among years in RE. Kendall’s coefficient of concordance *W* was obtained to study the degree of supra-annual synchrony among trees.

Wood dendrochronological cores were extracted from marked trees in population B in July 2005 to measure annual vegetative growth. Samples were mounted and ring growth from 2000 to 2005 was measured used standard dendrochronological techniques.

Physiological costs ($\delta^{13}C$)

As a surrogate of water use efficiency (WUE) we analyzed $\delta^{13}C$ isotopic discrimination by extracting a different set of cores from marked trees at population B in July 2005. Tree rings from year 2000 (previous to a masting year), 2001 (masting year) and 2004 (one of the lowest reproductive investment for both genders) were carefully separated. Cellulose was extracted following the protocol proposed by Freyer (1979). Samples were milled to dust and the cellulose extracted from each ring was analyzed at the Laboratorio de Isótopos Estables (Universidad Autónoma de Madrid, Spain) to determine the C isotope composition. After combustion of the samples in an elemental analyzer (EA 1108; CHNS, Carlo Erba, Milan, Italy) the $^{13}C/^{12}C$ ratios were determined with a continuous flow isotope mass ratio spectrometer (Micromass CF-Isocrom, Manchester, UK) with a measurement error of 0.1‰. The isotopic values are expressed in delta notation and calculated as:

$$\delta^{13}C(‰)=(R_{sample}/R_{standard}-1) \times 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the Pee Dee Belemnite carbonate standard, respectively.

Statistical Analyses

Linear Mixed Models (GMM) with autoregressive covariance structure of order one were run to test for differences between sexes and years and for correlations between variables. These models allow the study of time-series accounting for temporal autocorrelations among continuously repeated measures from the same tree. All the statistical analyses were performed by using SPSS 11.00.

Results

Reproductive and vegetative cycles

According to the indexes proposed by Herrera (1998) to quantify masting (CVp, CVi and W), reproductive variation in the studied populations presented a moderately high degree of inter-annual variability and population synchrony (Table 3.1), ranging from 110 to 180 for CV and from 0.34 to 0.61 for W. These indexes have been shown to range between 19 and 285 for variability (CV) and 0.02 and 0.65 for population synchrony (W) (Herrera 1998b).

Both populations presented a similar pattern of reproductive variation. In this pattern, flowering followed a similar biannual cycle for male and female trees (Fig. 3.1) which should have resulted in fruiting peaks two years later; but a biannual flowering pattern did not result in a biannual seed production pattern (Fr2 on Fig. 3.2).

Mean reproductive effort of females among years (1.4±3.2 g) was almost four times higher than that of males (0.4±0.8 g). However, reproductive effort was highly variable among years for both sexes (Fig. 3.3) and during some particular years male trees eventually invested a similar or even higher amount of resources to reproduction than female trees, resulting in a significant interaction (Table 3.2).

Table 3.1. Components of synchrony for the reproductive effort (RE) of male and female flowering (Fw); total flowering effort (Total Fw) and total reproductive effort of females (Total Female). CVp refers to the population coefficient of variation (SD/Mean) for RE among years. CVi refers to the mean of within-plant coefficients of variation among years in RE. *W* refers to the degree of supra-annual synchrony among trees by means of the Kendall's coefficient of concordance; this coefficient ranks from 0 (complete discordance) to 1 (complete synchrony).

	Male Fw	**Female Fw**	**Total Fw**	**Total Female**
Population A				
CVp	122	134	122	145
CVi	159	197	177	142
Kendall's *W*	0.52***	0.34**	0.59***	0.44***
Population B				
CVp	112	157	113	110
CVi	161	180	171	124
Kendall's *W*	0.47***	0.46***	0.61 ***	0.49 ***

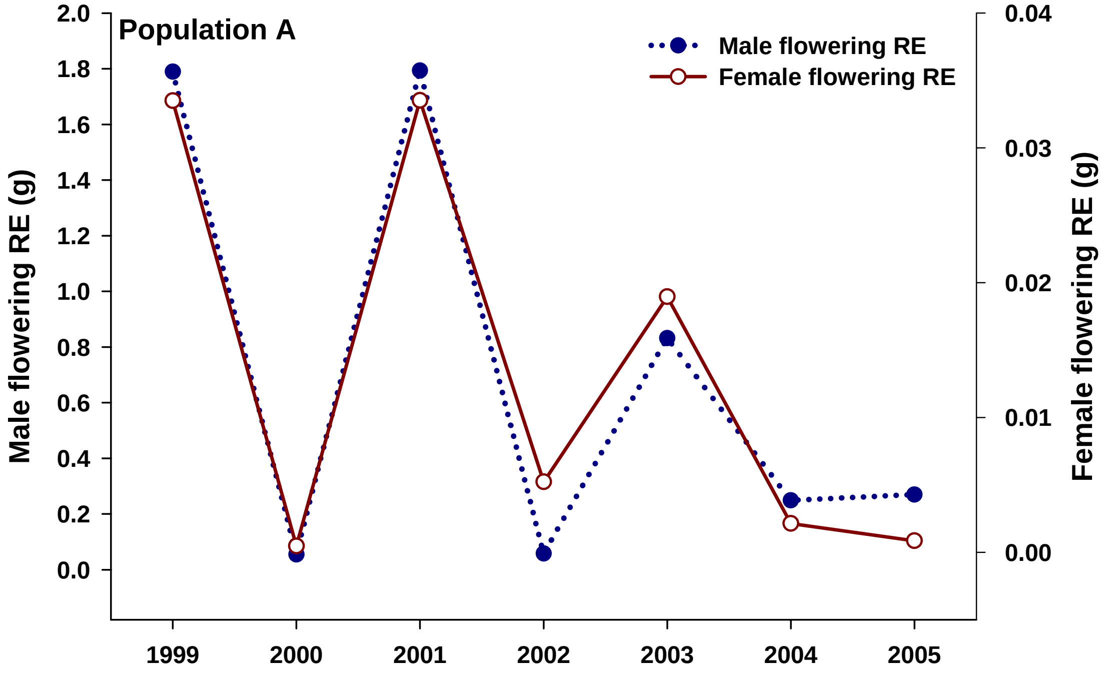
*p<0.05; **p<0.01; ***p<0.001

Table 3.2. Significance levels and F values are provided for the effect of sex and year on reproductive effort (RE) of both studied populations and on tree-ring growth and $\delta^{13}C$ (pop B).

	Sex	**Year**	**Sex x Year**
RE population A	28.4***	15.7***	12.0***
RE population B	8.22**	5.64***	4.67***
Tree-ring growth (pop. B)	0.05	26. 10***	0.77
$\delta^{13}C$ (pop. B)	1.58	1.29	0.13

*p<0.05; **p<0.01; ***p<0.001

Fig 3.1. Male and female trees followed a synchronic flowering pattern along the studied years. Plots A and B represent mean flowering reproductive effort (RE) from year 1999 to 2005 for populations A and B respectively.



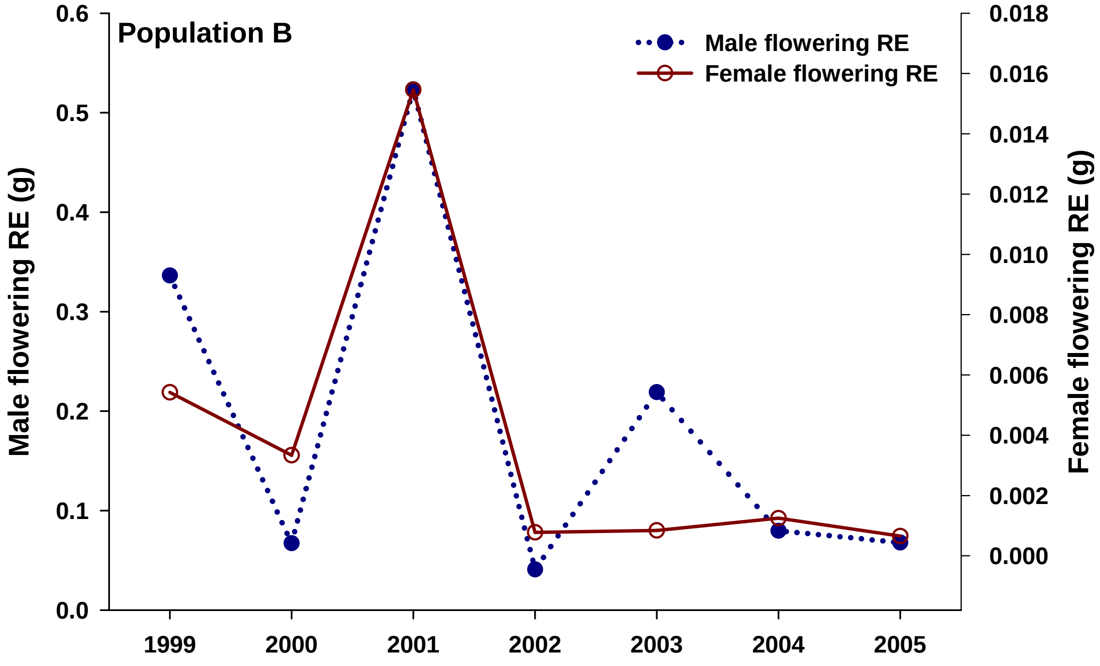
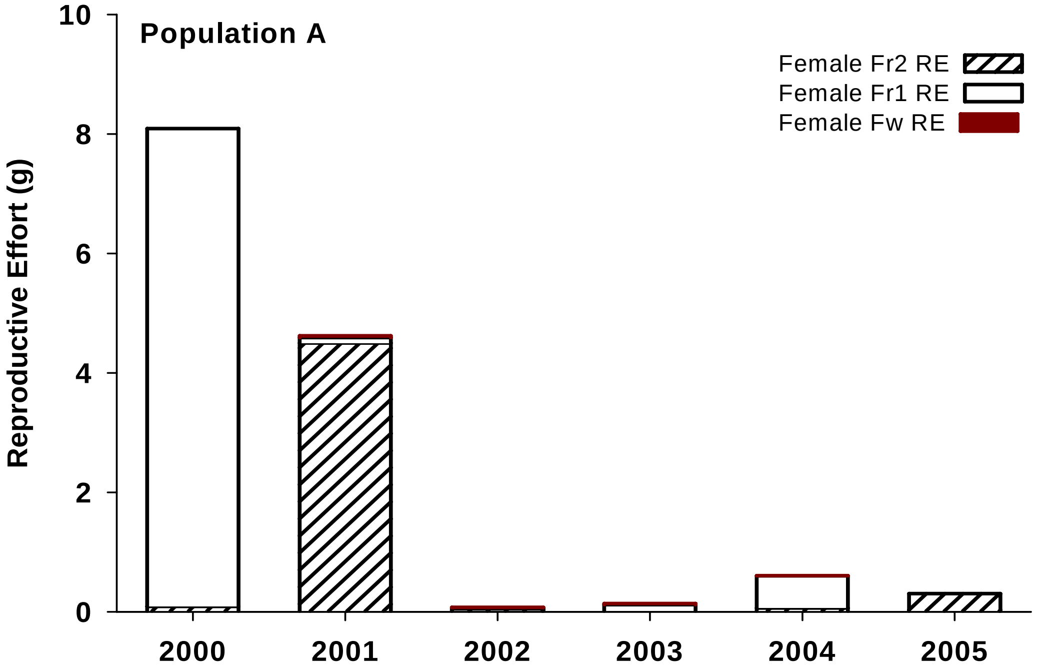


Fig. 3.2. Plots A and B represent females mean reproductive effort per branch (RE) from year 2000 to 2005 for populations A and B respectively. For each year RE column is divided into flowering (Fw), 12 months green cones (Fr1), and 22 months ripe cones (Fr2).



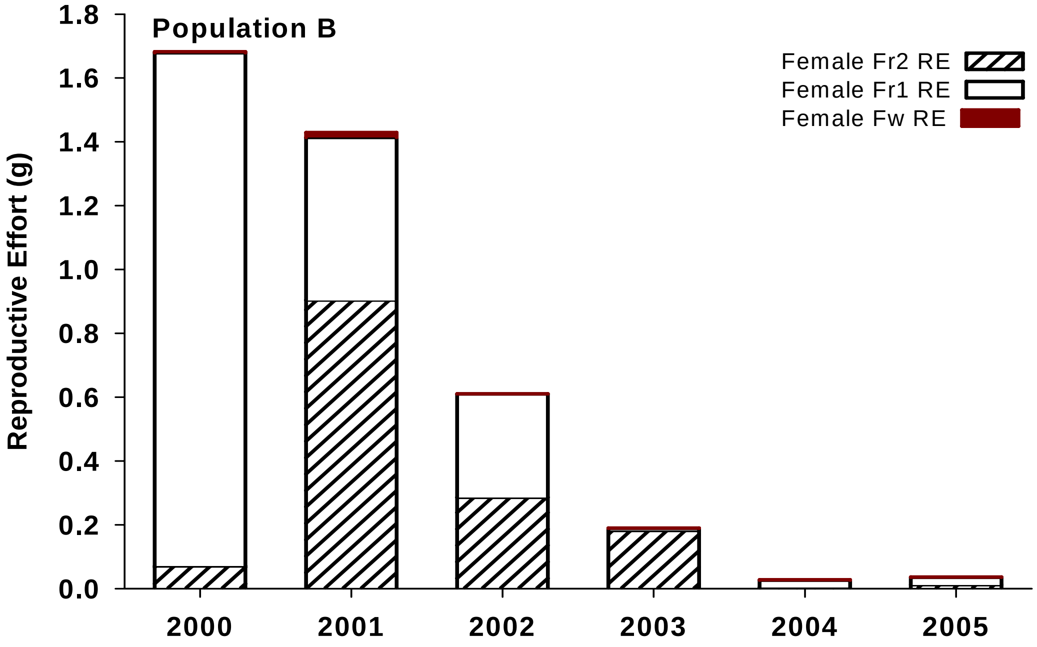
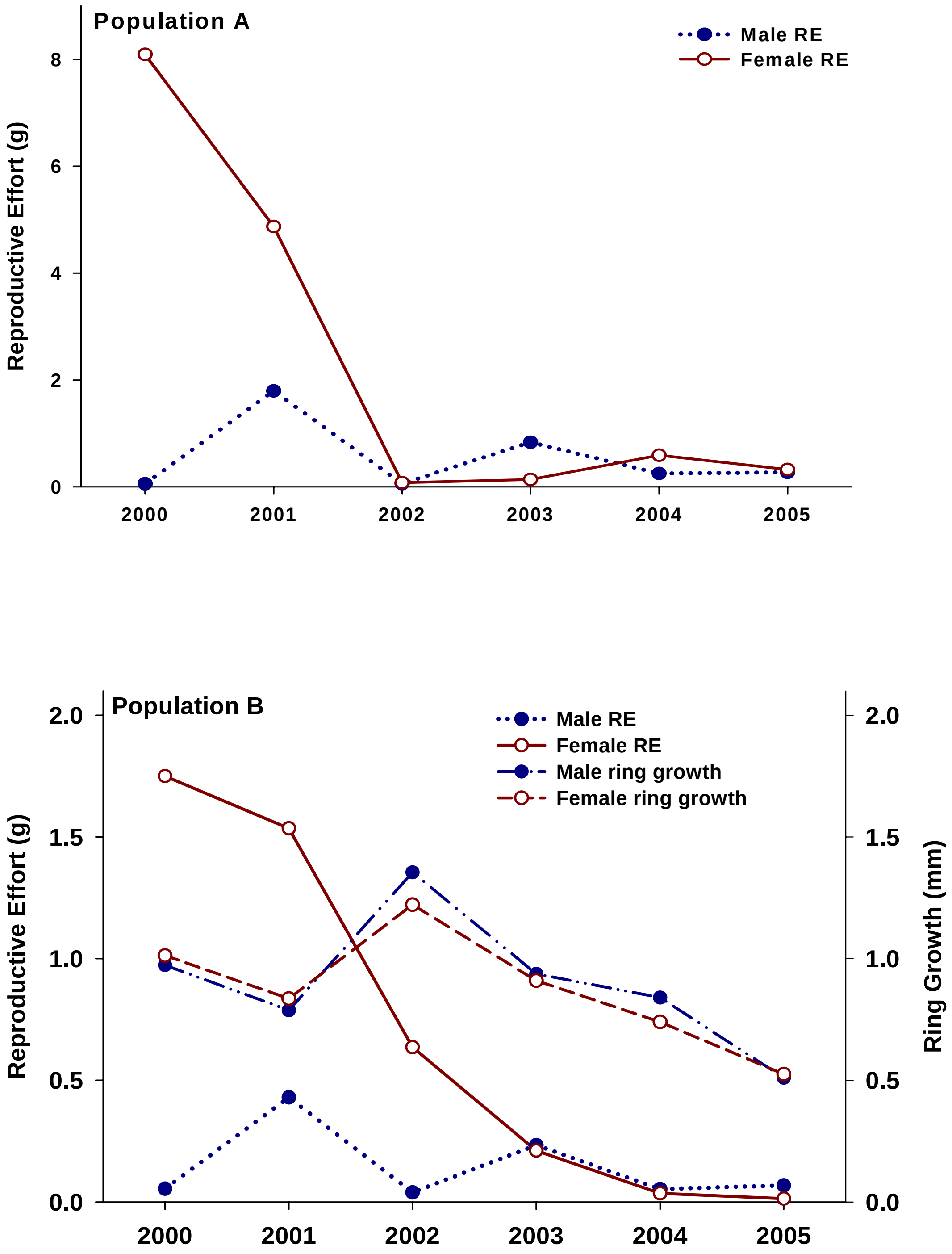


Fig. 3.3. Plots A and B represent mean reproductive effort per branch (RE) from year 2000 to 2005 for populations A and B respectively. For population B, males' tree-ring growth correlated negatively with reproduction whereas females' did not.



RE cycles differed for each sex: Male trees presented a biannual RE cycle, alternating years of high and low reproductive effort while females did not follow this pattern. Female flowering was coupled with that of males, but during the studied period females experienced a single mast-fruiting episode in 2001 (Fig. 3.2), since then females production of ripe reproductive cones (Fr2) decayed for at least four years. Female trees achieved their maximum reproductive investment during 2000 (Figs. 3.2 and 3.3) the year before the mast seeding event. This was due to the strong reproductive investment needed to increase the size of feminine unripe cones (Fr1, 12 months old). Ripening of feminine cones (Fr2, 22 months old) demanded a lower weight investment and investment in feminine flowers (Fw) was negligible.

Mean tree-ring growth for male trees (0.98±0.59 mm) was similar to that of female trees (0.94±0.53 mm) and temporal variations were similar for both sexes (Table 2). Tree-ring growth followed a similar biannual pattern for individuals of both sexes (Fig. 3-B). Male trees presented a negative correlation between reproduction and tree-ring growth (-0.34±0.12; F=8.41; df=52; p=0.005). In contrast, female trees showed no significant relationship between vegetative and reproductive investment (-0.01±0.03; F=0.01; df=74; p=0.990).

Physiological costs ($\delta^{13}C$)

Male (-24.99±1.34‰) and female trees (-24.54±1.26‰) showed no differences in $\delta^{13}C$ between sexes or years (Table 2). Interaction was not significant. Moreover δ^{13}C did not correlate with reproductive investment or tree-ring growth (statistical values not shown).

Discussion

One common assumption when studying masting is that masculine flowering intensity matches feminine flowering and that subsequent seed set will vary proportionally to flowering intensity in an efficient resource-use process; namely, that flowering and seeding masting cycles are coupled. Consequently with this assumption, most often only fruit-set is quantified (see reviews in Herrera et al. 1998; Koenig & Knops 2000; Kelly & Sork 2002).

J. thurifera flowering was synchronic between male and female trees. We recorded a mast seeding episode (2001) in both study populations. Mast seeding was preceded by a high flowering intensity for both sexes two years before (1999). However, not all mast flowering episodes resulted in mast fruiting two years later. We suggest that males would benefit from flowering regularly if females seeding cycle is unpredictable and some male fitness is achieved by residual female seeding. On the other hand feminine flowering had a negligible cost for female trees. Since weather variability and resource availability are difficult to predict, females can benefit from *bet hedging* if trees can abscise cones later in the reproductive cycle if costs become too high (Stephenson 1980; Sutherland 1986). As proposed by Stephenson(1981: 21): "The production of surplus flowers and their subsequent abscission appears to provide plants with some degree of control over the number and quality of their offspring". As far as we know such masculine "flowering wasting" has been described only once (Sork & Bramble 1993) based on flower and fruit counts of monoecious *Quercus* trees.

Fruit counts have been the traditional way to quantify reproductive intensity when evaluating masting (see reviews in Herrera et al. 1998; Koenig & Knops 2000). However in coniferous species maturation cycles of more than one year are common (e.g. Castroviejo et al. 1986; McDowell et al. 2000; Wesche et al. 2005), and plants often distribute their reproductive costs among years, resulting in long masting cycles (Norton & Kelly 1988; Herrera et al. 1998; Koenig et al. 2003). To study trade-offs between reproduction and growth, and to disentangle the ultimate functional and evolutionary causes underlying masting, an estimation of the RE will provide a more appropriate approximation of reproduction variability among years.

When we considered variation in total RE, male and female trees differed in their reproductive cycles: Male trees reproduced almost every year, high and medium intensity of reproduction alternated in a bimodal cycle which was negatively correlated with tree-ring growth. Bimodality is found in many species (Norton & Kelly 1988; Herrera et al. 1998) although it is always referred to fruit crop, since masculine flowering is rarely studied.

A negative correlation between growth and reproduction demonstrates *resource switching* (Kelly & Sork 2002) where plants allocate resources successively into, and then away from, reproduction (Koenig & Knops 2000).

On the other hand, reproductive effort of females of *J. thurifera* concentrated in scarce particular years and reproductive cycles lengthened for at least 6 years, which is a frequent cycle length in masting species (e.g. Herrera 1998a). As expected for a masting species, total reproductive effort was dramatically reduced for many years after masting (e.g. Kelly 1994; Kelly & Sork 2002). At last, females' reproduction was not correlated with tree-ring growth in the short term, although in the long term adult male trees presented higher tree-ring growth than females (Montesinos et al. 2006).

It has been suggested that plants store resources in the form of reserve carbohydrates or leaves during years with low reproduction and invest them during highly reproductive years (Kelly 1994; Antos & Allen 1999; Miyazaki et al. 2002; Sala 2006). Resources can be stored in form of starch (Miyazaki et al. 2002) or leaves (Sala 2006) and nutrients stored at leaves can be available to future reproduction (Henery & Westoby 2001).

This often results in undetectable trade-offs between growth and reproduction (Obeso 2002). In a different study in which females of the dioecious shrub *Rhamnus alpinus* had higher RE and males higher tree-ring growth in the long term (Bañuelos & Obeso 2004), both sexes presented the same short-term tree-ring pattern and there was no short-term correlation between tree-ring growth and RE. In our study only males showed a short-term trade-off between growth and reproduction, while females trade-off was detectable only in the long term (Montesinos et al. 2006). This suggests that female trees distribute costs of reproduction among years while males' reproduction depends on current year resources. Trade-offs between vegetative and reproductive functions are common among plants and particularly in coniferous trees (Koenig & Knops 1998). However, most often tree sex is not considered in conifer studies.

Since *J. thurifera* reproductive effort is much higher for female trees, and females control the population reproductive output, our study support the hypothesis that masting cycles are mediated by resource availability. Some studies modeling masting according to resource availability, like the "storage-size dependent strategy" (Yamauchi 1996) or the "resource balance of individual plants" (Isagi et al. 1997) successfully predicted reproductive synchrony, temporality and variability, and give credible proximate explanations for masting. Such models are of great interest and will win in realism if they include sexual dimorphism, flowering phenology of both sexes and "bet-hedging" strategies. Nevertheless, influence of resource availability on masting cycles does not invalidate other ultimate selective forces which can favor masting dynamics simultaneously, like predator satiation or pollination efficiency (Koenig et al. 2003) which could equally lead to flowering and seeding masting uncoupling.

Conclusions

Male and female plants of masting dioecious species often incur in different reproductive costs and are likely to be subject to different selective pressures, this can result in singular trade-offs resulting in different reproductive cycles. Even if flowering is synchronic between sexes, females can limit the reproductive success of most of the masculine flowering peaks leading to flowering and seeding masting uncoupling. Our work changes the perspective of studies of masting, particularly in species that store resources and distribute reproductive efforts along supra-annual reproductive cycles. In such species, quantification of yearly reproductive effort will provide a more accurate approximation to the costs of reproduction that the traditional fruit counts. Future studies should consider that different sexes and different reproductive stages (flowering; fruit maturation, predation and dispersal) can experience different selective pressures and functional constraints.

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IV - Los machos gastan mientras las hembras ahorran: la mitigación del estrés revela estrategias funcionales específicas de género en un árbol dioico.

IV - Males spend, females save: stress mitigation reveals gender-specific functional strategies in a dioecious juniper tree.

Los machos gastan mientras las hembras ahorran: la mitigación del estrés revela estrategias funcionales específicas de género en un árbol dioico.

Introducción En especies dioicas ambos sexos difieren a menudo en su inversión reproductiva y en ocasiones se han descrito adaptaciones fisiológicas y funcionales a estas diferencias. Las variables fisiológicas son muy plásticas y no está claro si las diferencias entre sexos aparecen como reacción a corto plazo a las demandas reproductivas o si son el resultado de diferencias innatas entre sexos. En cualquier caso, se desconoce si los mecanismos de compensación fisiológica están presentes durante los momentos en que el esfuerzo reproductivo es similar para ambos sexos. Las especies veceras son una buena oportunidad para testar esto, dado que durante los años no veceros ambos géneros invierten una cantidad similarmente baja de recursos a reproducirse.

Material y Métodos Para estudiar la respuesta de los sexos a diferentes niveles de disponibilidad y demanda se fertirrigaron (riego con fertilizante) durante dos años no veceros un grupo de machos y hembras del árbol dioico *Juniperus thurifera* con la expectativa de que esto incrementaría el esfuerzo reproductivo. Distintas medidas fisiológicas (Potencial hídrico, A, g_s y iWUE) se tomaron mensualmente durante los dos años experimentales. Otros datos más integradores (contenido en N de hojas, $\delta^{13}C$, crecimiento de ramas e inversión reproductiva por rama) se tomaron anualmente.

Resultados Los árboles control de ambos sexos presentaron inversiones reproductivas similarmente bajas pero aún así difirieron en su fisiología y las hembras presentaron mecanismos de compensación fisiológica. Los árboles fertirrigados incrementaron su inversión reproductiva pero su respuesta a la adición de recursos fue desigual. Los machos distribuyeron los recursos adicionales entre reproducción, crecimiento y fotosíntesis. Por su parte las hembras fertirrigadas solo mostraron un ligero incremento en la reproducción y un gran incremento en el crecimiento de ramas. Como consecuencia, las diferencias entre las tasa fotosintéticas de machos y hembras desaparecieron con la fertirrigación.

Conclusiones La estrategia fisiológica de hembras está asociada con el aumento precoz de superficies fotosintéticas y la mejora del aparato fotosintético mientras que los machos pagan sus costes reproductivos y biológicos únicamente con los productos fotosintéticos actuales. Nuestros resultados indican que las respuestas fisiológicas difieren entre géneros y representan una estrategia a largo plazo, no dependiendo exclusivamente de las demandas de recursos inmediatas.

Males spend, females save: stress mitigation reveals gender-specific functional strategies in a dioecious juniper tree.

Introduction In dioecious species genders often differ in reproductive investment and gender-specific physiological and functional adaptations have often been described. Physiological variables are very plastic and it is unclear if physiological differences between sexes arise as a short-term reaction to reproductive resource demands or can be the result of innate differences between the sexes. Anyhow, it is unknown if physiological compensation mechanisms are present during episodes of similar reproductive effort for both sexes. Masting species are a good opportunity to test this since during non-masting years both genders invest a similarly low amount of resources to reproduction.

Materials and Methods To study how sexes respond to different levels of resource availability and reproductive resource demands we fertirrigated (watering plus fertilization) during two non-masting years a set of male and female trees of the dioecious tree *Juniperus thurifera* with the expectation than this will increase reproductive effort. Physiological measurements (Water potential; A; g_s and iWUE) were taken monthly during the two experimental years. Other integrative data (branch nitrogen content, $\delta^{13}C$, branch growth and reproductive investment per branch) were taken yearly.

Results Control trees of both sexes presented similarly low reproductive investments but differed in their physiology: female trees presented compensation mechanisms even when their reproductive investment was similar or lower to that of males. Fertirrigated trees increased reproduction but their response to resource addition was unequal: Male trees distributed additional resources among reproduction, growth and photosynthesis. In contrast fertirrigated females only presented a slight increase in reproduction and a high increase in branch growth. As a consequence photosynthetic differences between sexes disappeared with fertirrigation.

Conclusions Females' strategy is associated with precocious increase of photosynthetic surfaces and photosynthetic system while males pay their reproductive and biological costs out of current photosynthesis. Our results indicate that physiological responses differ between genders represent long term strategies, not depending exclusively on immediate resource demands.

Introduction

Male and female trees of dioecious species have to satisfy different biological demands and therefore have to deal with different selective pressures. Female plants often need to invest more resources to reproduction than males in the long term (Obeso 2002). On the other hand, selection acts on size of male trees of anemophilous (wind-pollinated) species because taller males increase their pollen dispersal distance and pollination success (Burd & Allen 1988).

When resources are limited, different biological demands might result in different physiological adaptations for each sex. Thanks to physiological compensation mechanisms some plants might arrive to neutralize the costs of reproduction (Delph 1990). In a recent review (Obeso 2002) a series of physiological and functional mechanisms are enumerated as compensation of females' higher reproductive effort: development of photosynthetic reproductive structures; delayed age of reproduction; nutrient resorption from senescing structures; module specialization and female's higher photosynthetic rates.

In addition, plant's physiology might change over time due to seasonality, inter-annual variation and developmental changes (Dawson et al. 2004), and reproductive trade-offs might be detected under some environmental conditions but not in others due to phenotypic plasticity (Stearns 1989; Correia & Diaz Barradas 2000; Verdú et al. 2004). Due to its plasticity, it is unclear if physiological differences between sexes arise as a short-term reaction to resource availability (Dawson et al. 2004) and reproductive demands (Dawson & Ehleringer 1993b) or if they are the result of innate differences between the sexes (Nicotra 1999).

Long living masting species are a good opportunity to test for the reactive vs. innate physiology disjunctive, since during non-masting years both genders invest a similarly low amount of resources to reproduction. Physiological differences between sexes arising during years with similar or inverted reproductive demands would confirm the existence of long term gender specific strategies.

To experimentally study how variation in resource availability affected both physiological and reproductive performance of each sex, we fertirrigated (watering with fertilizer diluted) during two low-reproduction years a set of male and female trees of the dioecious *J. thurifera* with the expectation than this will increase reproduction of both sexes. Physiological measurements (Water potential; A; g_s and iWUE) were taken monthly during the two experimental years. Other integrative data (branch nitrogen content, branch isotopic discrimination of carbon, branch growth and reproductive investment per branch) were taken yearly.

With this experimental design we wanted to answer the following specific questions: (a) Do female *J. thurifera* trees exhibit physiological compensation mechanisms to offset reproductive costs? (b) Are such physiological mechanisms a long-term strategy or are regulated by immediate reproductive demands? (c) Does resource addition down-regulate such physiological compensation?

Materials and Methods

Study species

Juniperus thurifera is a dioecious long-lived tree with relictic distribution from the Tertiary throughout the calcareous high-mountains of the western Mediterranean Basin. Together with two closely related vicariant species with similar biology (*J. excelsa* and *J. procera*) they distribute along the Mediterranean basin, western Asia and eastern Africa. It forms low-density stands in which it can be the dominant species or can coexist with *Quercus ilex*, *Q. faginea* and *Pinus nigra*. Trees are 5-10m high and often live for centuries (Bertaudière et al. 1999). Female trees bear two kind of fleshy cones: unripe, green-coloured ones (hereafter Fr1) growing from the last flowering period, and mature, ripe dark-coloured ones (hereafter Fr2), growing from the flowering of two years before, which have been growing and ripening for 22 months in the tree’s branches. Ripe cones usually carry with 1-7 seeds (mean 3.5, N=2000). Viability and germination of seeds of *J. thurifera* are extremely low (Ceballos & Ruiz de la Torre 1979; Melero & García-Fayos 2001). Mean reproductive investment per branch during a typical non-masting year (2003) is 0.22±0.34 g (N=17) for males and 0.19±0.19 g (N=23) for females. During a masting year (2001) males invested an average of 0.52±0.42 g (N=17) and females 1.42±1.85 g (N=23) (Montesinos *et al.* unpublished data from a long-term masting study). This means approximately a seven fold increase for females and two fold for males.

Study site

Study was performed at La Puebla de San Miguel (Valencia, Eastern Spain). Study site is at 1500m a.s.l. and vegetation is dominated by *J. thurifera*. The study area has been used for agriculture, timber, and extensive livestock grazing for centuries. Narrow valleys were tilled for rye and barley until land abandonment in 1960 (Rodrigo 1999). In the last decades, forests have occupied many of these abandoned croplands (Lasanta 1996), though livestock grazing is still an important activity. The soils are limestones <30 cm deep, although cracks in the rock allow roots to go deeper than this (Verdú et al. 2004). Climate is Mediterranean, with cold and long winters (the duration of the freezing period is more than 120 days per year) and warm and dry summers. Mean annual precipitation is 486 mm being October the wettest month (58.6 mm) and July the driest (26.86 mm). Mean annual mean temperature is 13º C, being August the warmest month (mean 22.8º C) and January the coldest (mean 4.8º C).

Selection of trees and fertirrigation treatments

We selected 40 reproductive and healthy trees at the study site: 20 males and 20 females. Initial aboveground biomass of the selected trees was calculated from the equation described by Bertaudière (2001) and Montès (2002) for *J. thurifera*. To check for initial differences in size a GLM with sex and fertirrigation as fixed factors and estimated initial biomass as dependent variable was run. No significant differences were found between sexes (F=0.127, df=1, p=0.724) nor between fertirrigation treatments (F=0.009, df=1, p=0.925). Interaction was not significant (F=0.157, df=1, p=0.695). One of the males consistently presented disproportionate different values and was discarded of the study.

From April 2003 to March 2005, half of the male and female trees were fertirrigated monthly, except in June and July, the driest months, when they were fertirrigated fortnightly. On each fertirrigation date every tree was supplied with 100 L of 200 ppm fertilization solution (Universol Blue 18N:11P:18K plus oligoelements, Scotts, USA). This resulted in a yearly dose per tree of 1000 L of water and 2 Kg of fertilizer. Fertirrigation treatment was applied the day after collection of physiological measurements to avoid short-term interferences on tree physiology.

Shoot water potential and gas exchange measurements

Shoot water potential (Ψ), net photosynthetic rate (A) and stomatal conductance (g_s) were measured monthly from April 2003 to March 2005. Dawn and midday (6:00-8:00 and 12:00-14:00 h solar time, respectively) water potentials were determined with a Scholander chamber (SKPM 1400 Skye Instruments Ltd. Powys, UK) on one shoot from the south side of each tree. A and g_s were measured with a portable photosynthesis system (LCi ADC BioScientific Ltd. Herts, UK) immediately after Ψ measurements. Sampled shoots were very close to those sampled for Ψ. Intrinsic water use efficiency (iWUE) was calculated as:

$$\text{iWUE} = A/g_s \ (\mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}/\text{mmol H}_2\text{O m}^{-2}\ \text{s}^{-1})$$

From December 2004 to February 2005 we could not measure either Ψ or A or g_s because shoots were frozen and low air temperature hindered IRGA functioning.

Shoot $\delta^{13}C$ and N concentration

On January 2004 and on January 2005, five current-year shoots were cut from each tree all around the whole plant to avoid any effect of orientation. Samples from each tree were mixed, dried at 50 ºC for 48 h, and then ground with a mill. A 1 g subsample was used for total N determination following the standard Kjeldahl method. A second 20 mg subsample was analysed at the Laboratorio de Isótopos Estables (Universidad Autónoma de Madrid, Spain) to determine the C isotopic composition. After combustion of the samples in an elemental analyzer (EA 1108; CHNS, Carlo Erba, Milan, Italy) the $^{13}C/^{12}C$ ratios were determined with a continuous flow isotope mass ratio spectrometer (Micromass CF-Isocrom, Manchester, UK) with a measurement error of 0.1‰. The isotopic values are expressed in delta notation and calculated as:

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard}-1) \times 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the Pee Dee Belemnite carbonate standard, respectively.

Reproductive effort per branch

For two years (February 2003 to October 2005) we counted the number of male or female flowers (Fw), unripe female cones (Fr1, 10 months old) and ripe feminine cones (Fr2, 22 months old) in 10 branches randomly sampled all around each tree. The Fw, Fr1 and Fr2 mass was measured on 10 flowers (in February of each year) and in 10 Fr1 and Fr2 cones (in October of each year) randomly sampled from each tree after drying at 50 ºC for 48 h. Reproductive effort per branch was calculated as:

$$(Fw_{mass} \times Fw_{frequency})+(Fr1_{mass} \times Fr1_{frequency})+([Fr2_{mass}-\text{ (previous year }Fr1_{mass})] \times Fr2_{frequency})\ (g)$$

Branch and tree ring growth

In March 2003 we marked the tip of five branches in each of the experimental trees. Resulting branch growth from the marks was collected in January 2006. Samples were dried for 48h and weighted to the nearest 0.0001 g.

Dendrochronological cores were extracted at d.b.h. from the experimental trees in July 2005. After mounting and polishing the samples tree ring growth was measured to the nearest 0.01 mm for years 2003 and 2004 following standard dendrochronological methods.

Data analyses

Linear Mixed Models with an autoregressive covariance structure of order one and month and time of the day (dawn or midday) as repeated measures were fitted to the data to analyze the effect of fertirrigation and sex on tree's Ψ; A; g_s and iWUE.

Linear Mixed Models with an autoregressive covariance structure of order one and year as repeated measure were fitted to the data to analyze the effect of sex and fertirrigation on shoot $\delta^{13}C$; N concentration and reproductive effort.

A General Linear Model was fitted to the log transformed data to test the effect of sex and fertirrigation over branch growth.

A General Mixed Model with an autoregressive covariance structure of order one was fit to the log transformed data to test the effect of sex and fertirrigation on tree ring growth.

Results

Shoot water potential and gas exchange measurements

No differences were found for Ψ between sexes and it was not significantly affected by fertirrigation (Table 4.1 and Fig. 4.1A). Females had greater A and g_s than males when not fertirrigated. Fertirrigation increased A and g_s in males but not in females resulting in no significant gender differences in fertirrigated individuals (significant sex × fertirrigation interaction, see Table 4.1 and Figs. 4.1B and 4.1C). Neither gender nor fertirrigation significantly influenced iWUE (Table 4.1 and Fig. 4.1D).

Shoot $\delta^{13}C$ and N concentration

Females presented more negative $\delta^{13}C$ values than males independently of fertirrigation treatments (Table 4.1 and Fig. 4.2A) indicating a lower long-term WUE for this sex. Fertirrigation equally enhanced shoot N in both sexes, which did not present significant differences between them (Table 4.1 and Fig. 4.2B).

Reproductive effort per branch

During the studied years males invested significantly more resources to reproduction than females. Fertirrigation increased reproductive effort of both sexes (marginal p=0.09, Table 4.1 and Fig. 4.2C).

Branch and tree ring growth

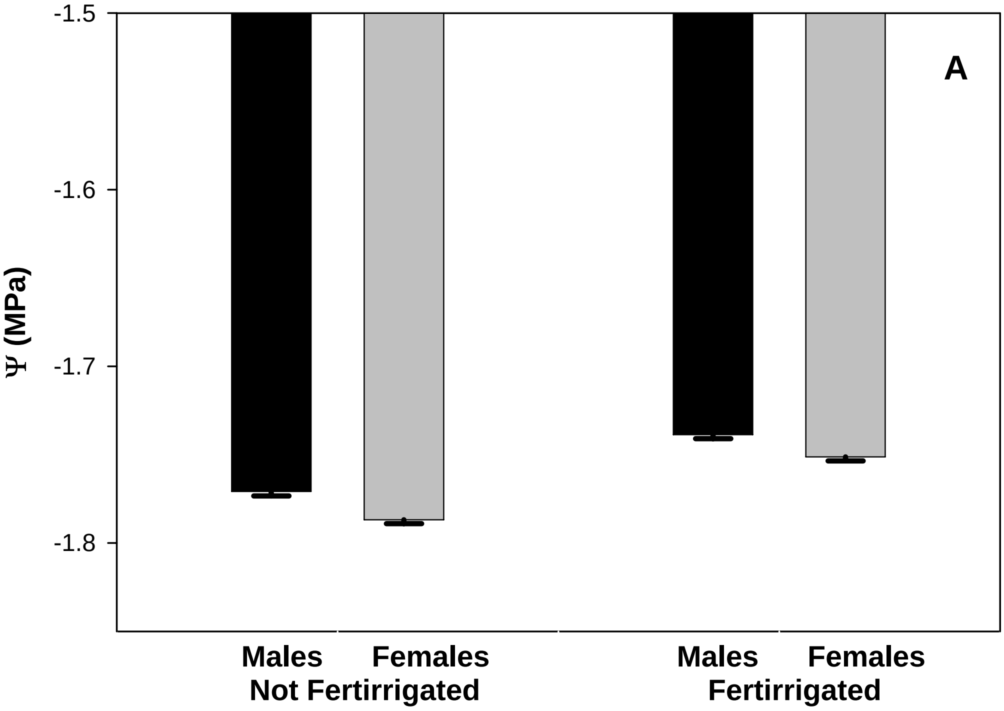
Fertirrigation significantly increased branch growth, furthermore, branch growth was increased proportionally more in female than in male trees (marginal sex x fertirrigation interaction p=0.066, Table 4.1 and Fig. 4.1D). In contrast, fertirrigation increased radial growth in males but reduced it in females (sex × fertirrigation interaction, see Table 4.1 and Fig. 4.1E).

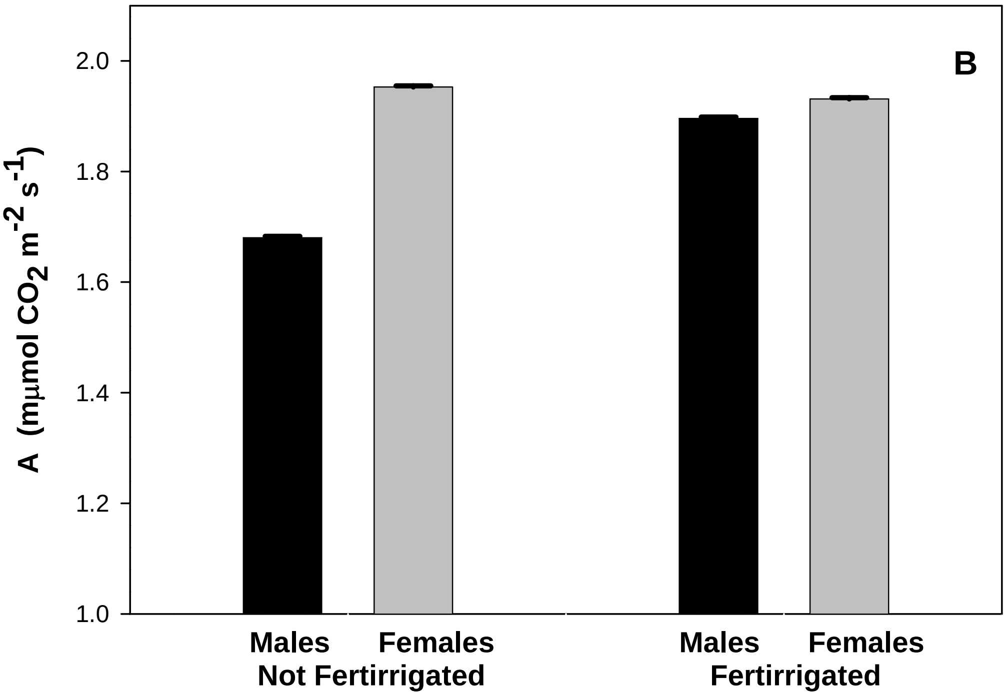
Table 4.1: Lineal Mixed Model F values for the studied variables.

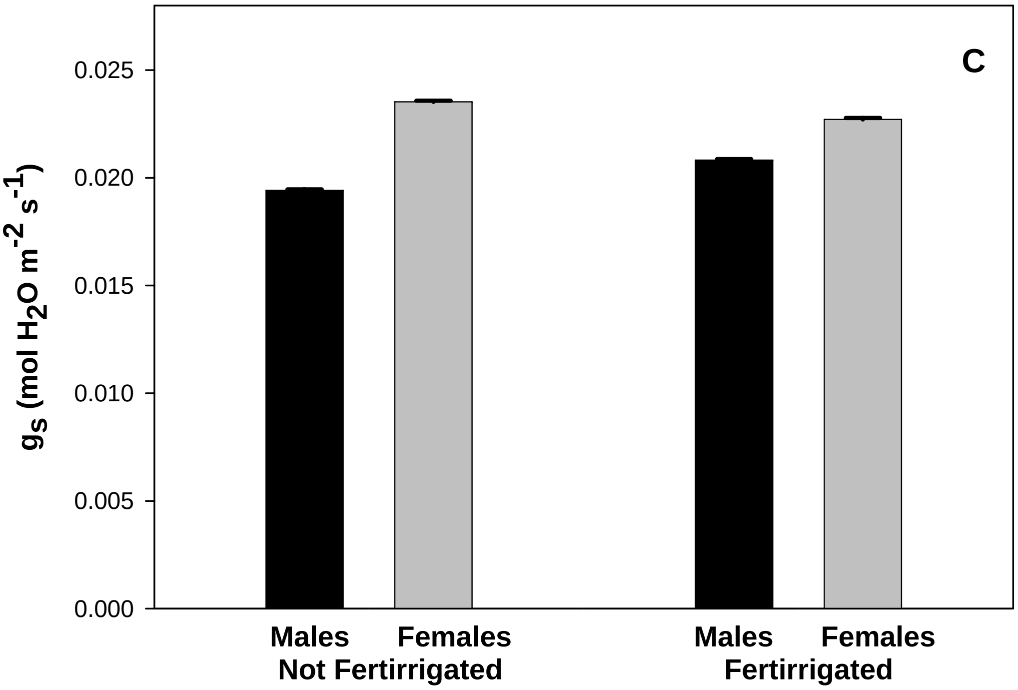
	Sex	**Fertirrigation**	**Sex x Fertirrigation**
Ψ	0.09	0.56	0.01
A	8.53**	3.93*	4.37*
gs	5.56*	0.12	4.22*
iWUE	0.99	1.37	0.21
$\delta^{13}C$	6.89*	0.61	0.63
N	0.05	33.48***	0.01
Reproductive effort	7.13*	2.97^{+}	0.17
Branch growth	1.50	7.04**	3.42^{+}
Ring growth	0.39	0.07	4.23*

$^{+}$, P≤0.09; *, P≤0.05; **, P≤0.01; ***, P≤0.001.

Figure 4.1: Mean values for: (A) water potential; (B) net photosynthesis; (C) stomatal conductance; (D) intrinsic water use efficiency. For each fertirrigation treatment, solid bars represent males and grey bars females. Error bars represent standard errors.


A
Ψ (MPa)
-1.5
-1.6
-1.7
-1.8
Males
Females
Not Fertirrigated
Males
Females
Fertirrigated


B
A (mμmol CO$_2$ m^{-2} s^{-1})
2.0
1.8
1.6
1.4
1.2
1.0
Males
Females
Not Fertirrigated
Males
Females
Fertirrigated



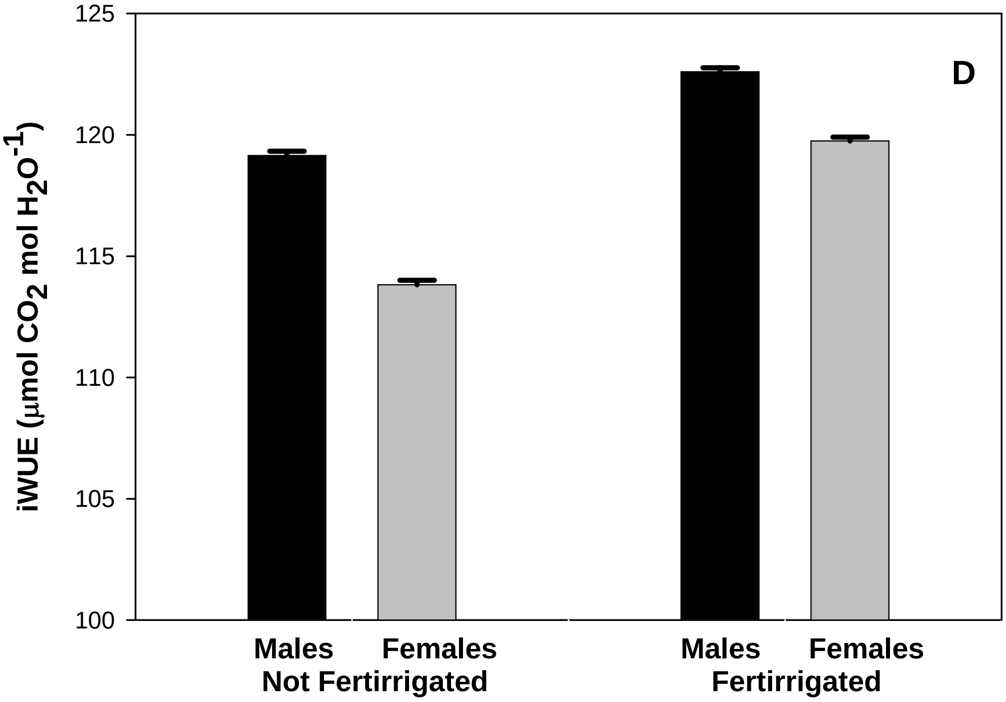
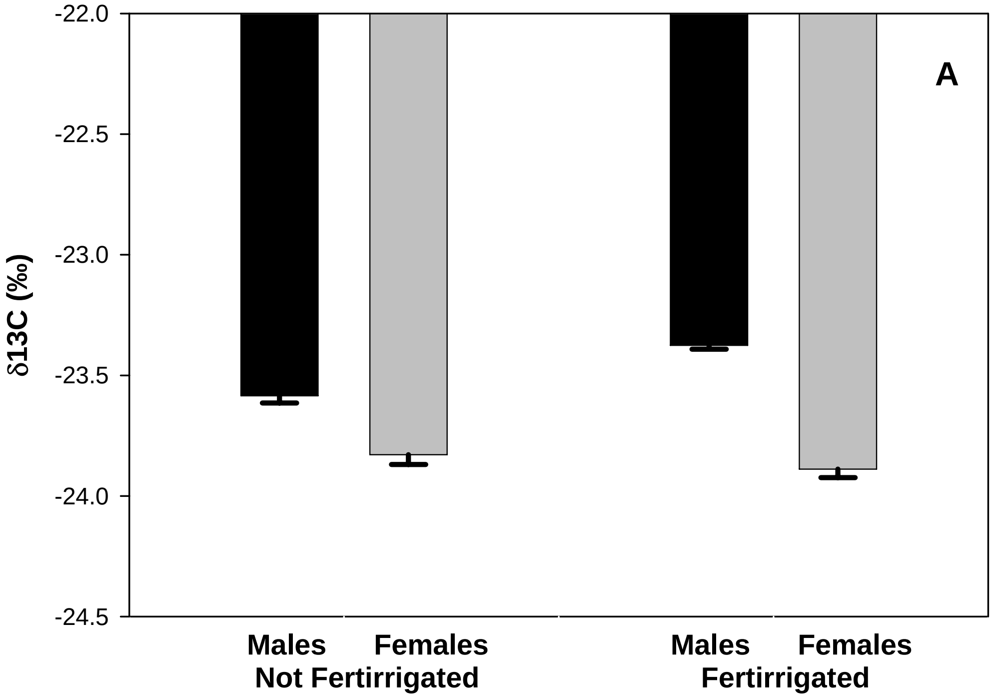
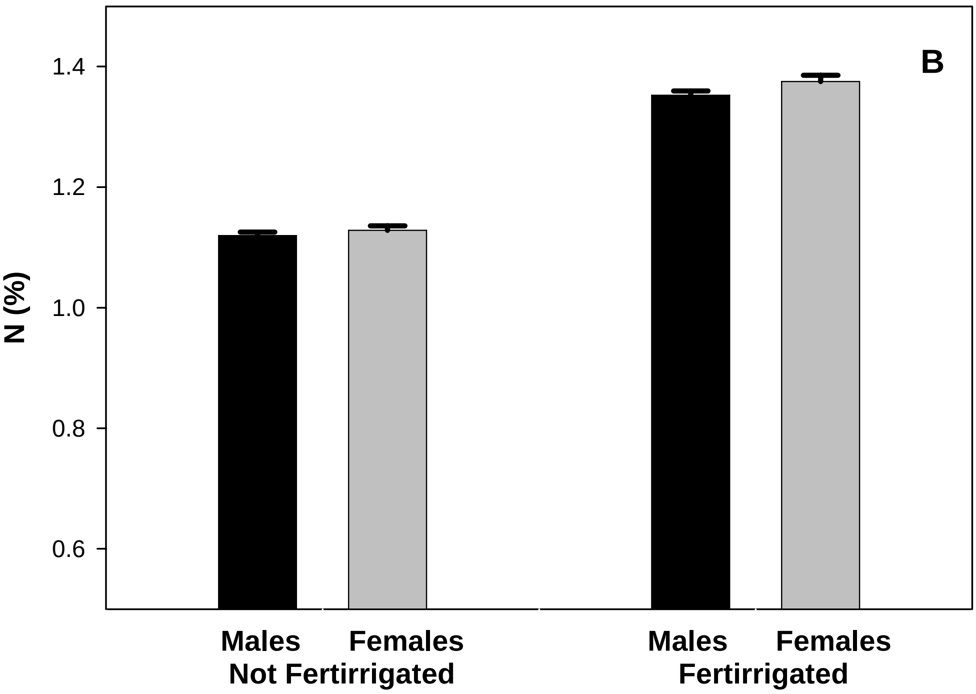
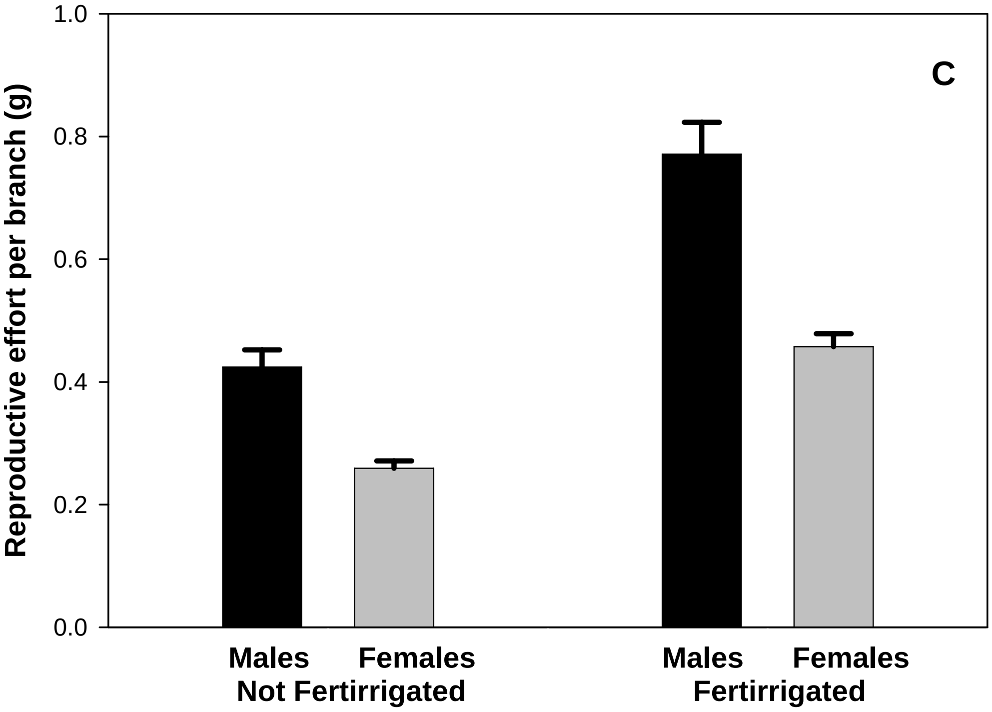


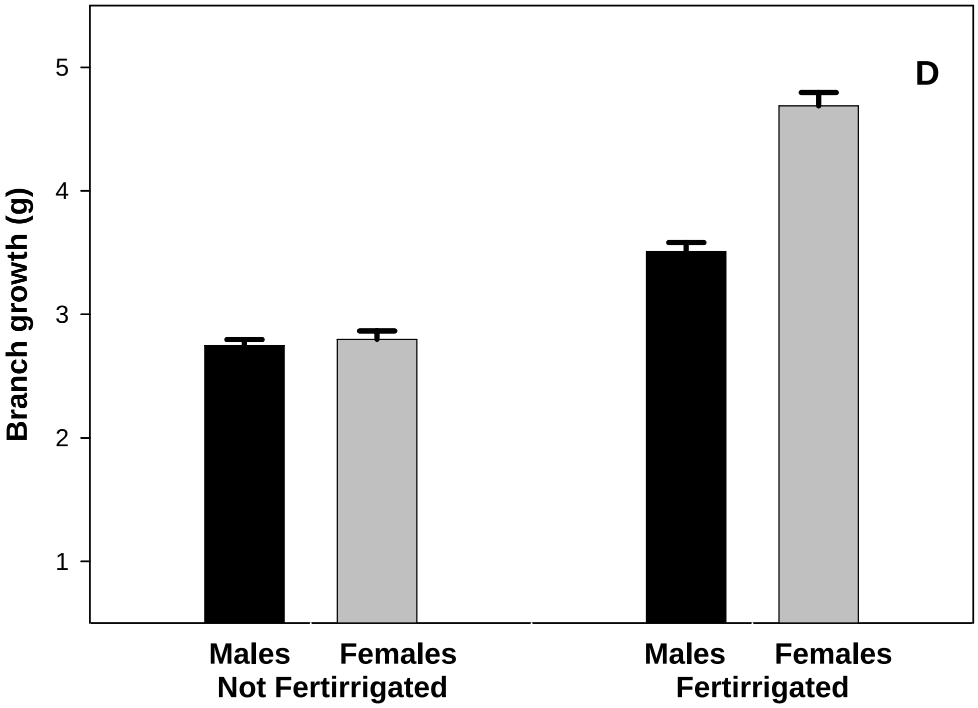
Figure 4.2: Mean values for: (A) shoot $\delta^{13}C$; (B) branch nitrogen content; (C) branch reproductive effort (D) branch growth; (E) tree ring growth. For each fertirrigation

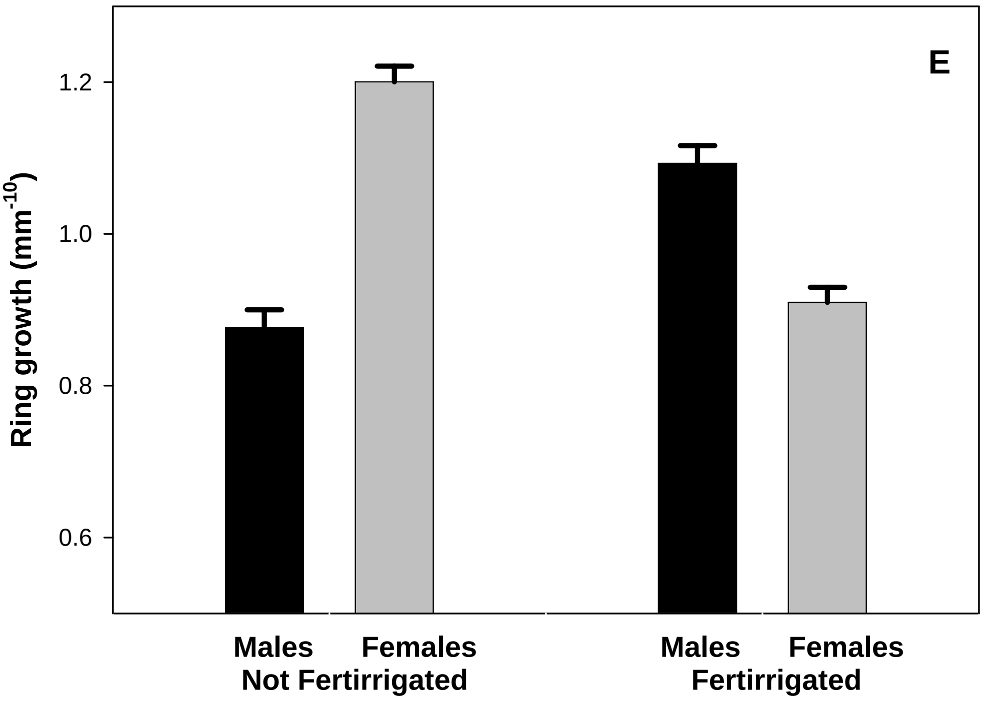
treatment, solid bars represent males and grey bars females. Error bars represent standard errors.






C
Reproductive effort per branch (g)
1.0
0.8
0.6
0.4
0.2
0.0
Males
Females
Not Fertirrigated
Males
Females
Fertirrigated


D
Branch growth (g)
5
4
3
2
1
Males
Females
Not Fertirrigated
Males
Females
Fertirrigated



Discussion

Male and female trees differed in their physiological behavior and in its response to variations in resource availability. During episodes of massive reproduction (masting) *J. thurifera* female trees allocate to reproduction seven fold more biomass than males (Montesinos et al. 2007 see M&M). Even if during the studied non-masting years male trees inverted slightly more resources to reproduction than females, overall inter-annual reproductive investment was significantly higher for females (Montesinos et al. 2007 see M&M). As a compensation of long-term higher reproductive investments, females of *J. thurifera* developed higher photosynthetic rates (A) and stomatal conductances (g_s). Higher photosynthetic performance has been described frequently as a compensation mechanism to higher reproductive effort (Dawson & Ehleringer 1993b; Obeso 2002) and He (2003) described higher photosynthetic rates for females of the cogeneric species *Juniperus sabina* (Syn: *Sabina vulgaris*). It is unclear if physiological differences between sexes arise as an immediate reaction to resource availability (Dawson et al. 2004) and reproductive demands (Dawson & Ehleringer 1993b) or if they represent a long-term strategy (Nicotra 1999). Our results showed that physiological differentiation was present during low-reproduction non-masting years, indicating the presence of long-term gender-specific physiological strategies.

Higher photosynthetic rates might result also in a lower WUE for female trees. However, we did not find differences between sexes for water potential or iWUE in the short term. Conversely $\delta^{13}C$ showed than males had a more efficient use of the water on the long term, in agreement with results for other juniper species (Hill et al. 1996). Females' higher photosynthetic rates imply higher transpiration and physiological specialization can be the cause of subsequent habitat partitioning between the sexes; when this occurs, the less demanding reproductive effort of males might enable them to colonize more stressful environments (Dawson & Bliss 1989; Dawson & Ehleringer 1993a; Hill et al. 1996; Dawson et al. 2004) although in other species there are no further consequences (Liebig et al. 2001). Juniper species acclimate to a relatively large range of environments (Moore et al. 1999) and Peñuelas (1999) found a spatial gradient related to water availability for a multi-species set including *J. thurifera* although without considering the sex of the studied trees. Therefore we do not have any evidence of habitat partitioning between sexes for the studied species.

Reproductive trade-offs might be detected under some environmental conditions but not in others due to phenotypic plasticity (Stearns 1989; Correia & Diaz Barradas 2000; Verdú et al. 2004) seasonality and inter-annual variation (Dawson et al. 2004). *J. thurifera* males and females responded differently to experimental addition of water and nutrients during the two studied years. Whereas photosynthesis in females remained unaltered when fertirrigated, the fertirrigation treatment increased the photosynthetic rates in males to reach that of the females, and also increased reproductive allocation proportionally more than females. The increase in reproductive allocation when water and nutrient availability was experimentally increased, suggests that reproduction in this masting species is mediated by resource matching (sensu Kelly & Sork 2002). Resource matching does not invalidate other selective factors which could be simultaneously explaining masting dynamics, like pollination efficiency or predator satiation (Kelly & Sork 2002; Montesinos et al. 2007).

Nutrients stored at leaves can be available also to reproduction (Henery & Westoby 2001). Some studies have detected increased females' branch and leaf growth compared to that of males (Nicotra 1999) and Sala (2006) already pointed to leaves as nutrient stores for species producing exceptionally large crops. Fertirrigation affected *J. thurifera* branch growth and reproduction in an opposite way: fertirrigated male trees increased reproduction in almost 0.4 g/branch and fertirrigated females increased reproduction in less than 0.2 g/branch. However, fertirrigated males increased branch growth less than 1 g/branch, while fertirrigated females increased branch growth 2g/branch. Increasing photosynthetic surface constitutes a wise strategy to devote resources to future reproductive events, and our results indicate that females are preparing future reproductive events by investing surplus resources in leaves. Male trees on the other hand seem to modulate their vegetative growth according to current resource availability.

Our measures of branch growth were based on biomass and some authors suggest that this could not be a representative physiological currency (Obeso 2002), although results from studies considering other currencies (N and P mainly) did not find different patterns that when considering biomass alone (Delph 1999 and references therein). Since in our study branch N content per gram was similar for both genders and lifted in a similar pattern when fertirrigated, we can confidently presume that leaves are an equally rich in essential nutrients for both genders.

Differences in the effects of reproduction and post-reproductive response have been previously described for species with sex biased reproductive investment (e.g. Delph & Meagher 1995; Saulnier & Reekie 1995; Nicotra 1999). However, describing physiological responses to resource addition among years and among levels of reproductive investment is a rarely used comprehensive approach to study the temporal extent of physiological and functional adaptations.

Conclusions

Our results show two well differenced physiological strategies for each sex, with physiological and functional specialization present along a range of reproductive investments. Male and female physiological output differed at low reproductive levels, and genders presented different strategies in the use of supplementary nutrients and water. Control females presented higher photosynthetic rates than control males. When resource availability was experimentally increased, male trees distributed surplus resources among reproduction, ring and branch growth and in improving their photosynthetic system. On the contrary, female trees reduced ring growth and maintained photosynthetic rates and only branch growth experienced a considerable increment while reproduction was slightly increased. However, the most remarkable is that physiological divergence between sexes responded not only to an immediate resource demand but to a well established long-term functional strategy. In *J. thurifera*, functional and physiological gender-specific strategies are present at any time, and might be genetically fixed to each sex.

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V - El coste de ser relíctico: la viabilidad de semillas se encuentra limitada por la altitud en *Juniperus thurifera*

V - On the cost of being relictual: altitude limits seed viability of the mountain tree *Juniperus thurifera*

V - El coste de ser relíctico: la viabilidad de semillas se encuentra limitada por la altitud en *Juniperus thurifera*

Introducción Las poblaciones de *Juniperus thurifera* se distribuyen a lo largo de la cuenca oeste mediterranea y son un relicto de su antigua distribución Terciaria, resultado del progresivo calentamiento del planeta desde la última glaciación.

Material y Métodos Para descubrir que factores son responsables de la extremadamente baja fertilidad de las poblaciones actuales de *J. thurifera* combinamos un estudio a macroescala, seleccionando 11 poblaciones a lo largo y ancho de la Península Ibérica, con un experimento a microescala, en una sola población, mediante reducción del estrés ambiental por fertirrigación de una selección de árboles.

Resultados La comparación de los patrones mostrados por la macroescala geográfica con los resultados experimentales a microescala evidencian que la fertilidad se encuentra limitada principalmente por la altitud y la actividad de insectos predadores de semillas. Incrementos en la cantidad de nutrientes y agua disponibles incrementaron significativamente el crecimiento de hojas y el tamaño de cosecha pero no modificaron la tasa de viabilidad de semillas.

Conclusiones El acelerado cambio climático contemporáneo forzará la migración altitudinal de este árbol, reduciendo aun más su fertilidad y comprometiendo el futuro de esta especie relíctica, aunque dicho efecto tardará en notarse dada la gran longevidad (>200 años) de esta especie.

V - On the cost of being relictual: altitude limits seed viability of the mountain tree *Juniperus thurifera*

Introduction *Juniperus thurifera* populations are scattered throughout the western Mediterranean basin and are relictual from the Tertiary distribution due to progressive climatic warming since the last glacial period.

Materials and Methods To disentangle the factors responsible for its present day extremely low fertility we combined a macroscale study, by selecting 11 populations across a geographical range, with a microscale experimental alleviation of environmental stress, by adding water and fertilizer to a set of trees.

Results Comparison of macroscale patterns and microscale experiments evidenced that fertility was mainly limited by altitude and seed predator activity. Increased nutrient and water availability significantly increased leaf growth and crop size but not seed viability rates.

Conclusions Global climate change might force upward altitudinal migration of this tree, reducing still more its fertility and compromising future regeneration of this relictual species.

Introduction

Altitudinal migration of high mountain flora is a general trend in response to global warming (Grabherr et al. 1994; Klanderud & Birks 2003; Walther 2003). Rise of lower distribution limits of mountain plants is not normally due to heat stress but rather to an increase of the upper altitudinal limit of competitor species with faster growth rates (Loehle 1998; Walther 2003; Alward et al. 2006). To rise their distribution limits plants have to face a set of increasingly harsh environmental factors characteristic of higher mountains, like higher radiation (IR, UV and visible); lower CO_2 and O_2 partial pressure; stronger winds which can destroy tissues mechanically and more frequent drought events (Barceló et al. 1992; De Lillis et al. 2004). These conditions can inhibit photosynthetic activity (Kofidis et al. 2003) and reduce net primary productivity (Luo et al. 2004). Under such unfavorable circumstances reproductive allocation tends to decrease with altitude (Hemborg & Karlsson 1998; Obeso 2002; Sakai et al. 2006) and plants can mitigate their reproductive effort by producing low quality seeds (Houle & Barbeux 1994). Seed predator species can also migrate altitudinally due to temperature warming, and raise their altitudinal distribution faster than plants do, increasing predation rates of previously unaffected populations and species (Hódar & Zamora 2004).

Plants present lower leaf growth rates and lower specific leaf areas (SLA) at higher altitudes (De Lillis et al. 2004; Angert 2006). Since SLA is positively correlated to longer leaf lifespan (Westoby et al. 2006), high altitude plants are expected to show lower leaf lifespan. In addition, increased UV radiation can be a significant stress factor for leaves (Robberecht & Caldwell 1980) and is likely to result also in reduced leaf lifespan.

Juniperus thurifera (Cupressaceae) is a dioecious tree living in semiarid cold environments of the Holartic region that originated during the Tertiary (Suárez Cardona et al. 1991). Since the last glacial period its distribution has been reduced to scattered populations restricted to the high mountains of the western Mediterranean Basin and its present day habitats represent a compromise between environmental harshness and low presence of competitor species (Gómez-Manzaneque 1997). Like many junipers, *J. thurifera* presents low seed viability rates (e.g. Roques et al. 1984; García 1998). However, geographical and environmental factors responsible for junipers low fertility are unclear and might be related to a series of environmental and geographical factors (García et al. 2000). Junipers seed viability can be limited by environmental harshness (García et al. 2000; Wesche et al. 2005), nutrient and water availability (Stephenson 1981; García et al. 1999; Drenovsky & Richards 2005) and pollination failure (Ortiz et al. 2002). Junipers typically present a very high proportion of parthenocarpic seeds (Fuentes 2000) and moderate to high rates of abortion and predispersal seed predation (Rappaport et al. 1993; García 1998; El Alaoui El Fels et al.

1999). Explanations for parthenocarpy include frost damage to the ovule, nutrient limitation and defense against seed predators (Traveset 1993; Verdú & García-Fayos 1998 and references therein).

In this work we try to infer the reproductive consequences of an upward migration of *J. thurifera* populations as a response to the predicted global warming (Schröter et al. 2005) by elucidating factors responsible for plants' fertility. We study variation in seed fate (viability, abortion, predation and parthenocarpy), leaf lifespan and leaf growth at two different scales of environmental variation: at macroscale, by sampling 11 populations along a latitudinal range across the Iberian Peninsula; and at microscale, by experimentally modifying water and nutrient availability in a set of trees at one population.

Material and Methods

Study species

Juniperus thurifera lives in the calcareous high-mountains of the western Mediterranean Basin. It is found in the Alps (France and Italy), across the mountains of the Iberian Peninsula (Spain and French Pyrenees), in the Atlas mountains (Morocco) and in two small populations in Corsica and Algeria. Together with two closely related vicariant species with similar biology (*J. excelsa* and *J. procera*) they are scattered all along the Mediterranean basin, western Asia and eastern Africa. It forms low-density pure stands or mixed open forests with *Quercus ilex*, *Q. faginea, Pinus nigra, P. sylvestris* and *P. pinaster*. Trees are 5-10m high and often live for centuries (Bertaudière et al. 1999). Cones take 22 months to develop and ripe and carry with 1-7 seeds (mean 3.5, N=2000). Viability and germination of *J. thurifera* seeds is extremely low due to high rates of parthenocarpy, abortion and predation (Ceballos & Ruiz de la Torre 1979; Melero & García-Fayos 2001).

Fleshy cones are attacked by a variety of pests, but main seed predators are the chalcid *Megastigmus bipunctatus* (hereafter *Megastigmus*) and the mite *Trisetacus quadrisetus* (hereafter *Trisetacus*). *Megastigmus* oviposites on unripe seeds on the first summer after pollination and larvae develop and grow for one more year before they emerge from the seed leaving a characteristic exit hole (Roques et al. 1984). *Trisetacus* oviposition occurs much earlier, often before pollination occurs. Mites live in colonies and use seeds as growth chambers often leading to the destruction of the totality of the seeds in a cone. Seeds affected by *Trisetacus* colonies present a characteristic brown "fibrous" morphology (Roques et al. 1984).

Study sites

From French Pyrenees to southern Spain eleven populations (see Fig. 5.1) were sampled for seeds during three years (2002-2004) and for leaf growth and longevity in 2004. Sampled populations cover a wide range of environmental characteristics (Table 5.1). In each population, we estimated tree density by counting every individual taller than 2 m in 10 transects each of 100 m long and 10 m wide randomly distributed across each population. Sex ratio was estimated by determination of the sex of the first 100 reproductive individuals along the same transects.

Simultaneously, we randonmly selected and marked a set of 20 female trees at population number 3 (Puebla de S. Miguel, Valencia) for the experiment. From April 2003 to March 2005, half of the trees were fertirrigated monthly, except in June and July, the driest months, when they were fertirrigated fortnightly. On each fertirrigation date every tree was supplied with 100 L of 200 ppm fertilization solution (Universol Blue 18N:11P:18K plus oligoelements, Scotts, USA). This resulted in a yearly dose per tree of 1000 L of water and 2 Kg of fertilizer.

Seed fate

We collected 10 ripe cones from each of 20 female trees randomly chosen across every population. Cones were collected from all around the tree to avoid orientation effects on seed viability or seed set. Ripe cones where collected in October 2002, 2003 and 2004. Seeds where cut to show embryo and visually assigned to one of five categories: viable seeds, aborted, parthenocarpic, preyed by *Megastigmus* and preyed by *Trisetacus*.

At the experimentally watered set of trees, three individual branches were marked in every tree and total number of flowers were counted in January 2002, before experimental addition of water with fertilizer started. At the end of the experiment, in October 2004, fruit and seed set were estimated and seeds collected to study viability.

Leaf growth and longevity

J. thurifera presents a continuous stem and leaf growth only limited by high temperatures in summer and low temperatures in winter (D. Montesinos pers. obs.). Natural defoliation of the oldest leaves results in leaves appearing only at branches' tips. We collected one branch from each of ten male and ten female trees from each population by cutting them up to the defoliation point in October 2004. Leaf lifespan was estimated following the approximation used by Mediavilla and Escudero (2003). Last year's branch growth is easily recognizable for a lighter green leaf color and because stems are not lignified. Leaves from the last year were manually separated from older leaves and dried at 60ºC for 48 h. The ratio between the dry weight of last year leaf growth and growth from previous years (darker leaves on dark lignified stems) is a convenient approximation to leaf longevity in scale-like leaved plants under the assumption that leaf growth is similar among years.

Statistical analyses

Linear Mixed Models with an autoregressive covariance structure of order one and year as repeated measure were fitted to the data to analyze the effect of populations' environmental characteristics (latitude, altitude, previous year and current year temperature and precipitation, mean annual temperature and precipitation, water deficit, tree density and sex ratio) on seed viability. Linear regressions were used to study the relationship between leaf longevity and leaf growth and different environmental variables (altitude, precipitation, etc.). General Linear Models were fitted to the data to determine if fruit and seed set, seed viability, abortion, parthenocarpy or predation were affected by regular watering with fertilizer (fertirrigation).

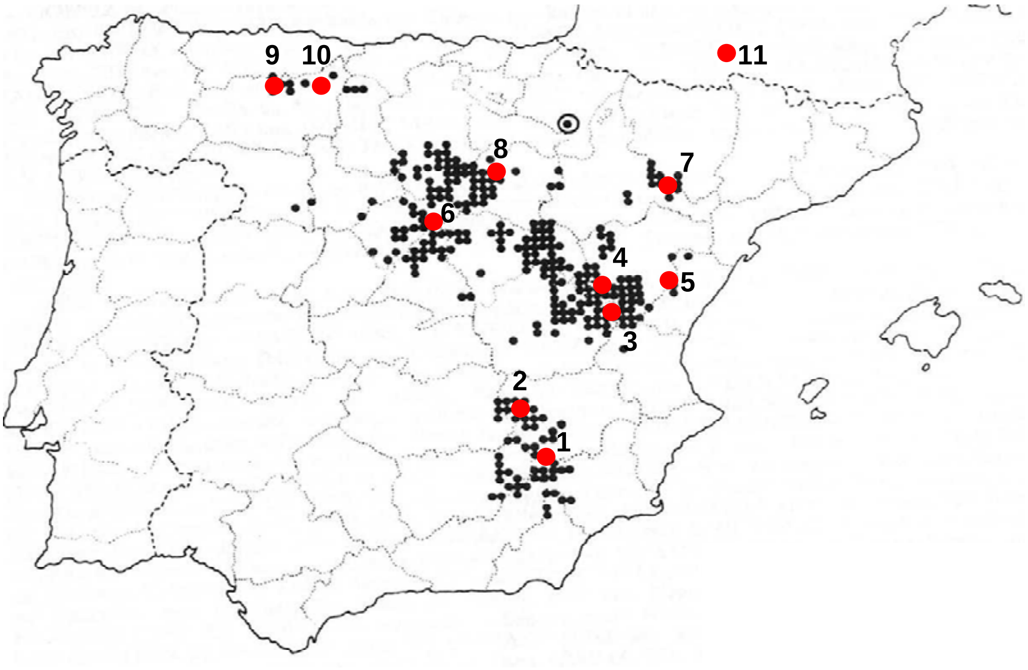

Fig. 5.1: *J. thurifera* Iberic distribution (small dots, based on Gómez Manzaneque et al. 1993) and studied sites (large dots): 1-Nerpio; 2-Ossa de Montiel; 3-Puebla S. Miguel; 4-Saldón; 5-Portell de Morella; 6-Hornuez; 7-Purburrell; 8-Calatañazor; 9-Barrios de Luna; 10-Crémenes ; 11-Mt. de Rie.

Population	Latitude (nearest º)	Altitude (m a.s.l.)	Mean annual temperature (º C)	Mean annual precipitation (mm)	Mean annual water deficit (mm)	Tree density (trees/ha)	Sex ratio (males/total)	Leaf growth (g) (2004)	Leaf longevity (years) (2004)
1.- Nerpio	38ºN	1285	14.6	466	-215	690	0.52	4.7	2.26
2.- Ossa de Montiel	39ºN	1000	12.8	448	-179	905	0.3	5.01	2.17
3.- Puebla de S. Miguel	40ºN	1500	12.6	535	-102	423	0.54	4.62	2.03
4.- Saldón	40ºN	1420	10.8	470	-112	596	0.45	5.2	2.71
5.- Portell de Morella	41ºN	1120	11.2	665	82	178	0.29	2.21	2.41
6.- Hornuez	42ºN	1130	11.4	510	-82	350	0.5	5.96	2.51
7.- Purburrell	42ºN	350	15.2	360	-352	104	0.5	6.1	3.23
8.- Calatañazor	42ºN	1100	9.8	700	143	429	0.5	3.69	2.44
9.- Barrios de Luna	43ºN	1200	9.4	848	298	267	0.56	6.29	2.46
10.- Crémenes	43ºN	1150	8.8	1274	751	327	0.57	8.42	2.38
11.- Mt. de Rie	43ºN	850	11.0	1050	441	925	0.56	-	-

Table 5.1: Sampled populations and their environmental characteristics ordered by latitude.

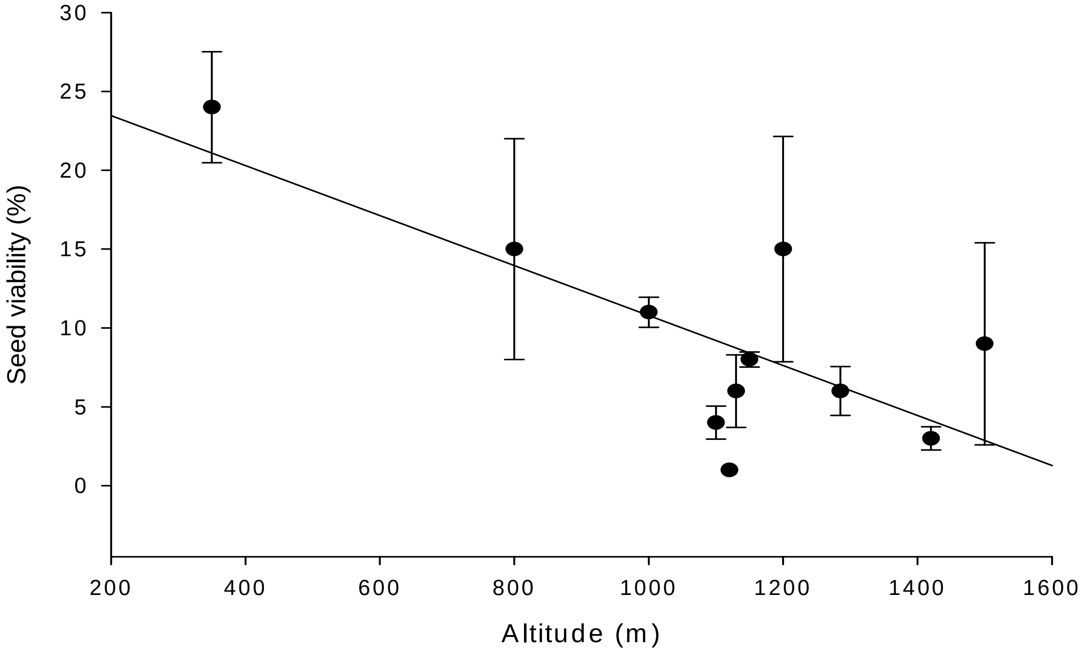

Fig. 5.2: Seed viability decreases with altitude along the Iberian Peninsula. Mean inter-annual values and their standard errors are shown (R^2=0.54; p=0.007).

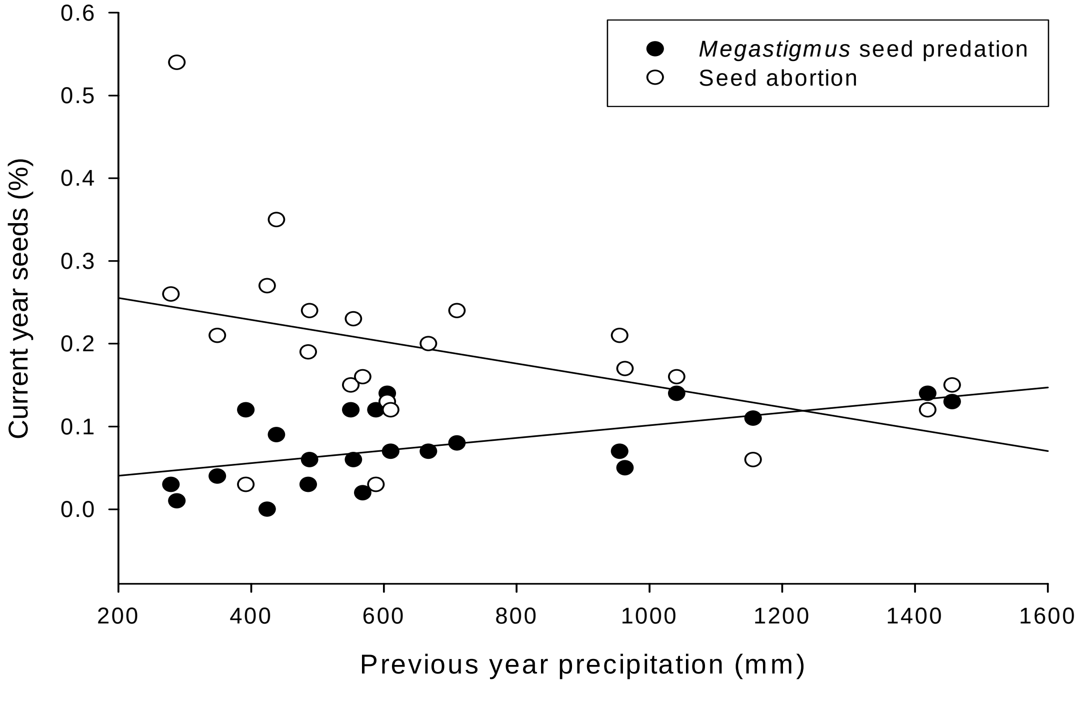

Fig. 5.3: Precipitation in a given year reduces seed abortion and increases seed predation in the following year. Open dots represent seed abortion rates, filled dots represent seed predation by *Megastigmus*.

Results

Seed fate

Seed viability averaged 9.5±8.8 % among populations and presented a negative relationship with altitude ($F_{1,20}$=9.0; p=0.007; see Fig. 5.2). There were no significant relationships between seed viability and any other environmental or geographical variable (data not shown).

Seed abortion averaged 19±11 % among populations and correlated negatively with previous year precipitation ($F_{1,20}$=4.16; p=0.055; Fig. 3). Seed predation averaged 13.6±8.7 % among populations. Two seed predators where identified: *Trisetacus* (6.3±10 %) and *Megastigmus* (7.3±4.6 %). The first did not show any relationship with climate while the second correlated positively with previous year precipitation ($F_{1,14}$=8.41; p=0.012; Fig. 3). The rate of parthenocarpy averaged 58.7±11.9 % and did not correlate with any other variable. There were no significant relationships between mean number of seeds per cone, proportion of filled seeds or seed fate with any other environmental or geographical variable (data not shown).

Regarding the watering experiment at population three, only fruit set showed significant differences between fertirrigation treatments. Fertirrigated trees produced three fold more ripe cones per flower (0.28±0.19 cones/flower) than controls (0.09±0.05 cones/flower; F=6.3; df=1; p=0.028). However, proportion of viable seeds was similar for both treatments.

Leaf growth and longevity

Across the geographical range there were no significant differences between sexes in leaf growth (F=0.228; df=1; p=0.639) or leaf longevity (F=0.755; df=1; p=0.396). Since our study was focused in seed production, subsequent analyses on leaf growth and longevity considered only females' leaves. Leaf growth and leaf longevity (see values on Table 1) were not influenced by any of the geographical or environmental variables (data not shown).

In contrast, experimental watering and fertilization significantly raised leaf growth from 2.8±2.6 g to 4.7±3.9 g (F=6.15; df=1; p=0.015) but mean leaf longevity (2±0.3 years) was not affected.

Discussion

As predicted, altitude reduced seed viability, although leaf growth and leaf lifespan was unaffected by it. Along the geographical range (macroscale), environmental variation associated with altitude was higher than that associated with latitude. As a result, latitude did not correlate straightforwardly with any variable and then altitude represented the main geographical gradient. Altitude significantly reduced seed viability of *J. thurifera* seeds, in agreement with other studies (Hemborg & Karlsson 1998; Sakai et al. 2006), and as expected for environments increasingly harsh (Caldwell & Robberecht 1980; Kofidis et al. 2003) with light levels which may induce photoinhibition (Close et al. 2003) and with low primary productivity (Luo et al. 2004). The congeneric *J. communis* also reduced fertility with increasing altitude (Ortiz et al. 2002).

Across the geographical range, precipitation in a given year significantly reduced seed abortion of the following seed crop, indicating that water stress was an important factor for seed viability. However, reduction in seed abortion did not result in an increase in seed viability; instead, reduction in seed abortion was paralleled by a similar increase in seed predation by *M. bipunctatus*. This pattern suggests that there is an optimum viability rate above which additional viable seeds have low probability of escaping from seed predators.

It is remarkable that in our study, both among the studied populations along the geographical range and between the experimental treatments, parthenocarpy rate was quite high and regular (58.7±11.9 %) and was not correlated with any geographical or environmental variable. Trees experimentally supplied with extra water and nutrients also maintained high parthenocarpy and low viability rates although they were able to increase global fertility by increasing fruit-set.

Parthenocarpy might be an exadaptive response to escape from seed predation (Traveset 1993; Verdú & García-Fayos 1998) being the result of a long evolutionary history in common which is genus specific for Cupressaceae (Roques 1983; Turgeon et al. 1994; Roux & Roques 1996). Parthenocarpy can reduce both pre and postdispersal seed predation because predators spend time and eggs in unviable seeds thus increasing survival probability of viable seeds (Traveset 1993; Verdú & Garcia-Fayos 2000). Our results indicate that *J. thrurifera* parthenocarpy rate will not be affected by climate change and that further reductions on seed viability will be due to seed abortion and predation.

On the macroscale (geographical range) neither leaf lifespan or leaf growth were limited by precipitation and did not correlate with any other variable. Microscale experiments of water with fertilizer addition significantly increased leaf growth. This suggests that nutrients, and not water, are the main limiting factor for leaf growth in this species. Nutrients, and particularly N, have been described to be a critical nutrient limiting plant growth in nutrient-limited environments (Drenovsky & Richards 2005) and forest productivity is affected more strongly by N availability than by any other nutrient (Saxe et al. 2001 and references). Finally, and in concurrence with other studies (e.g. Jonasson et al. 1997), leaf lifespan did not decrease with increased nutrient availability as previously predicted (Aerts 1995).

Conclusions

J. thurifera today's distribution is the result of dramatic climatic changes since the Tertiary and most of present day populations are set in relictual areas at high altitudes. However plants perform better within their original altitudinal range as their physiology is best adapted to it (Angert 2006). Our data indicates that altitude is the main environmental gradient responsible for the extremely low seed viability found. Increase of water availability also reduced abortion rates and increased fruit-set and leaf growth.

Climate change will affect Mediterranean plants more than in other areas(Sala et al. 2000; IPCC 2001; Schröter et al. 2005). Precipitation in Spain experienced a reduction of 30% in the last century and temperature experienced an increase of 0.8º C. Further increase in temperature and reduction of precipitation will shift competitive interactions, pushing upwards *J. thurifera* populations. However, a series of environmental factors associated with altitude are unaffected by climate change (high IR, UV and visible irradiance, low gas pressure, etc.) and might be the ultimate reason of low juniper fertility. Reproductive downsizing of migrated populations will compromise its future, although the immediate effect will be attenuated by the long lifespan (>200 years) of this tree species.

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Conclusiones

La dioecia es un factor significativo a lo largo de toda la historia vital de *J. thurifera*. Tanto los datos observacionales como los experimentales indican que machos y hembras modifican el ambiente de forma diferente, experimentan diferentes costes reproductivos y presentan diferentes estrategias fisiológicas y funcionales, respondiendo diferencialmente a los cambios ambientales.

En concreto, podemos enumerar las siguientes conclusiones particulares:

Capítulo I

- La estructura poblacional de adultos se encuentra agregada en el espacio.
- Las hembras atraen a las aves dispersoras y mejoran el microhábitat bajo su copa, facilitando el establecimiento de plántulas en este microhábitat más que bajo machos, y bajo estos más que al descubierto, generando un patrón de autofacilitación sexualmente sesgada. Este patrón se ve reforzado, además, por la deposición natural de frutos no dispersados bajo copa de hembras.
- A lo largo de todo el ciclo vital, todos los estadíos vitales favorecen la regeneración bajo copa de árbol, y particularmente bajo hembras. Por tanto, existe un acoplamiento total entre estadíos vitales (*stage coupling*). Esto es muy infrecuente y, hasta donde sabemos, este es el primer caso descrito en ambientes mediterráneos.

Capítulo II

- Machos y hembras de *J. thurifera* invierten una cantidad similar de recursos al crecimiento vegetativo durante los primeros treinta años de su vida, hasta alcanzar la madurez sexual.
- A partir de ese momento ambos reducen su inversión vegetativa, aunque las hembras presentan una reducción significativamente mayor. Los machos de esta especie anemófila se beneficiarían de una inversión vegetativa mayor, ya que esto incrementaría su éxito polinizador, mientras que las hembras aumentarían más su eficacia reproductiva invirtiendo en reproducción.
- Machos y hembras asignan sus recursos con un patrón temporal diferente, debido a que los machos realizan su máxima inversión reproductiva durante el periodo de floración, mientras que las hembras realizan su máxima inversión inmediatamente después de la floración, cuando deben crecer y madurar los gálbulos carnosos.

Capítulo III

- El esfuerzo reproductivo interanual de hembras fue cuatro veces mayor que el de los machos. Si consideramos únicamente un año vecero, entonces el esfuerzo de hembras fue siete veces mayor.
- Machos y hembras se reprodujeron sincrónicamente con un patrón bianual de floración vecera.
- Sin embargo, no todos los episodios de floración vecera resultaron en una fructificación vecera. Los ciclos de fructificación femeninos fueron más largos y desperdiciaron la mayoría de los eventos de floración, generando un desacoplamiento en la vecería.
- Para comprender las causas del comportamiento vecero, el análisis del esfuerzo reproductivo de machos y hembras y de la inversión dedicada a la floración, el crecimiento de gálbulos y la fructificación de gálbulos es una aproximación más informativa que los habituales conteos de frutos.
- Los ciclos de vecería pueden estar condicionados por fuerzas evolutivas, sin embargo, nuestro trabajo sugiere que las hembras se benefician de "apuestas de pérdidas limitadas" (*bet hedging*) y absciden los gálbulos polinizados si los costos reproductivos se tornan demasiado altos, lo que indica que los costes reproductivos diferenciales juegan un rol determinante el la modulación de los ciclos de vecería en especies dioicas.

Capítulo IV

- Machos y hembras de *J. thurifera* presentan diferentes estrategias fisiológicas y funcionales.
- Cuando la disponibilidad de recursos fue incrementada de forma experimental, los machos dividieron los recursos adicionales entre varias funciones biológicas diferentes (crecimiento vegetativo, reproducción, mejora del aparato fotosintético).
- Por el contrario, las hembras almacenaron la mayoría de los recursos en forma de hojas, y solo invirtieron una pequeña proporción de estos en incrementar ligeramente la reproducción inmediata.
- Estas diferencias están presentes incluso durante los periodos en los que las demandas reproductivas de ambos sexos son similares (años no veceros), por lo que deben estar genéticamente fijados a cada sexo.

Capítulo V

- La altitud es el principal factor geográfico determinante de la baja tasa de viabilidad de semillas de *J. thurifera*.
- La precipitación es capaz de reducir la tasa de aborto de semillas, pero esta reducción suele estar acompañada de un incremento de magnitud similar en la predación predispersiva de semillas, lo que resulta en una tasa de viabilidad máxima determinada por la actividad de predadores de semillas.
- Sin embargo, el incremento en la disponibilidad de recursos (fertirrigación) fue capaz de aumentar la fertilidad global de las hembras incrementando el tamaño de cosecha, aunque la tasa de viabilidad de estos gálbulos se mantuvo similar.
- Si el cambio climático incrementa la temperatura media y reduce las precipitaciones en la cuenca mediterránea los bosques de *J. thurifera* se verán invadidos en su límite altitudinal inferior por especies competidoras de crecimiento rápido y por predadores de semillas que previamente no eran capaces de sobrevivir a mayor altitud. Por la otra parte, su migración altitudinal se verá limitada por la reducción en la fertilidad asociada con el incremento en la altitud.

En resumen, la dioecia es un carácter extremadamente significativo en la biología de *J. thurifera*. Machos y hembras modifican el ambiente de forma diferencial, generando dinámicas poblacionales y estructuras espaciales determinadas por el sexo. La diferencia en costos reproductivos entre machos y hembras genera diferentes compromisos de inversión de recursos, estrategias fisiológicas y funcionales bien diferenciadas para ambos sexos e incluso diferencias entre los ciclos reproductivos de ambos sexos. Además, la variación en la disponibilidad de recursos modifica la eficacia reproductiva de machos y hembras de forma diferente: los machos suelen distribuir los recursos adicionales entre diversas funciones biológicas, utilizando los recursos disponibles de forma más o menos inmediata; por su parte las hembras almacenan la mayor parte de esos recursos, demostrando tener una estrategia a largo plazo que les permite optimizar el compromiso entre la eficacia reproductiva presente y la supervivencia futura. Las hembras controlan el éxito del proceso reproductivo y son, por tanto, de importancia crítica frente a los cambios ambientales naturales o inducidos por la actividad humana.